The using of essential oils in improving mycological status of some meat products

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A B S T R A C T

This work was carried out to evaluate the fungal quality of basterma and minced meat sold in local markets in as well as to evaluate the use of essential oil of clove and Cinnamon essential oil as antifungal agent (in vivo) in food preservation. The total mould counts in the examined samples ranged from 2.0 to 3.6 log cfu/g with a mean value of 2.9 log cfu/g for basterma, while the mean count in minced meat was 2.4 log cfu/g and ranged from 0.6 to 3.2 log cfu/g. While the total yeast counts in the examined meat samples ranged from 1.7 to 2.4 log cfu/g, with a mean value of 2.1 log cfu/g for basterma, while that in minced meat was 2.9 log cfu/g and ranged from 2.4 to 3.1 log cfu/g. In the examined samples, 9 mould and 3 yeast genera were identified. Minced meat used in these trials was treated with 0.5 %, 1% and 1.5% from each essential oil. The obtained results showed that the tested essential oils caused a highly significant inhibition on fungal growth. Clove oil (1.5 %) was needed for inhibition of yeast and mould growth. The authors concluded that these essential oils have the potential to be used in food as flavoring and natural preservatives. Thus, the essential oil of clove and cinnamon could be used to control food spoilage as a potential source of food preservative. The antifungal effects of clove oil were found to be more effective than those of cinnamon oil, but the odour of cinnamon is more palatable than the clove odour.

Keywords: Fungi, clove and Cinnamon essential oil, minced meat

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1. INTRODUCTION

Humans have eaten meat and meat products for thousands of years and our teeth have evolved so that they are shaped to tear apart and to chew meat. Countless highly valuable vitamins, minerals and trace elements are present in a concentrated form within meat and meat products remain part of a balanced and healthy diet today (Feiner, 2006). Aflatoxins are secondary toxic fungal metabolites produced by *Aspergillus flavus* and *A. parasiticus*. There are four naturally occurring aflatoxins, the most toxic being aflatoxin B_1 (AFB_1). Aflatoxins are not only contaminate our food stuffs, but also are found in edible tissues, milk and eggs after consumption of contaminated feed by farm animals (Fink-Gremmels, 1999; Bennett and Klich, 2003). The contamination of meat products with moulds may be attributed in part to the addition of spices, which were highly contaminated with mould specially aflatoxin producing strains (Raper et al., 1985). In addition, such high contamination of basterma with moulds may be attributed to the unsatisfactory hygienic condition during handling and transportation of meat from its arrival until reached the cold store in processing plant (Abdel-Rahman et al., 1984; Hamdy et al., 1990). Most important mycotoxins are produced by moulds belonging to Aspergillus, Penicillium and Fusarium genus. If some toxins display an important acute toxicity or chronic effects are probably more important in humans.

Mycotoxins are suspected to be responsible for several pathological syndromes in human: ochratoxin A and Balkan endemic nephropathy, oesophageal cancer and fumonisin B_1, etc. Mycotoxin exposure of human consumers is usually directly linked with alimentary habits. Mycotoxin toxicity is variable. Some are hepatotoxic (aflatoxins), immunotoxic (trichothecenes, fumonisins), others have an estrogenic potential (zearalenone), etc. Certain mycotoxins are considered as carcinogenic or suspected to have carcinogenic properties (Francis et al., 2009). Antimicrobial agents including food preservatives have been used to inhibit food borne bacteria, fungi and extend the shelf life of processed food. Many naturally occurring extracts like essential oils from edible and medicinal plants, herbs and spices have been shown to possess antimicrobial functions and could serve as a source for antimicrobial agents against food spoilage and pathogens (Oussallah, et al., 2006). Various plant materials are believed to have antifungal activity and many essential oils have been reported to have antifungal activities with no side effects on humans and animals. Previous in vitro and in vivo investigations suggested that the essential oils could be used as effective antifungal agents. The selection of plants for evaluation was based on traditional usage for treatment of infectious diseases. However, there are only limited data available on the antifungal activity of essential oils against human and plant fungal pathogens. Fungal species of the genera Aspergillus, Fusarium and Alternaria have been considered to be major plant pathogens worldwide (Nuzhat and Vidyasagar, 2013). The purpose of the present investigation was planned out to study the prevalence of fungi in basterma and minced meat and to evaluate the effect of the natural clove and Cinnamon essential oil as antifungal in contaminated minced meat.

2. MATERIAL AND METHODS

2.1. Samples Collection:

A total of 50 random samples of basterma with coat and minced meat (25 of each) were collected from different localities at Benha city under different trade names. The samples were taken aseptically in polyethylene bags. The samples were transferred to the laboratory in ice box without undue delay and mycologically examined.

2.2. Fungal isolation and identification:

The collected samples were prepared according to the technique recommended by ISO, 217-1-2:2008. Preparation of minced meat homogenate was done according to the method recommended by A.P.H.A. (2002). The isolated fungi were identified according to macro and microscopic characteristics as described by Pitt and Hoching (2009), while yeast isolates were identified according to some complementary tests used for final identification of the isolates as recommended by Kurtzman *et al*. (2003) and Pitt and Hoching (2009).

2.3. Evaluation of Clove and Cinnamon essential oil as antifungal in minced meat:

2.3.1. Essential oil:

The essential oil of clove and Cinnamon were obtained from pharmacognosy department, National Research Center, Dokki, Giza.

2.3.2. Preparation of minced meat:

Four thousands and two hundred twenty five grams (4225g) fresh minced meat were purchased from the butcher shop at Benha. It was transferred to the laboratory in an ice box. Then, the batch (3150 kg) was divided into 7 portions, each portion was divided into three sub-group of 150 g. 1st subgroup as control samples, 2nd ,3rd and 4th sub-groups were further mixed with the appropriate volume of Cinnamon essential oil concentrations (0.5, 1 and 1.5 % v / w), while 5th , 6th and 7th subgroup was further mixed with the appropriate volume of Clove essential oil concentrations (0.5, 1, and 1.5 % v / w). All samples were stored at 4°C. Sampling was carried out every 3 days until the end of storage (9 days).

2.3.3. Mycological analysis:

Twenty five grams from each sample were carefully and aseptically homogenized in blinder after mixing with 225 ml of sterile peptone water 0.1% to form a dilution of 1:10, from which tenfold dilutions were accomplished up to 10^6.

2.3.4. Preparation of minced meat for sensorial analyses:

One thousand and seventy five gram (1075g) minced meat were divided into 7 samples each sample average 150 g, 1st sample as control, 2nd, 3rd, 4th samples were further mixed with the appropriate volume of Cinnamon essential oil concentrations (0.5, 1, & 1.5 % v / w), 5th, 6th and 7th group were further mixed with the appropriate volume of Clove essential oil concentrations (0.5, 1, & 1.5 % v / w), spice mixture (0.1 black pepper + 1 g salt) were added to each sample and mixed well. Each minced meat sample was packed in polyethylene bag. Control sample, containing all components except Cinnamon and Clove essential oils. All samples were stored at 4°C. Sampling was carried out after 24 h. Minced meat samples used in sensorial evaluation were cooked in an oven at 180°C for 45 min.

2.3.5. Sensorial analyses:

Sensorial analysis of marinated minced meat were carried out according to general sensory analysis guidance, given in SRPS ISO 6658/2002. In order to evaluate the influence of different concentrations of Cinnamon and Clove oils on the sensory characteristics, samples were assessed by a trained panel of 8 members. Sensory quality evaluation was performed using analytical descriptive scoring system, with a scale of 1 - 5 points. Every point is defined descriptively (Table 1). Sensorial characteristics that were evaluated are: odour, taste, softness, juiciness and overall acceptability.

3. RESULTS

Results achieved in table (2) indicated that, mould counts in the examined meat products ranged from 2.0 to 3.6 log cfu/g with a mean value of 2.9 log cfu/g for basterma, while the mean count in minced meat was 2.4 log cfu/g and ranged from 0.6 to 3.2 log cfu/g. Regarding to the frequency distribution of moulds in examined samples (Basterma and Minced meat) based on their mould count (Table 3) it was evident that 71.5% of Basterma were in rang of 10^2-< 5×10^2, While in minced meat it was 47.6 %. Furthermore, the total yeast count in the examined meat samples in table (4) ranged from 1.7 to 2.4 log cfu/g, with a mean value of 2.1 log cfu/g for basterma, while that in minced meat was 2.9 log cfu/g and ranged from 2.4 to 3.1 log cfu/g. On the other hand, the results tabulated in table (5) the frequency distribution of yeast in the examined samples (Basterma and Minced meat) based on their yeast count, it was evident that 66.7% of Basterma were in rang of 10^2-< 5×10^2, while in minced meat it was 53.8 % in rang of 5×10^2-< 10^3. The data obtained from table (6) declared that 9 mould genera could be isolated and identified from the examined meat samples. The identified mould genera were; Acremonium, Alternaria, Aspergillus, Eupenicillium, fusarium, Geotrichum, Mucor, Penicillium, and Talaromyces species. In addition, that 6 Aspergillus species could be identified from meat product samples. The most predominant species were; *Aspergillus flavus* and *Aspergillus niger* which were present in 10 (21.7%), 13 (28.3%), 10 (27.0 %), and 8 (21.6%), respectively, in Basterma, and minced meat. *Aspergillus niveus, Aspergillus parasiticus, Aspergillus* *versicolor* and *Aspergillus ustus* were only present in minced meat. Incidence of Penicillium species isolated from examined meat samples revealed that *P. thomii* was the most predominant species isolated from basterma with frequency of 5 (10.9%).While *P. viridicatum* was the isolated from minced meat with frequency of 3 (8.1%). In addition, the result achieved in table (7) it is obvious that different yeast genera were isolated from the examined meat samples. These were Candida, Torulopsis, and Rhodotorula**.** Candida species was the most frequent yeast species isolated from the examined meat samples. Candida species was presented by 5 species, of which, *Candida gullermondii* and *C. krusei* which present in basterma only with frequency of 2 (5.4%) and1 (2.7%), respectively. While in minced meat *C. lipolytica, C. lusitaniae, C. tropicals* and *C. krusei* were present in minced meat with frequency of 6 (16.2%), 5 (13.5%), 2 (5.4%) and 3 (8.1%), respectively. Incidence of Rhodotorula species in the examined meat samples were; 3(8.1%) and 8 (21.6%), for basterma and minced meat. While incidence of Torulopsis species were; 4 (10.8%) and 3(8.1%). Results are presented in table (8), revealed that, the group of minced meat treated with Cinnamon1% and Clove 0.5% showed a higher organoleptic score of overall acceptability (17.9 ± 0.58 and 16.7±0.43). This ,followed by the Cinnamon 1.5%, Clove 1% and Cinnamon 0.5% (13.9±0.31, 12.3 ± 0.25 and 11.6 ± 0.32) then lowest one was obtained by Clove 1.5% and Control (untreated minced meat) (7.4 ± 0.32 and 6.2 ± 0.42), respectively. The results illustrated in table (9) showed powerful effects of clove essential oil in both concentrations (1 % and 1.5%) in days 3, 6 and 9 from the experiment (< 10^2) in case of total yeast count while in total mould count (<10) in 3rd day and no obvious growth in 6th and 9th days. While in control samples, at 6th day signs of deterioration (slimness, abnormal odour, and proteolysis) was appeared. When analysing the effect of Cinnamon essential oil on the inhibition of yeast and mould growth as in Table (10) showed that the yeast and mould counts detected in the control (non-treated) minced meat was $9.4x10^3±3.9x10^3$ and$7.6x10^2±1.1x10^2$, respectively at the 0 day of examination. The treatment of minced meat with Cinnamon essential oil lead to the inhibition and retardation of yeasts and moulds growth. The yeasts counts ranged from the beginning $5.1x10^2±1.3x10^2$ and $6.2x10^1±1.4x10^1$ to $2.3x10^2±5.7x10^1$ and $1.9x10^2±9.3x10^1$ at the end of storage period (9th day) in minced meat samples treated with Cinnamon essential oil 1% and 1.5%. While total mould counts ranged from beginning $1.4x10^2±1.0x10^2$ and <10^2 to $1.6x10^2±4.5x10^1$ and 0 at the end of storage period (9th day) in minced meat samples treated with Cinnamon essential oil 1% and 1.5%.

Table (1): Sensory quality evaluation of heat treated meat products

Point	Odor	Taste	Softness	Juiciness	Overall acceptability
1	Extremely bad	Extremely bad	Tough	Extremely bad (very dry)	Enough
2	Bad	Bad	Moderately tough	Bad (dry)	Neither good or bad
3	Neither good or bad	Neither good or bad	Moderately tender	Moderately	Good Juicy
4	Good	Good	Tender	Juicy	Very good
5	Very good	Very good	Very tender	Very juicy	Very good

Table (2): Mean values of total moulds count (log 10 CFU/g) of minced meat samples (N=25).

Examined samples	Total mould count *(log 10 CFU/g)*		
	Min	Max	Mean ±S.E
Basterma	2.0	3.6	2.9 ± 2.4
Minced meat	0.6	3.2	2.4 ± 2.0

Table (3): Frequency distribution of total mould count/g. of the examined meat products based on their total mould counts / g.

Examined samples / Frequency distribution	Basterma		Minced meat	
	No.	%	No.	%
<10^2	0	0	3	14.3
10^2-< 5×10^2	15	71.5	10	47.6
5×10^2-< 10^3	2	9.5	5	23.8
10^3 -< 5×10^3	4	19.0	3	14.3

Table (4): Mean values of total yeast count (log 10 CFU/g) of examined samples (N=25).

Examined samples	Total yeast count *(log 10 CFU/g)*		
	Min	Max	Mean ±S.E
Basterma	1.7	2.4	2.1 ± 1.5
Minced meat	2.4	3.1	2.9 ± 1.9

Table (5): Frequency distribution of total yeast count/g. of the examined meat products based on their total mould counts / g.

Examined samples	Basterma		Minced meat	
	No.	%	No.	%
<10^2	2	33.3	-	-
10^2-< 5×10^2	4	66.7	4	30.8
5×10^2-< 10^3	-	-	7	53.8
10^3 -< 5×10^3	-	-	2	15.4

Table (6): Incidence of the isolated mould genera in the examined meat product samples

Mould genera	Basterma		Minced meat	
	No.	%	No.	%
Aspergillus species	10	21.7	10	27.0
A. flavus				
A. niger	13	28.3	8	21.6
A. niveus	-	-	1	2.7
A. parasitcus	-	-	2	5.4
A. versicolor	-	-	2	5.4
A. ustus	-	-	2	5.4
Acremonium sp.	2	4.3	-	-
Alternaria sp.	3	6.5	-	-
Eupenicillium sp.	3	6.5	-	-
Fusarium sp.	-	-	2	5.4
Geotrichum sp.	-	-	3	8.1
Mucor sp.	1	2.2	-	-
Pencillium species	2	4.3	-	-
P. citreonigrum				
P. citrinum	3	6.5	-	-
P. decumbens	-	-	2	5.4
P. digitatum	1	2.2	-	-
P. simplicissimum	3	6.5	-	-
P. thomii	5	10.9	-	-
P. viridicatum	-	-	3	8.1
Talaromyces sp.	0	0	2	5.4

N.B: percentages were calculated in relation to the total number of isolated mould species of examined samples

Table (7): Frequency percentages of the isolated yeast genera in the examined meat products samples (N=37).

Yeast species	Basterma No.	Basterma %	Minced meat No.	Minced meat %
Candida species				
C. gullermondii	2	5.4	-	-
C. lipolytica	-	-	6	16.2
C. lusitaniae	-	-	5	13.5
C. tropicals	-	-	2	5.4
C. krusei	1	2.7	3	8.1
Rhodotorula spp.	3	8.1	8	21.6
Torulopsis spp.	4	10.8	3	8.1

N= Number of isolated yeast species

Table (8): Sensory evaluations of cooked minced meat treated with cinnamon and clove essential oils.

Essential oil	Odor	taste	softness	Juiciness	Over all acceptability
Control	1.4±0.186	2.1±0.2	1.5±0.19	1.3±0.16	6.2±0.42
Cinnamon 0.5%	2.6±0.18	3.1±0.13	3.1±0.13	2.8±0.16	11.6±0.32
Cinnamon1%	4.4 ±0.26	4.5±0.19	4.6±0.18	4.4 ±0.18	17.9±0.58
Cinnamon 1.5%	3.3±0.23	3.1±0.16	3.7±0.11	3.6 ±0.10	13.9±0.31
Clove 0.5%	4.1±0.09	4.1±0.18	4.2±0.21	4.0 ±0.16	16.7±0.43
Clove 1%	3.4±0.14	3.0±0.25	2.7±0.16	3.2 ±0.11	12.3±0.25
Clove 1.5%	2.1±0.14	1.8±0.09	1.9±0.08	1.6 ±0.18	7.4±0.32

4. DISCUSSION

Mould contamination of meat and meat products may occur during slaughtering of the animals, transportation, or during processing of meat products through the use of contaminated equipments or contaminated additives and spices which considered the most important source of mould contamination in meat products (Jay et al., 2005). The results in table (2) revealed that the total mould count of examined basterma samples were higher than that obtained by Maha and Sohad (2005) and Hussein (2008). While higher figures were recorded by Shaltout (1996), Nouman et al. (2001c) and Hafez (2003).The results obtained for basterma indicated that the examined basterma samples were contaminated with moulds which may be derived from the natural untreated spices and food additives used in their processing. From the above mentioned results, it is cleared that total mould counts were higher than that recommended by (EOSQC, 2005) which must be not more than 10^2. While the results of the examined minced meat samples were lower than that obtained by Shaltout (1996); Sayed et al. (2000); Saleh and Salah El-Dien (2006) and Salem et al. (2015) who reported that the mean total mould counts in examined minced meat were 4.21 CFU/g. While the results obtained for minced meat samples are agree with those reported by many investigators (Mansour et al., 1994; Shaltout and Salem, 2000 and Mohamed, 2004).

Table (9): Antifungal activity of various concentrations of clove essential oil against yeast& mould in minced meat during refrigerator storage at 4 ^{0}C.

Essential oils	Zero day		3 day		6 day		9 day	
	TYC*	TMC**	TYC	TMC	TYC	TMC	TYC	TMC
Control	$9.4x10^3±3.9x10^3$	$7.6x10^2±1.1x10^2$	$9.4x10^4±1.9x10^3$	$2.1x10^3±2.5x10^2$	Appearance signs of decomposition (slimness, abnormal odour, proteolysis			
0.5 % Clove	$7.5x10^3±2.8x10^3$	$1.4x10^2±1.0x10^2$	$7.6x10^2±3.4x10^1$	$5.7x10^2±2.3x10^1$	Appearance signs of decomposition (slimness, abnormal odour, proteolysis			
1% Clove	$1.2x10^3±2.5x10^2$	$6.7x10^1±3.3x10^1$	$< 10^2$	<10	$<10^2$	ND	$<10^2$	ND
1.5% Clove	$1.3x10^3±3.6x10^2$	$<10^2$	$<10^2$	<10	$<10^2$	ND	$<10^2$	ND

* TYC= Total yeast count.

* TMC= Total mould count

Table (10): Antifungal activity of various concentrations of Cinnamon essential oil against Yeast& mould in minced meat during refrigerator storage at 4 ^{0}C.

	Zero day		3 day		6 day		9 day	
	TYC	TMC	*TYC	*TMC	TYC	TMC	TYC	TMC
Control	$9.4x10^3±3.9x10^3$	$7.6x10^2±1.1x10^2$	$9.4x10^4±1.9x10^3$	$2.1x10^3±2.5x10^2$	Appearance signs of decomposition (slimness, abnormal odour, proteolysis			
0.5% Cinnamon	$4.1x10^3±1.5x10^3$	$3x10^2±1.9x10^1$	$7.6x10^2±3.4x10^1$	$5.7x10^2±2.3x10^1$	Appearance signs of decomposition (slimness, abnormal odour, proteolysis			
1% Cinnamon	$5.1x10^2±1.3x10^2$	$1.4x10^2±1.0x10^2$	$< 10^2$	$<10^2$	$1.3x10^2±8.9x10^1$	$9.7x10^1±4.2x10^1$	$2.3x10^2±5.7x10^1$	$1.6x10^2±4.5x10^1$
1.5% Cinnamon	$6.2x10^1±1.4x10^1$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	***ND	$1.9x10^2±9.3x10^1$	***ND

*TYC = Total yeast count. **TMC= Total mould count. ***ND= No mould growth

The obtained results in table (4) declared that the total yeast count in examined meat product samples were lower than those reported by Ouf et al. (2010); Saleh et al. (2012); Eleiwa and El-Diasty (2014). The data obtained in table (6) and (7) of mould and yeast identification declared that the most predominant mould and yeast genera in examined meat samples were; Aspergillus ,Penicillium and candida species which agree with the results obtained by many researchers as Edris et al. (1992), Hafez (2003), Saleh and Salah El-Dien (2006), Hussein (2008), Pitt and Hooking (2009), Samaha (2013) and Ismail- Seham et al. (2013), Eleiwa and El-Diasty (2014), Salem et al. (2015). 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 (Lattaoui and Tantaoui-Elaraki, 1994 and Koutsoumanis et al., 1998). There is an 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 (Tsigarida et al., 2000). Since EOs are considered as generally regarded as safe (GRAS) Kabara (1991), 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. Results are presented in table (8) and figure (1) A lot of researchers have documented the antimicrobial activity of essential oils including lemongrass, citronella, clove, peppermint, thyme, dill, Capsicum and oregano oils against different fungal species. This is reported by different investigators, (Mishra and Dubey, 1994; Viuda-martos et al., 2007; Eleiwa and El-Diasty, 2014) and Salem et al. (2015) who reported that minced meat treated with Dill oil 3% and 1% showed a higher organoleptic score of overall acceptability (19.3 and 19.1) along the experiment days rather than other groups. This is followed by the Capsicum oil 1% and Capsicum oil 3% (14.9 and 14.5) ,then lowest one was obtained by Clove oil 3% and Clove oil 1% (14.7, 13.7), respectively. The results illustrated in table (9 & 10) showed greater antifungal activity of whole essential oils have due to a synergistic effect with some active components; thus, they are more promising in commercial application than single compounds. The antifungal activity of the clove oil and its main component, eugenol (85.3%) were investigated by Eugénia et al., (2009) against Candida and Aspergillus. Also previous studies have reported antifungal activity for clove oil and eugenol against yeast and filamentous fungi, such as several foodborne fungal species (Lopez et al., 2005; Velluti et al., 2004). The antifungal activity can be attributed to the presence of some components such as Eugenol (a phenylpropanoid, is an ally chain-substituted guaiacol). Hashim et al. (2008) mentioned that the total inhibitory effect of Cinnamomum zeylanicum oil extract on the growth of A. flavus on PDA medium at the levels up to 650ppm, the antifungal activity can be attributed to the presence of Glycosides, Alkaloids, Resins, Saponins, Coumarins, Flavonoids, Terpens and Steroids. The authors concluded that treatment of minced meat by addition of Clove oil and Cinnamon oil inhibit the mould and yeast growth and extends the shelf-life of treated minced meat. Clove oil 1.5 % was needed for inhibition of yeast and mould growth. In addition, these essential oils have the potential to be used in food as flavoring and natural preservatives. Thus, the essential oils of clove and cinnamon could be used to control food spoilage as a potential source of food preservative. The antifungal effects of clove oil were found to be more effective than those of cinnamon oil, but the odour of cinnamon is more palatable than the clove odour.

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