ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Potential Sources of Aerobic and Anaerobic Spore Former Bacteria in Processed Cheese.

Zeinab I Sadek[1], Refaat BM[2], Abd el- shakour EH[2], Nayra SH Mehanna[1] and Hassan MS.

[1] Dairy Science Dept., National Research Centre, Dokki, Cairo, Egypt.
[2] Botany and Microbiology Dep., Faculty of science Al-Azhar University, Cairo, Egypt.

ABSTRACT

A total of 150 samples of locally made processed Cheese (70 triangle and 80 block) and some ingredients used in the manufacture of processed Cheese such as butter, skim milk powder, cheddar cheese, parmesan cheese, prolia, milk protein concentration, casein, lactosan. These samples were examined for isolation and enumeration of aerobic and anaerobic spore former bacteria and their related species. The obtained results achieved that *Bacillus spp*. could be detected in 41(51.2%), 32 (45.7%), 22 (73.3%), 6 (30%), 11 (55%), 8 (80%), 4 (40%), 3(30%), and 5 (50%) of the examined processed cheese blocks, triangle, skim milk powder, cheddar cheese, parmesan, prolia, milk protein concentrate, casein, and lactosan respectively. While, *clostridium spp* could be detected in 25(31.2%),19 (27.1%), 22 (73.3%),6 (30%), 9 (45%), 7 (70%), 2 (20%), 2(20%), and 3 (30%) of the examined processed cheese blocks, triangle, skim milk powder, cheddar, parmesan, prolia, milk protein concentrate, casein, and lactosan respectively. *B. cereus* and *clostridium perfringens* could be detected in all the tested sample with varying percentage (both types of processed cheese and the tested ingredients) except the tested samples of butter.

Keywords: *Bacillus*, *clostridium*, processed cheese.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Processed cheese is a very versatile dairy product because of the different textures and styles in which it is marketed .It has been growing popularly in all over the world with a history of safety and future of increased consumption rate. Large tons of natural cheese is converted to processed cheese. The global production of processed cheese products is estimated at ~1.5- 1.8 million ton/year (Giunee, 2003).

In Egypt it represented about one quarter of the total cheese imports in 1997 (Mohamed *et al* 2011); but recently, the production reached about 10 thousand ton either in spreadable or block forms (Awad, 2003).

The processed cheese is not a preserved food, but a 'semi-preserved food'(Schär and Bosset, 2002). Also, it could be estimated as microbiologically safe milk products due to the use of suitable heat treatment and preventive control measures during the manufacturing and packaging chain.

Nevertheless, Microorganisms may gain entrance to such products during processing, handling and distribution.

In some cases milk ingredients used for processed cheese mixture formulation could also be a source of microbial contamination (Bhowmick et al., 2006; Kumbhar et al., 2009).

Highly undesirable microbial contaminates of processed cheese are rod- shaped endospore – forming bacteria of the genera Bacillus and clostridium, formation of the spores allows it to be resistance of heat chemicals and other adverse environments that undergoes during processing preparation of the product. *Bacillus spp*. are the most common isolated Gram-positive according to their physiological characteristics, they belong to the mesophilic and thermophilic psychrotrophic strains (Samarzija et al, 2012).

The combination of psychrotrophic and thermoduric properties in the same microorganism represents a great potential to spoil food (Montanhini and Bersot, 2013). Also, growth temperature for some species Clostridia can vary from 3.3 ^{0}c to 80 ^{0}c (Alureli and Fanciosac, 2002).

The heat treatment may activate spore germination and the subsequent slow/insufficient cooling or hot holding of food at temperatures too low may then allow germination out growth and multiplication, so there is still potential for food borne illness.

Additionally some of the species are food borne pathogens (Gleeson *et al*, 2013), *Bacillus cereus* may be the single most common cause of food borne illnesses worldwide, but rarely reported to health agencies because symptoms tend to be short-lived and self-limited(Abu Elnaga*et al*, 2014).

Moreover, *Clostridum perfringens* causes a toxin mediated disease represents the 3rd most commonly reported food-borne illness (Grass al, 2013). Some *Cl. Perfringens* strains produce important toxin named *Cl. Perfringens* enterotoxin (CPE), which is responsible for several human gastrointestinal diseases (Lohti*et al*, 2008).

Examination for the presence and number of specific micro-organisms is an integral part of any quality control or quality assurance plan and it may be applied to a number of areas: raw materials, intermediate samples and finished products.

So, this study aimed to evaluate the prevalence of aerobic and anaerobic spore formers, identifying and verifying some characters of the isolated strains from processed cheese samples commonly available at consumer level and the possible source of contamination from ingredient used for helping for a strategy of prevention of food borne diseases.

ISSN: 0975-8585

MATERIALS AND METHODS

Sample collection:

A total of 150 samples of locally made processed Cheese (70 triangle and 80 blocks) were randomly purchased from different local markets in Cairo and Giza. Some ingredients used in the manufacture of processed Cheese such as butter, prolia, milk protein concentration, casein, lactosan (10 samples each) skim milk powder (30), cheddar cheese and parmesan cheese (20 for each) were collected kindly from company of dairy product, sterile techniques were used during samples collection, packaging and microbiological analysis. Each was analyzed for total bacterial, total aerobic spore forming, total anaerobic spore forming, and total psychrotrophic spore forming counts and, identification of the isolate.

Microbiological analysis:

- **Total bacterial counts:**

Total bacterial counts were carried out with plate count agar according to APHA, 1992.

- **Spore former counts:**

Spore formers were determined by heating the sample (10^{-1})in a water bath for 10 min at 80-85°C(Meer *et al* 1991) then was plated on plate count agar (PCA) supplemented with 0.1 % soluble starch , incubated at 37°C/18h for total aerobic spore formers and at 10 °C for 7 days for psychrophilic spore formers, and with addition of agar layer and incubated in gas pack anaerobic jar using kits of anaerobic gas generating kit oxoid for anaerobic spore former. The numbers of colonies per countable plates was determined and expressed as cfu/g.

- **Isolation and identification of *Bacillus spp.*:**

Colonies (2-3) were randomly picked from each plate of total aerobic spore forming count. Each colony was isolated for further purification and identification and Characteristics of *bacillus spp*.

A single representative colony was isolated and inoculated into 10 ml nutrient broth, incubated at 37 °C for 24 hrs. after that used to inoculate the various biochemical test media and identified according to Varadarj (1993).

- **Isolation and identification of *Clostridium spp.*:**

Colstridia was counted on Reinforced *clostrididum* medium (RCM) (Oxoid) and the isolates were identified according to Varadaraj (1993) and Cato *et al* (1986).

- **Detection of haemolysin activity**:

Haemolysin activity of the isolates was assayed on sheep blood agar plates (5%) by plating a loopful of 24-hrs. old culture of the isolates.

The plates were then incubated at 30 °C for 24 hrs. and checked for hemolysis surrounding the growth (Nour et al 2002)

RESULTS AND DISCUSSION

The distribution of total bacterial counts in block and triangle samples is presented in table (1), from a quantitative point of view, of the 70 samples of triangle cheese only 13 samples (18.5%).

ISSN: 0975-8585

Table (1): Total bacterial, total aerobic spore forming, total psychrotrophic spore forming, and total anaerobic spore forming counts in two type of processed cheese (blocks and triangles).

Type of tested samples	Number Of samples	Bacterial groups	Distribution of the counts Cfu/g					Counts (Cfu/g)		
			Number of samples					Min.	Max.	Average
			-ve	10	10^2	10^3	10^4			
processed cheese blocks	80	Tbc	-	18	44	18	-	5×10	2.8×10^3	5.6×10^2
		Tasc	39	7	23	11	-	3×10	1.8×10^3	2.8×10^2
		Tpsc	52	3	25	-	-	3×10	5×10^2	7.1×10
		Tansc	55	4	19	2	-	3×10	1.2×10^3	1.4×10^2
processed cheese triangles	70	Tbc	10	13	34	13	-	3×10	2.1×10^3	4.5×10^2
		Tasc	38	11	14	7	-	2×10	1.5×10^3	2.3×10^2
		Tpsc	55	2	13	-	-	3×10	6×10^2	4.9×10
		Tansc	51	5	14	-	-	2×10	9×10^2	1×10^2

- Tbc: total bacterial count
- Tasc: total aerobic spore forming count
- Tpsc: total psychrotrophic spore forming count
- Tansc: total anaerobic spore forming count

While, it was 18 sample out of 80 sample (22.5 %) of block cheese samples showed values of Total bacterial counts of 10^3 Cfu/g These results are in the line of the spreads cheese samples produced in mechanized factory which showed only a small percentage (9%) of total aerobic counts slightly higher than 10^3 CFU/g (Palmas et al 1999).

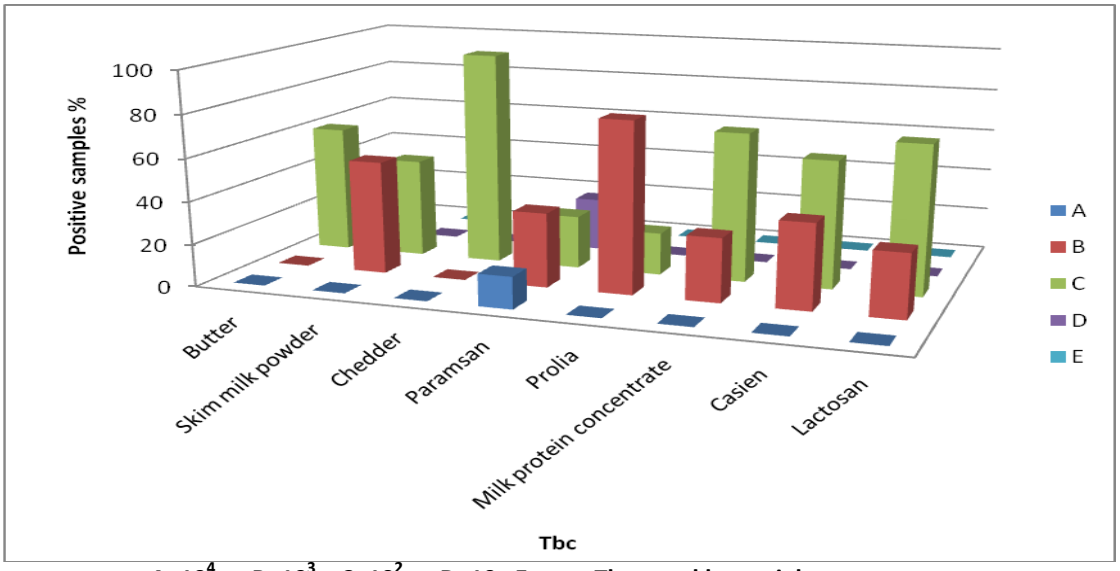
Also , results revealed that total aerobic spore former count were detected in 51.2% from samples of processed cheese blocks and 45.7% from samples of triangle cheese, while total psychrotrophic were not detected in52 and 55 samples of processed cheese blocks and triangle cheese samples respectively. Ranging from 3×10 to 5×10^2 cfu/g in processed cheese blocks. and 3×10 to 6×10^2 cfu/g in processed cheese triangle. The obtained results are lower than those reported by (Nazemet *al.*, 2010) and (Nour Eldiam and Elzubeir, 2006)

It is evident from the results in Table (1) that anaerobic spore former count could be detected of the processed cheese blocks and triangle in 31.25 % and 27.1% respectively. Higher percentage was reported by Abd Alla *et al*., (1996) and El-Tukhy (2007) they found that 75 % of the processed cheese samples contain anaerobic spore former. And Abed El-Raheem (2009) who found 90 % of the processed cheese was positive for presence of anaerobic. While, Sadek (2005) detected anaerobic spore former in 40 % of the processed cheese samples.

Some ingredients which used in processed cheese manufacture might be of low quality from the start which has an effect on the microbiological properties of final product, so Fig. (1, 2, 3, 4) show total bacterial, total aerobic spore forming, total psychrotrophic spore forming and total anaerobic spore forming counts of some ingredients.

None of the surveyed butter samples contained detectable levels of total aerobic, psychrotrophic and anaerobic spore former counts, but 70 % of the samples positive for total bacterial counts Fig (1) The microbiota of butter reflects the quality of cream, sanitary conditions of the equipment and the environmental and sanitary conditions during packaging and handling (Varge, 2007).

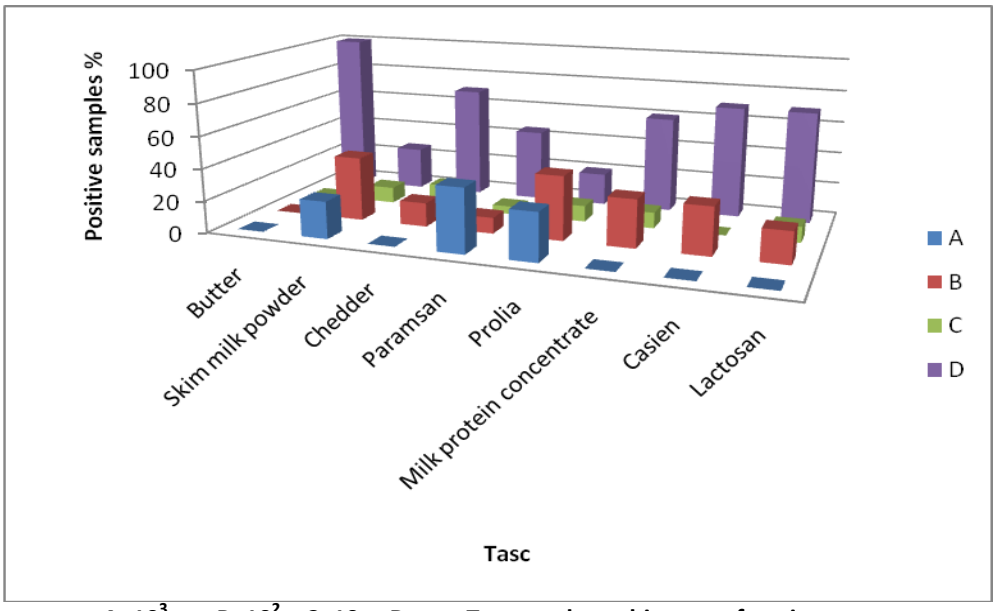
ISSN: 0975-8585



A: 10^4 B: 10^3 C: 10^2 D: 10 E: -ve, Tbc: total bacterial count

Fig (1):Distribution of total bacterial count in some raw materials ingredients of processed cheese.

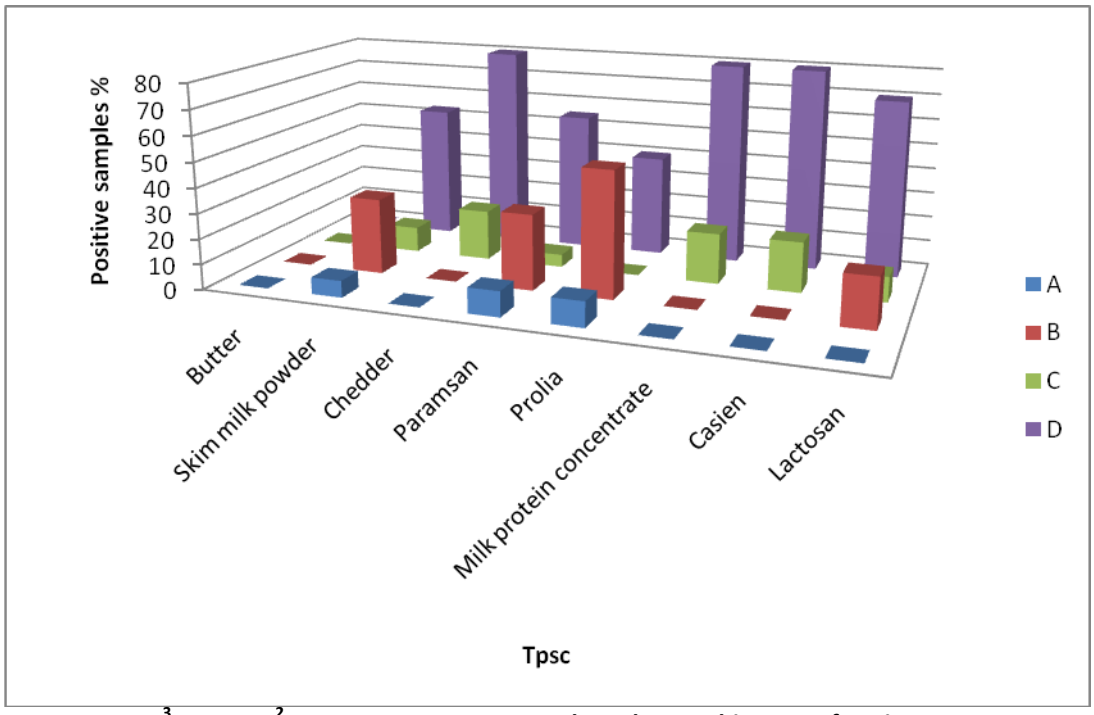
Results of skim milk powder samples raveled that 73.3%positive for both total aerobic and anaerobic spore forming counts, and 46.6% positive total psychrotrophic spore forming count. Nearly similar finding were reported by Abed El-Hameed (2004) for aerobic spore former (74.3 %) in milk powder. While, in another investigation, Saad and Ahmed (2013) reported 40 % of the milk powder were positive for aerobic spore formers.



A: 10^3 B: 10^2 C: 10 D: -ve, Tasc total aerobic spore forming count

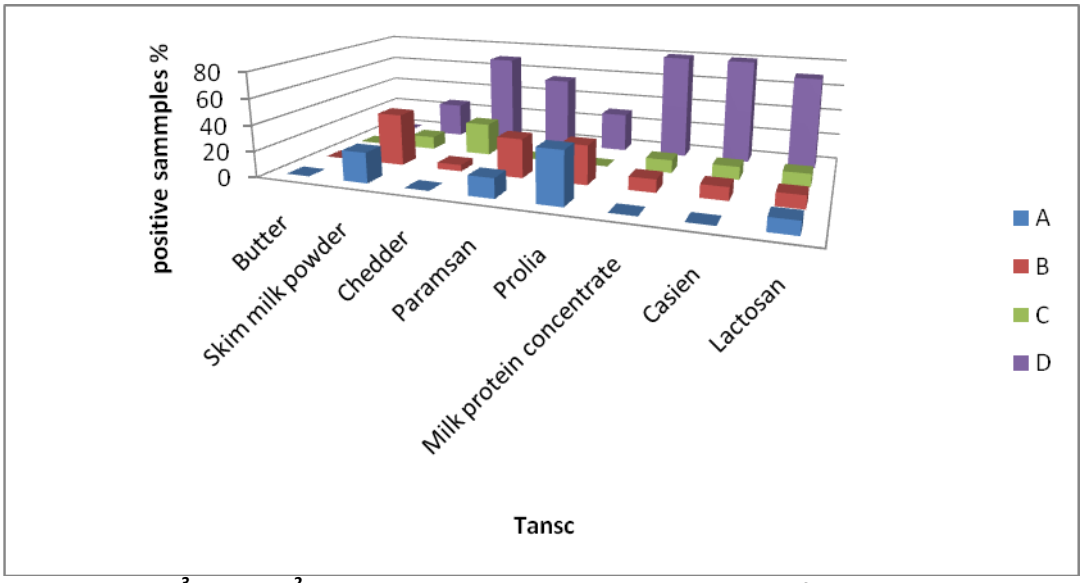
Fig (2): Distribution of total aerobic spore forming counts in some raw materials ingredients of processed cheese.

ISSN: 0975-8585



A: 10^3 B: 10^2 C: 10 D: -ve, Tpsc total psychrotrophic spore forming count

Fig (3): Distribution of total psychrotrophic spore forming counts in some raw materials in ingredients of processed cheese.



A: 10^3 B: 10^2 C: 10 D: -ve, Tansc total anaerobic spore forming count

Fig (4): Distribution of total anaerobic spore forming counts in some raw materials ingredients of processed cheese.

Regarding to the cheddar cheese (as ingredient) total psychrotrophic spore observed in low percentage 20 % (4 out 20), and 30 %(6 out 20) of the samples were positive for both total aerobic and anaerobic spore-forming counts respectively. On the other hand in parmesan cheese total aerobic spore forming count detected in55% of the samples, while total anaerobic and psychrophilic spore forming counts could be detected in equal

ISSN: 0975-8585

percent 45%of the samples as shown in the Fig. (2, 3, 4). In this concern, El-Toukhy (2007) reported 30 % samples of the Ras cheese were positive for anaerobic spore former.

On the contrary, milk protein concentrate and casein only 40 %of samples were positive for total aerobic spore forming count and 80 % (8 of 10) samples were free from total psychrotrophic and anaerobic spore forming counts.

While, in lactosan 50 % of samples were positive for total aerobic spore forming count and 30% were positive for both total anaerobic and psychrotrophic spore forming counts Fig. (2, 3, 4).

Generally, all of the tested ingredients were positive for total bacterial counts except butter. The high bacterial count in dried milk powders, milk protein concentration, casein and lactosan, may be result the high number bacteria present in the raw milk or milk by-product, preheating temperatures, operating conditions of the evaporator & dryer, and low plant hygiene.

Moreover, incidence of spore former, which form spores during unfavorable growth conditions, these spores are heat resistant and can survive during processing, pasteurization and cooking (Saad and Ahmed 2013).

Data in Fig (5) reports the frequency distribution of different Bacillus species from the examined sample.

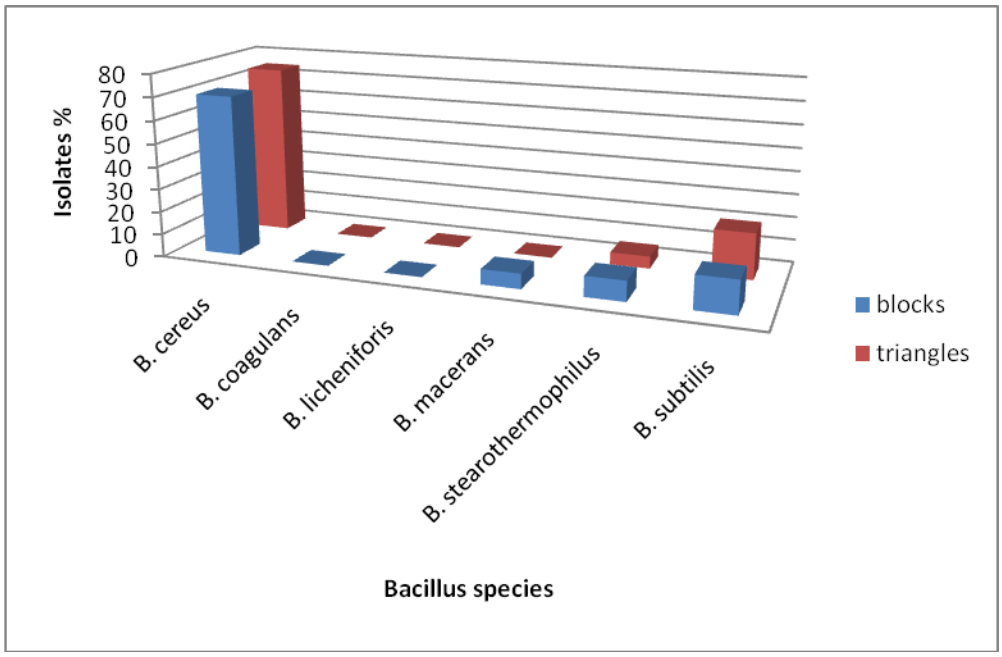


Fig (5): distribution of different total Bacillus species in processed cheese (blocks and triangles).

Among Bacillus species, *B. cereus* was isolated in all the tested sample with varying percentage (both types of processed cheese and the tested ingredients) except the tested samples of butter, the identified isolates were mainly consisted of *B. cereus* (70, 74.6 %) and *B. subtilis* (14.4, 20 %) followed *B. sterothermophilus* (8.8, 5.3 %) in blocks and triangles processed cheese respectively, while *B. coagulas* was recovered only from prolia and casein samples isolates. Also *B. macerance* was isolated only in block cheese represent 6.6 % of the isolates.

Moreover, *B. licheniformis* was recovered from skim milk powder, lactosan and cheddar cheese isolates with percentage 8.3, 33.3, 20 % respectively (Fig6).

ISSN: 0975-8585

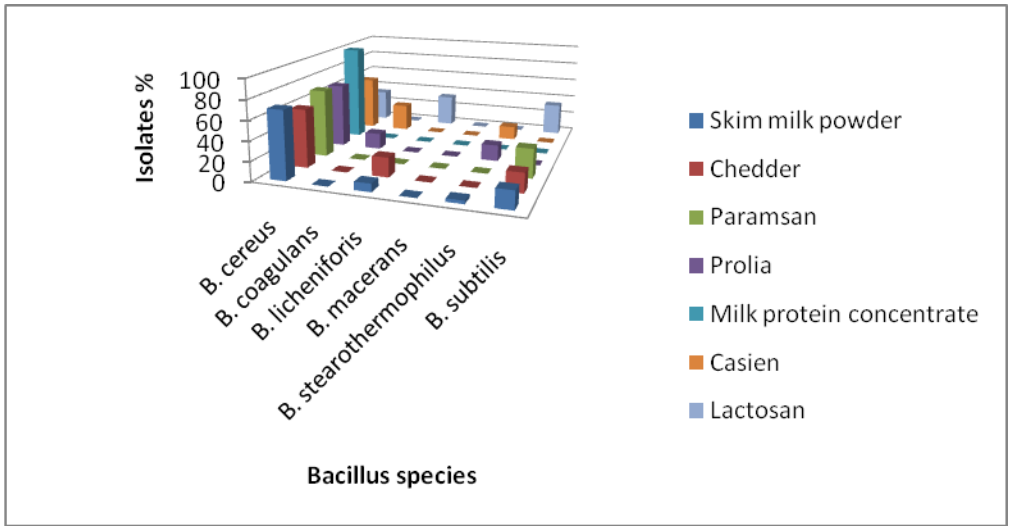


Fig (6): distribution of different total Bacillus species in some raw

Material ingredients of processed cheese. The results were confirmed with Sadek *et al*., (2006) who found that *Bacillus cereus* has been isolated from 25 % of processed cheese samples.

Also, (Abed el Hameed, 2004) reported that, *B. cereus, B. subtilis, B. licheniformis* followed by *B. mycoides, B. polymyxa, B. pumilus, B. macerans and megaterium* were isolated from some dairy products including processed cheese and dried milk all in the Egyptian market.

It could also be observed from (Fig 6) that, the most isolates from skim milk powder and milk protein concentration were *B. cerues* in high ratio of 70% and 100 % respectively.

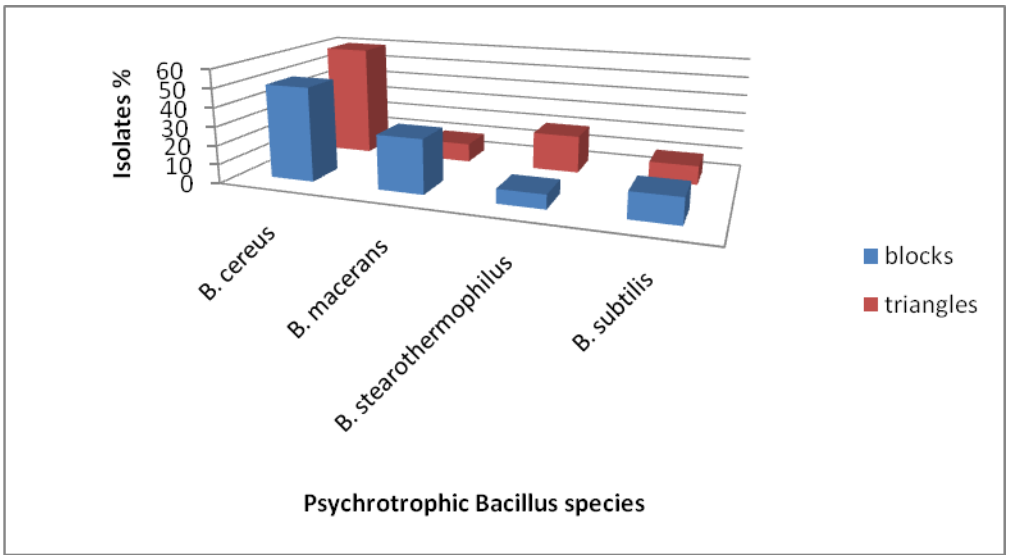


Fig (7): Distribution of Psychrotrophic Bacillus species in processed cheese (blocks and triangles).

In this respect, Saad and Ahmed (2013) and (Sadek, *et al*., 2006) reported that spore former in milk powder samples consisted mainly of *B. cerues* followed by *B. subtilis* in the local market.

ISSN: 0975-8585

Psychrotrophic bacteria are defined as group of different bacterial species that are able to grow at 7 ^{O}C or less regardless to their optical temperature of growth (Samarzija *et al.,* 2012) positive samples showed visible growth at 7 ^{O}C within 10 days and thus fitted the generally accepted definition of psychrotrophs.

Data given in Fig. (7) revealed that the tested isolates belonged to the following *bacillus* species: *B. cereus*, *B. subtilis, B. macerans*, and *B. sterothermophilus*. Also, notice that *B. cereus* was the principal species representing as Psychrotrophic about 50 % and 60 % of isolates from the positive samples for *Bacillus spp*. of the blocks and triangles processed cheese respectively.

Followed by *B. macerans* 28.7 % and *B. subtilis* 13.6 % from the total of 66 isolates from the positive samples of psyhrotrophic *bacillus spp*. of processed cheese (blocks). While, from 40 isolates processed cheese (triangles) *B. stearothermophilus* representing 20 % of the isolates whereas *B. macerans*and *B. subtilis* representing 10 %.The results are in the harmony with Griffiths and Phillhps (1990) who found that 50 % of the *Bacillus spp*. strains have ability to survive even at temperature of 2 ^{O}C.

In the same trend, the isolated of *Bacillus spp.* from the different ingredients used for processing, *B. cereus* were recovered at varying of percentage (40-100 %) and being the most aerobic species except for butter samples which were free from psyhrotrophic spore former bacteria. Fig. (8).

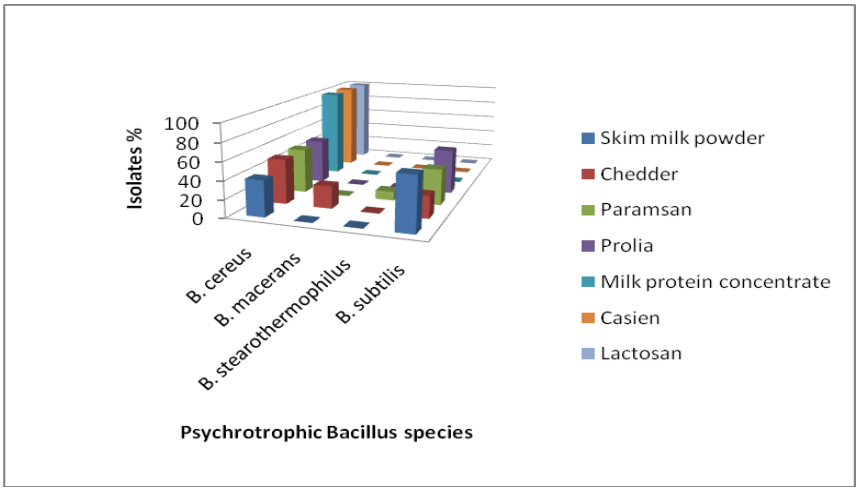


Fig (8): Distribution of Psychrotrophic Bacillus species in some raw materials ingredients of processed cheese.

Among the bacteria belonging to genus *Bacillus* from dairy products *B.cereus, B.subtilis, B.stearothermophilus, B. licheniformis, B.coagulans* and *B. circulans* that were thermo resistant psychrotrophic aerobic or facultative anaerobic are commonly isolated species (Samarzija et al, 2012).

In this respect Vaisainin *et al*., (1991) reported that majority of the strains isolated from dairy product were able to grow at a temperature below 10 °C.

The isolates of *clostridium spp.* recovered from processed cheese samples were classified to *Cl. perfringen* (70 and 76.6 %) , *Cl. Butyricum* (12.5, 15 %), *Cl. tyrobutyricum* (10, 5 %), and *Cl. sporagenes* (7.5, 3.3 %), in blocks and triangles processed cheese samples respectively and other species failed to detected from the two tested types of processed cheese Fig. (9).

ISSN: 0975-8585

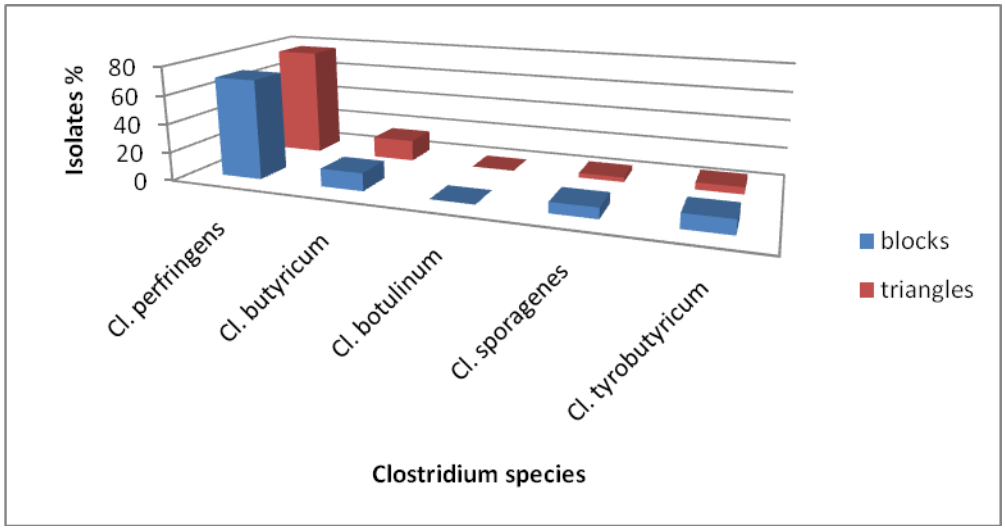


Fig (9): Distribution of total Clostridium species in processed cheese (blocks and triangle).

The same isolates were also recovered from processed cheese samples at varying percentages (Sadek 2005). However, different types with different percentage of *Clostridia spp*. were isolated from Romy, Ras, processed cheese and dried milk (Ibrahim, 1986, El-toukhy 2007 and Abed El-Raheem 2009).

It could be noticed from Fig. (9) *Cl. perfringen* was the most frequent clostridia in the examined samples similar finding were reported by El-Basiony, 1980 and Nazem and A man (1994), in the Egyptian market.

In contrast, Varga, 2007 recorded that none of the processed cheese samples surveyed contained food borne pathogens at detectable level.

Nearly, the same trend of distribution of the isolates with different percentage were obtained from the tested ingredients Fig. (10) except for isolates from skim milk powder where 3 isolate out from 50 (6 % were identified as *Cl. Botulinum*.

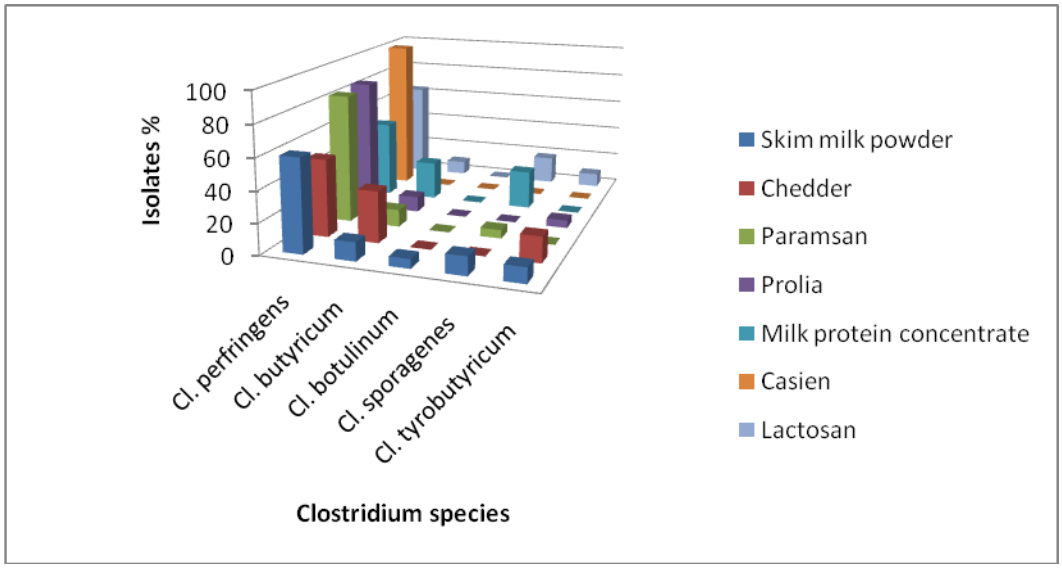


Fig (10): Distribution of total Clostridium species in some raw materials ingredients of processed cheese.

In general *B. cerues* and *Cl. perfringens* were the most frequent species in both the examined cheese samples and ingredients. Used low quality of ingredients produced low quality of processed cheese. Then, we must recommend the applying HACCP analysis and risk management system in food chain, which related with all milk and milk products industries. Hereupon, the increasing incidence of *B. cereus* in the processed cheese and ingredients emphasizes that such products may constitute a public health hazard and need much attention.

Type of test samples	No. of isolates	Positive isolates	
		No.	%
Processed cheese blocks	63	51	80.9
triangles	56	42	75
Total	119	93	78.1

Table (2): Hemolytic activity of *B.cereus* isolates from processed cheese (blocks and triangles).

Type of test samples	No. of isolates	Positive isolates	
		No.	%
Skim milk powder	42	35	83.3
Cheddar	6	4	66.6
parmesan	14	9	64.2
prolia	12	9	75
milk protein concentration	8	5	62.5
casein	4	3	75
lactosan	4	4	100
Total	90	69	76.6

Table (3): Hemolytic activity of *B.cereus* isolates from some raw materials ingredients of processed cheese.

Bacillus cereus produce a large number of potential virulence factors, tripartite hemolysin is one of them (Schoeni and Wang, 2005). As shown in table (2, 3) 93 out of 119 isolates (78.1 %) in processed cheese, while 69 out of 90 isolates (76.6%) show hemolytic activity in ingredients of processed cheese. This result due to the risk analysis system is not apply in the most dairy factories. This result is lower than obtained by (Wong et al, 1988) who recorded 98 % from market dairy products lysed erythrocytes. Also Nour*et al*, (2002) which recorded that 89.74% and 86.29% show hemolysis surrounding the growth of the isolates from market dairy products and farm samples respectively. While, Sadek, *et al* (2006) reported that 88.4%isolates from processed cheese and some dairy products show hemolytic activity.

Table (4): Hemolytic activity of *Cl.perfringens* isolates from processed cheese (blocks and triangles).

Type of test samples	No. of isolates	Positive isolates	
		No.	%
Processed cheese blocks	56	48	85.7
triangles	46	33	71.7
Total	102	81	79.4

ISSN: 0975-8585

Table (5): Hemolytic activity of *Cl.perfringens* isolates from some raw materials ingredients of processed cheese.

Type of test samples	No. of isolates	Positive isolates	
		No.	%
Skim milk powder	30	27	90
Cheddar	9	6	66.6
parmesan	15	14	93.3
prolia	17	17	100
milk protein concentration	4	3	75
casein	6	6	100
lactosan	7	7	100
Total	88	80	90.9

*C. perfringens*causes a toxin-mediated disease represents the 3rd most commonly reported food-borne illness (Grass *et al., 2013).*

Results in Table (4, 5) reveal that most of isolates of *C. perfringens* show hemolysin production, which one of the virulence factors of the organisms. 81 out of 102, 80 out of 88 isolates (79.4, 90.9 %) in processed cheese and ingredients isolates respectively show hemolytic activity. These results are not far from those obtained by Sadek, (2005) who recorded that 81.8 % from processed cheese samples were showed hemolytic activity. Colonies usually show a double zone of hemolysis on blood agar plates with a clear inner theta-toxin zone and a hazy outer zone caused by alphatoxin production (Brynestad and Granum, 2002). These results mean that it has to be the application of HACCP in processed cheese factories or any of its components.

REFERENCES

[1] Abd Alla, E.A.M. .,El-Shafei, K.,Ibrahim, G.A. and Sharaf, O.M (1996). Changes in microflora and biogenic amines of some market processed cheese during storage. Egyptian J. dairy Sci., 24:217.

[2] Abed el Hamid (2004).Studies on Bacillus cereus and related species in heat treated milk and some milk product. Thesis M.V.SC.Fac.Veterinary Assuit University.

[3] Abed El- Raheem (2009). Studies on anaerobic bacteria in some dairy product in Assuit City. Thesis PHD.V.SC.Fac. Veterinary Assuit University.

[4] Abu Elnaga (2014), Bacterial aspect of Food Poisoning .Life Science Journal 2014;11(3).

[5] American Public Health Association (A.P.H.A) (1992). Compendium of methods for microbiological examination of food. 3rd Edition, pp. 843-845. American Public Health Association, Washington D.C., USA.

[6] Aureli, P. and Franciose, G (2002). Clostridia spp. In : Encyclopedia of Dairy Science (Eds. H. roginski, J.W.Fuquay and P.F.Fox) London, UK, 1:456- 463.

[7] Awad, R.A. (2003). Impact of potato puree as a cheese base replacement in the manufacture of processed cheese. Egyptian Journal of Dairy Science, 31, 375–387.

[8] Bhowmick, B.K., M.L. Saha and M.R. Khan, (2006). Microbial study of some milk with special reference to coli form bacteria. Int. J. Dairy Sci., 1(1): 57-62.

[9] Brynestad, s. and Granum, P.E. (2002). Clostridium perfringens and foodborne infections. Int. J. Food Microbiol. 74:195.

[10] Cato, E.P., Lance, G. and Finegold, S.M. (1986). Bergey's Manual of Systematic Bacteriology (10th Ed.), the section 13: Endospore- forming Gram-positive Rods and Cocci, Williams and Wilkins, Baltimore, MD, USA.

[11] El-Bassiony, T.A. (1980) . Occurrence of Clostridium perfringens in milk and dairy products. J. Food Prot. 43:537.

[12] EL-toukhy (2007). Isolation and identification of Clostridia in milk and some dairy product especially stormy fermenter species. Thesis, M.V.SC. Fac. Veterinary Mansoura university.

[13] Giunee, T.P.(2003). Pasteurized processed cheese products . in :Encyclopedia of Dairy Science , VOL1, Roginski, H; Fuquay .J.W. and Fox. P.E Eds. Academic Press, 411- 418.

[14] Gleeson, D; O′ connell, A. and Jordan, k. (2013). Review of potential sources and control of thermoduric bacteria in bulk- tank milk.
[15] Grass, JE.; Gould, L.H. and Mahon, B.E. (2013). Epidemiology of food borne disease outbreaks caused by *Clostridium perfringens*, United States, 1998-2010. Food borne Pathog Dis. 10(2):131-6
[16] Griffiths, M.W. and Phillips, J.D. (1990). Incidence, source and some properties of psychrotrophic Bacillus found in raw and pasteurized milk, J. soc. Dairy technol., 43, 62, 1990.
[17] Ibrahim, A.A. (1986). Incidence and significance of anaerobic organisms in market cheese. M.V. Sc. Thesis, Fac. Vet. Med., Cairo Univ.
[18] Kumbhar, S.B., J.S. Ghosh and S.P. Samudre, (2009). Microbiological analysis of pathogenic organisms in indigenous fermented milk products adv. J. Food Sci. Technol., 1(1): 35-38.
[19] Lahti, P.; Heikinheimo, A.; Johansson, T. and Korkeala, H. (2008). *Clostridium perfringens* type A isolates carrying the plasmid-borne enterotoxin gene (genotype IS1151-cpe or IS1470-like-cpe) are a common cause of food poisoning. J. Clin. Microbiol. 46:371–373.
[20] Meer, R. R., Baker, J., Bodyfelt, F. W., and Griffiths, M. W. (1991). Psychrotrophic *Bacillus* spp. In fluid milk products: a review. *Journal of Food Protection, 54*, 969–979.
[21] Mohamed. M. O., Suleiman T.A. ; Mohammed N.H. (2011). Chemical and microbiological evaluation of processed cheese available in Khartoum market, Sudan. Am. J. Food. Nutr, V, 1(1): 28-33.
[22] Montanhini. M. Tand Bersot L.S (2013). Evaluation of psychrotrophic behavior and lipolytic and proteolytic activity of Bacillus cereus isolated from refrigerated dairy products. Acta Scientiarum. Technology, v. 35, n. 1, p. 163-167,
[23] Nasem, AM. And Aman, IM(1994). Late gas problem in processed cheese , Vet .Med. J; 42:107.
[24] Nazem, A.M., El- Moghraby .M.M, Amer A.A; and Hassan ,E.O.M (2010) . quality assessment of different varieties of processed cheese of Alexandria markets. Alex. J Vet. Sci,VOL.30, (1)13- 19.
[25] Nour El Diam, M.S.A and El Zubeir, I.E.M (2006). Comparison of microbiological quality of processed and non-processed Sudanese white cheese. Res. J. Microbiol. 1(3):273-279. Palmas, F., Cosentino, S., Fadda, M.E., Deplano,
[26] Nour, M.A., Tohami, M. and Kahter, A.A. (2002): Identification and characterization of Bacillus cereus group isolated from different sources.
[27] Saad and Ahmed (2013), Quantitative, Qualitative and Enzymatic Activities of Aerobic Spore forming Bacteria in Foods of Animal Origin. International Proceedings of Chemical, Biological & Environmental SOURCE TYPE Academic Journal, Vol. 55, p 43- 48.
[28] Sadek, Z . I (2005), Clostridium perfringens in feta and processed cheese and its behavior with lactic acid bacteria. Egyptian J. Dairy Sci., 33:187-200.
[29] Sadek, Z . I, Fathi, F.A. and Salem, M.M.E (2006), Incidence, Survival and Biocontrol of Psychrotrophic Bacillus cereus and its Toxin in Milk and Tallaga Cheese. Polish of food and nutrition sciences VOL 15/56(4) : 419-425.
[30] Samarzija, D, Zamberlin. S . and P Ogacic. T. (2012) pschrotrophic bacteria and milk and dairy products quality . Mijekarstro 62 (2),77:95.
[31] Schar, W. and J.O. Bosset, (2002). Chemical and physicochemical changes in processed cheese and readymade fondue during storage. A Review. Lebensm.- Wiss. u.-Technol., 35: 15-20.
[32] Schoeni J.L., Wang A.C., (2005), *Bacillus cereus* food poisoning and its toxins. J. Food Prot. V. 68, 636–648.
[33] Vaisainin O.Ml, Mwaisumo N.J., Salkinoja-Salonen M.S., (1991) Differentiation of dairy strains of *Bacillus cereus* group by phage typing, minimum growth temperature and fatty acid analysis. J. Appl. Bacteriol., V, 70, 315–324.
[34] Varadaraj M.C., (1993) Methods for detection and enumeration of food born bacterial pathogenes. A critical evaluation. J. Food Sci. Technol., V, 30, 1–13.
[35] Varga .L, (2007), Microbiological quality of commercial dairy products. FORMATEX.P 487-494.
[36] Wong, H. C., Chen, Y. L. and Chen,C. L. F. (1988). Growth, germination and toxigenic activity of Bacillus cereus in milk produces. J. Food Protect. 5 1(9):707- 710.