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ANAEROBIC SPORE FORMERS IN DIFFERENT VARIETIES OF CHEESE

By

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ABSTRACT

One hundred and five cheeses (Twenty-five each of White pickled soft cheese, Ras cheese, processed cheese and thirty of Kariesh cheese) were collected from dairy shops and supermarkets in Cairo and Giza governorates, Egypt and examined microbiologically. The mean titratable acidity % of Kariesh cheese, White soft cheese, Ras cheese and Processed cheese samples were 0.67 ± 0.045, 1.0 ± 0.075, 0.6± 0.047 and 0.83 ± 0.064 %, while the mean values of salt % were 0.59 ± 0.044, 5.79 ± 0.12, 3.5 ± 0.098 and 1.024 ± 0.061%, respectively. Anaerobic spore formers were detected in 56.7, 28 and 48% with mean counts of 21.7 ± 8.5, 30.7 ± 11.7 and 48.9 ± 15.3 in the examined Kariesh, Ras and Processed cheese samples, respectively, while couldn't be detected in white soft cheese.

Key words:

Salt, Titratable acidity, Anaerobes.

INTRODUCTION

Some undesirable microorganisms, like spores of certain species of the genus Clostridium are able to survive milk pasteurization and cheese-making processes. They remain in cheese paste during ripening and they can germinate into vegetative cells. They subsequently metabolize lactate producing organic acids, mainly butyric acid also some gases such as Co_2 and H_2 that cause abnormal aroma, flavor, cracks and slits in the cheese paste. **Le Bourhis *et al.*, 2007 and Garde *et al.*, 2013 found that** such change is known as late blowing defect (LBD) and that is a major cause of spoilage in semi hard and hard cheeses. The risk of late blowing varies with salt, pH, water activity and temperature **(McSweeney and Fox, 2004),** as well as pharmaceutical technologies such as bacterial centrifugation and the addition of nitrates **(Stadhouders, 1990).**

MATERIAL AND METHODS

Collection of samples:

One hundred and five cheeses (Twenty-five each of White pickled soft cheese, Hard cheese (Ras), processed cheese and thirty of Kariesh cheese) were randomly collected from dairy shops, supermarkets and street hawkers in Cairo and Giza governorates.

Chemical examination:

Titratable acidity percentage was determined according to **(AOAC, 2000).** Determination of NaCl content according to what have been mentioned by Volhard method **(APHA, 2004).**

Microbiological examination:

Decimal dilution preparation and anaerobic spore formers count (MPN/g) were determined according to **(APHA, 2004).** Identification of isolated clostridium species was according to **(Bergey's Manual, 2009).**

Statistical analysis:

The collected data were analyzed using SPSS statistics 17 programe.

RESULTS

Table (1): Statistical analytical results of acidity percentage in the examined samples of cheese

Type of samples	Total No. of samples	Min.	Max.	Mean ± S.E.M.
Kariesh cheese	30.0	0.18	1.2	0.67 ± 0.o45
White soft cheese	25.0	0.36	1.76	1 ± 0.075
Ras cheese	25.0	0.2	0.97	0.6 ± 0.047
processed cheese	25.0	0.36	1.5	0.83 ± 0.064

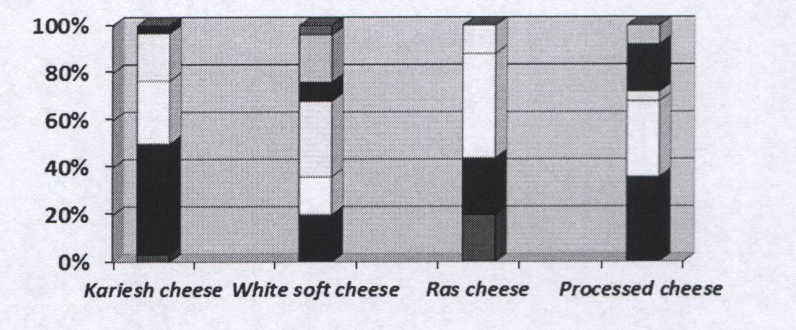

Fig. (1): Frequency distribution of the examined samples based on their acidity percentages

Table (2): Statistical analytical results of salt percentage in the examined cheese samples

Type of samples	Total No. of samples	Min.	Max.	Mean ± S.E.M.
Kariesh cheese	30.0	0.14	1.02	0.59 ± 0.044
White soft cheese	25.0	4.75	6.41	5.79 ± 0.12
Ras cheese	25.0	2.4	4.4	3.5 ± 0.098
Processed cheese	25.0	0.48	1.5	1.024 ± 0.061

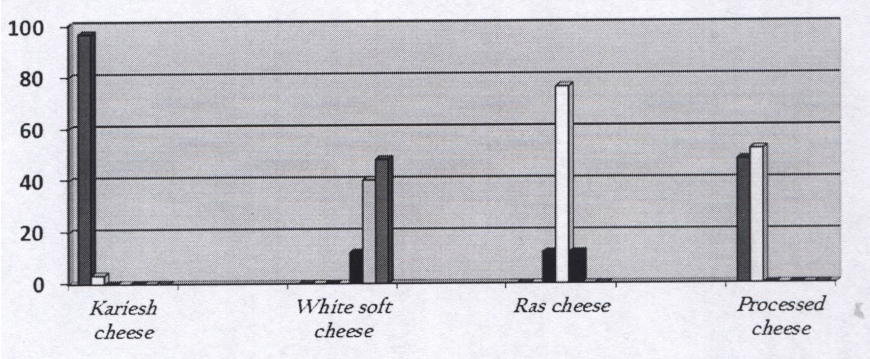

Fig.(2): Frequency distribution of the examined cheese samples based on their salt percentage

Table (3): Statistical analytical results of anaerobic spore formers count (MPN/g) in the examined cheese samples

Type of samples	Total No. of samples	Positive samples		Anaerobic spore formers count (MPN/g)		
		No.	%	Min.	Max.	Mean± S.E.M.
Kariesh cheese	30.0	17.0	56.7	4.0	150.0	21.7 ± 8.5
Ras cheese	25.0	7.0	28.0	4.0	75.0	30.7 ± 11.7
Pressed cheeseoc	25.0	12.0	48.0	4.0	150.0	489 ± 15.3

Fig. (3): Frequency distribution of the examined cheese samples based on their anaerobic spore formers count (MPN/g)

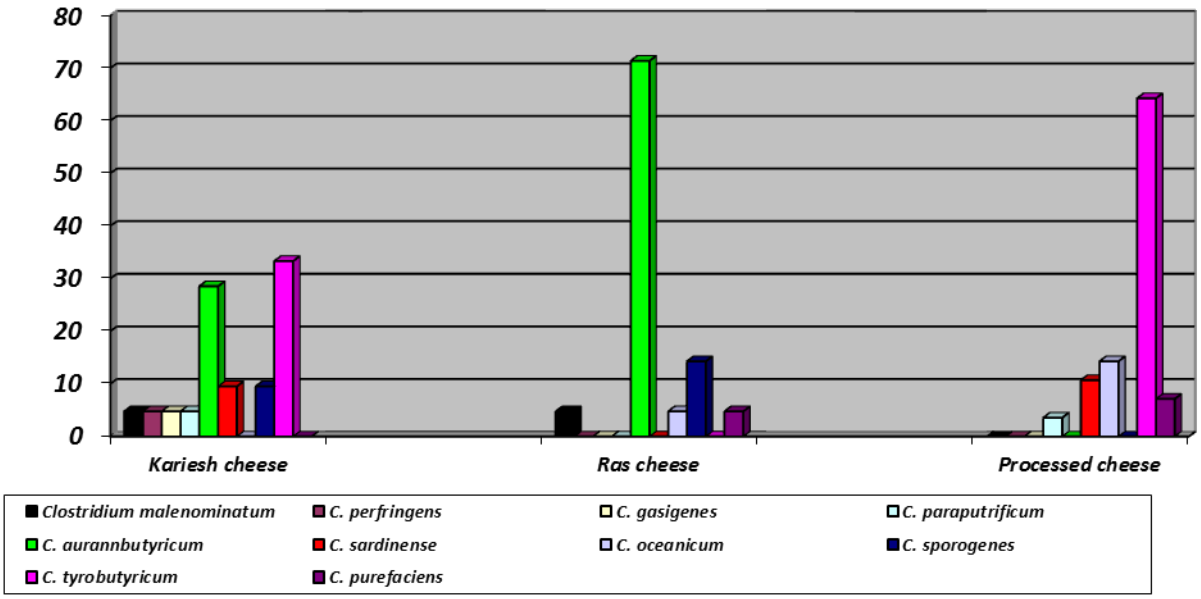

Fig. (4): Incidence of isolated anaerobic spore formers from examined samples of cheese

DISCUSSION

Titratable acidity percentage:

Titratable acidity is a measure for freshness and bacterial activity in milk. However fresh milk does not contain any appreciable amount of lactic acid therefore an increase in acidity is a rough measure of its freshness and bacterial activity **(Popescu and Angel, 2009).** Meanwhile lowering pH of milk from 6.8 to less than 4.6 protects the fermented dairy products against the risk of contamination by pathogens and rendering it safe **(Al-Kadamany *et al.*, 2001 and Wilton, 2004)**. Data recorded in (Table 1), Fig. (1). showed that, the mean titratable acidity percentage in the examined Kariesh cheese, White soft cheese, Ras cheese and Processed cheese samples were 0.67 ± 0.045, 1.0 ± 0.075, 0.6± 0.047 and 0.83 ± 0.064 % respectively. The highest frequency distribution of kariesh cheese (46.67%), White soft cheese (32%), Ras cheese (44%) and Processed cheese (36%) lies within the ranges of (0.35 - 0.6), (0.85 - 1.1), (0.6-0.85), and (0.45 - 0.6), respectively**. Mennane *et al.* (2007)** recorded nearly similar findings in Kariesh cheese**.** Higher findings were obtained by **Hassan and Samia (2007) and Sonia and Metwalli (2011),** while lower results were recorded by **Jana (2010) and Arzu *et al.* (2012)** Nearly similar results of White soft cheese were obtained by **El-Owni and Hamed (2009) and Kheir *et al.* (2011)**. Higher results were recorded by **Ercan (2009) and**

Ercan *et al.* (2011), while lower results were reported by **Karakaş and Korukluoğlu (2006) and Nour El - Dian and El Zubeir (2010). Karima (2012)** obtained nearly similar findings of Ras cheese**. Hussein *et al.* (2011)** reported higher results of processed cheese**, whereas Tayseer *et al.* (2011) and Karima (2012)** obtained lower findings of processed cheese**.**

Salt percentages:

Salting is a very important step in most cheese varieties; it plays a role in enhancing flavor, drawing the whey out of the curd and in the control of the undesirable microorganisms**.** The salt content of cheese differs markedly with cheese variety ranging from about 0.5 to 0.7% (w/w) in acid curd varieties, such as cottage cheese to about 4 to 6% (w/w) in pickled cheeses **(Guinee, 2004 and Metwaly, 2006)**. Regarding the salt percentage in examined cheese samples (Table 2), it was ranged from 0.14 – 1.02 , 4.75 - 6.41, 2.4 - 4.4 and 0.48 - 1.5 with a mean value of 0.59 ± 0.044, 5.79 ± 0.12, 3.5 ± 0.098 and 1.024 ± 0.061% in the examined samples of Kariesh cheese, White soft cheese, Ras cheese and Processed cheese, respectively. **Sonia and Metwalli (2011) and Salwa *et al.* (2012) obtained nearly similar findings in Kariesh cheese.** Nearly similar results in White soft cheese were reported by **Turkoglu1 *et al.* (2003), Hamid, and El- Owni (2007). Sert *et al.* (2007) and Kheir *et al.* (2011) recorded lower results, while Ercan (2009) obtained higher results and Bakirci *et al.* (2011). Karima (2012)** recorded nearly similar results in processed cheese, while **Karima (2012)** recorded lower figures with Ras cheese**.** Inspection of Fig. (2) Showed that, the highest frequency distribution of Kariesh cheese (96.67%) lies within the range of (0-1), whereas 40 and 48% of White soft cheese present in the intervals of (5 - 6) and (6 - 7), respectively. On the other hand 76% of Ras cheese present in the interval of (3 - 4), in Processed cheese 48% and 52% lie within the range of (0 - 1) and (1 - 2), respectively. The apparent variation among salt content in the examined cheese samples are due to the fact that added salt relies upon individual dairies, and there is no standard concerning the addition of sodium chloride to cheese and the amount of salt added depends on discretion of cheese maker himself **(Metwaly, 2006)**. The high salt content in some investigated samples may be to counteract the poor bacteriological quality of the raw milk and to prevent the formation of gas holes and abnormal flavor especially in summer **(Ismail, 2005).**

Total anaerobic spore formers count:

Results reported in (Tables 3) showed that anaerobic spore formers were detected in 56.7%, 28% and 48% of the examined Kariesh cheese, Ras cheese and Processed cheese samples, respectively, with mean counts of 21.7 ± 8.5, 30.7 ± 11.7 and 48.9 ± 15.3 MPN/g, respectively, while couldn't be detected in white soft cheese samples. The highest frequency distribution of Kariesh (47.1%), Ras (71.4%), and Processed cheese (66.67%) samples lies within the range of 10-102 Fig. (3). nearly similar results of Kariesh cheese were recorded by **El-Leboudy (1985). Abd El-Raheem, Manal (2009) and Aya (2014**) recorded higher findings**,** while **Walaa (2008) reported lower results. Torkar and Teger (2006)** reported lower findings of Ras cheese, while **Karima (2012), Thoraya (2013) and Aya (2014)** reported higher counts**. Lycken and Borch (2006) and Aya (2014) recorded** higher counts in processed cheese were reported by**,** while **Karima (2012) and Thoraya (2013)** recorded lower counts**.**It is evident from the obtained data; there is a reverse relationship between the titratable acidity percentage and the mean count of anaerobic spore formers in the examined cheese samples while a strong reverse relationship between the salt percentage and the same mean count of the microorganisms. **Karima (2012) and Thoraya (2013)** obtained nearly similar results**.** The high incidence of anaerobic organisms may be attributed to neglected hygienic measures during production and handling of milk /or dairy products. as most of the anaerobic organisms are saprophytes and normally grow in soil, silage and water, some of them are commensals of the animal and human intestine, and therefore, presence of such anaerobes in cheese may be indicative of manure and soil contamination **(Salih *et al.*, 2010).**

Isolated Clostridium spp:

Results depicted in Fig. (4) Revealed that *Clostridium tyrobutyricum* was the most frequent one in Kariesh cheese (33.33%) and processed cheese samples (64.29%). *C. aurannbutyricum* was high in Ras cheese (71.43%) samples then *C. aurannbutyricum* (28.55%), *C. sporogenes* (9.52%) and *C. sardinense* (9.52%)**;** *C. sporogenes* (14.29%)**;** *C. oceanicum* (14.29%)*, C. sardinense* (10.71%) and *C. purefaciens* (7.14%) in the examined Kariesh, Ras and Processed cheese samples, respectively.

Bergere and Lenoir, 2000 found that *Clostridium tyrobutyricum*, as an acid-resistant, grew well in pH zone of 4.5–7.5. As a relatively salt-tolerant, it also tolerates as much as 55-60 g L-1 NaCl. *C.butyricum* and *C.sporogenes* have been identified as a major species associated

with late blowing of hard and semi-hard cheeses as outgrowth of them during cheese manufacture. It affects both flavor by producing butyric acid and cheese structure by gas formation which resulted in cracks and cavities. These organisms have also been isolated from spoiled processed cheese, as the heat-resistant spores survive the treatments involved in pasteurization, processing, and canning and even low numbers of spores can cause cheese spoilage **(Mayer *et al.*, 2010 and Kalac, 2011).**

CONCLUSION

From the obtained results, we can conclude that some of examined cheese samples exposed for sale in Cairo and Giza governorates contain anaerobic spore formers that affect cheese quality (Late blowing defect). Therefore, to save cheeses from being spoiled on the market, the following suggestions may be considered.

- Manufacturing of dairy products using high quality raw milk produced from healthy animals under strict hygienic conditions, water supply must be clean and comply with the standard requirements, good cleaning and sanitizing of food processing plant and food contact surfaces is essential to produce safe and high quality product.

-Reduction of the number of spores present in milk by centrifugation, bactofugation, membrane filtration or using safe food grade preservatives as nisin to prevent the growth and germination of spore forming microorganisms and other pathogens.

-Applying effective technological measures (pasteurization, sterilization or acidification) in technological processes prolongs the product shelf life and decreases or eliminates pathogens in milk and milk products.

-Using specific high quality starter cultures in production of cultured dairy products that exert antagonistic action to pathogens through production of bacteriocins.

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