INFLUENCE OF SOME TREATMENTS ON THE FUNGAL GROWTH AND MYCOTOXIN INCIDENCE IN SOME CHEESE VARIETIES

BY

RAGIA OMAR YOUSSEF MOHAMED

B.Sc. Agric. Sc. (Dairy Science), Ain Shams University, 1991 M.Sc. Agric. Sc. (Dairy Science), Ain Shams University, 1997

A thesis submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Agricultural Science (Dairy Science and Technology)

Department of Food Science Faculty of Agriculture Ain Shams University

Approval Sheet

INFLUENCE OF SOME TREATMENTS ON THE FUNGAL GROWTH AND MYCOTOXIN INCIDENCE IN SOME CHEESE VARIETIES

BY

RAGIA OMAR YOUSSEF MOHAMEDB.Sc. Agric. Sc. (Dairy Science), Ain Shams University, 1991

M.Sc. Agric. Sc. (Dairy Science), Ain Shams University, 1997
This thesis for Ph.D. degree has been approved by:
Prof. Dr. Nabil M.Mehana
Prof. of Dairy Science, Faculty of Agric. Kafr El-Sheikh, Tanta Univ.
Prof. Dr. Saad M.Khalafalla
Prof. Emeritus of Dairy Science and Microbiology, Fac. of Agric., Ain Shams Univ.
Prof. Dr. Atef El-Sayed Fayed
Prof. of Dairy Science and Technology, Fac. of Agric., Ain Shams Univ.
Dr. Rezk A. Awad

Date of Examination: / /2005

INFLUENCE OF SOME TREATMENTS ON THE FUNGAL GROWTH AND MYCOTOXIN INCIDENCE IN SOME CHEESE VARIETIES

BY

RAGIA OMAR YOUSSEF MOHAMED

B.Sc. Agric. Sc. (Dairy Science), Ain Shams University, 1991 M.Sc. Agric. Sc. (Dairy Science), Ain Shams University, 1997

Under the supervision of:

Prof. Dr. Atef El-Sayed Fayed

Prof. of Dairy Science and Technology, Faculty of Agriculture, Ain Shams University.

Dr. Rezk A. Awad

Associate Prof. of Dairy Science and Technology, Faculty of Agriculture, Ain Shams University.

Dr. Mostafa A. Zedan

Head of Research, Dairy Research Department, Food Technology Institute, Agricultural Research Center

تأثير بعض المعاملات على نمو الفطر ووجود السموم الفطرية في بعض أنواع الجبن

رسالة مقدمة من راجية عمر يوسف محمد

بكالوريوس علوم زراعية (علوم وتكنولوجيا الألبان)، جامعة عين شمس، 1991 ماجستير علوم زراعية (علوم وتكنولوجيا الألبان)، جامعة عين شمس، 1997

للحصول على درجة دكتور فلسفة في العلوم الزراعية (علوم وتكنولوجيا الألبان)

قسم علوم الأغذية كلية الزراعة _ جامعة عين شمس

جامعة عين شمس كلية الزراعة

رسالة دكتوراه

وسف محمد	اسم الطالبة : راجية عمريو		
المعاملات على نمو الفطر ووجود السموم عض أنواع الجبن			
عص الواع الجبن في العلوم الزراعية (علوم وتكنولوجيا الألبان)			
	,		
له الإشراف	لجن		
	أ.د. عاطف السيد فايد		
ان ، قسم علوم الأغذية ، كلية الزراعة ،	أستاذ علوم وتكنولوجيا الألب جامعة عين شمس		
	د. رزق عزب عواد		
ألبان المساعد ، قسم علوم الأغذية ، كلية	أستاذ علوم و تكنولوجيا الأ الزراعة ، جامعة عين شمس		
	د. مصطفى عبد المنعم زيدان		
لبان ، معهد بحوث تكنولوجيا الأغذية، مركز	رئيس بحوث، قسم بحوث الأا البحوث الزراعية		
تاريخ البحث: 1998/9/21			
الدراسات العليا			
أجيزت الرسالة بتاريخ 1/ 2005/10	ختم الإجازة		
موافقة مجلس الجامعة	موافقة مجلس الكلية		
2005 / /	2005 / /		

صفحة الموافقة على الرسالة تأثير بعض المعاملات على نمو الفطر ووجود السموم الفطرية في بعض أنواع الجبن

رسالة مقدمة من راجية عمر يوسف محمد

بكالوريوس علوم زراعية (علوم وتكنولوجيا الألبان)، جامعة عين شمس، 1991 ماجستير علوم زراعية (علوم وتكنولوجيا الألبان)، جامعة عين شمس، 1997

للحصول على درجة دكتور فلسفة في العلوم الزراعية (علوم وتكنولوجيا الألبان) وقد تمت مناقشة الرسالة والموافقة عليها

تاريخ المناقشة / 2005/

الجنة:
د. نبیل محمد یوسف مهنا
أستاذ الألبان ، كلية زراعة كفر الشيخ ، جامعة طنطا
د. سعد الدين محمد خلف الله
أستاذ علوم وميكروبيولوجيا الألبان غير المتفرغ ، كليـة الزراعـة ، جامعة عين شمس
د. عاطف السيد فايد
أستاذ علوم وتكنولوجيا الألبان ، كلية الزراعة ، جامعة عين شمس
. رزق عزب عواد
أستاذ علوم وتكنولوجيا الألبان المساعد، كلية الزراعة ، جامعة عين شمس

ACKNOWLEDGEMENT

First and foremost, I'm indicated to **ALLAH** forever, the most beneficent and merciful.

I would like to convey my profoundly gratitude to Prof. **Dr. A. E. FAYED**, Professor of Dairy Science and Technology, Food Science Department, Faculty of Agriculture, Ain Shams University, for his close supervision, unlimited guidance and valuable assistance during all stages of this Thesis.

I would like to express my thanks to **Dr. R.A. AWAD** Associate Prof. of Dairy Science and Technology, Food Science Department, Faculty of Agriculture, Ain Shams University, for his fruitful supervision, facilities offered during study.

Appreciation should be extended to departed **Dr. S. G. OSMAN**, the previous Chief of Researchers, Institute of Food Research and Technology, Agriculture Research Center, and to **Dr. M. A. ZEDAN**, Chief of Researchers of Dairy Science, Food Technology Institute, Agricultural Research Center for supervising of this investigation.

My special thanks would be expressed to **Prof. Dr. El-Sayed A. M. AbdAlla**, Professor of Mycotoxin and Food Contaminants Department National Research Center, for helping with the microbiological facilities and examinations.

I'm very much indebted also to the Staff Members of Food Science Department, Faculty of Agriculture, Ain Shams University, and of Institute of Food Research and Technology, Agriculture Research Center, as well as to National research center, Cairo for their faithful help during all stages of this study.

My deep grateful and appreciation to my father and mother for their continuous encouragements, patience and support.

ABSTRACT

Ragia Omar Youssef Mohamed, Influence of Some Treatments on The Fungal Growth and Mycotoxin Incidence in Some Cheese Varieties. Unpublished Ph.D. Thesis, Department of Food Science, Faculty of Agriculture, Ain Shams University, 2005.

The aim of this study was to diagnosis the common molds infecting local market Ras cheese and to evaluate the antifungal efficiency of eugenol, propolis and the substitution of Yoghurt Starter Culture (YSC) with *Lactobacillus* one (LSC) for avoiding the fungal infection and hence aflatoxin production in plastic uncoated Ras cheese wheels.

Twenty four samples of mature Ras cheese were randomly surveyed from different four locations in Cairo and Giza governorates during the period extending from March to August 2002. Experimentally Ras cheese wheels were finished with surface spraying with 200 ppm eugenol, 5 or 10% propolis solution instead of plastic coating. On the other hand, the normal Ras cheese starter culture, namely YSC was substituted with nil, 50 and 100% of mixed LSC of *L. Casei* and *L.reuteui* (1:1) for making plastic uncoated Ras cheese. Furthermore aflatoxin containing mature Ras cheese was exposed for making processed cheese spread using different cooking temperature (75-95°C).

The results of surveying part revealed that each of the dry matter (DM), fat / DM and protein contents conformed to the Egyptian legal standard while the yeasts and molds counts of all samples were over the legal standard. Five *penicillum* spp.were found and *P.fumiculosium* was the most dominant

followed by six *Asperigillus* spp. from which *A. Parasiticus* was the most dominant of this genus whilst, *Fusarium* spp. was not detected.

Three types of aflatoxins (AF) were found namely AF.B₁, G_1 and G_2 were the maximum mycotoxins found in market Ras cheese with sum concentration reached sometimes to 2.25 ppb.

Experimentally, as the plastic coating forbode, the wheels surface spraying either with 200 eugenol or 10% propolis solution prevented completely the fungal growth and aflatoxins production of A. *Parasiticus* on Ras cheese wheels. Likewise the substitution of YSC with LSC at a level of 50% at least led also to inhibit the purposely artificial fungus to grow. Moreover, all experimented trails of plastic coating substitution did not exhibit any abnormal behavior either in all gross composition criteria namely, DM, Fat/DM protein / DM and ash / DM contents or the repining indices studied namely, titratable acidity, pH value, water soluble nitrogen / total nitrogen and total volatile fatty acid of Ras cheese along maturation period (three months). Moreover, the conversion of aflatoxin containing mature Ras cheese into processed cheese spread led to disappear the aflatoxin regardless the cooking temperature mainly due to the presence of emulsifying salts.

Keywords: Ras cheese, processed cheese spread, gross composition, ripening indices, *L. Casei*, *L. reuteri*, *A. Parasiticus*, *Penicillium* spp., *Aspergillus* spp, Aflatoxin.

CONTENTS

			page	
1. IN	ΓROD	UCTION	1	
11. R	EVIEV	W OF LITERATURE	3	
1-	- Overview of health hazard of mycotoxins			
2-	2- Occurrence of mycotoxinic species in dairy products			
	2.1.	Aspergillus species	5	
	2.2.	Penicillium species	9	
	2.3.	Further fungal genera other than Aspergillus and	15	
		Penicillium species		
3.	Facto	ors allowing aflatoxins incidence in dairy products	17	
4.	Aflat	oxin diffusiveness into cheese matrix	21	
5.	Preve	ention and detoxification of aflatoxins	24	
	5.1.	Food additives as toxigenic-molds preventive and aflatoxin detoxifier	25	
	5.2.	Bacterial treatments for aflatoxins districtation	34	
	5.3.	Further antimycotic treatments or	34	
		antimycotoxinogenic		
111. N	ИАТЕ	RIALS AND METHODS	35	
1- Ma	terials	S	35	
	1-1	Cheese milk	35	
	1-2	Market Ras cheese	35	
	1-3	Butter oil	35	
	1-4	Sodium Chloride	35	
	1-5	Emulsifying salts	35	
	1-6	Microbial starter culture	36	
		1.6.1. Yoghurt starter culture	36	
		1.6.2. <i>Lactobacillus</i> strains	36	
		1.6.3. Aspergillus parasiticus	36	
	1.7.	Rennet	36	
	1.8.	Honey Bee gum (propolis)	36	
	1.9.	Eugenol oil	36	

	1.10.	Aflatoxins standards	37
	1.11.	Plastic coat	37
2	Expe	rimental procedures	37
	2.1.	Activation of bacterial starter	37
	2.2	Preparation of propolis	37
	2.3.	Ras cheesemaking	37
	2.4.	Processed cheese spread making	38
3- A	Analytica	ıl methods	41
	3.1.	Chemical analyses	41
		3.1.1. Determination of dry mater content	41
		3.1.2. Determination of fat content	41
		3.1.3. Determination of ash content	41
		3.1.4. Determination of total nitrogen and water	41
		soluble nitrogen	
		3.1.5. Determination of total volatile fatty acids content	41
		3.1.6. Determination of titratable acidity	41
		3.1.7. Extraction and determination of aflatoxins	41
	3.2.	Physical properties	42
		3.2.1. Measurement of pH value	42
		3.2.2. Determination of the meltability	42
		3.2.3. Determination of oil separation index	42
		3.2.4. Penetration measurement	42
	3.3.	Microbiological analyses	43
		3.3.1. Count yeasts and molds	43
		3.3.2. Isolation and identification of fungi	43
RE	SULTS A	AND DISCUSSION	44
Part	t I : Cha	racteristics of market Ras cheese with emphasis on	
		mycotic and mycotoxigenic situations	44
	1.	Chemical and microbial properties	44
	2.	Detailed mycotic and mycotoxigenic situations	48
		2.1. Mycotic profile	48
		2.2. Mycotoxigenic situation	52

		ves against Aspergillus parasiticus growth on Ra e wheels
1.		iency of the plastic coating
	1.1.	Effect on the gross composition
	1.2.	Effect on the ripening indices
	1.3.	Effect on the mycotic growth
	1.4.	Effect on the aflatoxin incidence
2.	Effic	iecy of Eugenol surface sprayed
	2.1.	Effect on the gross composition
	2.2.	Effect on the ripening indices
	2.3.	Effect on the mycotic growth
	2.4.	Effect on the aflatoxin incidence
3.	Effic	iency of propolis surface spayed
	3.1.	Effect on the goss empossition
	3.2.	Effect on the ripening indices
	3.3.	Effect on the mycotic growth
	3.4.	Effect on the aflatoxin incidence
4.	Effic	iency of some lactobacillus species as yoghurt starte
	cultu	re substitute
	4.1.	Effect on the gross composition
	4.2.	Effect on the ripening indices
	4.3.	Effect on the mycotic growth
	4.4.	Effect on the mycotoxigenic incidence
5.	Effic	iency of the temperature of cooking processing
	5.1.	Effect on the gross composition
	5.2.	Effect on the keeping quality indices
	5.3.	Effect on the physical properties
	5.4.	Effect on the aflatoxin persistence
SUM	MARY	Y AND CONCLUSIONS

List of Tables

No.	Title	Page
1	Mycotoxins produced by species of the genus	
	Aspergillus	8
2	Mycotoxins produced by species of the genus Penicillium	11
3	Mycotoxins produced by various genera other than	11
	Aspergillus and Penicillium species	16
4	The blend formulas (Kg /100g) of processed cheese	20
	spread	39
5	Chemical properties and some microbial qualities of Ras	
	cheese samples surveyed during period extending from	
	March to August 2002. Six samples for each group	46
6	Statistical analysis of the data given in Table (5)	47
7	Frequency distribution percent of identified fungal	
	genera and species among Ras cheese samples surveyed during period extending from March to August 2002.	
	Six samples for each group	49
8	Statistical analysis of the data given in Table (7)	77
	according to the surveying area	50
9	Statistical analysis of the data given in Table (7)	
	according to isolation medium.	51
10	Frequency distribution percent of Aflatoxins, their kind	
	and concentration (ppb) of Ras cheese samples surveyed	
	during period extending from March to August 2002.	7 0
11	Six samples for each group.	53
11	Properties of Ras cheese brushed purposely on the surface with <i>Aspergilus parasiticus</i> spores as affected by	
	the plastic coating step and ripening prolonging for 3	
	months	56
12	Factorial analysis of data given in Table (11)	
	i actorial aliarysis of data given in Table (11)	57

13	Properties of plastic-uncoated Ras cheese during ripening as affected by the eugenol level of the solution	
	sprayed on cheese wheels prior the purposely surface brushing with <i>Aspergillus parasiticus</i> spores	63
14	Factorial analysis of data given in Table (13)	64
15	Properties of plastic-uncoated Ras cheese during ripening as affected by the propolis level of the solution sprayed on cheese wheels prior the purposely surface brushing with <i>Aspergillus parasiticus</i> spores	68
16	Factorial analysis of data given in Table (15)	69
17	Properties of Ras cheese during ripening as affected by the substitution level of yoghurt starter culture (YSC) with Lactobacillus one (LSC) in its making and the purposely surface brushing with <i>Aspergillus</i>	
18	parasiticus spores.	75
10	Factorial analysis of data given in Table (17)	77
19	Properties of processed cheese spread made using starting mature Ras cheese containg aflatoxins at a level of 3.00 ppb as affected by the cooking temperature	84
20	Factorial analysis of data given in Table (19)	85

List of Figure

No.		Page
1	Flow scheme of experimental Ras cheesemaking	40