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 Unpublished Ph.D. Thesis, Department of Food Science, Faculty of Agriculture, Ain Shams University, 2005.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.B_1, 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.

SUMMARY AND CONCLUSIONS

Ras cheese is very susceptible to mold growth and mycotoxins formation. Little quantities of Ras cheese wheels are plastic coated because coating is expensive. Most Ras cheese handicraftmen neglect the wheel plastic coating step depending on the hard rind, which naturally formed surrounding cheese wheels due to the surface moisture vaporization and dry salting. Therefore this study was aimed to investigate market Ras cheese sample to find out the most common toxigenic molds infecting them and to evaluate experimentally the antifungal efficiency of some natural preservatives whether obtaincal (eugenol) or insectile (propolis) and some strains of *lactobacillus* species against the growth and mycotoxins production of one of the most common fungal strains artificially grown on Ras cheese.

Part I : Characteristics of market Ras cheese with emphasis on mycotic and mycotoxigenic situations.

In this part twenty four samples of mature Ras cheese were randomly collected from different markets in Cairo and Giza governorates during the period extending from March to August 2002.

The first group (six samples) was obtained from the marketing service of Misr for Milk and Food Co. at Amiriya, while the second (also six samples) was gained from wholesale markets at Ben El-Soyrain. Whilst the third and fourth groups were surveyed from the retail markets in Cairo and Giza, respectively.

The obtained results could be summarized as follow:

1 - The dry matter (DM) and fat/DM contents of all samples conformed to The Egyptian legal standards and did not differ among the four surveying locations.

2 - Likewise, the protein content of all samples was conformed to the Egyptian legal standard. However Ras cheese of Misr for Milk and Food Co. (group 1) had a protein content lower than those of the other three groups.

3 - The ash content of Giza governorate possessed the highest level, while those of Cairo governorate contained the lowest one.

4 - There were no significant differences among the four surveying locations in titratable acidity (TA%), pH value and total volatile fatty acids (TVFA).

5 - The yeasts and molds counts as a microbial quality indicators confirmed the unsafety of all samples for the human consumption according to the Egyptian legal standard. The count reached to $3x10^2 - 9x10^4$ without any significant differences between them.

6 - *Penicillium spp* was the dominant genus in all isolates.The frequency distribution (FD)% fluctuated from 72 to 85% regardless the surveying location. The 4^{th} location group exhibited the highest figure followed by group I, III and II respectively. Five *Penicillium spp.* were found among Ras cheese samples with the following range of FD% namely, *P.fumiculosium* (16-25%),*P.roqueforti*(15-17%)*P.brevicompactum* (12-18%), *P.chresogenum* (12-16%) and *P.spectes* (11-13%) vary as the surveying area and isolation medium differed.

7 - *Aspergillus spp* came in the 2nd order of the common mold contaminated Ras cheese samples, its corresponding figures ranged from 56 to 65%. Six *Aspergillus spp* were isolated from Ras cheese samples with the following range of FD% namely, *A.parasiticus* (8-20%), *A.niger* (16-29%), *A.flavus* (8-18%), *A.versicolor* (4-6%), *A.terreus* (3-8%) and *A.achraceus* (12-6%) varying as the surveying area and isolation medium differed.

8 - For both strains Potato dextrose agar medium was found to be more specific for fungal isolation as compared with Rose bengal agar on.

9 - All Ras cheese samples were free from *Fusarium spp.*

10 - Three types of aflatoxins (Af) were found in the 2nd group namely Af.B_1 (at a level of 1.45 ppb), AfG_1 (at a level of 0.5 ppb) and AfG_2 (at a level of 0.3 ppb) with a sum FD% of 33.3%. Both of the 1st and 3rd groups possessed FD% of 16.6%, however, the 1st group contained two Af. types (B_1 and G_2) while the 3rd group contained AfB_1 only. Nevertheless the 4th group was aflatoxin free.

The foregoing results indicated that *A.parasiticus* which a considerable mycotoxic producer was the main common fungus found in surveyed Ras cheese.

Part II : Protective efficiency of some treatments and food additives against *Aspergillus parasiticus* growth and mycotoxins production in Ras cheese.

In this part, five items (I – V) were experimentally studied to evaluate the antifungal efficiency of the wheel plastic coating (I) or the spraying with eugenol (II) or propolis solution (III) instead of plastic coating. In details, Ras cheese wheel were divided after dry salting step into five portions. The first one was plastic coating, the second portion was left without coating or sparying, while the third part was sprayed with 200 ppm eugenol solution. Whilst the fourth and fifth portions were sprayed with solutions containing 5 and 10% propolis respectively. Then, all cheese wheels were surface brushed with *A.parasiticus* spores. Three replicates for each treatment wree carried out.

Besides, the partial (50%) or complete (100%) substitution (IV) of yoghurt starter culture (YSC) with mixed culture (LSC) of *Lactobacilus casei* and *L.reuteri* (1:1) in the ripening of Ras cheese milk were also experimented with regard to their antifungal role then Ras cheese wheels were brushed with *A.parasiticus* spores or not. Three replicates were carried out for each treatment.

Furthermore, aflatoxins-containing mature Ras cheese (at a level of 3 ppb) was converted as a supply of aroma and hydrolyzed protein into processed cheese spread (V). The blends were cooked at 75, 85 and 95°C for 7 min. three replicates for each treatment were carried out.

The obtained results could be summarized as follow:

1 - **Efficiency of the plastic coating**

1.1 - Reduction in the DM, protein/DM and ash/DM% as well as an increase in fat/DM% of Ras cheese were associated with plastic coating of cheese wheels.

1.2 - TA%, pH value, water soluble nitrogen (WSN)/total nitrogen (TN)% and TVFA of Ras cheese were not affected by plastic coating, which led to reduce the counts of yeasts and molds in Ras cheese.

1.3 - Plastic coated Ras cheese was completely aflatoxin free along three months of ripening period. While, the absence of plastic coat allowed the cheese to contain Af.G_2 at a level of 23.17 ppb after two months and to contain Af.B_1, G_1 and G_2 at levels of 4.69, 15.36 and 9.9 ppb respectively at the end of the experimental period (3 months).

2 - **Efficiency of eugenol surface sprayed.**

2.1 - Application of eugenol solution (200 ppm) for cheese wheels spraying resulted in a reduction in the DM, and protein/DM% of cheese, while both of fat/DM and ash/DM% were not influenced.

2.2 - Except of TVFA, all other ripening indices were not affected by wheels eugenol spraying, which led to increase in the TVFA of Ras cheese.

2.3 - Yeasts and molds counts of Ras cheese did not exhibit any significant differences among the treatment with eugenol solution.

2.4 - A complete absence of aflatoxin production was achieved in Ras cheeses during ripening when the cheese wheels were sprayed with 200 ppm eugenol solution.

3. - **Efficiency of propolis surface sparyed**

3.1 - Compositionally gradual DM and ash/DM% decrement, as well as protein/DM% increment in Ras cheese were associated with the propolis level of wheel spraying solution. However, fat/DM% of Ras cheese did not exhibit any significant responses due to the propolis level used.

3.2 - TA%, pH value and TVFA were not affected by propolis spraying solution but WSN/TN % decreased when the propolis level raised from 5 to 10%.

3.3 - Yeasts and molds counts of Ras cheese did not differ as the level of propolis in the spraying solution varied.

3.4 - The use of 5% propolis spraying solution delayed the incidence of Af.G_2 to the 3rd month of ripening and forbade completely the production of Af.B_1 andAf.G_1, those occurred beside Af.G_2 in the control cheese (without propolis spraying). While the wheels spraying with 10% propolis solution prevented totally the aflatoxin production in cheese until the end of ripening period (3 months).

4 - **Efficiency of some *Lactobacillus* species as yoghurt starter culture substitute.**

4.1 - Although DM and ash/DM% of Ras cheese were not influenced, fat/DM% raised and protein/DM% decreased due to the YSC replacement with LSC at

any level. Moreover, Ras cheese brushed with *A.parasiticus* spores appeared lower fat/DM% regardless the starter culture used, however, both of DM, protein/DM and ash/DM% were not affected by the presence of the fungus brushed on Ras wheels surface.

4.2 - The complete YSC substitution (100%) with LSC raised TA% and hence reduced pH value of Ras cheese, while other ripening indices namely WSN/TN% and TVFA were not affected. The purposely brushing of Ras cheese wheel surface with *A.parasiticus* spores led to decrease in WSN/TN% to increase TVFA of Ras cheese, while both of TA% and pH value did not show any significant responses.

4.3 - Neither YSC substitution with LSC nor the brushing of cheese wheels with *A.parasiticus* spores led to any significant difference in yeasts and molds count of Ras cheese.

4.4 - The YSC substitution with LSC at any level resulted in a complete prevention of aflatoxin production along the3 months of Ras cheese ripening period.

5. - **Efficiency of cooking processing.**

5.1. - Although both of fat/DM and protein/DM% were not influenced the DM% of processed cheese was gradually increased by exceeding the cooking temperature than 75°C.

5.2 - Once the mixed blends were cooked at any temperature, TA% increased, while pH value exhibited proportional reduction as the cooking temperature raised. However, WSN/TN% and TVFA of processed

cheese spread did not vary as the cooking temperature varied.

5.3 - Although the penetration value of processed cheese spread was not affected, its meltability property was raised once it was cooked regardless the temperature at which was cooked, while the property of oil separation did not vary providing that the cooking temperature should be not exceeded 85°C. whilst the processing at 95°C led to significant increase in this property.

5.4 - Mycotoxically, the processing of cheese blend at any temperature led to complete disappearance of aflatoxin previously contained in the mature Ras cheese.

Finally, the foregoing results of this part led to conclude that, it could prevent the mycotic growth and aflatoxin production in plastic uncoated Ras cheese, even when purposely brushed with mycotoxigenic fungal strains, either by wheel surface spraying with 200 ppm eugenol, 10% propolis solution or YSC substitution with at least 50% LSC in its milk ripening. Besides the cooking temperature applied for processed cheese spread production possessed an aflatoxin districtation effect.