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Occurrence of *Escherichia coli* and coliforms in processed cheese

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Abstract: One hundred and twenty samples of processed cheese representing: 60 samples of processed cheese cubes, 30 samples blocks and 30 samples spread were randomly collected from different super markets in Cairo, Giza, Alexandria and Behira Governorates, Egypt. Collected samples were subjected to Coliform count as well as biochemical and serological identification of the isolated strains. The results reveal the presence of Coliforms, in percentages of 25.8%. On the other hand, *Escherichia coli* (*E. coli*) could be detected in in percentages of 2%. Only three isolated of E. coli could be serologically identified as *E. coli* O27. The degree of acceptability of the processed cheese samples vs Egyptian standards was determined the economic and public health significance of contamination of processed cheese products was discussed.
Key words: Coliforms, *Escherichia coli* and processed cheese.

Introduction

Cheese is an important integral part of diet consumed in Egypt. It is consumed almost daily in Egypt as an excellent food that contains a wide variety of easily digested nutrients; its important in human nutrition not only in supplying the consumer with a high quality protein but also it is rich in the essential amino acids, high fat% which supplies consumer with essential fatty acids and phospholipids, it also a good source of vitamin A and B_2 as well as higher mineral content specially calcium and phosphorous. The initial idea of processed cheese production was to increase the shelf life of natural cheese and find alternative uses for natural cheese that was difficult to sell[1,2].

Processed cheese is one of the most popular foods in all over the world. It is basically melted cheese which is manufactured from a complex mix of ingredients including natural cheese, water, stabilizers, flavorings, emulsifying agents (mostly sodium polyphosphates) and optional agents such as butter or spices, followed by heating of the blend under a partial vacuum with constant agitation until a homogenous product obtained[3,4].

For a typical processed cheese production, the principal raw material is semi mature cheese of shorter structure with partially hydrolyzed protein, and the processing markedly changes the structure of the natural cheese and results in the development of new structures in the processed cheese [5 and 6]. Nowadays, Production and consumption of processed cheese increased steadily as large tonnages of natural cheese are converted to processed cheese[7,8].

The popularity of processed cheese is due to its numerous end use applications. Processed cheese is one of the leading cheese varieties in the world that is used as an ingredient in various food preparations (food

processing and food servicing establishments). Processed cheese is produced and sold in various forms such as loaves, slices, shreds, blocks or spreads and is used as an ingredients in numerous products. The versatility of processed cheese can be attributed to its unique functional properties, the functional properties of a cheese during all stages of preparation and consumption of the food that would eventually contribute to the taste as well as the aesthetic appeal of the prepared food. The desired functional properties of the processed cheese can be made into two major categories; unmelted texture and melted texture properties. Due to the technological progress in cheese making, process cheese has three sub groups, ***pasteurized process cheese***, that is most closely related to the natural cheese; ***pasteurized process cheese food*** that resembles pasteurized process cheese but cream, milk or other components may be added and ***pasteurized process cheese spread***. Both pasteurized process cheese food and spread normally have higher water content, lower fat content and considerably higher carbohydrate content[9,10,11]**.** Although processed cheese is considered an important food source, however sometimes it may become unmarketable or even harmful when it is subjected to contamination during its production, handling or storage[12,13].

The spoilage organisms may gain entrance to processed cheese as a result post-manufacturing contamination or even due to unhygienic conditions during production. Undesirable microbes that can cause spoilage of processed cheese include coliforms may contaminate the product causing public health hazards as enteropathogenic strains of *Escherichia coli*. Processed cheese was implicated in much food-borne illness [14,15]**.**

Coliforms count is used as a hygienic indicator to reflect the general microbiological quality in routine testing of processed cheese, although coliform organisms can be easily destroyed by heat; however they are used as an indicator of heat treatment failure as well as post heat treatment contamination[16,17,18]. Diarrheagenic *Escherichia coli* can be divided into 5 at least categories based on differences in the pathogenic mechanisms as enteropathogenic, enterotoxifgenic, enterohaemoragic, enteroinvasive and enteroaggregative *E. coli*. Entertoxigenic *E. coli* produces either or both heat labile enterotoxin (LT) and heat stable entertotoxins causing food borne outbreaks [19,20.]

Due the increasing demands, of consumers of all ages, for consumption of processed cheese and as processed cheese is a semi preserved product; which may be contaminated by different types of microorganisms during processing, handling, storage and distribution; resulting in spoilage of the product or may constitute public health hazard [21,22,23]. Therefore, this study was planned to estimate the Coliform Count (MPN/g.) and isolate and identify coliform strains from all the examined samples.

Material and Methods

One hundred and twenty random samples of locally manufactured processed cheese (sixty cubes, thirty blocks and thirty spread samples) representing 12 different brands were collected from different retailers at Cairo, Giza, Alexandria and Behara Governorates. Collected samples were transferred directly to the laboratory with a minimum of delay to be immediately examined.

Preparation of samples and decimal serial dilution :

Aseptically 11 grams of each sample were emulsified in sterile poly ethylene bag with 99 ml of sterile Sodium citrate 2% solution using stomacher (Lab stomacher 400) for 1-3 minutes to obtain homogenate (1/10 dilution).Tenth fold serial dilutions were prepared by adding one ml of the prepared homogenate to 9 ml of sterile 2% aqueous solution of sodium citrate. All prepared samples were subjected to the following microbiological examinations [24,25.]

Determination of Coliform Count MPN/g.[26,27]

1. Determination of presumptive coliform count (MPN/g.)[28,29]

One ml from each of the previously prepared decimal dilutions was inoculated into a series of 3 fermentation tubes containing lauryl sulphate tryptose broth (Difco™ 22415) supplemented with inverted Durham's tubes for collection of gas. Inoculated tubes, as well as, the control one were incubated at 35ºC for 48 hours + 2 hours, and then examined for gas production. From the results obtained, Presumptive MPN / g. was computed.

2. Determination of confirmed coliform count (MPN/g.)[30.]

3. Identification of isolated coliform strains according to [31,32].

3.1. Biochemical examinations of the *E.coli* isolates using API 20E System [33, 34.]

3.2. Biochemical examinations of the isolated *E.coli* using VITEK 2 System according to [35.]

3.3. Serological typing of *E.coli* strains [36,37.]

Results and Discussion

The Coliforms group includes species from the genera Escherichia, Klebsiella, Enterobacter and Citrobacter. Coliforms were historically used as indicator microorganisms to serve as a measure of faecal contamination, and thus potentially, of the presence of enteric pathogens in food. Although some coliforms are found in the intestinal tract of man, most are found throughout the environment and of high sanitary significance [38,39].

Table (1): Statistical analytical results of examined processed cheese samples based on their Total Coliforms count (CFU/ g.)

Type of Samples	No. of examined samples	Positive samples		Min.	Max.	Mean	S.E.M±
		No	%				
Cubes	60	19	31.7%	3	$11x10^2$	65.6	7.25
Blocks	30	7	23.3%	4	15	8.12	2.65
Spread	30	5	16.6%	7	$2.1x10^2$	72.6	5.21
Total	120	31	25.8%	3	$11x10^2$	69.8	6.98

Table (2): Frequency distribution of examined processed cheese samples based on Total coliforms count (CFU/g.)

Intervals	Types of Samples							Total
	No. of samples	%	No. of samples	%	No. of samples	%	No. of samples	%
<10	49	81.7	27	90	27	90	103	85.8
10- $<10^2$	6	10	3	10	3	10	12	10
10^2- $<10^3$	3	5	0	0	0	0	3	2.5
10^3- $<10^4$	2	3.3	0	0	0	0	2	1.7
Total	60	100	30	100	30	100	120	100

As shown in **Table (1)** Coliforms were detected in 31.7%, 23.3% and 16.6 % of the examined processed cheese samples of cubes, blocks and spread, respectively. The total incidence of Coliforms was 25.8%, with a mean value of 56.6 ± 7.25, 8.12 ± 2.65 and 72.6 ± 5.21 of the examined processed cheese samples of cubes, blocks and spread, respectively, and a total mean value was 69.8 ± 6.98 MPN/g.

Data presented in **Table (2)** show that the highest frequency distribution of Coliform content in the examined processed cheese cubes, blocks and spread lies with the range <10 cfu/g. The obtained results of processed cheese are nearly similar to obtained by[40]. Higher results were recorded by [(39)], while lower results were reported by [19, 20,29.]

The public health importance of coliform bacteria is it implicated in gastrointestinal illness as gastroenteritis, epidemic diarrhea in children and cases of food poisoning [17,37]. Contamination of cheese with

Coliforms gives indication of bad hygienic conditions during production, handling, distribution and the possible presence of enteric pathogens [29,34].

Counts of Coliform are the traditional indicator of possible faecal contamination, microbial quality and wholesomeness which reflect the hygienic standards adopted in the food operation. A lack of good sanitary practices may result in loss of quality, spoilage or, in some cases, create a health hazard [11, 13,36.]

Coliforms contamination could be attributed to poor quality ingredients and is considered essentially a plant problem and the presence of any member of the family Enterobacteriacae is undesirable in pasteurized dairy products, which suggests post – pasteurization contamination [10,28,31] .

Table (3): **Frequency of isolated Enterobacteriacae strains based on their identification (No. of isolates 144).**

Isolated spp		**Processed**		**Processed**		**Processed**		**Total**	
Citrobacter species		No.	%	No.	%	No.	%	No.	%
	braakii	5	7.3	6	13.3	1	3.3	12	8.4
	freundii	18	26	0	0	4	13.3	22	15.3
	murliniae	2	2.9	0	0	2	6.7	4	2.8
	sedlaki	1	1.5	10	22.2	3	10	14	9.7
Edwardsiella tarda		6	8.7	3	6.7	5	16.7	14	9.7
Enterobacter species	**pyrinus**	5	7.3	0	0	1	3.3	6	4.2
	sakazakii	0	0	3	6.7	0	0	3	2
Escherichia species	**coli**	2	2.9	0	0	0	0	2	1.3
	Frequosnii	2	2.9	1	2.2	0	0	3	2
Ewingella Americana		4	5.8	5	11.1	0	0	9	6.3
Klebsilla species	**oxytoca**	0	0	5	11.1	2	6.7	7	4.9
	pneumoniae	2	2.9	0	0	3	10	5	3.5
Kluyvera species	**cryoerse**	8	11.6	0	0	0	0	8	5.6
	oscorbat	7	10	0	0	0	0	7	4.9
Providencia species	**alcalifaciens**	2	2.9	0	0	3	10	5	3.5
	rustigiani	0	0	4	8.9	3	10	7	4.9
	stuarttii	0	0	3	6.7	0	0	3	2
Shigella species	**flexnetti**	3	4.4	5	11.1	1	3.3	9	6.2
	sonnervi	2	2.9	0	0	2	6.7	4	2.8
Total		69	100	45	100	30	100	144	100

Results presented in **Table (3)** show that biochemical identification of isolated Enterobacteriacae strains from processed cheese samples reveal that Citrobacter freundii was the most common one in a percentage of (15.3%), followed by *Citrobacter sedlak (9.7%), Edwardsiella tarda (9.7%), Citrobacter braakii (8.4%), Ewingella Americana (6.3%), Shigella flexnetti (6.2%), Kluyvera cryoerse (5.6%) and (Citrobacter murliniae, Enterobacter pyrinus, Enterobacter sakazakii, Escherichia coli, Escherichia Frequosnii, Klebsilla oxytoca, Klebsiella penumoniae, Kluyvera oscorbat, Providencia alcalifaciens, Providencia rustigiani, Providencia stuarttii and Shigella sonnervi)* could be isolated from the examined processed cheese samples with an incidence ranged from 1.3 % - 4.9 %.

Nearly similar results for Enterobacteriacae species isolated from examined processed cheese samples were obtained by [29.37,40]**.** *Klebsiella penumoniae* it is a world wide spread bacteria that can be responsible for arthritis, meningitis, appendicitis, cystitis and septicemia outbreaks in kids and newborns, but is more frequent responsible for pneumonia and necrotic damage of the lungs, while Enterobacter species were incriminated in urinary tract infection and septicemia [20,33]**.**

Citrobacter can cause a wide spectrum of infections in humans such as infection in the urinary tract, respiratory tract, wounds, bone, peritoneum, endocardium, meninges, and bloodstream among the various sites of infection, the urinary tract is the most common, followed by the respiratory tract, and skin/ soft tissues [9,21,39.]

Table (4): Frequency of isolated *E.coli* based on seriological identification.

Species	Number	Percentage (%)
***E.coli* O27**	**3**	**100.00**

The isolated *E. coli* strains could be serological identified as (3) *E.coli* O27 **Table (4).** The results are nearly similar to those obtained by[24,26] while higher incidence of the isolated *E.coli* obtained by [33,36,38]. *Escherichia coli* is a bacterium that is present in the gastrointestinal tract of man and dairy animals. Milk collected for human consumption can become contaminated with E. coli directly via animal feces or indirectly via contaminated farm and dairy parlor environments, equipment, and workers. While most strains of *E. coli* are harmless commensals, some are able to cause human gastrointestinal disease with mild to severe symptoms that may progress to long-term squeal or fatal outcomes in high-risk individuals. Also E. coli was found to be responsible for cases of cystitis, pyelitis, pyelonephritis as well as appendicitis and peritonitis[10,16.]

E. coli, Citrobacter spp., *Klebsiella penumoniae* and Enterobacter spp. were implicated in gastrointestinal illness as cholera-like syndrome, gastroenteritis, epidemic diarrhea, traveler's diarrhea, cystitis, pyelitis, pyelonephritis, appendicitis, peritonitis, food poisoning, lobar pneumonia and other infections of respiratory tract, meningitis, pyaemia, appendicitis and cystitis, Urinary tract infections and septicemia [33,35.]

Table (5): Degree of acceptability of examined processed cheese samples vs. Egyptian standards for the presence of Total coliforms and *E.coli*.

Type of samples	Acceptable				Not acceptable			
	Coliforms		*E.coli*		Coliforms		*E.coli*	
	No.	%	No.	%	No.	%	No.	%
Cubes	41	68.3%	58	96.7%	19	31.7%	2	3.3%
Blocks	23	76.7%	29	96.7%	7	23.3%	1	3.3%
Spread	25	83.3%	30	100%	5	16.7%	0	0%
Total	89	74.2%	117	97.5%	31	25.8%	3	2.5%

* Critical limit for total Coliforms was 10 CFU/g. and absent for *E.coli*.

On studying the degree of acceptability of the examined processed cheese samples vs. Egyptian Standards for the presence of Total Coliforms and *Escherichia coli*, data presented in **Table (5)** show that 74.2 and 97% of the total examined processed cheese samples were acceptable. Nearly similar findings were reported by[1,2,40], while lower findings were obtained by [11, 29,34].

Conclusion

Contamination of the examined processed cheese samples by Coliforms indicated inferior quality and fecal pollution as well as pathogenic Escherichia coli O27 leads to food borne illness. Therefore to safe guard public health hazards strict hygienic measures should be imposed for production, handling and storage.

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