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RESEARCH ARTICLE

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Antifungal Activity Of Dill Essential Oil (*Anethum graveolens L.*) In Minced Meat

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ABSTRACT

The objective of the present study was to evaluate the use of essential oil of dill (*Anethum graveolens L.*) as antifungal agent (in vivo) in food preservation. Minced meat used in these trials was treated with 0.5% and 2.0% dill essential oil. The samples were packed into plastic bags and stored in refrigerator at 4°C. The samples were examined every 2 days until appearance of deteriorative changes on minced meat under examination. Minced meats samples were evaluated for sensorial properties and mycological counts on days 0, 2, 4, 6 and 8 of storage. The obtained results showed that the tested essential oil caused a highly significant inhibition on fungal growth, where total mould counts ranged from 4.01 to 4.93 log10 *cfu/g*. While in control total mould counts ranged from 8.00 to 9.78 log10 cfu/g .It was found that essential oil of dill has antifungal activity against *A. flavus* in vivo. Also, the results indicated that the application of dill oil on the minced meats improves the mycological quality and extends the shelf-life of meat samples. Thus, the essential oil of dill could be used to control food spoilage as a potential source of food preservative as well as it considered as an eco-friendly antifungal agent.

Keywords: *Dill Essential Oil (Anethum graveolens L.), Meat, A. flavus.*

Introduction

Minced meat is one of meat products widely consumed in Egypt. Microbial quality of meat products plays an important role in increasing public health issue all over the world. During the last decade, there were great improvements in hygienic technique for production of meat products with attention of a lot of consumers towards healthy nutrition. The use of antimicrobial ingredients is one of the widely used methods to maintain microbiological safety and prolong the shelf-life of food products [1].

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Minced meat is not only highly susceptible to spoilage, but also it frequently involved in the spread of food-borne illnesses. During slaughtering and processing of animal, all potentially edible tissues are subjected to microbial contamination from various sources. It is suggested that yeast and mould play an important role in causing spoilage of meat [2-3].

The increased demand for safe and natural food, without chemical preservatives, provokes many researchers to investigate the antimicrobial effects of natural compounds. Numerous investigations have confirmed the antimicrobial action of essential oils (EOs) in model food systems and in real food [4-5], evidence that EOs are more strongly antimicrobial than is accounted for by the additive effect of their major antimicrobial components; minor components appear, therefore, to play a significant role [6]. Since EOs are considered as Generally Regarded As Safe (GRAS) [7], the possibility of reinforcing their

natural antimicrobial effects by the addition of small amounts of other natural preservatives may be a way to attain a balance between sensory acceptability and antimicrobial efficacy.

Anethum graveolens L. (dill), an important member of the Umbelliferae family native to southwest Asia or southeast Europe, is widely used for flavoring foods and beverages, and for the treatment of many pathological conditions such as disease of the uterus, cervical ectropio, flatulence, indigestion, stomachache, colic, and gas in the intestinal tract **[8]**. Dill has been reported to possess antibacterial [9], antihyperlipidemic, and antihypercholesterolemic properties [10]. As a traditional medicine, dill increases milk production and promotes menstruation for women [11]. Based on previous work, it is assumed that dill oil can be a potential source of ecofriendly antifungal drugs and food preservatives [12-13] .The purpose of the present investigation was to study the prevalence of yeasts and moulds in minced meat and to evaluate the effect of the natural dill essential oil (*Anethum graveolens L.*) as antifungal on growth of contaminated minced meat.

Figure 1: *Anethum graveolens L.*

Materials and Methods

Part I: enumeration of fungi in minced meat

Samples collection:

A total of twenty-five samples of minced meat (each sample 250 gm) were collected from butcher's shops. These samples were obtained and preserved in an ice box then transferred to the laboratory under complete aseptic condition without undue delay and examined as rapidly as possible.

Fungal isolation and identification:

Preparation of food homogenate according to the technique recommended by [14]. Twenty five grams of the mined meat samples were homogenized in stomacher with 225 ml of sterile buffered peptone water and to give 10^{-1} dilution. However, one ml of the clear food homogenate was mixed with 9 ml of buffered peptone water in a test tube and the contents were mixed carefully, then tenfold serial dilutions were prepared. Duplicated petri dishes of Dichloran rose bengal chloramphenicol (DRBC) media were inoculated with 1 ml from each dilution and were left to solidify at room temperature and incubated at 25 ˚C for 5-7 day. The isolated fungi were identified according to macro and microscopic characteristics as described in [15], while yeast isolates identifications were performed by using rapid miniaturised system API 20 C AUX (bioMérieux, France). Some complementary tests used for final identification of the isolates as recommended by [15-16].

Part II: evaluation of dill oil (*Anethum graveolens*) as antifungal in minced meat:

Essential oil:

The essential oil of *Anethum graveolens* Leaves was obtained from pharmacognosy department, National Research Center, Dokki, Giza.

Preparation of test microorganisms and cultures:

The essential oil of *Anethum graveolens* leaves was assayed for antifungal activity against the fungal strains of *A. flavus* obtained from Mycology Department at Animal Health Research Institute, Giza. Confirmation of *A. flavus* was done by subculture onto Malt Extract Agar and Czapek yeast agar [17]. Furthermore, confirmation of *A.flavus* was carried out by Single Spore method, using the identification keys of [15]; these fungi were stored in tubes of PDA acidified at 4˚C. The inoculum was prepared by adding 4 ml of sterile distal water to the agar slant, and shake vigorously to get the cell or spore suspension. The

test organism with the inoculum size of 1 × 10^8 colony-forming units (CFU)/ mL [15] was used for the study.

Preparation of minced meat:

A total of 600 g fresh meat was obtained from super markets, preserved in an ice box then transferred to the laboratory under complete aseptic condition without undue delay. Meat samples were minced under aseptic condition and divided into six groups. All samples were packed in polyethylene bags separately. The experimental design and different treatments have been shown in Table 1. All samples were kept in the refrigerator (4 ± 1 °C). The samples of 5th and 6th were group subjected to organoleptic examination and examined every 2 days until appearance of deterioration according to [18].

Results and Discussion

All examined samples were proved to be contaminated with mycotic organisms, and this may be attributed to contamination of such type of food (minced meat) during all procedures of preparation, manufacturing until purchasing. Moulds lead to great economic losses and constitute a major production of mycotoxins [19]. Contamination of such product with fungal species or genera plays an important dangerous role, for partially the economic losses due to the possible spoilage of such food which may render it unfit for human consumption or even unmarketable [20]. Results achieved in Table 2, indicated that mould and yeast counts in examined mined meat were log 3.48 and log 4.08 CFU/g, respectively. Nearly similar findings were recorded by [21-24]. Saleh and Salah El-Dien [24] who examined 20 minced meat samples for detection of total mould count, the incidence of positive samples were 85 % and the mean mould count / g were 2.1x 10^6 ± 6.1x10^5.

The obtained result in Table 3, show presence of 19 species of fungi have already been isolated from mined meat. However, only 6 of them are reported to be toxigenic [15]. Toxigenic moulds are able to produce toxic metabolites known as mycotoxins. Since not all genera of moulds are toxigenic, the presence of microscopic filamentous fungi in meat products does not confirm the presence of mycotoxins. The most common isolated fungal genera were Aspergillus, Penicillium, Cladosporium, Mucor, Acremonium, Fusarium, Candida, Rhodotorula and Saccharomyces species. In all instances, *Aspergillus niger* was the predominant one, similar isolates were obtained by [22-26].

Members of the genus *Penicillium* are reported to produce the widest range of mycotoxins. Among them patulin; citrinin; cyclopiazonic and roquefortin C. *Aspergillus flavus* produces aflatoxins and cyclopiazonic acid. The growth of *Aspergillus niger* can be accompanied by the production of ochratoxin A [15].

The presence of yeast and mould in meat products are objectionable, as they grow at a wide range of temperature and pH values, resulting in spoilage of the product [15]. Such yeast and mould might cause gas and off flavor in chilled food on account of their proteolytic activity, rancidity of cold-stored meat products spoiled by yeast is mainly the cause of lipolytic activity by yeast [27]. Meanwhile some pathogenic yeast found in meat products, makes these products unsuitable for human consumption [28].

Table 1: *Formulations of EO additive and different treatments*

Treatments	
1st treatment	Minced meat Free from anything (control negative).
2nd treatment	Minced meat + *A. flavus* 10^8spore/ ml.(control positive).
3th treatment	Minced meat + 0.5% Dill oil + A. flavus 10^8spore/ ml.
4th treatment	Minced meat + 2% Dill oil + A. flavus 10^8spore/ ml..
Organoleptic examination	
5th treatment	Minced meat + 0.5% Dill oil
6thtreatment	Minced meat + 2% Dill oil

Table 2: Statistical analytical results of total moulds and yeast count (log 10 CFU/g) of minced meat samples (N=25)

Type of sample	No. of examined	No. of +ve sample	Mould count (log CFU/g)	Yeast count (log CFU/g)
Minced meat	25	25	3.48	4.08

As in all foods, the organoleptic tests are generally the final guide of the quality from the consumers' point of view. The results recorded in Table 4, are the data of sensory evaluation carried out on minced meat treated with Dill oil (0.5 % and 2 %) and stored at 4 ˚C during 0, 2, 4, 6 and 8 days of storage. It is evident that minced meat containing dill oil was significantly different from control one and was more acceptable. It could be observed that sensory properties of minced meat (taste, odour, texture and overall acceptability) were significantly affected by constituents of dill oil. The degree of these changes increased gradually until the eight day of storage in control sample had signs of decomposition (slimness, abnormal odour and proteolysis). On the other hand the dill oil –treated minced meat (0.5 % and 2 %) showed an improvement of shelf-life extended up to the 8th day of storage. The minced meats which contain dill oil concentration 2 % had the higher score, while the other concentration (0.5 %) had the lesser scores in all sensory attribute.

Essential oils, which are aromatic volatile products of the secondary metabolism of plants, have been applied in natural remedies, perfumes and make-up products, sanitary products, in dentistry, in agriculture, as food preservatives, and as additives [29]. Generally, whole essential oils have greater antifungal activity due to a synergistic effect with some active components; thus, they are more promising in commercial application than single compounds. The major components detected in dill oil are carvone (41.5%), limonene (32.6%), and apiol (16.8%). The antifungal activities exhibited by dill oil might be attributed to these major components [30].

The results presented in Table (5) showed that the mould and yeast counts detected in the control (non-treated) minced meat was2.60 log cfu/g at the 0 day of examination. This count increased during storage and reached to the highest level (7.80 log cfu/g) by the end of storage period. The treatment of minced meat with dill oil lead to the inhibition and retardation

Table 3: Prevalence of individual members of fungi in minced meat samples (25).

Isolated fungal species	Minced meat samples	
	No. of isolates	%
Aspergillus spp.		
A. niger	9/25	13.04
A. flavus	6/25	8.69
A.terrus	3/25	4.34
A. ochraceus	2/25	2.89
Penicillium spp.		
P. corylophilum	3/25	4.34
P. chrysogenum	5/25	7.24
P. citrinum	1/25	1.44
P. funiculosum	2/25	2.89
P. fellutanum	1/25	1.44
Cladosporium cladosporioides	6/25	8.69
Mucor spp.	4/25	5.79
Acremonium spp,	1/25	1.44
Fusarium spp.	1/25	1.44
Yeast spp.		
Candida genera		
C. krusei	3/25	4.34
C. parapasilosis	6/25	8.69
C. tropicals	6/25	8.69
Rhodotorula mucilaginosa	7/25	10.14
Saccharomyces cerevisiae	3/25	4.34
Total	69	100

of moulds and yeasts growth and lowered the maximum growth levels in the minced meat. The moulds and yeasts counts ranged from the beginning 4.01to 4.93 log cfu /g at the end of storage period in minced meat samples treated with dill oil 2%, while in samples treated with dill oil 0.5% the count ranged from 5.95- 8.81 log cfu /g. From the achieved results, it is clear that the addition of dill oil at concentration of 2% is relatively more effective than 0.5% in suppressing the moulds and yeasts growth in minced meat.

Jun *et al.* [30] reported that the antifungal activity of dill oil results from its ability to disrupt the permeability barrier of the plasma membrane and from the mitochondrial dysfunction-induced reactive oxygen species (ROS) accumulation in *Aspergillus flavus*.

The chemical analysis of dill seed oil was carried out by gas chromatography, which revealed the presence of various fatty acids namely capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleaic acid, linoleic acid, linolenic acid and arachidac acid. Fatty acids are saturated and unsaturated depending upon the double bond. Anethum graveolens oil contained saturated fatty acids namely: capric (decanoic), lauric (dodecanoic), myristic (tetradecanoic), palmitic (hexadecanoic) and stearic acids (octadecanoic), while unsaturated fatty acids were oleaic, linoleic, linolenic and arachidoic acids. The differences in geometry between various types of unsaturated fatty acids, as well as between saturated and unsaturated fatty acids, play an important role in biological processes and in the construction of biological structures such as cell membranes. Fatty acids act as anionic detergents and literature on this effect is as far back as reported by [31]. In subsequent years the antifungal and bactericidal properties of fatty acids have been extensively investigated [32].

Conclusion

The results obtained in this study indicate that treatment of minced meat by addition of dill oil inhibits the mould and yeast growth and extends the shelf-life of treated minced meat. Dill oil concentration of 2 % was needed for inhibition of yeast and mould growth. We concluded that these dill essential oils have the potential to be used in food as flavoring and natural preservatives. Thus, the essential oil of dill could be used to control food spoilage as a potential source of food preservative.

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Table 4: Sensory evaluation of minced meat with dill (0.5 % and 2) of different treatments at 8th day of refrigeration storage of meat.

Treatments	Evaluation of the sensory properties				
	Taste	Texture	Odour	Colour	Overall acceptability
Control	Appearance signs of decomposition (slimness, abnormal odour, proteolysis)				
5th treatment	6.70	6.70	5.86	6.50	6.44
6thtreatment	8.90	7.10	9.00	6.50	7.88

Table 5: Effect of different concentrations of dill oil on mould (counts in log10 cfu/g) minced meat stored at 4°C.

Dill extract (%)	Sampling time (days)				
	0	2	4	6	8
1st treatment (control negative)	2.60	3.51	4.52	5.80	7.80
2nd treatment (control positive).	8.00	8.49	8.72	9.32	9.78
3thtreatment (M.M+0.5% EO +A. flavus)	5.95	6.00	6.54	7.75	8.81
4th treatment (M.M+2.0% EO +A. flavus)	4.01	3.63	3.75	4.84	4.93

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