Food and Public Health 2014, 4(3): 110-122
DOI: 10.5923/j.fph.20140403.07

In vivo Effect of Essential Oils from Laurus Nobilis, Anethum Graveolens and Mentha Piperita on Mycobiota Associated with Domiati Cheese During Storage

Mady A. Ismail[1], Al-Zahraa M. Darwish[2], Nemmat A. Hussein[1], Soumia M. Darwish[3,*]

[1]Department of Botany & Microbiology, Faculty of Science, Assiut University, Egypt
[2]Department of Dairy Science, Faculty of Agriculture, Assiut University, Egypt
[3]Department of Food Science and Technology, Faculty of Agriculture, Assiut University, Egypt[1]

Abstract Three concentrations (3, 5, and 7 ml/100g retentate) of each of three natural oils were added during manufacturing of low salt white cheese. The mycobiota of cheese were assessed after 8 hours, 3 weeks, 7 weeks and 11 weeks. Twelve species were isolated (*Aspergillus flavus, A. fumigatus*, *A. niger*, *A. versicolor*, *Penicillium aurantiogriseum, P. camembertii, P. griseofulvum, P. islandicum, P. oxalicum, P. restrictum*, *Ulocladium atrum* and the yeast species *Debaryomyces hansenii*). The total counts of fungi increased in *Laurus nobilis* oil-treated cheese at the three concentrations after 3 weeks compared with control, but decreased after 7 weeks in treatment with 3 and 7% of oil concentrations. Cheese treated with *Anethum graveolens* oil at 3% concentration showed the highest fungal counts after 7 weeks of storage. The fungal counts decreased by increasing all concentrations of *Mentha piperita* oil (3, 5 and 7%). Generally, treatment of cheese with *M. piperita* oil significantly decreased the total counts of fungi. On the other hand, oils of *L. nobilis* and *A. graveolens* at 3 to 7% concentrations caused an increase of total counts after 3 and 11 weeks but *L. nobilis* and *A. graveolens* oils caused a decrease after 7 weeks at 5 and 7% concentrations compared to control,. Isolates of *Aspergillus flavus* screened for aflatoxin production using Coconut agar medium (CAM) were positive foraflatoxin B production when observed at 365 nm UV light.

Keywords Domiati cheese, Essential oils, Laurus nobilis, Anethum graveolens, Mentha piperita, GC-MS, Fungi, Aspergillus, Penicillium, Aflatoxin

1. Introduction

Cheese is highly nutritious as it provides protein, calcium, zinc and vitamins that are vital to good health (Kosikowski & Mistry, 1997). Domiati cheese is one of the most popular soft white cheeses in Egypt which is consumed fresh or after pickling for some months. This type of cheese is possibly made at home by small milk producers since it does not need complicated equipments. The pickled cheese could be stored at room temperature up to 6 months without deterioration (AbdEl-Kader, 1999). In Egypt, the salt content in cheese could reach up to 7 g salt/100 g (Ceylan *et al.*, 2003; Elsanhoty *et al.*, 2009). Salt (sodium chloride) is an important factor for two major roles: it acts as preserving agent and it contributes to the sensory (quality) of the cheese. Recently with increased prevalence of heart disease and blood pressure, increasing trend consumers to foods which was containing a small amount of salt so manufacturing tended to produce low salt products. In low salt content cheese, some microorganisms may grow and produce uncharacteristic visual appearance and diminish the commercial value of the cheese, and result in a reduction in quality and quantity (Soliman & Badeaa, 2002), therefore preservatives should be added to stop the activity of these microorganisms (Pintado *et al.* 2010).Additionally, fungi can grow in cheese and defect the color and aroma cheese (Erdogan *et al.*, 2001).Growth of fungi causes spoilage and occurs sporadically on surface of hard cheese and packaged cheese (Hocking & Faedo, 1992). One of the modern ways to improve the hygienic safety of manufactured food products is to exploit the antimicrobial properties of natural plant extracts, allowing for the reduction of the use of preservatives, which constitute a potential human health hazard. Essential oils have been known for a long time and continue to be the subject of several studies that evaluate their microbial potential as alternatives to chemical agents in food industries (Djenane *et al.*, 2012). Laurel (*Laurus nobili* L.) is rich in essential oil which is reported as toxic to many microorganisms (Hassiotis & Dina, 2011). Dill (*Anethum graveolens* L.) oil has also been reported to possess antibacterial (Rafii and Shahverdi, 2007) and antifungal

* Corresponding author:
soumiadarwish123@hotmail.com (Soumia M. Darwish)
Published online at http://journal.sapub.org/fph

drugs and food preservatives (Tian*et al*., 2011). Mint (*Mentha piperita* L.) oil also showed antifungal activity against *Aspergillus niger, Alternaria alternata* and *Fusarium chlamydosporum* (Lirio *et al.*, 1998; Aqil *et al.*, 2001).

Yeasts are found within the surface microflora of many cheese types. Within 2 or 3 days of ripening, growth of salt-tolerant yeasts appears on the cheese surface. However, occasionally fungal growth discolours the cheese surface and affects the flavour. During fungal growth a risk of mycotoxin production in the cheese exists. For example Northolt *et al.* (1980) investigated cheeses infected by *Aspergillus versicolor* and contained sterigmatocystin.

Taxonomic and phylogenetic overviews of the most important filamentous cheese fungi were presented by Roparset *al.* (2012). Some fungi, such as, *P. camemberti, F. domesticum*, *Scopulariopsis flava* and *S. casei*, are only known from cheeses and are probably adapted to this particular habitat, which is extremely rich in protein and fat. Other cheese fungi are ubiquitous, such as, *P. roqueforti, Scopulariopsis candida* and *Scopulariopsis fusca* (Roparset *al.* 2012).

The present study aimed at evaluating the effect of three essential oils from *Laurus nobilis*, *Anethum graveolens* and *Mentha piperita* on the organoleptic characteristics and mycobiota that might be associated with low salt white soft cheese during storage. Chemical analysis of these oils to evaluate their major components was also targeted.

2. Materials and Methods

Source of *Laurus nobilis, Anethum graveolens* and *Mentha piperita* oils

The oil extracts of Laurel (*Laurus nobili* L., Family Lauraceae) *L. nobilis,* Dill (*Anethum graveolens* L., Family Apiaceae) and Mint (*Mentha piperita* L., Family Lauraceae) were purchased from plants medical group, Cairo, Egypt.

Cheese making

Fresh raw buffaloe's milk having 13.4% total solids, 4.90% fat, 3.60% protein, and 0.18% titratable acidity was obtained from private farm in Assiut Governorate during the month of November 2012. Commercial liquid calf rennet and commercial salt were obtained from local markets. Domiati cheese was made as described by Fahmi and Sharara (1950). Milk retentate was divided into 10 portions; each portion was salted to a concentration of 3%, well mixed and pasteurized at 73°C for 15 sec. First portion was served as control and for the other nine portions, different concentrations of *L. nobilis, A. graveolens* and *M. piperita* essential oils (3, 5, and 7 ml / 100g retentate) were added at 40°C to prepare cheese treatments. Curds were hold at the same temperature after adding the rennet, cheeses samples were taken for analysis at intervals during storage in refrigerator (5±1°C) for up to 11 weeks. Three replicates were prepared for each cheese sample to determine their mycological analysis and organoleptic properties were also assessed.

Chemical analysis of oils using Gas chromatography-mass spectrometry (GC-MS)

The essential oils were analyzed using Gas Chromatography/Mass Spectrometry (GC/MS). A Hewlett-Packard (HP) system 6890 series gas chromatograph coupled with a HP model 5975B quadruples mass spectrometer; cross-linked 5% phenyl methyl siloxane capillary column (HP-5MS, 30 m x 0.25 mm x 0.25 µm film thickness) was used. GC operating conditions were as follows: initial temperature 40ºC (1 min hold), increased at 20ºC min^{-1} to 210ºC, then increased at 1.5ºC min^{-1} to 215ºC (4 min hold); injector temperature 240ºC; carrier gas Helium (99.999%), flow-rate 1.3 ml^{-1} min; ion source temperature 270ºC; operated in the split less mode; purge off time 1 min; injection volume 1 µl. MS operating conditions were: solvent delay 6 min; electron-impact (EI) mode ionization voltage 70 eV using selected ion monitoring (SIM); dwell time for each ion 100 ms (EPA 1998).

Sensory analysis

Fifteen panelists (7 males and 8 females, aged between 25 and 45 years) who have experience with white cheese and regularly used its descriptive vocabulary, were participated. The cheese samples were scored for flavor (50 points), body and texture (40 points) and appearance and colour (10 points) (Larmond 1977). Panel members were also instructed to report any defects or unpleasant flavor.

Fungal assessment

Three concentrations (3, 5, and 7 ml/ 100g retentate) of each of three natural oils were added at the beginning of manufacturing of low salt white cheese. A sample of cheese without natural oils was used as a control. Cheese samples were analysed firstly at the beginning of manufacturing (8 hours after adding oil), then after 3, 7 and 11 weeks (until cheese got spoiled). Dilution-plate method was used for isolation of fungi. Dichloran rose-bengal chloramphenicol agar (DRBC) was used as a general medium for isolation of fungi (King et al. 1979). 1 ml of an appropriate dilution of an in vitro manufactured low-salt white cheese sample was transferred into sterilized petri dish, then 15-20 ml sterilized molten DRBC medium were poured. The plates were incubated at 28ºC for 7-10 days. The fungal colonies were identified based on morphological and microscopic characters according to Pitt and Hocking (2009). Microscopic examination of preparations of the different fungal species stained with lactophenol cotton blue were carried out. Yeasts were identified by molecular methods. The ITS sequences of nuclear rDNA were amplified using ITS1 ((5' - TCC GTA GGT GAA CCT GCG G - 3'),, and ITS4 ((5'- TCC TCC GCT TAT TGA TAT GC -3') primers in SolGent company (Daejeon, Stouth Korea). Contigs were created from the sequence dat using CLC Bio Main Workbench program. The sequence obtained from the isolate was further analyzed using BLAST from the National Center of Biotechnology Information (NCBI) website. Sequences

obtained were subjected to Clustal W analysis using MegAlign (DNAStar) software version 5.05 for the phylogenetic analysis and the phylogenitic tree was constructed.

Table 1. The major components and their percentages of *Laurus nobilis* oil, as revealed byGC- MS analysis

	Compound	% of total constituents	Fungi*	Reference
1	**γ- cadinene**	10.673	dermatophytes *Candida tropicalis* *Candida albicans* *C. krusei*	Policegoudra *et al.*(2012) Costa *et al.*(2013) Rodrigues *et al.*(2012)
2	**Caryophylla- 3,8 (13)-Dien-5-α**	8.618	*Candida albicans*	Kurtulmus *et al.*(2009)
3	**trans-caryophyl lene**	6.385	*Aspergillus niger* *A. ochraceus* *A. ustus*	Bhuiyan *et al.*(2011)
4	**7,8- Epoxy- α-Ionone**	2.913	*Candida glabrata* *C. kensei* *C. parapsileosis*	Liouane *et al.*(2010)
5	**β–Citronellol**	2.320	Antifungal	Kalemba and kunicka (2003)
6	**(Z - E) Alpha – Farnesene**	2.140	Antifungal	El-Sayed *et al.*(2013)
7	**Methoxyeugenol**	2.064	*Aspergillus flavus* *A. flavus* *A. niger* *A. ochraceus* *A. terreus* *Penicillium citrinum* *P. viridicatum*	Sudhakar *et al.*(2009) Singh *et al.*(2005)
8	**3,7Guaiadiene**	1.715	*Aspergillus niger* *A. ochraceus* *A. ustus*	Bhuiyan *et al.*(2011)
9	**Germacra- 1(10),4,11(13)-Trien-12-Oic Acid**	1.532	--	
10	**α- Humulene**	1.493	Servel phytopathogens	Hossain *et al.*(2008)
11	**camphor**	1.382	*Aspergillus flavus* *A. fumigatus* *A. niger*	Zuzarte *et al.*(2009)
12	**Palmitic acid**	1.321	*Aspergillus niger* *A. terreus*	Agoramoorthy *et al.*(2007)
13	**Tetradecanoic Acid**	0.923	Antifungal	Ogunlesi *et al.*(2010)
14	**β-elemene**	0.897	*Aspergillus niger* *A, ochraceus* *A. ustus*	Bhuiyan *et al.*(2011)
15	**Elemol**	0.754	*Aspergillus niger* *A. ochraceus* *A. ustus* .	Bhuiyan *et al.*(2011)
16	**Cis-α-Copaene- 8-Ol**	0.725	--	
17	**β–Eudesmol**	0.651	*Aspergillus fumigatus* *A. niger*	Ben Mansoura *et al.*(2013)
18	**4-Nonylphenol**	0.643	--	Guart *et al.*(2013)
19	**Vitamin E Tocopherol**	0.637	*Aspergillus flavus* *A. fumigatus* *A. niger* *A.terreus*	Arnusch *et al.*(2012)
20	**caryophyllene oxide**	0.546	*Aspergillus flavus* *A. fumigatus* *A. niger* *A. restrictus* *Penicillium digitatum* *P. chrysogenum* *P. italicum*	Licina *et al.*(2013)
21	**Elemenal**	0.529	--	

* The fungi that have been documented to be affect by the different components of *Laurus nobilis*oil.

Table 2. The major components and their percentages of *Anethum graveolen* oil, as revealed byGC- MS analysis

	No. Compound	% of total constituents	Fungi*	Reference
1	Parsley Camphor	31.946	*Aspergillus flavus* *A. fumigatus* *A.niger*	Zuzarte *et al.*(2013)
2	Carvone	8.279	*Asperigallus niger* *Penecillium digitatum*	El- Seedi *et al.*(2012)
3	α- Bergamotene	3.147	*Mucor* sp.	Malele *et al.*(2003)
4	β-Sesquiphellandrene	2.525	*Several Human pathogens*	Bisht *et al.*(2010) Ibrahim *et al.*(2009)
5	Dihydrocarvone	1.535	*Candida albicans* *C. parapsilosis*	Orhan *et al.*(2012)
6	γ- Tocopherol	1.530	*Penicillium verucosum* *var. cyclopium*	Reis *et al.*(2012)
7	Linoleic Acid	1.428	*Aspergillus Flavus*	Al–Qarawi *et al.*(2013)
8	Anise Camphor (Anethole)	1.303	*Aspergillus flavus* *A. niger* *A. ochraceus* *A. parasiticus* *A. ustus*	Rahman *et al.* (2012)
9	Oleic Acid	1.155	*Penicillium verrucosum* Several phytopathogens *Aspergillus niger*	Moro *et al.*(2013) Rahman and Amzad (2010) Matan and Ketsa (2012)
10	Oleic Acid	1.155	Several phytopathogens	Rizzello *et al.*(2013)
11	Gingerol	0.756	*Several phytopathogens*	Mostafa *et al.*(2012)
12	Farnesene Epoxide	0.758	antifungal	Arkoudis and Stratakis (2008)
13	α- Patchoulene	0.718	*Candida albicans.*	Oladosa *et al.*(2009)

* The fungi that have been documented to be affect by the different components of *Anethum graveolen* oil.

Table 3. The major components and their percentages of *Mentha peprinta* oil, as revealed by GC- MS analysis

	No. Compound	% of total constituents	Fungi*	Reference
1	Menthol	43.436	Antifungal Several phytopathogens	Pattnaik *et al.*(1997) Chessa *et al.*(2013) Al- Mughrabi *et al.*(2013)
2	P-Propenylanisole	16.298	Antifungal	Al-Fatimi *et al.*(2010)
3	Anise Camphor	16.241	Several phytopathogens	Basile *et al.*(2013)
4	Carvone	7.847	*Aspergillus tubingensis* *A. carbonarius* *Penicillium* sp.	Morcia *et al.*(2012)
5	L -Menthone	4.738	*Aspergillus flavus* *A. niger* *A. ochraceus* *A. terreus* *A. versicolor* *Penicillium ochrochloron* *P. funiculosum*	Sokovic *et al.*(2009)
6	Tranes-Carane	2.220	*Candida albicans*	Nikitina *et al.*(2012) Nikitina *et al.*(2010)
7	L-Caryophyllene	1.206	*Candida tropicalis*	Costa *et al.*(2013)
8	Valerenol	0.979	Antifungal	Majiene *et al.*(2004)
9	α-Copaene	0.790	*Aspergillus flavus* *A. niger* *A. ochraceus* *A. parasiticus* *A. ustus*	Rahman *et al.* (2012)
10	α–Cumyle Alcohol	0.676	*Aspergillus. ochraceus* *Penicillium viridicatum* -	Singh *et al.*(2004)
11	Terpineol	0.638	*Aspergillus ochraceus* *Penicillium viridcatum* *Aspergillus niger* *Penicillium chrysogenum*	Singh *et al.*(2013) Shah *et al.*(2013)
12	Valencene	0.621	Antifungal	Mazimba *et al.*(2012)

* The fungi that have been documented to be affect by the different components of Mentha peprinta oil.

Table 4. Organoleptic characteristics of domiati cheese furcated with different concentration of ***Laurus nobilis, Anethum graveolens*** and ***Mentha piperita*** oils

Cheese	**zero time**				**after three weeks**				**after seven weeks**				**after eleven weeks**			
	Flavor	Body and Texture	Appearance and Color	Total	Flavor	Body and Texture	Appearance and Color	Total	Flavor	Body and Texture	Appearance and Color	Total	Flavor	Body and Texture	Appearance and Color	Total
	(50 Points)	(40 Points)	(10 Points)	(100)	(50 Points)	(40 Points)	(10 Points)	(100)	(50 Points)	(40 Points)	(10 Points)	(100)	(50 Points)	(40 Points)	(10 Points)	(100)
								***Laurus nobilis* oil**								
Control	45±1.67	37±1.63	10±0.41	92	42±1.63	36±1.26	10±0.52	88	38±1.10	32±1.10	9±0.89	79	32±1.67	28±2.45	7±1.26	67
3 %	42±2.10	35±1.10	9±0.89	86	39±1.55	35±2.10	9±0.63	83	36±2.07	30±2.10	8±1.26	74	31±1.67	28±1.26	6±1.26	65
5 %	36±1.79	35±1.41	9±1.17	80	33±2.45	34±1.79	9±0.89	76	30±2.19	29±1.67	8±1.41	67	20±1.10	25±2.28	6±1.26	51
7 %	28±2.53	32±0.98	9±1.10	69	30±1.55	33±1.79	9±0.63	72	27±1.90	20±2.19	7±1.51	54	15±2.53	18±1.26	5±1.26	38
								***Anethum graveolens* oil**								
Control	45±1.67	37±1.63	10±0.41	92	42±1.63	36±1.26	10±0.52	88	38±1.10	32±1.10	9±0.89	79	32±1.67	28±2.45	7±1.26	67
3 %	47±1.79	37±1.79	9±1.26	93	46±1.51	36±2.51	9±0.89	91	40±2.10	32±2.28	9±0.89	81	30±2.20	32±1.79	6±1.26	68
5 %	42±0.82	36±2.32	9±0.63	87	41±2.97	34±2.83	9±0.89	84	38±1.55	30±1.67	8±0.89	76	29±1.26	28±2.74	6±1.26	63
7 %	40±1.67	35±1.67	9±0.89	84	37±1.17	33±3.85	8±1.41	78	35±2.42	29±2.93	7±1.26	71	25±1.26	25±2.83	5±1.67	55
								***Menthapeprinta* oil**								
Control	45±1.67	37±1.63	10±0.41	92	42±1.63	36±1.26	10±0.52	88	38±1.10	32±1.10	9±0.89	79	32±1.67	28±2.45	7±1.26	67
3 %	40±1.41	35±3.03	9±2.10	84	38±1.10	33±2.53	9±1.79	80	31±1.67	27±1.86	7±0.89	65	28±1.47	25±2.19	6±1.67	59
5 %	34±2.10	35±2.28	9±1.26	78	30±1.26	33±4.03	9±1.90	72	29±1.72	24±3.03	7±1.26	60	20±1.67	20±1.26	6±1.47	46
7 %	25±1.26	30±2.90	8±2.10	63	23±1.79	30±3.03	8±1.41	61	18±2.88	20±2.10	6±1.26	44	15±1.86	19±2.19	5±1.10	39

±SD = standard Deviation

Screening for aflatoxins

Seven isolates of *Aspergillus flavus* (3) and some other fungal species (4) collected in this study were screened for aflatoxin production using coconut agar medium (CAM). CAM was prepared according to Davies *et al.* (1987) as follows; 100 g of shredded coconut was homogenized for 5 min with 300 ml hot distilled water, then the homogenate was filtered through four layers of cheese cloth, completed into 1000 ml by distilled water, the pH of the clear filtrate was adjusted to pH 7 and 20 g agar were added. The medium was autoclaved for 15 min at 121ºC, cooled to about 40-45ºC, and poured while being stirred into sterile Petri-dishes. Fungal isolates were inoculated at the center of CAM agar plates and incubated at 25ºC in the dark for 7 days. Cultures were observed for blue fluorescence under long-wave (365 nm) UV light. An uninoculated plate was observed as a reference.

Statistical analysis

Statistical analysis of experimental data was performed by analysis of variance (ANOVA) producers using SPSS version 9.0 program to examine statistical significance differences of sensory analysis means of experimental data. Results were considered statistically significant when $p < 0.05$. Mean ± standard deviation values were also presented.

3. Results and Discussion

GC-MS analysis of oils

Using the essential oil (EO) as a natural preservative have been increased the researches provided that the biologically active compounds in EO have been seen as a potential way to control fungal contamination. The present study had assessed potential antifungal activity of essential oils *Laurus nobilis*, *Anethum graveolens* and *Mentha piperita*.

GC-MC analysis of *L. nobilis* oil resulted in 21 major components with concentrations ranging from 10.7% (for γ-cadinene) to 0.5% (for elemenal). These components and their concentrations as revealed by GC-MS analysis in the current study along with their antifungal effects (Kurtulmus *et al*., 2009 & Rodrigues *et al*., 2012 & Policegoudra *et al.*, 2012 & Costa *et al*., 2013).

Also, GC-MS analysis of *A. graveolens* oil resulted in 13 major components with concentrations ranging from 31.9% (for parsley camphor) to 0.7% (for α- patchoulene). These components and their concentrations as revealed by GC-MS analysis are presented in table (2). The antifungal effects reported earlier by these components were also presented in table (2).

GC-MC analysis of *M. piperita* oil resulted in 12 major components with concentrations ranging from 43.4 % (for menthol) to 0.6% (for valencene). These components and their concentrations as revealed by GC-MS analysis in the current study along with their antifungal effects as revealed by different authors are reported in table (3).

Organoleptic properties of domiati cheese treated with *Laurus nobilis*, *Anethum graveolens* and *Mentha piperita* oils.

Statistical analysis of organoleptic properties of domiati cheese treated with *L. nobilis* showed that there were significant ($P<0.05$) differences in flavor among all *L. nobilis* concentration and among all storage time. Body and texture showed significant ($P<0.05$) differences among all *L. nobilis* concentration and among all storage time except between zero time and 3 weeks had no significant differences. Appearance and color showed no significant differences among all *L. nobilis* concentrations but there were significant ($P<0.05$) differences between all storage times except between zero time and 3 weeks.

Statistical analysis of organoleptic properties of domiati cheese treated with *Anethum graveolens* revealed significant ($P<0.05$) differences in flavor among all *A. graveolens* concentration and among all storage time. In body and texture, there were significant ($P<0.05$) differences among all *A. graveolens* concentrations except between control and 5%, and there were significant ($P<0.05$) differences between all storage time. On the other hand, there were significant ($P<0.05$) differences in appearance and color among all *A. graveolens* concentration except among 3% & 5% and control & 3%, and there were significant ($P<0.05$) differences among all storage time except between zero time and 3 weeks storage.

Statistical analysis of organoleptic properties of domiati cheese treated with *Mentha piperita* revealed significant ($P<0.05$) differences in flavor among all *M. piperita* concentrations and between all storage periods for cheese. In body and texture, there were significant ($P<0.05$) differences among all concentrations and among all storage periods except between zero time and 3 weeks storage. On the other hand, appearance and color showed significant ($P<0.05$) differences among all concentrations except between concentrations 3% and 5%, and there were significant ($P<0.05$) differences among all storage periods except between zero time and 3 weeks.

Fungal assessment

Twelve species belonging to four genera of fungi were isolated and identified during this work. These were *Aspergillus* (represented by *A. flavus, A. fumigatus*, *A. niger* and *A. versicolor*), *Penicillium* (*P. aurantiogriseum, P. camembertii, P. griseofulvum, P. islandicum, P. oxalicum, P. restrictum*), *Ulocladium atrum* and the yeast species, *Debaryomyces hansenii*. The yeast species was identified molecularly and a phylogenitic tree was constructed (Figure 1). Our isolate (AUMC 9300) has similarities of 98.03-99.67% with Genbank numbers JN851059, HF545662, GQ458041, AF210327 and JQ425358). Two genera, *Aspergillus* (3 species) and *Debaryomyces* (*D. hansenii*) were isolated in control samples, while species of *Penicillium* and *Ulocladium* were not detected. Members of *Penicillium* (*P. brevicompactum, P. chrysogenum, P.*

*paxilli*and *P. waksmanii*) and *Aspergillus* (*A. flavus, A. niger*and *A. oryzae*) were the most prevalent fungi on cheese samples together with unknown yeasts (Nasser, 2001). *Debaryomyces hansenii* was reported as the most frequently isolated yeast in cheese and yoghurt samples and it was stated that it plays an important role in the stages of the ripening process of cheese and production of taste and flavor (Beresford *et al*. 2001 and Fadda *et al.* 2004), however, Moreira, *et al.* (2001) found that there was no association between presence and absence of flavouring of yoghurt and level of yeasts.

Fadda, *et al.* (2004) found that mean $\log_{10}$ yeast counts in Fiore Sardo cheese increased less than 1 unit during the first 48 h of production with respect to the initial number in the milk (from 2.64±1 to 3.06±0.9 $\log_{10}$ CFU g^{-1}), then they gradually decreased to a level of 0.65±1 $\log_{10}$ CFU g^{-1} at 9 months of ripening and the most numerous yeast species were detected in 48-h-old cheese. Yeasts are also found within the surface microflora of many cheese types. Yeasts either are characterized by the ability to ferment glucose, to utilize lactate and to increase pH values and responsible for alcoholic, acidic, fruity or fermented odours (*Clavispora lusitaniae, Pichiajadinii* and *Williopsis californica*), or non-fermenting species which utilize lactate (*Galactomyces geotrichum, Trichosporon ovoides* and *Yarrowia lipolytica*). These yeasts yield a cheesy aroma (Wyder and Puhan, 1999). In principal, increased diversity could lead to increased mutualism in breaking down and utilizing the food substrate and thus enhancing spoilage (Morier*aet al.* 2001).

Effect of *Laurus nobilis* oil on the mycobiota of domiati cheese

Three genera and 6 species were isolated from cheese treated with *L.nobilis* oil. The most common species was the yeast *D. hansenii* followed by *Aspergillus* (*A. flavus*, *A. fumigatus* and *A. niger*) and *Penicillium* (*P. griseofulvum* and *P. islandicum*) (Table 5). Table (8) showed highly significant increase in total counts of fungi in treatments after 3 and 11weeks comparing with control due to the large numbers of yeast species appeared. After 7 weeks, the total fungal counts were decreased significantly and high significantly in cheese treated with 7 and 3% of oil concentrations, respectively. 5% concentration showed increase in the number of yeasts, Also, yeast numbers increased by treatment with 7% oil after 11 weeks comparing with control (Tables 5 & 8).

Several investigators have reported that the dominant fungi isolated from cheese were *Penicillium* spp. Northolt *et al.* (1980) identified 62% of 208 isolates as *Penicillium* and the most commonly identified species was *P. verrucosum* var. *cyclopium.* Aran and Eke (1987) also found *P. verrucosum* var. *cyclopium* as the dominant species on cheese. However, Tsai *et al.* (1988) identified 67% of 263 *Penicillium* isolates from cheese as *P. roqueforti* and Northolt and Soentoro (1988) reported that the dominant species was *P. aurantiogriseum.* Lund and Filtenborg. (1995) found that the fungi isolated from cheese belonged to the genera *Penicillium, Aspergillus, Verticillium, Mucor, Fusarium, Cladosporium* and *Scopulariopsis* and 88% of 371 isolates collected belonged to species of *Penicillium* and *Aspergillus versicolor*. Aran and Eke (1987) and Kivanc (1992) recorded high frequency isolation of *Penicillium* spp. from cheeses. *Aspergillus versicolor* often totally dominated the airborne conidia in the ripening rooms but rarely grew on the cheeses. *Penicillium* was isolated also from yoghurt (Moreira, *et al.,* 2001).

Effect of *Anethumgraveolens oilon* the mycobiota of domiati cheese

Aspergillus (A. flavus, A. fumigatus and A. niger), Penicillium (P. aurantiogriseum and P. restrictum), Ulocladium atrum and Debaryomyees hansenii were isolated from cheese treated with A. graveolens oil. The highest fungal count was detected at 3% oil concentration after 7 weeks of storage, although, the lowest fungal count was also detected at 3% oil, but after 11 weeks concentration (Tables 6 & 8). Extracts of 43 plants exhibited varying degrees of inhibition activity against the fungi. Among these extracts, the extract from Grewia arborea showed maximum activity (Bobbarala et al. 2009). As shown in table (8), the total counts were highly significant increased in cheese treated with 3, 5, 7% after 3 weeks.

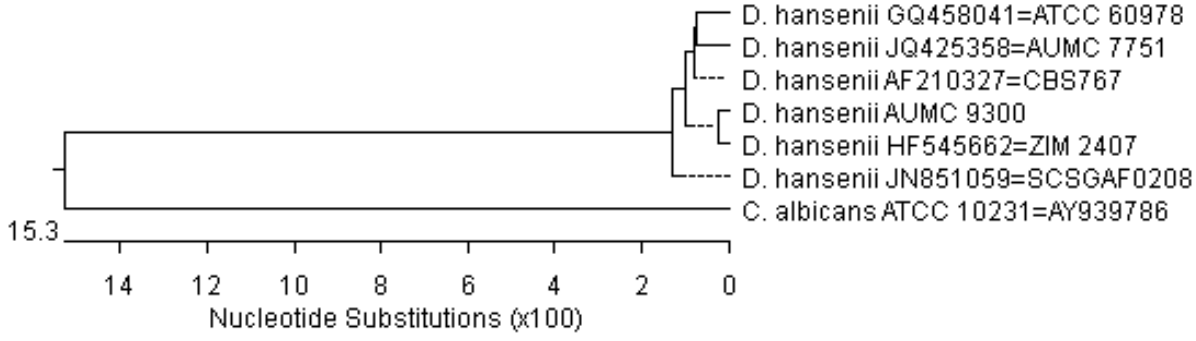

Figure 1. The phylogenetic tree of *Debaryomyces hansenii* AUMC 9300 based on partial nucleotide sequences (bp) of the ribosomal DNA internal transcribed spacer regions. The scale indicates the number of nucleotide substitution per site

Table 5. Fungi isolated on dichloran rose-Bengal agar (DRBC), from domiati cheese samples, supplemented with three concentrations (3, 5, 7%) of *Laurus nobilis* oil

Fungal taxa	Control				3%				5%				7%			
	Zero time	After three weeks	After seven weeks	After eleven weeks	Zero time	After three weeks	After seven weeks	After eleven weeks	Zero time	After three weeks	After seven weeks	After eleven weeks	Zero time	After three weeks	After seven weeks	After eleven weeks
Aspergillus	+	+	+	-	+	-	+	+	-	-	-	-	-	-	+	-
A. flavus	+	-	+	-	-	-	-	+	-	-	-	-	-	-	+	-
A. fumigatus	+	-	+	-	+	-	-	-	-	-	-	-	+	-	+	-
A. niger	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Penicillium	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+	-
P. griseofulvum	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-
P. islandicum	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-
Debaryomyces hansenii	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
No. of genera (3)	1	2	2	1	2	1	1	2	1	1	1	1	2	1	1	1
No. of species (6)	3	2	2	1	2	1	1	2	1	1	1	2	2	1	1	1

Table 6. Fungi isolated on Dichloran Rose-Bengal Agar (DRBC), from domiati cheese samples, supplemented with three concentrations (3, 5, 7%) of *Anethum graveolens* oil

Fungal taxa	**Control**				**3%**				**5%**				**7%**			
	Zero time	**After three weeks**	**After seven weeks**	**After eleven weeks**	**Zero time**	**After three weeks**	**After seven weeks**	**After eleven weeks**	**Zero time**	**After three weeks**	**After seven weeks**	**After eleven weeks**	**Zero time**	**After three weeks**	**After seven weeks**	**After eleven weeks**
Aspergillus	+	+	+	-	+	-	+	+	+	-	+	-	+	-	+	-
A. flavus	+	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-
A. fumigates	+	-	+	-	+	-	+	-	+	-	+	-	+	-	-	-
A. niger	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Penicillium	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-
P. aurantiogriseum	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-
P. restrictum	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Ulocladium atrum	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Debaryomyces hansenii	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
No. of genera (4)	1	2	2	1	2	2	2	3	3	1	1	1	2	1	1	1
No. of species (7)	3	2	2	1	2	2	2	3	3	1	1	1	2	1	1	1

Table 7. Fungi isolated on Dichloran Rose-Bengal Agar (DRBA), from domiati cheese samples, supplemented with three concentrations (3, 5, 7%) of Mentha piperita oil

Fungal taxa	Control				3%				5%				7%			
	Zero time	After three weeks	After seven weeks	After eleven weeks	Zero time	After three weeks	After seven weeks	After eleven weeks	Zero time	After three weeks	After seven weeks	After eleven weeks	Zero time	After three weeks	After seven weeks	After eleven weeks
Aspergillus	+	+	+	-	+	+	-	+	+	+	-	+	+	-	+	-
A. flavus	+	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-
A. fumigatus	+	-	+	-	+	+	-	+	+	+	-	+	+	-	-	-
A. niger	+	+	-	-	-	+	-	-	-	-	-	-	+	-	-	-
versicolor	-	-	-	-	-	-	-	-	-	-	-	-	+			
Penicillium	-	-	-	-	-	+	-	-	+	+	-	-	-	+	-	-
P. aurantiogriseum	-	-	-	-	-	-	-	-	+	-	-	-	-			
P. camembertii	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
P. griseofulvum	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
P. oxalicum	-	-	-	-	-	+	+	-	-	+	-	-	-	+	-	-
Debaryomyces hansenii	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-
No. of genera (3)	1	2	2	1	2	3	3	2	3	3	3	1	2	2	2	0
No. of species (9)	3	2	2	1	2	8	8	2	3	3	3	1	4	2	2	0

Table 8. Mean, standard deviation (SD) and percent of inhibition in total counts of fungi isolated from Domiati cheese on DRBC medium

Treatments	Zero time		After 3 weeks		After 7 weeks		After 11 weeks	
	Mean$x10^3$ ± SD	% Inhibition	Mean$x10^3$ ± SD	% Inhibition	Mean$x10^3$ ± SD	% Inhibition	Mean$x10^3$ ± SD	% Inhibition
Control	1.07±0.12	0.00	4.17±0.76	0.00	159.67±2.52	0.00	1.23±0.25	0.00
				Laurus nobilis oil				
3%	3.83±0.76	-2.58	122.33±4.51	-28.34	13.67±0.58	91.44	70.67±9.02	-56.23
5%	4.17±1.26	-2.90	127.00±7.55	-29.46	80.67±15.01	49.94	38.67±8.08	-30.21
7%	2.83±0.76	-1.65	75.67±3.06	-17.15	18.67±1.15	88.31	229.67±24.34	-185.72
				Anethum graveolens oil				
3%	3.33±0.58	-2.11	76.17±3.75	-17.27	416.67±43.92	-1.61	2.33±0.58	-0.89
5%	31.83±4.07	-28.75	127.00±7.94	-29.46	95.33±3.51	40.30	4.00±1.00	-2.25
7%	8.33±1.53	-6.79	127.00±5.00	-29.46	140.67±5.13	11.90	264.00±20.78	-213.63
				Mentha piperita oil				
3%	9.33±0.58	-7.72	5.67±0.29	-0.36	36.67±3.06	77.03	3.00±1.00	-1.44
5%	5.83±0.76	-4.45	1.67±0.58	59.95	22.67±3.06	85.80	10.00±2.00	-7.13
7%	2.17±0.29	-1.03	0.50±0.00	88.01	5.00±1.00	96.87	0.00±0.00	100.00

±SD = standard Deviation

Effect of *Mentha piperita* oil on the mycobiota of domiati cheese

The fungal counts decreased by increasing the concentration of *M. piperita* oil in comparison with control (Tables 7 & 8). Medicinal plants represent a rich source of antimicrobial agents. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs (Mahesh and Satish 2008). Highly significant decrease in total counts was observed after 7 weeks at the three concentrations used (Table 8).

Aflatoxin screening on CAM

Isolates of *Aspergillus flavus* (3 strains) showed blue fluorescence at 365 nm indicating aflatoxin B production; *P. oxalicum* strain produced also blue fluorescence. On the other hand, isolates of other species (one strain of *A. niger*, 2 strains of *D. hansenii*) showed negative results. In this respect, all *P. verrucosum* isolates from cheese produced ochratoxin A (Aran and Eke 1987). Frisvad and Filtenborg (1989) detected cyclopiazonic acid in culture extracts of half of 46 isolates collected from cheese identified as *P. verrucosum* var. *cyclopium*, while those from cereals often produce citrinin as well as ochratoxin A. Isolates of some *Penicillium* species such as *P. chrysogenum* and *P. roqueforti* produced the metabolites, Meleagrin, Isohtmigaclavine A, PR-toxin, roquefortine C (Frisvad and Filtenborg 1989). *Aspergillus flavus* could produce aflatoxins B_1 and G_1 on Cheddar cheeses (Lieand Marth 1967).

4. Conclusions

Generally, results showed that treatment of cheese with *M. piperita* oil significantly decreased total counts of fungi. On the other hand, oils of *L. nobilis*and *A. graveolens* caused either increase or decrease in fungal counts. Twelve species appertaining to 4 genera of fungi were isolated from cheese and cheese treated with essential oils. The results revealed that the Domiati cheese under investigation exhibited low level of contamination and most of the samples tested, were free of fungi, where the total fungal counts ranged from 95 and 125 colonies/160 cheese pieces.

ACKNOWLEDGEMENTS

This research was financially supported by Assuit University, Egypt.

REFERENCES

[1] AbdEl-Kader, Y.I. (1999) Effect of cooling or freezing process on some properties of cow's milk and Domiati cheese. Egypt Journal for Applied Science14(2).

[2] Agoramoorthy, G., Chandrasekaran, M., Venkatesalu, V. & Hsu, M.J. (2007) Antibacterial and antifungal activities of Fatty Acid Methyl Esters of the blind-your-eye mangrove from India. Brazilian Journal of Microbiology 38(4), 739-742.

[3] Al-Fatimi, M., Wurster, M., Schroder, G. & Lidequist, U. (2010) In vitro antimicrobial, cytotoxic, and radical scavenging activities and chemical constituents of the endemic Thymus laevigatus (Vahl). *Rec Nat Prod,* 4 (1**):** 49-63.

[4] Al-Mughrabi, K.I., Coleman, W.K., Vikram, A., Poirier, R. & Jayasuriya, K.E. (2013) Effectiveness of Essential Oils and Their Combinations with Aluminum Starch Octenylsuccinate on Potato Storage Pathogens. Journal of Essential Oil Bearing Plants 16(1): 23-31.

[5] Al-Qarawi, A.A., Abd Allah, E.F. & Abeer, H. (2013) Effect of Ephedra Alata Decne. On Lipids Metabolism of Aspergillus Flavus Link. Bangladesh Journal of Botany 42(1): 45-49.

[6] Aqil, F., Beg, A.Z. & Ahmad, I. (2001) In vitro toxicity of plant essential oils against soil fungi. J. Med. Aro. Plant Sci 22(23): 177-181.

[7] Aran, N. & Eke, D. (1987) Mould mycoflora of KaÅŸar cheese at the stage of consumption. Food Microbiology 4(2): 101-104.

[8] Arkoudis, E. & Stratakis, M. (2008) Synthesis of cordiaquinones B, C, J, and K on the basis of a bioinspired approach and the revision of the relative stereochemistry of cordiaquinone C. Journal of Organic Chemistry 73(12): 4484-4490.

[9] Arnusch, C.J., Ulm, H., Josten, M., Shadkchan, Y., Osherov, N., Sahl, H.-G. & Shai, Y. (2012) Ultrashort Peptide Bioconjugates Are Exclusively Antifungal Agents and Synergize with Cyclodextrin and Amphotericin B. Antimicrobial Agents and Chemotherapy 56(1), 1-9.

[10] Basile, A., Rigano, D., Sorbo, S., Conte, B., Rosselli, S., Bruno, M. & Senatore, F. (2013) Antibacterial and antifungal activities of Otanthus maritimus (L.) Hoffmanns. & Link essential oil from Sicily. Natural Product Research 27(17): 1548-1555.

[11] Ben Mansoura, M., Balti, R., Rabaoui, L., Bougatef, A. & Guerfel, M. (2013) Chemical composition, angiotensin I-converting enzyme (ACE) inhibitory, antioxidant and antimicrobial activities of the essential oil from south Tunisian Ajuga pseudoiva Rob. Lamiaceae. Process Biochemistry 48(4), 723-729.

[12] Beresford, T.P., Fitzsimons, N.A., Brennan, N.L. & Cogan, T.M. (2001) Recent advances in cheese microbiology. International Dairy Journal 11(4), 259-274.

[13] Bhuiyan, M. N. I., Varshney, V. K., Shiam C. Varshney, Tomar, A. & Akter, F. (2011) Composition of essential oil of the leaf and inflorescence of Pogostemon benghalensis (Burm.f.) Kuntze. *International Research Journal of Plant Science* 2(9)**,** 271-275.

[14] Bisht, D.S., Padalia, R.C., Singh, L., Pande, V., Lal, P. & Mathela, C.S. (2010) Constituents and antimicrobial activity of the essential oils of six Himalayan Nepeta species. Journal of the Serbian Chemical Society 75(6), 739-747.

[15] Bobbarala, V., Katikala, P.K., Naidu, K.C. & Penumajji, S. (2009) Antifungal activity of selected plant extracts against phytopathogenic fungi *Aspergillus niger* F2723. Indian Journal of Science and Technology 2(4), 87-90.

[16] Ceylan, Z.G., Turkoglu, H. & Dayisoylu, K.S. (2003) The microbiological and chemical quality of sikma cheese produced in Turkey. Pak. J. Nutr 2(2), 95-97.

[17] Chessa, M., Sias, A., Piana, A., Mangano, G.S., Petretto, G.L., Masia, M.D., Tirillini, B. & Pintore, G. (2013) Chemical composition and antibacterial activity of the essential oil from *Mentha requienii* Bentham. Natural Product Research 27(2), 93-99.

[18] Costa, E.V., da Silva, T.B., Alencar Menezes, L.R., Gonzaga Ribeiro, L.H., Gadelha, F.R., de Carvalho, J.E., Barizon de Souza, L.M., Navarro da Silva, M.A., Theodoro Siqueira, C.A. & Salvador, M.J. (2013) Biological activities of the essential oil from the leaves of Xylopia laevigata (Annonaceae). Journal of Essential Oil Research 25(3), 179-185.

[19] Davis, N., Lyer, S. & Diener, U. (1987) Improved method of screening for aflatoxin with a coconut agar medium. Applied and environmental microbiology 53(7), 1593-1595.

[20] Deep, A., Phogat, P., Kumar, M., Kakkar, S., Mittal, S.K. & Malhotra, M. (2012) Newtetradecanoic acid hydrazones in the search for antifungal agents: synthesis and in vitro evaluations. Acta Poloniae Pharmaceutica 69(1), 129-133.

[21] Djenane, D., Aider, M., Yangueela, J., Idir, L., Gomez, D. & Roncales, P. (2012) Antioxidant and antibacterial effects of *Lavandula* and *Mentha* essential oils in minced beef inoculated with E. coli O157:H7 and S. aureus during storage at abuse refrigeration temperature. Meat Science 92(4), 667-674.

[22] El-Sayed, A.M., Cole, L., Revell, J., Manning, L.-A., Twidle, A., Knight, A.L., Bus, V.G.M. & Suckling, D.M. (2013) Apple Volatiles Synergize the Response of Codling Moth to Pear Ester. Journal of Chemical Ecology 39(5), 643-652.

[23] El-Seedi, H. R., Khalil, N. S., Azeem, M., Taher, E. A., Goransson, U., Palsson, K. & Borg-Karlson, A. K. (2012) Chemical Composition and Repellency of Essential Oils From Four Medicinal Plants Against Ixodes ricinus Nymphs (Acari: Ixodidae). *Journal of Medical Entomology,* 49**,** 1067-1075.

[24] Elsanhoty, R.M., Mahrous, H. & Ghanaimy, G.A. (2009) Chemical, microbial counts and evaluation of biogenic amines during the ripening of Egyptian soft Domiati cheese made from raw and pasteurized buffaloes milk. International Journal of Dairy Science 4(2), 80-90.

[25] Erdogan, A., Gurses, M., Turkoglu, H. & Sert, S. (2001) The determination of mould flora of some Turkish cheese types (Kasar, Civil, Lor, Tulum). Pakistan J Biol Sci 4(7), 886-7.

[26] EPA (1998) Method 8270D: Semivolatile organic compounds by gas chromatography/mass spectrometry (GC/MS). In: Draft update IVA of SW-846 on-line. U.S. Environmental Protection Agency. Office of Solid Waste.

[27] Fadda, M., Mossa, V., Pisano, M., Deplano, M. & Cosentino, S. (2004) Occurrence and characterization of yeasts isolated from artisanal Fiore Sardo cheese. International journal of food microbiology 95(1), 51-59.

[28] Fahmi, A. H. & Sharara, H. A. (1950) 429. Studies on Egyptian Domiati cheese. *Journal of Dairy Research,* 17**,** 312-328.

[29] Frisvad, J.C. & Filtenborg, O. (1989) Terverticillate penicillia: chemotaxonomy and mycotoxin production. Mycologia 81(6), 837-861.

[30] Guart, A., Wagner, M., Mezquida, A., Lacorte, S., Oehlmann, J. & Borrell, A. (2013) Migration of plasticisers from Tritan (TM) and polycarbonate bottles and toxicological evaluation. Food Chemistry 141(1), 373-380.

[31] Hassiotis, C.N. & Dina, E.I. (2011) The effects of laurel (Laurus nobilis L.) on development of two mycorrhizal fungi. International Biodeterioration & Biodegradation 65(4), 628-634.

[32] Hocking, A.D. & Faedo, M. (1992) Fungi causing thread mould spoilage of vacuum packaged Cheddar cheese during maturation. International journal of food microbiology 16(2), 123-130.

[33] Hossain, M.A., Ismail, Z., Rahman, A. & Kang, S.C. (2008) Chemical composition and anti-fungal properties of the essential oils and crude extracts of Orthosiphon stamineus Benth. Industrial Crops and Products 27(3), 328-334.

[34] Ibrahim, H., Aziz, A.N., Syamsir, D.R., Ali, N.A.M., Mohtar, M., Ali, R.M. & Awang, K. (2009) Essential oils of Alpinia conchigera Griff. and their antimicrobial activities. Food Chemistry 113(2), 575-577.

[35] Kalemba, D. & Kunicka, A. (2003) Antibacterial and antifungal properties of essential oils. Current Medicinal Chemistry 10(10), 813-829.

[36] King, A. D., Hocking A. D. & Pitt J. I. (1979) Dichloran rose bengal medium for enumeration and isolation of molds from foods. Appl. Environ. Microb. 37, 959-964.

[37] Kivanç, M. (1992) Fungal contamination of Kashar cheese in Turkey. Food/Nahrung 36(6), 578-583.

[38] Kordali, S., Kotan, R. & Cakir, A. (2007) Screening of antifungal activities of 21 oxygenated monoterpenes in-vitro as plant disease control agents. Allelopathy Journal 19(2), 373-391.

[39] Kosikowski, F. & Mistry, V.V. (1997) Process cheese and related products. In: Cheese and Fermented Milk Foods: Origins and Principles (edited by F.V.Kosikowski). Pp. 328–352. Westport: F.V. Kosikoswki & Associates.

[40] Kurtulmus, A., Fafal, T., Mert, T., Saglam, H., Kivcak, B., Ozturk, T., Demirci, B. & Baser, K.H.C. (2009) CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OILS OF THREE Anthemis SPECIES FROM TURKEY. Chemistry of Natural Compounds 45(6), 900-904.

[41] Larmond, E. (1977). Laboratory Methods for Sensory Evaluation of Food. Publication no. 1637. Ottawa: Canadian Department of Agriculture.

[42] Licina, B.Z., Stefanovic, O.D., Vasic, S.M., Radojevic, I.D., Dekic, M.S. & Comic, L.R. (2013) Biological activities of the extracts from wild growing Origanum vulgare L. Food Control 33(2), 498-504.

[43] Lie J. L. &Marth E. H. (1967) Formation of Aflatoxin in

Cheddar Cheese by *Aspergillus flavus* and *Aspergillus parasiticus.* Journal of Dairy Science 50(10), 1708–1710.

[44] Liouane, K., Saidana, D., Edziri, H., Ammar, S., Chriaa, J., Mahjoub, M.A., Said, K. & Mighri, Z. (2010) Chemical composition and antimicrobial activity of extracts from Gliocladium sp. growing wild in Tunisia. Medicinal Chemistry Research 19(8), 743-756.

[45] Lirio, L.G., Hermano, M.L. & Fontanilla, M.Q. (1998) Note antibacterial activity of medicinal plants from the Philippines. Pharmaceutical Biology 36(5), 357-359.

[46] Lund, F. & Filtenborg, O. (1995) Associated mycoflora of cheese. Food Microbiol 12, 173-180.

[47] Mahesh, B. & Satish, S. (2008) Antimicrobial activity of some important medicinal plant against plant and human pathogens. World journal of agricultural sciences 4(5), 839-843.

[48] Majiene, D., Trumbeckaite, S., Grunoviene, D., Ivanauskas, L. & Gendrolis, A. (2004) [Investigation of chemical composition of propolis extract]. Medicina (Kaunas, Lithuania) 40(8), 771-4.

[49] Malele, R.S., Mutayabarwa, C.K., Mwangi, J.W., Thoithi, G.N., Lopez, A.G., Lucini, E.I. & Zygadlo, J.A. (2003) Essential oil of Hyptis suaveolens (L.) Poit. from Tanzania: Composition and antifungal activity. Journal of Essential Oil Research 15(6), 438-440.

[50] Marei, G.I.K., Abdel Rasoul, M.A. & Abdelgaleil, S.A.M. (2012) Comparative antifungal activities and biochemical effects of monoterpenes on plant pathogenic fungi. Pesticide Biochemistry and Physiology 103(1), 56-61.

[51] Matan, N., Matan, N. & Ketsa, S. (2012) Effect of heat curing on antifungal activities of anise oil and garlic oil against Aspergillus niger on rubberwood. International Biodeterioration & Biodegradation 75, 150-157.

[52] Mazimba, O., Masesane, I.B., Majinda, R.R.T. & Muzila, A. (2012) GC-MS Analysis and Antimicrobial Activities of the Non-polar Extracts of Mundulea sericea. South African Journal of Chemistry-Suid-Afrikaanse Tydskrif Vir Chemie 65, 50-52.

[53] Morcia, C., Malnati, M. & Terzi, V. (2012) In vitro antifungal activity of terpinen-4-ol, eugenol, carvone, 1,8-cineole (eucalyptol) and thymol against mycotoxigenic plant pathogens. Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment 29(3), 415-422.

[54] Moreira, S.R., Schwan, R.F., Carvalho, E.P.d. & Wheals, A.E. (2001) Isolation and identification of yeasts and filamentous fungi from yoghurts in Brazil. Brazilian Journal of Microbiology 32(2), 117-122.

[55] Moro, A., Libran, C.M., Isabel Berruga, M., Zalacain, A. & Carmona, M. (2013) Mycotoxicogenic fungal inhibition by innovative cheese cover with aromatic plants. Journal of the Science of Food and Agriculture 93(5), 1112-1118.

[56] Mostafa, A.A., Al-Rahmah, A.N., Abdel-Megeed, A., Sholkamy, E.N., Al-Arfaj, A.A. & El-shikh, M.S. (2012) Fungitoxic Properties of Some Plant Extracts Against Tomato Phytopathogenic Fungi. Journal of Pure and Applied Microbiology 6(4), 1889-1898.

[57] Nasser L.A., 2001. Fungal contamination of white cheese at the stage of consumption in Saudi Arabia. Pakistan Journal of Biological Sciences 4: 733-735.

[58] Nikitina, L. E., Startseva, V. A., Artemova, N. P., Dorofeeva, L. Y., Kuznetsov, I. V., Lisovskaya, S. A., Glushko, N. P. & Kutyreva, M. P. (2012) Synthesis and antifungal activity of monoterpenoids of the carane series. Pharmaceutical Chemistry Journal, 45**,** 664-667.

[59] Northolt M.D. and Soentoro P.S.S. (1988): Fungal growth on foodstuffs related to mycotoxin contamination. In Introduction to food-borne fungi (Eds Samson R.A. and van Reenen-Hoekstra E.S.) pp. 231-238. Baarn, Netherlands, Centraalbureau voor Schimmelcultures.

[60] Northolt M.D., van Egmond H.R., Soentoro R and Deijll E. (1980): Fungal growth and the presence of sterigmatocystin in hard cheese. J. .Assoc. Off. Anal. Chem. 63: 115-119.

[61] Oladosu, I.A., Zubair, M.F., Ali, M.S. & Olawore, N.O. (2009) Anticandidal activity of volatile compounds from the Root Bark of *Ficus exasperata* Vahl-Holl (Moraceae). Journal of Essential Oil Bearing Plants 12(5), 562-568.

[62] Orhan, I.E., Ozcelik, B., Kartal, M. & Kan, Y. (2012) Antimicrobial and antiviral effects of essential oils from selected Umbelliferae and Labiatae plants and individual essential oil components. Turkish Journal of Biology 36(3), 239-246.

[63] Pitt, J.I. & Hocking, A.A.D. (2009) Fungi and food spoilage. Springer.

[64] Pintado, C. M. B. S., Ferreira, M. A. S. S. & Sousa, I. (2010) Control of pathogenic and spoilage microorganisms from cheese surface by whey protein films containing malic acid, nisin and natamycin. Food Control, 21**,** 240-246.

[65] Policegoudra, R.S., Goswami, S., Aradhya, S.M., Chatterjee, S., Datta, S., Sivaswamy, R., Chattopadhyay, P. & Singh, L. (2012) Bioactive constituents of Homalomena aromatica essential oil and its antifungal activity against dermatophytes and yeasts. Journal De Mycologie Medicale 22(1), 83-87.

[66] Rafii, F. & Shahverdi, A.R. (2007) Comparison of essential oils from three plants for enhancement of antimicrobial activity of nitrofurantoin against enterobacteria. Chemotherapy 53(1), 21-25.

[67] Rahman, M.A., Chakma, J.S., Bhuiyan, N.I. & Islam, M.S. (2012) Composition of the Essential Oil of Clausena Suffruticosa Leaf and Evaluation of its Antimicrobial and Cytotoxic Activities. Tropical Journal of Pharmaceutical Research 11(5), 739-746.

[68] Reis, F.S., Stojkovic, D., Sokovic, M., Glamoclija, J., Ciric, A., Barros, L. & Ferreira, I.C.F.R. (2012) Chemical characterization of Agaricus bohusii, antioxidant potential and antifungal preserving properties when incorporated in cream cheese. Food Research International 48(2), 620-626.

[69] Rodrigues, L., Duarte, A., Figueiredo, A.C., Brito, L., Teixeira, G., Moldao, M. & Monteiro, A. (2012) Chemical composition and antibacterial activity of the essential oils from the medicinal plant Mentha cervina L. grown in Portugal. Medicinal Chemistry Research 21(11), 3485-3490.

[70] Ropars, J., Cruaud, C., Lacoste, S. & Dupont, J. (2012) A taxonomic and ecological overview of cheese fungi.

International journal of food microbiology 155(3), 199-210.

[71] Shah, W.A., Dar, M.Y., Zagar, M.I., Agnihotri, V.K., Qurishi, M.A. & Singh, B. (2013) Chemical composition and antimicrobial activity of the leaf essential oil of Skimmia laureola growing wild in Jammu and Kashmir, India. Natural Product Research 27(11), 1023-1027.

[72] Singh, G., Marimuthu, P., Catalan, C. & Delampasona, M. P. (2004) Chemical, antioxidant and antifungal activities of volatile oil of black pepper and its acetone extract. Journal of the Science of Food and Agriculture, 84**,** 1878-1884.

[73] Singh, G., Marimuthu, R., De Heluani, C. S. & Catalan, C. (2005) Antimicrobial and antioxidant Potentials of essential oil and acetone extract of Myristica fragrans Houtt. (Aril part). *Journal of Food Science,* 70**,** M141-M148.

[74] Sokovic, M.D., Vukojevic, J., Marin, P.D., Brkic, D.D., Vajs, V. & van Griensven, L.J.L.D. (2009) Chemical Composition of Essential Oils of Thymus and Mentha Species and Their Antifungal Activities. Molecules 14(1), 238-249.

[75] Soliman, K.M. & Badeaa, R.I. (2002) Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food and Chemical Toxicology 40(11), 1669-1675.

[76] Sudhakar, P., Latha, P., Sreenivasulu, Y., Reddy, B.V.B., Hemalatha, T.M., Balakrishna, M. & Reddy, K.R. (2009) Inhibition of Aspergillus flavus colonization and aflatoxin (AfB1) in peanut by methyleugenol. Indian Journal of Experimental Biology 47(1), 63-67.

[77] Tian, J., Ban, X., Zeng, H., Huang, B., He, J. & Wang, Y. (2011) In vitro and in vivo activity of essential oil from dill (Anethum graveolens L.) against fungal spoilage of cherry tomatoes. Food Control 22(12), 1992-1999.

[78] Tsai, W., Liewen, M. & Bullerman, L. (1988) Toxicity and sorbate sensitivity of molds isolated from surplus commodity cheeses. Journal of food protection 51.

[79] Walters, D., Raynor, L., Mitchell, A., Walker, R. & Walker, K. (2004) Antifungal Activities of Four Fatty Acids against Plant Pathogenic Fungi. Mycopathologia 157(1), 87-90.

[80] Wyder, M.-T. & Puhan, Z. (1999) Role of selected yeasts in cheese ripening: an evaluation in aseptic cheese curd slurries. International Dairy Journal 9(2), 117-124.

[81] Zuzarte, M., Gonã§Alves, M. J., Cavaleiro, C., Dinis, A. M., Canhoto, J. M. & Salgueiro, L. G. R. (2009) Chemical composition and antifungal activity of the essential oils of Lavandula pedunculata (Miller) Cav. *Chemistry & biodiversity,* 6, 1283-1292.

[82] Zuzarte, M., Goncalves, M.J., Cavaleiro, C., Cruz, M.T., Benzarti, A., Marongiu, B., Maxia, A., Piras, A. & Salgueiro, L. (2013): Antifungal and anti-inflammatory potential of *Lavandula stoechas* and *Thymus herba-barona* essential oils. Industrial Crops and Products 44, 97-103.

[1] Web: http://www.aun.edu.eg/membercv.php?M_ID=1261