Vet.Med.J.,Giza. Vol.48, No.4. (2000): 447-456.

EFFECT OF SOME SPICE OILS ON *ASPERGILLUS PARASITICUS* GROWTH AND AFLATOXIN PRODUCTION DURING RIPENING OF RAS CHEESE.

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Received: 20.9.2000.
Accepted :12.10.2000.

SUMMARY

Clove oil (100&200ppm) , cumin oil (100&200ppm) and nigella sativa seeds oil (N.S.S.O.) (100&200ppm) as spice oils (natural preservatives) were evaluated for potential inhibition of Aspergillus parasiticus growth and subsequent aflatoxins production in Ras cheese. All the tested spice oils decreased the growth rate of A.parasiticus and aflatoxins production in variable degrees. Clove oil at concn. 200ppm achieved the results as a best natural preservative since it eliminated A.parasiticus after 16 days and reduced aflatoxin B1& total aflatoxin contents to 0.001and 0.6 ppb after 90 days. Moreover, the detoxifying effect of the three spice oils were evaluated against AFB1 in broth medium. None of the tested oils showed a detoxifying effect against AFB1. The tested oils had no marked effect on the chemical properties of Ras cheese. Although clove and cumin oils slightly increased protein degradation, N.S.S.O. decreased protein degradation and also slightly increased total volatile fatty acids (TVFA) content in comparison with the control.

Results indicated that certain flavourings used for speciality cheeses can also be effective in controlling growth rate and aflatoxins production of A.parasiticus in Ras cheese.

INTRODUCTION

Spoilage molds represent a major problem for cheese manufacturers and marketers. A.parasiticus is wide spread as cheese contaminant (EL-Essawy et al.,1984 ; Abouzeid et

al.,1996 and Abdel-Hakiem & EL-Kosi,1999) and is known by its high efficiency in producing aflatoxins (Aly,1979 ; Abu-Sree, 1997 and Bars-Bailly et al.,1999).

Ras cheese is one of the most popular type of hard cheese in Egypt. It is characterized by its strong pungent and sharp flavour which meet the taste of the Egyptian consumer. It is likely to be exposed to many sources of contamination with A.parasiticus during manufacturing, storage and marketing (Bullerman,1979).

With the improvement of people's living standard, consumers have higher demands for the safety of food. The use of natural preservatives to control fungal growth and aflatoxin production in milk and dairy products has been a subject of a considerable concern in recent years for the importance of milk products in human diet (Bullerman et al.,1977 and Wendorff & Wee,1997). Spices were recognized by the Egyptians over 3000 years ago as having preservation possibilities which mainly present in their essential oils. The first reference in the literature to essential oils of spice appears in the latter part of the thirteenth century (Farrell,1985). In Egypt, cumin oil, clove oil and nigella sativa seeds oil , N.S.S.O., are used as flavouring agents. Moreover, cumin oil has pharmaceutical purposes as a carminative, and antispasmodic; clove oil has some antiseptic qualities (Farrell,1985),and N.S.S.O. is used for treatment of indigestion and asthma (EL- Faham,1994).

In Egypt, there is little information about the antimycotic and antimycotoxigenic effects of spice oils. Accordingly, three spice oils were chosen to be tested for their effectiveness against the growth rate of A.parasiticus and subsequent aflatoxins production during ripening of Ras cheese, and also evaluation of their detoxifying effect against AFB1 in broth medium.

MATERIAL AND METHODS

A-Material:

1- Aspergillus parasiticus strain used:

It was obtained from Animal Health Research Institute. Potato Dextrose Agar (PDA) was used for mold growth (Marshall,1992).

2- Preparation of spice oils:

Emulsions of clove oil, cumin oil and N.S.S.O., obtained from local markets, were prepared by emulsifying the oil in water and tween 80 (Sherine,1996). Sufficient volume of each oil emulsion was added to milk to achieve 100 & 200 ppm.

3- Aflatoxin B1 standard (AFB1):

AFB1 standard was obtained from Sigma Co.

B- Methods:

1- Preparation of spore suspention:

A.parasiticus strain was grown on PDA slants at 25(C for 7 days. The spores were collected using

sterile 0.05 % tween 80 to provide suspension containing about $0.9x10^9$ spores/ml.

2- Laboratory experimental work:

Raw A.parasiticus free buffaloe's milk (90 Kg) was pasteurized at 63°C / 30 min. and cooled to 4°C. The milk was experimentally contaminated with the A.parasiticus strain to obtain a final concn. of $7x10^3$ CFU/ml. The milk was divided into 7 batches:

1) Batch "A" : Contained clove oil 100 ppm.
2) Batch "B" : Contained clove oil 200 ppm.
3) Batch "C" : Contained cumin oil 100 ppm.
4) Batch "D" : Contained cumin oil 200 ppm.
5) Batch "E" : Contained N.S.S.O. 100 ppm.
6) Batch "F" : Contained N.S.S.O. 200 ppm.
7) Batch "G" as a control : contained no oil.

Three replicates were prepared from each treatment. The batches were manufactured into Ras cheese according to the method adopted by Abdel-Tawab (1963). Cheese samples were analysed when fresh(zero time) and at 6, 9 and up to 90 days after manufacture for A.parasiticus count; at 6 , 9 , 16 and up to 90 days for estimation of AFB1 and total aflatoxin contents (TAC); and at zero time, 30, 60 and 90 days for chemical examination.

3- Aspergillus parasiticus count:

Samples from the manufactured product were examined for A.parasiticus count by culturing on (PDA) using the technique adopted by (Marshall,1992).

4- Aflatoxins analysis:

a- Estimation of AFB1 & Total aflatoxin contents in Ras cheese:

AFB1 and total aflatoxin contents were extracted and detected in Ras cheese using AflaTest*. The technique was conducted as follows:

I- Sample extraction: Fifty gram of each sample, 5 gram NaCl and 100 ml methanol : water (80 : 20) were blended at high speed for 1 minute. The extract was poured into fluted filter paper, and the filtrate was collected in a clean vessel.

II- Extract dilution: Ten ml of the filtered extract was diluted with 40 ml purified water, mixed well and then filtered through glass microfibre filter into glass syringe barrel using markings on barrel to measure 10 ml.

III- Column chromatography: Ten ml filtered diluted extract was passed through Afla Test® - P affinity column at a rate of about 1 - 2 drops / second until air comes through column. Ten ml of purified water was passed through the column at the same previous rate (two times). Affinity column was elluted by passing 1.0 ml HPLC grade methanol through column at a rate of 1 - 2 drops / second and all the sample elute was collected (1 ml) in a glass cuvette. One ml of AflaTest developer was added to elute in the cuvette and mixed well. The cuvette was placed in a calibrated fluorometer and read aflatoxin concentration after 60 seconds.

* The AflaTest was supplied by Vicam Co., MA. USA.

b- Evaluation of the detoxifying effect of spice oils against AFB1 in broth medium:

The detoxifying activity of spice oils against A.parasiticus was evaluated by adding a sample of spice oils to a flask containing 100 ml of Yeast Extract Sucrose (YES) medium for a final concn. of 100 & 200 ppm for each oil. The broth cultures containing spice oils were autoclaved at 121°C for 15 min. After cooling, the flasks were inoculated with AFB1 to obtain a final concn. of 40 ppb and incubated at 25°C. Samples were analysed at 30 and 45 days for AFB1 content using AflaTest.

5- Chemical examination of the product:

Cheese samples were analysed for moisture % , fat % , soluble nitrogen % (SN) and total nitrogen % (TN) according to Ling (1963); titratable acidity % according to Atherton and Newlander (1977), and for total volatile fatty acids (TVFA) according to Kosikowski (1978).

RESULTS AND DISCUSSION

Data presented in Tables (1&2) reveal that A.parasiticus was able to grow and produce aflatoxins in the inoculated Ras cheese. A.parasiticus count in the control batches increased during the interval between the zero time (4. $3x10^3$) and the three months of ripening (6.4×10^3). Moreover, AFB1 and (TAC) increased (approximately fourfold for AFB1 and ninefold for TAC) during the interval between the six and ninetieth day of ripening period. The concn. of AFB1 in cheese samples, regardless of the A.parasiticus count or period of ripening , would probably be considered hazardous to human health (0.5 ppb for AFB1 according to China and Cyprus standards) (FAO/WHO 1996).

The growth of mold and production of AFB1 in control cheese is in agreement with Aly (1979) who concluded that fresh cheese is a good medium for A.flavus growth and aflatoxin production. Aspergillus spp. is one of the most common groups of molds causing defects in cheese (Bars-Bailly et al.,1999). Aflatoxins seldom occur naturally in milk products other than cheese (Scott,1989). The contamination of milk by aflatoxins is a major problem of those countries where the sanitary conditions allow the growth of A.parasiticus and the contaminated milk and milk products are not discarded because of the critical economical and social situation. This kind of contamination may represent a source of chronic exposure to an important risk factor for hepatocarcinogenesis, AFB1 being the most toxic and carcinogenic one (Gilli et al.,1989 and Hassan and Bullerman,1995). Moreover, Ali et al.(1971) declared that children may be exposed to aflatoxins even before weaning because mothers comsuming aflatoxins in their food may secrete AFM1 in their milk.

Cheese batches treated with clove oil (100ppm) was ovoid of A.parasiticus count within 30 days of ripening, while the count was completely reduced by 16 days using (200ppm). Furthermore, using the concn. 100ppm, AFB1 and total aflatoxin contents were detected after 6 days of ripening (1.4 &13 ppb), reached a maximum after 16 days (3.6 & 23 ppb) and then declined and reached (0.13 & 1.0 ppb) after 90 days. The concn.200ppm showed a powerful antimycotoxigenic activity on AFB1 and total aflatoxin contents (0.001 & 0.6 ppb) after 90 days of ripening (Table 1&2). Lie and Marth (1967) reported that, the highest amounts of AFB1 & AFG1 produced by A.parasiticus in chedder cheese were formed after 7 weeks. According to Karaioannoglou (1990), AFB1was detected in a concn. ranged from 22-40 ppb of cheese.Furthermore,Abu Sree (1997) detected AFB1 in 6.6% of Ras cheese samples in a level ranged from 1.4 - 10.5 ppm.

Spice oils reportedly have antimycotic and antimycotoxigenic properties. Bullerman et al. (1977) reported that clove oil at concn. 200-250ppm inhibited growth and aflatoxin production by A.parasiticus.Moreover, Farag et al. (1989) reported that, clove oil decreased aflatoxin production from A.parasiticus by 99 %. According to Sherine (1996), clove oil exhibited a high inhibition power against A.parasiticus. The antimycotic activity of clove oil may be attributed to the effect of eugenol (Doerr and Fiddler,1970 and Farrell,1985).

Using cumin oil (100ppm), at zero time, the A.parasiticus count was $2.6x10^3$ and increased to reach $2.8x10^3$ after 6 days of ripening then completely inhibited after 3 months of ripening, while with 200ppm, the count was completely reduced after 30 days of ripening. The AFB1 and total aflatoxin contents in cumin oil cheese batches increased during the interval between the six and thirtieth day of ripening and then sharpely decreased to reach 0.28 &2.3 ppb using 100 ppm, and 0.14 & 0.9 ppb using 200ppm after three months (Tables 1 & 2). Abouzeid et al. (1996) detected A.parasiticus in 20% of hard (Roume) cheese (40 - $6x10^2$ /g),and AFB1 in 20% of the samples in a concn. ranged from 100-176ppb. Moreover, Aly(1979) concluded that,Ras cheese was ovoid of AFB1&AFG1 within 60 days of ripening. An excellent indicator of endemic exposure to aflatoxins in the environment is their presence as residue in animal tissue or fluids. Aflatoxins-contaminated milk is a risk factor for the consumer, especially when processed into cheese. Farrell (1985) suggested the preservation activity of cumin oil to the effect of cumin aldehyde in its chemical composition. Therefore, foods containing cumin oil may not readily support A.parasiticus growth and / or aflatoxin production.

Nigella sativa seeds oil showed a moderate effect. The A.parasiticus count gradually decreased during the ripening period and reached $1.1x10^2$ and 2.0x10 after 90 days using 100 & 200ppm,respectively. While both AFB1 and

(TAC) increased during the interval between the first and fourth week of ripening, and then decreased to reach 0.4 and 5.0 ppb using 100 ppm, and 0.21 and 3.0 ppb using 200 ppm after three months (Tables 1&2).The obtained results are in agreement with those reported by Nadia and Waffa (1993) who concluded that N.S.S.O. had inhibited fungal growth by 88.21 %. Moreover, EL-Sayed et al.(1994) reported that N.S.S.O. had reduced yeast and mold count in processed cheese by 76 and 80 % for 0.2 and 0.3 % N.S.S.O., respectively.While, Sherine (1999) concluded that, N.S.S.O. encouraged A.flavus growth and subsequent AFB1 production. Aflatoxins remain a threat to human health due to their continuing intermittent occurrence in food.

Sherine (1999) recommended to use spice as flavourant, accelerating, antimycotic and antimycotoxigenic agent in Ras cheese which prevented A.flavus growth and subsequently mycotoxins production. Clove was the most antimycotic spice against A.flavus which prevented the mold growth at all concentrations used. Moreover, Darwish (1995) stated that clove extractions were more effective against A.flavus and A.parasiticus than the other ones.

The detoxifying effect of the three spice oils was evaluated against AFB1 in broth medium. None of the tested oils showed a detoxifying effect against AFB1 (Table 3).

Data presented in Table (4) summarize the chemical composition of Ras cheese treatments as affected by spice oils throughout the ripening period. As clearly shown, no big differences could be observed between the six treatments and the control either when fresh or at the end of ripening period. Upon ripening, all chemical parameters increased throughout the ripening period due to the moisture loss. Nearly similar findings were reported by Ahmed et al.(1999) and Sherine (1999). Moreover, Wendorff and Wee (1997) indicated that, the intensities of flavours in cheese treated with spice oils were acceptable. Titratable acidity (T.A.), soluble nitrogen (SN), soluble nitrogen/total nitrogen(SN/TN) and total volatile fatty acids (TVFA) were taken as the ripening parameters. The presented data in Table (4) clearly indicate that T.A. values rapidly increased in all treatments. The highest values were recorded with clove and cumin oils treatments, which could be considered as an indicator for a stimulation effect of both clove and cumin oils to lactic acid bacteria (Sherine, 1999).

SN and its ratio to TN are usually taken as an indication to the degree of proteolysis that occured in cheese during ripening. The recorded values in Table (4) show a progressive increase throughout ripening period in all treatments. However, the relatively higher values were recorded with clove and cumin oils at level 200ppm. These findings indicate that, clove and cumin oils might have a stimulation effect to proteolytic bacteria, which contribute to the acceleration of protein degradation during cheese ripening. These results

Table (1) : Aspergillus parasiticus count as affected by different spice oils during ripening of Ras cheese.

Treatment	Storage period (days)						
	0	6	9	16	30	60	90
Control	$4.3x10^3$	$4.7x10^3$	$5.4x10^3$	$6.0x10^3$	$6.1x10^3$	$6.3x10^3$	$6.4x10^3$
Clove oil (100ppm)	$2.8X10^3$	$0.7X10^3$	$4.0X10^2$	5.0X10	NMG	NMG	
Clove oil (200ppm)	$2.8X10^3$	$0.2X10^3$	2.0X10	NMG	NMG		
Cumin oil (200ppm)	$2.6X10^3$	$2.8X10^3$	$1.0X10^3$	$6.0X10^2$	$1.0X10^2$	2.0X10	NMG
Cumin oil (200 ppm)	$2.5X10^3$	$1.6X10^3$	$6.0X10^2$	4.0X10	NMG	NMG	
N.S.S.O.* (100ppm)	$2.6X10^3$	$0.9X10^3$	$3.1X10^2$	2.0X10	$1.5X10^2$	$1.2X10^2$	$1.1X10^2$
N.S.S.O.* (200ppm)	$2.6X10^3$	$0.2X10^3$	$1.5X10^2$	$1.0X10^2$	$0.5X10^2$	$0.4X10^2$	2.0X10

* N.S.S.O. : Nigella sativa seeds oil.

Table (2) : Effect of spice oils on aflatoxins production by Aspergillus parasiticus during ripening of Ras cheese.

Treatment	Storage period (days)											
	6		9		16		30		60		90	
	AFBI	TAC*	AFBI	TAC*	AFBI	TAC*	AFBI	TAC*	AFBI	TAC*	AFBI	TAC*
Control	12	87	16	112	19	130	30	295	4.1	580	50	790
Clove oil (100ppm)	1.4	13	2.4	17	3.6	23	0.86	8.1	0.27	3	0.13	1.0
Clove oil (200ppm)	1.2	13	1.8	16	2.7	20	0.61	7.2	0.002	2.5	0.001	0.6
Cumin oil (200ppm)	1.2	13	2.7	20	3.5	28	3.7	27	0.51	3.4	0.28	2.3
Cumin oil (200 ppm)	1.1	12	2.6	18	3.1	23	3.3	23	0.17	2.6	0.14	0.9
N.S.S.O.** (100ppm)	1.0	11	2.3	17	2.8	21	3.5	25	0.6	5.5	0.4	5.0
N.S.S.O.** (200ppm)	0.73	9.3	1.4	14	1.8	14	2.2	16	0.34	4.3	0.21	3.0

*TAC : Total aflatoxin contents.
**N.S.S.O. : Nigella sativa seeds oil.
Aflatoxin contents were detected in (ppb).

Table (3): Detoxifying effect of spice oils against AFB1 in broth medium.

Treatment	Storage period (days)		
	0	30	45
Control	40	40	40
Clove oil (100ppm)	40	40	40
Clove oil (200ppm)	40	40	40
Cumin oil (200ppm)	40	40	40
Cumin oil (200 ppm)	40	40	40
N.S.S.O.** (100ppm)	40	40	40
N.S.S.O.** (200ppm)	40	40	40

AFBI content was detected in (ppb)

Table (4): Effect of spice oils on some chemical composition parameters of Ras cheese during ripening period.

Treatments	Ripening period (days)	Chemical composition						
		Moisture %	Fat%	T.A.%	SN.%	TN..%	SN/TN. %	TVFA*
Control	0	42.49	26.00	0.62	0.38	4.78	7.95	10.00
	30	39.12	28.20	1.26	1.15	5.04	22.28	14.90
	60	37.54	29.50	1.75	1.39	5.37	25.89	17.60
	90	35.35	30.70	2.11	1.62	5.42	29.90	23.00
Clove oil (100ppm)	0	42.95	26.20	0.65	0.36	4.80	7.50	9.90
	30	39.50	28.00	1.48	1.32	5.10	25.88	13.70
	60	37.94	29.00	2.02	1.46	5.22	27.97	16.80
	90	35.90	30.20	2.62	1.82	5.39	33.77	19.20
Clove oil (200ppm)	0	43.10	26.33	0.70	0.36	4.74	7.59	9.80
	30	39.00	28.50	1.52	1.38	5.08	27.16	12.50
	60	37.70	29.70	2.11	1.52	5.19	29.29	14.70
	90	38.00	30.90	2.80	2.02	5.32	37.97	18.00
Cumin oil (10ppm)	0	43.21	26.80	0.68	0.38	4.72	8.05	10.00
	30	39.50	28.70	1.54	1.29	5.03	25.65	12.20
	60	37.46	29.90	1.99	1.44	5.17	27.85	16.00
	90	35.85	30.50	2.60	1.62	5.28	30.68	19.40
Cumin oil (200ppm)	0	43.35	26.70	0.72	0.37	4.84	7.64	10.30
	30	39.68	28.90	1.62	1.29	5.15	25.05	12.00
	60	37.84	29.10	2.13	1.53	5.28	28.98	15.20
	90	36.20	30.20	2.78	1.89	5.39	35.06	18.70
N.S.S.O. (100ppm)	0	43.07	26.33	0.65	0.37	4.82	7.68	10.50
	30	40.25	28.50	1.45	1.10	5.10	21.57	15.70
	60	38.10	29.90	1.82	1.22	5.28	23.11	18.90
	90	36.40	30.00	2.05	1.42	5.35	26.54	24.70
N.S.S.O. (200ppm)	0	43.18	26.50	0.68	0.37	4.64	7.97	9.90
	30	40.41	28.60	1.35	1.10	4.94	27.27	14.00
	60	38.25	29.40	1.70	1.12	5.07	22.01	19.80
	90	36.54	31.50	1.98	1.35	5.27	25.61	25.70

*T.A. % : Titratable acidity%(as lactic acid).
*SN% : Soluble nitrogen%.
*TN% : Total nitrogen%.
*TVFA : Total volatile fatty acids expressed as ml 0.1N Na OH / 10 g. cheese.

are in agreement with Ahmed et al.(1999) and Sherine (1999). Regarding the total volatile fatty acids (TVFA); it is clear that the values gradually increased in all treatments in variable rates. The highest values were recorded with N.S.S.O. treatment, while the lowest values were recorded with clove oil treatment (Table 4). These results might be due to the inhibition effect of clove and cumin oils to molds and lipolytic bacteria. These results are in agreement with Farag et al.(1989); Singh (1991) and Sherine (1996).

In conclussion, A.parasiticus growth and subsequent aflatoxins production can be inhibited in a commercially-made Ras cheese when it is treated with clove oil (200ppm) or cumin oil (200ppm) as natural preservatives.

REFERENCES

Abdel-Hakiem,E.H. and EL-Kosi,O.H.R.(1999): Occurrence of aflatoxin-producing molds in cheese. Assiut Vet. Med. J., 40, 80, 177-186.

Abdel-Tawab,G.(1963): Manufacturing Ras cheese from pasteurized milk . Cited from Yousseff,A.M. (1966). M. Sci. Thesis, Ain-Shams Univ., Egypt.

Abouzeid,A.M. ; Hassan,A.A. and Ragheeb,R.R.(1996): Mycological studies on hard (Roume) and skim milk soft cheese (Kareish) with quantitative evaluation of the existing mycotoxins. Vet. Med. J., Giza. 44, 2, 113-121.

Abu Sree,Y.H.(1997): Effect of cheese processing and ripening of hard cheese on formation and stability of some mycotoxins. Ph.D.Thesis. Fac. Agric. Cairo Univ.

Ahmed,H.F. ; EL-Bardisy,M.M. and Ismail,A.M.(1999): Potential growth and control of Clostridium perfringens type (A) during ripening of Ras cheese. Alex. J. Vet. Sci. 15,4,733-743.

Alma,I. ; Kamala,C.S. and Sreenivasmurthy,V.(1971): Cirrhosis in children from peanut meal contaminated by aflatoxin. Am. J. Clin. Nutr. 24,609-614.

Aly,A.A.(1979): Factors affecting aflatoxins B1&G1 formation in Ras cheese by Aspergillus flavus. Ph.D.Thesis. Fac. Agric., Cairo Univ.

Atherton,H.V. and Newlander,J.A.(1977): Chemistry and testing of dairy products. 4th Ed. AVI Publishing Co.. INC. Westport. Connecticut.

Bars-Bailly,S.LE.;Bailly,J.D. and Brugere,H.(1999): Defects in cheesemaking caused by moulds. Revue de Medecine Veterinaire. 150,5,413-430.

Bullerman,L.B.(1979): Significance of mycotoxins to food safety and human health. J. Food Prot., 42, 1, 65-86.

Bullerman,L.B. ; Lieu,F.Y. and Seier,S.A.(1977): Inhibition of growth and aflatoxin production by cinnamon and clove oils, cinnamic aldehyde and eugenol. J.Food Sci. 42,1107-1116.

Darwish, S.M.(1995): Effect of clove,cinnamon and thyme on the growth and aflatoxins production by some molds. J.Agric.Res. Tanta Univ., 21,110-115.

Doerr,R.C. and Fiddler,W.(1970): Partition ratios of some wood smoke phenols in two oil:water systems. J.Agric. Food Chem. 18,937-939.

EL-Essawy,H.D. ; Said, A.M. ; Mahmoud,S. and Morgan,S.D.(1984): Fungal contamination of hard cheese. Assiut Vet. Med. J. 11, 125-129.

EL-Faham,S.Y.(1994): Comparative studies on chemical composition of nigella sativa L. seeds and its cake. J. Agric. Sci. Mansoura Univ. 19,7,2283-2289.

EL-Saved,M.M. ; Hanaa,A.E. and Fatma,A.F.(1994): The use of Nigella sativa oil as a natural preservative agent in processed cheese spread. Egypt. J. Food Sci. 22,3,381-396.

FAO/WHO(1996): Worldwide regulations for mycotoxins. FAO of the united nations, Rome.

Farag,R.S. ; Daw,Z.Y. and Abo-Raya,S.H.(1989): Influence of some spices, essential oils on Aspergillus parasiticus growth and production of aflatoxins in synthetic medium. J. Food Sci. 54,74-76.

Farrell,K.T.(1985): Spices, Condiments, and Seasonings. Van Nostrand Reinhold Co. New York.

Gilli,G. ; Scursatone,E. and Petitti,G.(1989): Presence of aflatoxin in products intended for human consumption: the choice of methods and the results obtained. Annali de Igiene. 1,1-2&223-235.

Hassan,G. and Bullerman,L.B.(1995): Aspergillus flavus and Aspergillus parasiticus: Aflatoxigenic fungi of concern in food and feed. A review. J. Food Prot. 58,1395-1404.

Karaioannoglou,P.G.(1990): Aflatoxin production on Kefalotyri cheese. Lebensmittel-Wissenschaft & Technologie. 23,1,4-6.

Kosikowski,F.V.(1978): Cheese and fermented milk foods. 2 nd printing. Booktondale, New York, 14817.

Lie,J.L. and Marth,E.H.(1967): Formation of aflatoxin in Cheddar cheese by Aspergillus flavus and Aspergillus parasiticus. J. Dairy Sci. 50,1708-1710.

Ling,E.R.(1963): A text Book of Dairy Chemistry Vol 2 3rd Ed. Chapman and Hall Ltd. London.

Marshall,R.T.(1992): Standard methods for the examination of dairy products. 16th Ed.American Public Health Association.Washington,D.C.

Nadia,A.A. and Waffa,H.M.(1993): Studies on the keeping quality of butter using Nigella sativa oil. Minufia J. Agric. Res. 18,4,2403-2420.,

Scott,P.M.(1989): Mycotoxigenic fungal contaminants of cheese and other dairy products. In Mycotoxins in dairy products [edited by Egmond,H.P.van]. London.UK ; Elsevier Appl.Sci. 193-259.

Sherine,A.I.(1996): Use of spices as natural preservatives for some dairy products. Master Agric. Sci. Thesis (Dairy Sci.), Cairo Univ.

Sherine,A.I.(1999): Use of some spices in Ras and Roquefort cheese making and its effect on mycotoxins production. Ph.D. Agric. Sci. Thesis (Dairy Sci.), Cairo Univ.

Singh,R.K.(1991): Fungitoxic activity of cumaldehyde main constituent of the cuminum cyminum oil Dept Chem. Univ. Gorakhpur Iawa USA. 507pp.

Wendorff,W.L. and Wee,C.(1997): Effect of smoke and spice oils on growth of molds on oil-coated cheeses. J. Food Prot. 60,2,153-156.