STUDIES ON ANTIMICROBIAL EFFECT OF MARJORAM PLANT IN FOOD PRESERVATION

BY

AMIRA MOHAMED SHOKRY RIAD

B.Sc. Agric. Sc. (Food Technology), Cairo University, 1999

Under the supervision of:

Prof. Dr. Ahmed Yossef Gibrel

Professor of Food Science and Technology, Dep. of Food Science, Faculty of Agriculture, Ain Shams University (Principal supervisor)

Dr. Amal Ahmed Mohamed

Associate Prof. of Food Science and Technology, Dep. of Food Science, Faculty of Agric., Ain Shams University

Prof. Dr. Fathy Abd Elrazik Ali

Professor of Food Science and Technology, Agri Industrialization Unit, Desert Research Center, Cairo, Egypt.

STUDIES ON ANTIMICROBIAL EFFECT OF MARJORAM PLANT IN FOOD PRESERVATION

BY

AMIRA MOHAMED SHOKRY RIAD
B.Sc. Agric. Sc. (Food Technology), Cairo University, 1999

A thesis submitted in partial fulfillment
of
the requirements for the degree
of

MASTER OF SCIENCE

in

Agricultural Science
(Food Science and Technology)

Department of Food Science
Faculty of Agriculture
Ain Shams University

2005

Approval Sheet

STUDIES ON ANTIMICROBIAL EFFECT OF MARJORAM PLANT IN FOOD PRESERVATION

BY

AMIRA MOHAMED SHOKRY RIAD

B.Sc. Agric. Sc. (Food Technology), Cairo University, 1999

This thesis for M.Sc. degree has been approved by:

Prof. Dr. Salah Kamel El-Samahy ……
Prof. of Food Science& Technology, Faculty of Agric., Suez Canal Univ., Ismalia.

Prof. Dr. Yehia Abdel Razik Heikal …….
Prof. of Food Science & Technology, Faculty of Agric., Ain Shams Univ.

Dr. Amal Ahmed Mohamed ……..
Associate Prof. of Food Science & Technology, Faculty of Agric., Ain Shams Univ.

Prof. Dr. Ahmed Youssef Gibrel ……...
Prof. of Food Science & Technology, Faculty of Agric., Ain Shams Univ.

Date of examination: 06/07/2005

ACKNOWLEDGMENT

The author wishes to express her gratitude to **Prof. Dr. A.Y. Gibrel** Prof. of Food Science and Technology, Faculty of Agriculture, Ain Shams University, for suggesting the problem, continuous supervision, guidance and help through the investigation course and during the preparation of the manuscript.

I would like to express my deepest gratitude to **Prof. Dr. Fathi Abdel-Razik Ali,** Prof. of Food Science and Technology, Agri Industrialization unit, Desert Research Center for his supervision and valuable help during the investigation and preparation of the manuscript.

Thanks are also due to **Dr. Amal A. Hassan,** Associate Prof. of Food Science & Technology, Faculty of Agriculture, Ain Shams University for her valuable help, kind advice, her help during the laboratory work and her effort to finish up this work.

My deepest thanks to staff members of fermentation laboratory, Department of Food science and Technology for their cooperation and friendly atmosphere.

ABSTRACT

Amira Mohamed Shokry Riad. Studies on antimicrobial effect of marjoram plant in food preservation. Unpublished Master of Science Thesis in Agriculture (Food Science & Technology), Ain Shams University, Faculty of Agriculture, Department of Food Science, 2005.

The uses of chemical synthetic agents may be considered less desirable by a segment of the consuming public than are natural antimicrobial compounds. Thus, interests in using of natural antimicrobial compounds in foods have increased because of the growing demand in so called natural food.

From such point of view this study was undertaken to evaluate the chemical composition and antimicrobial activities of *Origanum majorana L.* (marjoram essential oil). The effect of storage conditions on physico-chemical properties of marjoram essential oil was investigated. The effect of addition marjoram essential oil concentrations on improving sensory properties of pickled cucumber was also studied. The obtained data showed that:

Marjoram essential oil was found to be rich in linalool (20.98%), limonene (16.78%), β-pinene (12.49%), *P*-cymene (10.88%), α-pinene (9.69%) and 1,8 cineol (6.84%). Also, It contained smaller quantities of terpinene-4-ol (1.92%), linalyl acetate (1.82%), α-terpinene (1.03%) and eugenol (0.99 %) . The identified compounds are representing 83.42% of the total essential oil.

Marjoram essential oil totally inhibited *Aspergillus niger, Aspergillus flavus, Fusarium moniliform, Penicillium expansum* at 300-400 ㎕ /100 ml, while 100 and 120 ㎕ / disc was required for *Pichia anomala* and *Rhodotorula minuta,* respectively and 100 and 160 ㎕ / disc for *Bacillus cereus* and *Escherichia coli*, respectively.

It was found that the oil samples, which were stored in brown glass bottles at refrigerator temperature were well preserved where the

values of specific gravity, refractive index, optical rotation, acid number and ester number were 0.8980, 1.458, 21º3`, 0.40 and 13.5, respectively.

A sensory testing of pickled cucumber proved that addition of marjoram essential oil concentrations (100-160 µl/ 100ml) gave higher scores for taste, texture, appearance, flavor and color than that of lower concentrations (60 and 80 µl/ 100ml). On the contrary, the control sample (without addition of marjoram essential oil) had the lowest scores for most sensory properties.

Key words: Marjoram essential oil, Antimicrobial activity, Physical and chemical properties, Cucumber pickling.

CONTENT

	Page
1. INTRODUCTION…….	1
2. REVIEW OF LITERATURE……	3
2.1. *Marjoram* (history, uses and medicinal action)……	3
2.2. Chemical composition of marjoram oil…….....	4
2.3. Antimicrobial activity of marjoram oil…….	10
2.4. Storage of marjoram essential oil …….	19
2.5. Use of marjoram essential oil in food processing…….	20
3. MATERIAL AND METHODS…….	26
Material …….	26
3.1. Plant material……	26
3.2. Chemical composition of marjoram essential oil……..	26
3.2.1. Extraction of marjoram essential oil……	26
3.2.2. Separation and identifications of marjoram essential oil components by GC ……	26
3.3. Antimicrobial activity of marjoram essential oil……..	27
3.31. Microorganisms …….	27
3.3.2. Media……..	27
3.3.3. Antifungal activity……..	28
3.3.4. Antibacterial and anti yeast activity……	29
3.4. Effect of storage condition on marjoram essential oil..	30
3.4.1. Type of containers……...	30
3.4.2. Storage temperature……	30
3.4.3. Determination of the physico-chemical properties.	30
3.5. Effect of marjoram essential oil on cucumber pickle…	31
3.5.1. Brining and fermentation procedures …….	31
3.6. Statistical Analysis……	32
4. RESULTS AND DISCUSSION……	33
4.1. Marjoram oil composition …….	33
4.2. Antimicrobial activity of marjoram essential oil……..	36
4.2.1. Antibacterial activity……...	36

Page

4.2.2. Antifungal activity of marjoram essential oil …… 39
4.3. Effect of the storage conditions on the physical and chemical characteristics of sweet marjoram essential oil during storage…… 46
4.3.1. Effect of the temperature on the physical and chemical properties of marjoram oil during the storage for 8 months…….. 49
4.3.1.1. Specific gravity:……... 49
4.3.1.2. Refractive index ……..... 51
4.3.1.3. Optical rotation ……... 54
4.3.1.4. Ester number ……... 56
4.3.1.5. Acid number……. 58
4.4. Utilizations of marjoram oil in cucumber pickling…... 58
4.4.1. Effect of marjoram oil concentrations on titratatble acidity and pH values …… 58
4.4.1.1. Titratatble acidity of cucumber pickles 58
4.4.1.2. pH values of cucumber pickles 63
4.4.2. Effect of marjoram oil on microflora of pickled cucumber……... 66
4.4.2.1. Lactic acid bacteria count…….. 67
4.4.2.2. Yeasts and molds counts …….. 67
4.4.3. Organoleptic evaluation of pickled cucumber…… 72
5. SUMMARY……... 78
6. REFERENCES……... 83
ARABIC SUMMARY……...

LIST OF TABLES

Table No.		Page
1	Percentage composition of the marjoram essential oil compounds……	35
2	Zones of growth inhibition (mm) showing by the antibacterial activity of marjoram essential oil at different concentrations……..	37
3	Percentage of inhibition of growth of the mycelium by marjoram essential oil at different concentrations in the culture medium (µl/ 100/ml) against fungal strain…….....	42
4	Zones of growth inhibition (mm) showing by activity of marjoram essential oil at different concentrations µl/disc against yeast strain……	42
5	Effect of storage period, storage temperature and type of container on specific gravity of marjoram oil……	50
6	Effect of storage period, storage temperature and type of container on refractive index of marjoram oil……...	52
7	Effect of storage period, storage temperature and kind of container on optical rotation of marjoram oil……..……..	54
8	Effect of storage period, storage temperature and type of container on ester number of marjoram oil……	56
9	Effect of storage period, storage temperature and type of container on acid number of marjoram oil……..	59
10	Changes in titratable acidity (%) during storage of pickled cucumber samples containing different concentrations (ml/100 ml) of marjoram essential oil……...	61
11	Changes in pH values during storage of pickled cucumber samples containing different concentrations (ml/100 ml) of marjoram essential oil……..	64

Table No.		Page
12	Effect of marjoram essential oil concentrations on lactic acid bacterial counts (cfu/ml brine) of pickled cucumber during storage at room temperature…….....	68
13	Effect of marjoram essential oil concentrations on yeast and mold counts (cfu/ ml brine) of pickled cucumber during storage at room temperature…...	70
14	Effect of marjoram essential oil concentrations (ml/100 ml) on taste of pickled cucumber during storage at room temperature……...	73
15	Effect of marjoram essential oil concentrations (ml/100 ml) on texture of pickled cucumber during storage at room temperature……...	74
16	Effect of marjoram essential oil concentrations (ml/100 ml) on appearance of pickled cucumber during storage at room temperature……...	75
17	Effect of marjoram essential oil concentrations (ml/100 ml) on flavor of pickled cucumber during storage at room temperature……...	76
18	Effect of marjoram essential oil concentrations (ml/100 ml) on color of pickled cucumber during storage at room temperature……...	77

LIST OF FIGURES

Fig. No.		Page
Fig. 1	Chemical composition of marjoram essential oil by gas chromatography…….	34
Fig. 2	Effect of marjoram essential oil on the inhibition of *Bacillus cereus* growth at different concentrations…….	38
Fig. 3	Effect of marjoram essential oil on the inhibition of *Escherichia coli* growth at different concentrations.	38
Fig. 4	Effect of different concentrations of marjoram essential oil on growth of bacterial strains (in vitro) ……..	40
Fig. 5	Effect of marjoram essential oil on the inhibition of molds growth at different concentrations, A: *Aspergillus niger* and B.: *Penicillum expansum*…….....	43
Fig. 5. Cont.	Effect of marjoram essential oil on the inhibition of molds growth at different concentrations,, C: *Fusarium moniliform* and D.: *Aspergillus flavus*……..	44
Fig. 6	Effect of different concentrations of marjoram essential oil on growth of fungal strains (in vitro)……...	45
Fig. 7	Effect of marjoram essential oil on the inhibition of yeasts growth at different concentrations, E: *Pichia anomala* and F: *Rhodotorula minuta*……..	47
Fig. 8	Effect of different concentrations of marjoram essential oil on growth of yeast strains (in vitro)…….....	48

Fig. No.		Page
Fig. 9	Effect of storage period and type of container on specific gravity of marjoram essential oil at room temperature……	50
Fig. 10	Effect of storage period and type of container on specific gravity of marjoram essential oil at refrigerator temperature……..	51
Fig. 11	Effect of storage period and type of container on refractive index of marjoram essential oil at room temperature……	53
Fig. 12	Effect of storage period and type of container on refractive index of marjoram essential oil at refrigerator temperature……..	53
Fig. 13	Effect of storage period and type of container on optical rotation of marjoram essential oil at room temperature……	55
Fig. 14	Effect of storage period and type of container on optical rotation of marjoram essential oil at refrigerator temperature……..	55
Fig. 15	Effect of storage period and type of container on ester number of marjoram essential oil at room temperature……	57
Fig. 16	Effect of storage period and type of container on ester number of marjoram essential oil at refrigerator temperature……..	57
Fig. 17	Effect of storage period and type of container on acid number of marjoram essential oil at room temperature……	60
Fig. 18	Effect of storage period and type of container on acid number of marjoram essential oil at refrigerator temperature……..	60

Fig. No.		Page
Fig. 19	Changes in titratable acidity of pickled cucumber containing different concentrations of marjoram essential oil……....	62
Fig. 20	Changes in pH values of pickled cucumber containing different concentrations of marjoram essential oil…….	65

1. INTRODUCTION

Recently, there has been an increasing interest in the finding of new natural antimicrobial compounds due to negative consumer perceptions of artificial preservatives.

The leafy part of plants belong to the *lamiaceae* family, has been added to meat, fish and food products for years. In addition to improving the flavor, certain spices and essential oils prolong the storage life of foods by an antimicrobial activity. Being natural foodstuffs, they appeal to consumers who tend to question the safety of synthetic additives **(Farag *et al* 1989).**

Essential oil extracts of various plants have been reported to have inhibitory effects against diverse types of microorganisms including gram-positive bacteria, gram-negative bacteria, fungi and viruses **(Morris *et al* 1979).**

The safe use of herbs and spices and their essential oils has led to their current status of Generally Recognized as Safe (GRAS) Food ingredients **(Beuchat, 1994)**.

Marjoram (*Origanum majorana* L.) is a widely known, old spice. It was cultivated as the secret plant of Osiris in ancient Egypt. It was the symbol of happiness in the Greek and Roman empires.

Marjoram is hardly perennial and herbaceous plant which grows wild in its natural areas: Egypt and eastern Mediterranean countries. It belongs to mint family (*lamiaceae*). It has dark green oval leaves and small grayish white flowers in cluster **(Furia and Bellance, 1971).**

Commercial *Origanum majorana* L. is used as a spice and condiment. The volatile aromatic compounds are employed in the food industry as a spice in sausages. It is also in baked goods, processed vegetables, condiments soups, snake foods and gravies. Also, Egyptian marjoram oil used in perfumery for its herbaceous notes, pharmacology medical (as one of the best antispasmodics, antiviral, analgesic, antiseptics, marjoram tea helps case of bad colds, has tranquilizing

effect on nerves, and helps settle upset stomachs) and clinical microbiology, phytopathology and food preservation **(Reineccius, 1994 and Circella *et al* 1995).**

The aim of this study was as follows:

1- Determination of the chemical composition of marjoram oil.
2- Study the antimicrobial activity of marjoram oil.
3- Study the best conditions for storage marjoram oil.
4- Utilization of marjoram essential oil in real food system (cucumber pickling).