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ANTIBACTERIAL AND ANTIFUNGAL EFFECT OF CARDAMOM, MASTICHE OILS AND HOT GREEN PEPPER EXTRACT ON SOME UNDESIRABLE BACTERIA AND MOULDS

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ABSTRACT

Cardamom and mastiche oils were extracted from cardamom capsules and from mastiche gum using steam distillation, while hot green pepper extract (HGPE) was prepared in the form of a sterilized juice. They were tested as inhibitors against some pathogenic and spoilage microorganisms that may be present as contaminants in milk and other dairy products, namely Staphylococcus aureus, Micrococci spp., E. coli, B. cereus, B. subtilis and Ps. aeruginora. The fungicidal effect of each of these spices against the growth of A. flavus and A. parasiticus, the common contaminants of cheeses, was also studied. The microbial growth rate and the minimum inhibitory concentration (MIC) were the parameters used to assess the inhibitory effect of tested spices. The obtained results indicated that the inhibition of the growth of tested bacteria except B. cereus by cardamom and mastiche oils was in the following descending order; Staph. aureus> Micrococci spp. > Ps. aeruginosa > E. coli > B. subtilis, while B. cereus growth was stimulated by cardamom oil and inhibited by mastiche oil. HGPE inhibited only the growth of Staph. aureus and Micrococci spp. and stimulated that of all other tested bacteria. Cardamom and mastiche had antifungal effect against A. flavus and A. parasiticus, while HGPE showed stimulation effect on both. The MIC values of the tested spices pointed out that cardamom had a more powerful inhibitory effect on tested bacteria and moulds.

INTRODUCTION

It is well known that spices and herbs have been used from prehistoric times not only for flavouring food but also for their antioxidative, preservative and medicinal properties. Food scientists indicated that intact spices, their essential oils and their extracts are superior substances which can be used as a flavouring materials and as a substitute for the artificial hazardous preservatives in food processing (Tsimidou and Baskron, 1994).

Some dairy products as a part of the human diet are no exception, therefore spices have been used in their processing. Davis (1965) listed caraway, cumin, aniseed, pepper and clove as the spices added to certain cheese during processing. Ghosh *et al*. (1986) and Patel and Abd El-Salam (1986) have used cardamom and mastiche in the manufacture of ice-cream mix. and shrikhand. Moreover, Hassan (1996) has used cardemom and mastiche as antioxidants in butter manufacture. Black cumin was used as an antimicrobial agent in some Syrian hard cheese (Abou Donia and Abd El-Kader, 1979), Karish chese (Abou Dawood, 1996) and butter processing (Salmerson *et al*., 1990; Abu Zeid and Mahmoud, 1993; Darwish, 1995 and Abou Dawood, 1996). Clove oil (euganol) was used as antifungal in Karish

cheese (Abou Dawood, 1996) and Ras cheese contaminated with Penicillium spp. (Abu Sree, 1997). Green pepper (sweet or hot) was pickled with Domiati cheese (Ismail *et al*., 1972 and Kamaly, 1978) and Karish cheese (Ezz El-Dine, 1978) to improve its quality. It was found that green pepper had a bactericidal effect against spoilage microorganisms. In addition, adding capsicum tincture to Domiati cheese milk gave similar results (Shehata *et al*., 1984).

Although, much attention has been given nowadays to the use of natural antimicrobial and antioxidants since it is considered safer and linked with fewer adverse reaction (Tsuda *et al.*, 1993), few studies were carried out on cardamom, mastiche and hot green pepper extract as antibacterial and antifungal agents.

Therefore, It was thought worthwhile to study their antibacterial and antifungal effect on some spoilage and pathogenic bacteria and some undesirable moulds.

MATERIALS AND METHODS

I. Materials:

1. Cardamom and Mastiche oils:

Cardamom capsules and mastiche gum were separately ground in a procelian mortar and steam distilled for 3 hr. The oil of each was collected in a glass vessel and dried over anhydrous sodium sulphate, then kept in a deep freezer until usage. 0.5 ml of oil was transferred to 20 ml volumetric flask followed by one ml of tween-80, then completed to the mark with distilled water. On the basis of specific gravity of oil (0.9 g/ml.), the resultant emulsion contains 22.5 mg oil/ml. Suitable volumes from this emulsion were used to get the desired oil concentration in the different media.

2. Hot green Pepper extract:

Hot green pepper pads were crushed using kitchen machine into a homogeuous juice. The resultant juice was poured in cleen test tubes, covered with cotton plugs and sterilized at 120°C/15 min. A clear supernatant and green precipitate were obtained after outoclaving. The supernatant was used as the hot green pepper extract.

3. Media and Microbial Cultures:

Nutrient broth, nutrient agar, violet red bile agar, Staphylococcus media (Oxoid), were used for enumarting studied bacteria. Potato dextrose agar (PDA) and yeast extract sucrose (YES), media (Davis *et al*., 1966) were used for the growth of studied moulds.

Pure cultures of some undesirable organisms namely Bacillus subtilis, Bacillus cereus, Esherishia coli, Micrococcus spp., Pseudomonas aeruginosa and Staphylococcus aureus were obtained from the Department of Microbiology, Faculty of Agriculture, Cairo University. Aspergillus flavus and Aspergillus parasiticus were obtained from Mycotoxin laboratory, National Research Center, Cairo, Egypt.

II. Methods of Analysis:

Determination of minimum ihibitory concentration (MIC) of cardamom, mastiche oils and hot green pepper extract against tested microorganisms:

The effect of tested essential oils and hot green pepper extract on the growth of tested undesirable microorganims was carried out according to Janssen *et al*. (1987). Different concentrations of each oil or pepper extract were thoroughly mixed with 10 ml of sterilized suitable media, then poured into petri dishes containing 1 ml. of a suitable broth culture dilution. The same steps were repeated with the rest of the tested microorganisms. The petri dishes were left at room temperature for solidification, then incubated at 32°C for 24-48 hr. The grown colonies were counted and the growth rate (increase or decrease as compared to a control) was calculated and the lowest concentration of the tested oils or pepper extract required to inhibit the growth of the microorganisms completely was designated as the minimum inhibitory concentraton (MIC).

Determination of fungicidal effect and MIC of cardamom, mastiche oils and pepper extract against Aspergillus flavus and Asp. parasiticus:

One. Preparation of spore suspentions:

The mold was grown on potato dextrose agar (PDA) slants at 25°C/7 days. The mold spores were collected by sterile 0.05% tween-80 to provide suspensions containing about 10^6 spores/ml.

Two. Evaluation of antimycotic activity of cardamom, mastiche and pepper extract:

The antimycotic activity against Asp. flavus and Asp. parasiticus was carried out following the method described by Bullerman (1974). Different concentrations of the tested oils and pepper extract were added to 50 ml. of yeast extract sucrose (YES) medium to a final concentration of 0.0 – 0.8% and 0.0 – 1.5% (v/v) for tested oils and pepper extract respectively. The flasks containing spices and YES medium were autoclaved at 121°C/15 min., cooled to room temperature and inoculated with 0.5 ml. of spore suspension containing about 10^6 spores/ml, then incubated at 25°C for 7 days. After the incubation period, the flasks were put in an oven at 100°C for 10 min. to stop the biological activity of mold. The mycellium mat was separated by filtering through a preweighed whatman No. 1 filter paper. The mycellium was rinsed with distilled water, allowed to air drying and then dired at 100°C for 3 hr. Mycellium dry weight was determined gravimeterically and the inhibition or stimulation percent was calculated as follows:

$$\text{Inhibition or stimulation \%} = \frac{B - A}{A} \times 100$$

Where: A = mycellium dry weight of control
B = mycellium dry weight of treatment

RESULTS AND DISCUSSION

Preliminary experiments indicated that the least effective concentration of cardamom and mastiche oils was 0.1 ml of their oil emulsion for Micrococci spp. and Staph. aureus, 0.25 ml. for other tested bacterial cultures and 0.18 ml for tested molds respectively. While that of hot green pepper extract (HGPE) was 0.25 ml. for all tested cultures. Therefore, these values were taken as a guide when planning the different experiments.

1. Effect of cardamom, mastiche oils and HGPE on the growth rate of Micrococci spp. and Staph. aureus:

The effect of various concentrations of cardamom and mastiche oils on the growth of Micrococci spp. and Staph. aureus is shown in Table 1. It is clear from the presented results that the increase in the concentration of cardamom and mastiche oils in the microbiological growth medium of Micrococci spp. and Staph. aureus led to a proportional inhibition of their growth. Complete inhibition of Staph. aureus growth was noticed in the presence of 450 and 1125 ppm of cardamom and mastiche oils in the medium, respectively. The above mentioned concentration of cardamom or mastiche oils can, therefore, be considered the minimum concentration required to induce complete inhibition of the Staphylococcus growth and is designated as the minimum inhibitory concentration (MIC).

Table (1): Effect of different concentrations of cardamom and mastiche oils on the growth rate of *Micrococci spp.* and *Staph. aureus*

Concentration (in the media)		Cardamom oil				Mastiche oil			
		Micrococci spp.		*Staph. aureus*		*Micrococci spp.*		*Staph. aureus*	
ml	ppm	TC 10^8	Red %*	TC 10^8	Red. %	TC 10^8	Red. %	TC 10^8	Red. %
Nil	Nil	473.0	0.00	553.0	0.00	383.0	0.00	181.0	0.00
0.10	225.0	198.4	58.06	111.0	79.93	262.4	31.50	92.5	48.93
0.20	450.0	75.3	84.08	ND	100.00	183.0	52.22	79.5	56.01
0.30	675.0	ND**	100.00			133.0	65.27	56.4	69.61
0.40	900.0					94.0	75.46	34.2	81.10
0.50	1125.0					53.5	86.03	ND	100.00
0.60	1350.0					ND	100.00		

*** Red. = reduction**
**** ND = not detected**

Furthermore, the MIC of cardamom and mastiche oils for Micrococci spp. was found to be 675 and 1350 ppm of both oils, respectively (Table 1 and Fig. 1), which suggests that Staph. aureus is more suceptible to cardamom and mastiche oils than Micrococci spp. Moreover, it is obvious that cardamom oil has intensive inhibitory effect on the growth of the aforementioned bacteria as compared with that of mastiche oil. This might be due to the presence of antimicrobial components (P-cymene, 1-8 cineol and linalool) which constitute 61.94 and 13.59% of the cardamom and mastiche chemical composition, respectively as indicated by Hassan (1996). The

obtained results (Table 1) are in accordance with those obtained by Abou Dawood (1996) who determined the MIC of black cumin, cinnamon, clove oils and garlic extract for Micrococci spp. and Staph. aureus. However, the MIC of cardamom and mastiche for Micrococci and Staph. aureus was much lower than that obtained by Abou Dawood (1996) for black cumin, cinnamon, clove or garlic, which indicates that cardamom and mastiche have a powerful bactericidal effect than the abovementioned spices.

Regarding hot green pepper extract (HGPE), data in Table (2) indicate that a very high concentrations of HGPE (20000 – 60000 ppm) were required to produce inhibition similar to that produced by cardamom and mastiche oils (Table 1). This indicates its lower inhibitory effect as compared with cardamom and mastiche. It is also worthy to note that the inhibitory effect of HGPE on Staph. aureus was more pronounced than that on Micrococci spp., which constitute a trend similar to that of cardamon and mastiche. The MIC of HGPE (Table 2 and Fig. 2a) was 55000 and 60000 ppm for Staph. aureus and Micrococci spp., which is equal to 5.5 and 6.0% of HGPE in the microbiological medium (v/v), respectively.

Fig. (1): Minimum Inhibitory concentration (MIC) of cardamom and mastiche oils against some microorganisms

Fig2

Table (2): Effect of different concentrations of hot green pepper extract on the growth rate of *Micrococci* spp. and *Staph. aureus*.

Concentration (in 10 ml medium)		*Micrococci* spp.		*Staph. Aureus*	
ml.	Ppm	TCx10^8	Red.* %	TCx10^8	Red. *%
0.0	0.0	383.0	-	181.0	-
0.2	20000	280.0	26.89	148.2	18.20
0.3	30000	215.0	43.83	93.6	48.30
0.4	40000	108.8	71.60	52.5	70.99
0.5	50000	30.5	92.10	19.9	89.00
0.55	55000	7.0	98.20	ND**	100.00
0.60	60000	ND**	100.00	-	

*** Red. = reduction.**
**** ND = not detected**

2. Effect of cardamom, mastiche oils and HGPE on the growth of B. cereus, B. subtilis, E. coli and Ps. aeruginosa:

It is obvious from the data in Table (3) that although cardamom oil has inhibited the growth of B. subtilis, E. coli and Ps. aeruginosa, it has stimulated the growth of B. cereus. These results are in line with those obtained by Abou Dawood (1996) who indicted that black cumin stimulated the growth of *B. megatherium* and *Micrococci spp.* when added at concentration lower than 1000 and 2000 ppm, respectively, while it inhibited the growth of E. coli and Staph. aureus. Furthermore, clove, cinnamon and garlic extract at concentration of 200 ppm showed stimulation effect on one or more of the experimented microorganisms (E. coli, Micrococci spp. and Staph. aureus), while higher concentrations resulted in inhibition.

Results in the same Table also illustrate that mastiche oil on the other hand has inhibited the growth of all tested microorganisms when added to their media including B. cereus. The MIC of cardamom and mastiche for B. subtilis, E. coli and Ps. aeruginosa (Table 3 and Fig. 1) was 1912.5 and 2475, 1800 and 2250, and 1125 and 2025 ppm, respectively. This further illustrate that cardamom has a greater inhibitory effect against these organisms than that of mastiche.

Regarding the effect of different concentrations of HGPE on the growth of the above mentioned four organisms, data in Table 4 show that all concentrations of HGPE added to the suitable media of tested organisms caused a very high stimulatory effect on their growth. This effect was more pronounced with E. coli and Ps. aeruginosa, since the increase (%) in their total count was much greater than that of B. cereus and B. subtilis as shown in Table 4.

Table (4): Effect of different concentrations of hot green pepper extract on the growth rate of some undesirable microorganisms.

Concentration		Microorganisms							
		B. cereus		*B. subtilis*		*E. coli*		*Ps. aeruginosa*	
Ml	Ppm	TCx10^9	Incr.* %	TCx10^9 %	Incr.%	TCx10^9 %	Incr.%	TCx10^9 %	Incr. %
0.0	0.0	28.4	-	39.5	-	38.0	-	27.3	-
0.25	25000	65.3	+130.0	102.7	+160.0	114.0	+200.0	81.0	+196.0
0.50	50000	90.8	+255.0	161.9	+310.0	187.0	+392.1	130.5	+378.0
0.75	75000	119.7	+321.5	210.1	+432.0	258.0	+578.9	202.0	+639.9
1.00	100000	149.4	+426.0	233.1	+490.0	UC**	-	UC**	-

*** Incr = increase**
**** UC = uncounted**

These results are contradictory to those obtained for Domiati cheese pickled in salted whey containing green pepper (Ismail *et al*., 1972) or capsicum tincture (Shehata *et al.,* 1984). These differences might be due to the presence of high concentrations of salt which greatly inhibit the growth of spoilage microorganisms. In addition, the presence of lactic acid bacteria in the pickling medium as well as in the cheese itself is expected to produce lactic acid as well as some antibacterial substances which might be considered as inhibitors for spoilage organisms (Bacillus, Esherishia and Pseudomonas).

1. Effect of different concentrations of cardamom, mastiche oils and HGPE on the growth rate of Aspergillus flavus and A. parasiticus.

The results presented in Table 5 and Fig. 2(b) indicate that cardamom and mastiche oils added to the suitable medium inhibited the growth of A. flavus and A. parasiticus. The inhibitory effect was proprortional to the added oil concentration. It is also evident that cardamom has a greater inhibitory effect than that of mastiche. This finding might be due to the presence of antimicrobial components (P-cymene, 1-8 cineol and linalool) which constitute 61.94 and 13.59% of the cardamom and mastiche oils chemical composition, respectively as reported by Hassan, (1996).

Table (5): Effect of different concentrations of cardomom and mastiche oils onthe growth rate of *A. flavus* and *A parasiticus*

Concentration (in 10 ml media)		Cardamom				Mastiche			
		A. flavus		*A. parasiticus*		*A. flavus*		*A. parasiticus*	
ml	ppm	Wt[1]	R[2]%	wt	R%	Wt	R%	wt	R%
0.0	0.0	1.7889	-	1.8235	-	1.7889	-	1.8235	0.0
0.18	400	1.5580	12.91	1.6874	7.46	1.7854	0.20	1.9433	+6.5
0.36	800	1.1932	36.10	0.7084	61.32	1.6248	5.67	1.4036	-23.03
0.54	1200	0.8821	50.70	0.1771	90.30	1.4814	17.18	1.3668	-25.04
0.72	1600	0.3151	82.40	N.D.	100.0	0.8820	50.70	0.648	-64.46
0.90	2000	N.D[3].	100.0			0.5249	70.66	0.118	-93.53
1.08	2400					0.2130	88.09	N.D.	-100.0
1.26	2800					N.D.	100.0		

1. wt = dry weight of molding.

2. R% = reduction in weight of molds (%)
3. N.D. = not detected

The obtained results are in general agreement with those of several invistigators who worked on some other spices to inhibit the growth of moulds in butter (AbouZeid and Mahmoud, 1993, Darwish, 1995 and Abou Dawood, 1996) or to prevent aflatoxin formation in Ras cheese (Abu Sree, 1997 and Abou Dawood 1999). Furthermore, Hassan (1996) has found that the use of cardamom and mastiche as antioxidants in butter during storage led to slow growth of moulds and yeasts.

Unlike cardamom and mastiche, HGPE added to the liquid yeast extract sucrose medium of either A. flavus or A. parasiticus at concentrations of 50000, 100000 and 150000 ppm resulted in increase in their growth rate as shown in Table (6). It is obvious that the stimulatory effect of HGPE on both moulds was similar and it was pronounced at concentrations more than 50000 ppm in their media.

Table (6): Effect of different concentrations of HGPE on the growth rate of *A. lavus* and *A. parasiticus*

Concentration of HGPE in 10 ml medium		*A. flavus*		*A. parasiticus*	
Ml	**Ppm**	**wt***	**I**%**	**wt**	**I%**
Nil	nil	0.3843	-	0.4135	-
0.5	50000	0.3893	+1.30	0.4262	+3.08
1.0	100000	0.5388	+40.20	0.6015	+45.47
1.5	150000	0.5758	+49.83	0.6322	+52.90

*** wt = weight of mould growth (g).**
**** I% = increase % in mould growth.**

These results are in agreement with those of Salmerson *et al*., (1990) who observed a progressive increase in the growth of A. flavus and A. parasiticus when oregano or thyme were added to their media as compared with their respective control. It is also in line with the results of Abou Dawood (1996) who indicated that black cumin oil has stimulated the growth of A. flavus and A. parasiticus, and also with those of Abou Dawood (1999) who found that black pepper has a potent stimulatory effect on the growth of A. flavus and P. roqueforti when separately added to their respective media.

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التـأثير المثبط لنمـو بعض البكتريـا والفطريـات غير المرغوبـة بفعل زيت الحبهـان وزيت المستكة ومستخلص الفلفل الأخضر الحار

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تم الحصول على زيت الحبهان Cardamom oil وزيت المستكة Mastiche oil بواسطة عملية التقطير ببخار الماء لمدة 3 ساعات. بينما تم الحصول على مستخلص الفلفل الأخضر الحار (الشطة) Hot green pepper extract في صورة عصير فلفل معقم على 120°م/15 د. وذلك لدراسة تأثيرها التثبيطي على بعض الميكروبات المرضية وغير المرضية غير المرغوب في تواجدها باللبن ومنتجاته وتشمل: Staph. aureus, Micrococci spp., E. coli, B. cereus, B. subtilis and Ps. aeruginosa.

كما تم دراسة التأثير المثبط لنمو أكثر الفطريات شيوعاً في تلوث الجبن وهي Asp. flavus and Asp. parasiticus بواسطة هذه التوابل. وتم تقييم هذا التأثير المثبط بواسطة تقدير معدل النمو لهذه الكائنات الحية الدقيقة Growth rate إلى جانب تقدير قيمة أقل تركيز من هذه المواد يسبب وقف كامل لنمو هذه الكائنات ويعبر عنه بالـ MIC اختصار الـ Minimum inhibitory concentration.

وقد أوضحت النتائج أن القوة التثبيطية لنمو جميع الميكروبات المستخدمة ما عدا B. cereus بواسطة زيت الحبهان وزيت المستكة يمكن ترتيبها ترتيباً تنازليا كما يلي:

Staph. aureus > Micrococci spp. > Ps aeruginosa > E. coli> B. subtilis

في حين أدى استخدام تركيزات مختلفة من زيت الحبهان (صفر – 3375 جزء في المليون) إلى تشجيع نمو بكتريا الـ B. cereus ولكن استخدام تركيزات مماثلة من زيت المستكة أدى إلى تثبيط نموها.

كما أظهرت النتائج أن استخدام مستخلص الفلفل الأخضر الحار أدى إلى تثبيط نمو الـ Staph. aureus and Micrococci spp فقط وتنشيط نمو باقي الميكروبات المستخدمة في هذه التجربة.

كما وجد أن استخدام زيت الحبهان، وزيت المستكة يثبطان نمو Asp. flavus, Asp. parasiticus وعلى العكس من ذلك فإن استخدام مستخلص الفلفل الأخضر الحار يؤدي إلى تنشيط نموها.

وقد أوضحت قيم الـ MIC لهذه المواد المستخدمة أن زيت الحبهان كان ذو قوة تثبيطية عالية بالمقارنة بزيت المستكة أو مستخلص الفلفل الأخضر الحار.

Table (3): Effect of different concentrations of cardamom and mastiche oils on the growth rate of some microorganisms.

Concentration (in 10 ml med.[1])		Cardamom								Mastiche							
		B.cereus		B. subtilis		E. coli		Ps. aeruginosa		B. cereus		B. subtilis		E. coli		Ps. aeruginosa	
ml.	ppm	TCx10[9]	Incr.[2] %	TCx10[8]	Red. %	TCx10 [8]	Red. [3] %	TCx10[8]	Red. %	TCx10[8]	Red. %	TCx10[8]	Red. %	TCx10[8]	Red. %	TCx10[8]	Red. %
0.0	0.0	28.40	0.0	395.0	0.0	380.0	0.0	273.0	0.0	500.0	0.0	352.0	0.0	380.0	0.0	273.0	0.00
0.25	562.5	36.45	+28.4	365.0	7.59	249.0	34.47	8.3	96.96	410.0	18.0	280.0	20.45	312.0	17.89	114.0	58.24
0.50	1125.0	39.04	+37.5	177.0	55.20	105.0	72.37	ND	100.00	380.0	24.0	236.0	32.92	279.0	26.58	64.0	76.86
0.75	1687.5	41.52	+46.2	68.5	82.66	15.0	96.05			260.0	48.0	196.0	44.32	134.0	64.74	24.0	91.21
0.80	1800.0			5.0	96.05	ND	100.00			266.0	46.6	106.2	69.88	87.0	77.10	3.0	98.90
0.85	1912.5			ND[4]	100.00					252.0	49.6	83.4	76.40	53.9	85.80	ND	100.00
0.90	2025.0									180.0	64.0	56.5	83.90	34.0	91.05		
1.00	2250.0	58.00	+104.2							106.0	78.8	26.6	92.44	ND	100.00		
1.10	2475.0									87.2	82.6	ND	100.00				
1.25	2812.5									41.0	91.8						
1.50	3375.0	108.20	+281.0							ND	100.00						

1- med. = medium
2- Incr. = increment
3- Red. = reduction
4- N.D. = not detected