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Biohazards and fat deterioration associated with fresh cream and cream filled pastries.

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This Research addressed that ingestion of contaminated dairy food causes foodborne diseases (FBD) that poses a significant, but often unrecognized threat to public health and worldwide, in addition to lipid oxidation that not only affects the organoleptic properties and the nutritional value of the fatty products, but also forms toxic byproducts. Consequently, this study throws light on the microbiological aspect of a Fresh cream and Cream filled pastries with the incidence of fat deterioration. Sixty random samples of Fresh cream and Cream filled pastries (thirty of each) were collected from dairy and pastry shops in Egypt and subjected to microbiological & chemical examination for determination of their hygienic safety and quality. Microbiological examinations revealed that most of the examined samples contained high numbers of Total aerobic mesophiles, Aerobic spore formers, Coliform, Staphylococci, Yeast and Mold. Some biohazards were detected, Agarose gel electrophoresis showed positive amplification for *E.coli* 16s rRNA gene at 585 bp isolated from cream filled pastries with 6.9%, whereas *Staphylococcus aureus* was detected in 11.4 & 6.25% of cream filled pastries and fresh cream samples, respectively. Chemical examination showed that all examined samples contained free fatty acids in accordance with the Egyptian standard, while only (20 & 10%) of the examined fresh cream and cream filled pastries were in accordance related to peroxide value. In conclusion, low hygienic conditions of production and storage mandate the promotion of hygienic regulations and guarantee of safety and quality during the production and storage.

Keywords: Fresh cream, Cream filled pastries, Free fatty acids, Peroxide value, *E.coli*, *Staphylococcus aureus*

INTRODUCTION

Foodborne diseases are considered to be among the foremost economic and public health concerns, particularly in susceptible people, such as infants, pregnant women and elderly throughout the world. Cream as a dairy product beside cream filled pastries which are milk-based bakery products with high production and consumption rates in the sweets are highly at risk to microbial contamination. Annually, hundred millions of people all over the world get poisoned with these products because of contamination either of raw materials, equipment or final products in the production process, transportation and distribution and non-observance of good manufacturing practices (GMP) by workers (OECD, 2005; Hoffmann, 2011; Pajohi-Alamotia et al. 2016).

Pathogens causing foodborne outbreaks that associated with the consumption of milk and dairy products include *E. coli* O157:H7, *Staphylococcus aureus, Bacillus cereus and Listeria*

monocytogenes, they represent a major public health hazard, especially for persons who still drink raw milk & raw milk products (Coia et al., 2001; Oliver et al., 2005).

A useful indicator for assessing the overall quality and safety of dairy products and monitoring the sanitary conditions applied during the production, collection and handling is the Standard Plate Count (ICMSF, 2009). Microbiological quality of dairy products & post heat treatment contamination can be also determined using coilform count (Asamenew et al. 2012), among these group *E. coli* which is considered the most common pathogen causing milk and dairy products borne outbreaks. Spoilage by fungi (yeast and mold) was regarded as a quality concern rather than a food safety issue since 50 years ago, later, many common mold species were discovered to be dangerously toxigenic due to mycotoxins production which represent a threat to public health (Ahmed et al. 2014). Presence of aerobic spore formers in milk and its products were evidenced to be of great importance, as some of them are of public health effect such as *Bacillus cereus* and *Bacillus megaterium* which involved in cases of food poisoning (Lawley et al. 2008), and the others were proved to be of economic importance because of production of heat stable proteinases which induce certain undesirable changes rendering the product of inferior quality or even unmarketable (De Jonghe et al. 2010; Madslien et al.2012).

Two major types of fat deterioration are there during dairy food (particularly the fatty one) storage and processing, each causes different changes in the lipid constituent with different mechanism of action, but at the end they produce off-flavors that lower the product sensory acceptability, and this is what is called rancidity which affects the sensory properties and nutritional value of the fat products beside producing toxic free radicals. Extent of fat deterioration can be estimated by determining acid and peroxide value of fat. Acid value indicates the amount of free fatty acids produced as a result of fat hydrolysis, while peroxide value which is one of the most important indicators of oil quality indicates the amount of hydro-peroxides formed as a result of the oxidation process (Schwartz & Parks, 1974; Dugan, 1976).

Because of all mentioned before and due to presence of few numbers of research articles cover this point, especially, the fat deterioration of dairy cream and cream filled products which are consumed with higher rates and has significant effect on public health.This study was conducted to throw the light to what extent these products were in accordance with the Egyptian standard and hygienically safe for the Egyptian consumers and what are the most common contaminating microorganisms especially those considered a biological hazard to search for a suitable control for these microorganisms in the future work.

MATERIALS AND METHODS

Sample Collection

Sixty random samples of Fresh cream on small scale and Cream filled pastries (plain type) (thirty of each) were collected from local dairy and pastry shops in two cities of Egypt, in the period from September / 2018 to June / 2019 and were transmitted to the laboratory in an insulating ice - box as soon as possible for examination.

Chemical examination

Titratable acidity percentage, Acid value and Free fatty acids % were determined according to (AOAC, 2000), while peroxide value according to (AOAC, 1995).

Microbiological examination:

Preparation of decimal dilutions of the examined samples according to (APHA, 2004).Total aerobic mesophilic count was applied according to (ISO, 2002), Aerobic Spore Formers according to (APHA, 2004) with identification of the isolated Aerobic Spore Formers according to (De Vos et al. 2009). Coliform content (MPN/g) was assessed according to (APHA, 2004) with biochemical identification of the isolated Coliform according to (De Vos et al. 2009), Molecular identification of isolated *E.coli* by polymerase chain reaction (PCR) for *E.coli* 16S rRNA gene by ECO-1 and ECO-2 primers (Table, 1)

Table (1): Molecular identification of isolated *E.coli* by polymerase chain reaction (PCR) for *E.coli* 16S rRNA gene by ECO-1 and ECO-2 primers.

Primers name	Sequence	bp	Target gene
ECO-1	ACCTCGGTTT AGTTCACAGA	585	16S rRNA
ECO-2	ACACGCTGA CGCTGACCA		

According to the standard procedure designated by (Schippa et al. 2010). Total Staphylococci count with biochemical

identification of the isolated strains were determined according to (APHA, 2004). Total Yeast and Mold count was assessed according to (ISO, 2012).

Statistical analysis

Results were calculated in the form of mean ± standard deviation using the program Statistical Package for Social Science (SPSS), version 17.

RESULTS

Results of the chemical analysis presented in (Table, 2) indicated that the maximum values of Titratable acidity % of the examined samples were 0.23 and 0.25% for fresh cream and cream-filled pastries samples with mean values of 0.11 and 0.14 %, respectively. The acid value of the examined samples of Fresh cream and Cream filled pastries ranged from (0.07 – 0.45) & (0.07 – 0.61) with a mean value of (0.18 ± 0.02 and 0.31 ± 0.03), respectively. Free fatty acid % was between 0.04 and 0.22 with a mean value of 0.09 % in fresh cream samples, whereas 0.04 & 0.31 with a mean value of 0.17% in cream filled pastries samples, while the mean peroxide value was 1.38 ± 0.19 and 2.2 ± 0.43 Meq/kg, respectively.

Microbiological examination presented in (Table, 2 & Fig., 1) illustrated that all examined samples of Cream-filled pastries and 83.3% of Fresh cream samples were contaminated with microorganisms with mean count of $42 \times 10^5 \pm 0.37 \times 10^5$ and $3.5 \times 10^7 \pm 1.55 \times 10^7$ cfu/g, respectively, while 50 and 70 % of fresh cream and cream filled pastries were contaminated with aerobic spore formers with mean count of 5.74×10^3 and 7.4×10^4 cfu/g, respectively.

Biochemical identification of aerobic spore formers isolates in fresh cream samples revealed that *B.mycoides* 19.4%, was the most frequent, followed by *B.subtilis* 14.3%, followed by *B.alvei* 9.5%, *B.macerans* 9.5%, *B.pantothenticus* 9.5%, *Sporolactobacillus inulinus* 9.5% and *Sporosarcina ureae* 9.5%, then *B.circulans, B.coagulans, B.insolitus and B.megaterium,* each one was 4.8%. In cream-filled pastries samples, *B.sphaericus* 22.2% was the most frequent, followed by *B.mycoides* 19.4%, then *Sporosarcina ureae* 16.7% and *B.subtilis* 13.8, while the lowest frequent one was *B.alvei* 2.8% (Fig., 2*). B.cereus* couldn't be detected in the examined samples.

Regarding the results presented in (Table,2 & Fig.,1) it is evident that coliforms could be detected in 83.3 and 90% of the examined fresh cream and cream filled pastries samples with a mean value of 9.8×10^4 and 6.1×10^3 MPN/g, respectively. Biochemical identification of coliform organisms in the examined fresh cream samples revealed that *Citrobacter diversus* 32.2% was the most common one followed by *Enterobacter intermedius* 19.4% and *Klebsiella oxytoca* 19.4% then *Citrobacter freundii* 16.1%, *Serratia fonticola* 9.7% and *Enterobacter amnigenus* 3.2%, while *E. coli* couldn't be detected in these samples. Whereas in cream-filled pastries, *Citrobacter diversus* 31.1%, *Citrobacter freundii* 24.2% and *Klebsiella oxytoca* 17.2% were the most frequent followed by *Enterobacter intermedius* 13.8% and *E. coli* 6.9%., presence of *E.coli* was confirmed using molecular identification by polymerase chain reaction (PCR) that showed positive amplification for *E.coli* 16s rRNA gene at 585 bp (Fig., 3 & 4).

Results presented in Table (2) and Fig. (1) Indicated that staphylococci were present in 80 and 93.3% of the examined samples of fresh cream and cream-filled pastries with mean count of 8.3×10^4 and 25×10^4 cfu/g, respectively. It was obvious that the incidence of *S. aureus* in the examined samples of fresh cream and cream-filled pastries depending on the results of the coagulase test were 12.5% and 20.5% while depending on the results of TNase test were 39.6% and 47.7%, respectively.

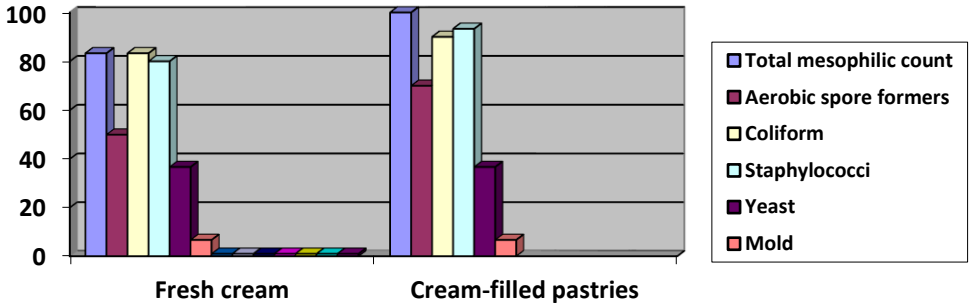

Figure 1: Incidence of the examined microbiological parameters in the examined samples.

Table 2: Statistical analytical results of the determined parameters in the examined samples (n=30).

Parameters	Total No. of samples	Min.	Max.	Mean ± S.E.M.
Chemical analysis				
Titratable acidity%	Fresh cream	0.01	0.23	0.11 ± 0.0096
	Cream filled pastries	0.03	0.25	0.14 ± 0.01
Acid value	Fresh cream	0.07	0.45	0.18 ± 0.02
	Cream filled pastries	0.07	0.61	0.31 ± 0.03
Free fatty acid%	Fresh cream	0.04	0.22	0.093 ± 0.0095
	Cream filled pastries	0.04	0.31	0.17 ± 0.011
Peroxide value (Meq/kg)	Fresh cream	0.1	3.6	1.38 ± 0.19
	Cream filled pastries	0.4	8.8	2.2 ± 0.43
Microbiological Examination				
Total aerobic mesophilic count (cfu/g)	Fresh cream	3.5×10^3	4.4×10^8	$3.5 \times 10^7 \pm 1.55 \times 10^7$
	Cream filled pastries	5×10^2	7.5×10^7	$42 \times 10^5 \pm 0.37 \times 10^5$
Aerobic Spore Formers (cfu/g)	Fresh cream	10^2	1.1×10^5	$5.7 \times 10^3 \pm 3.7 \times 10^3$
	Cream filled pastries	10^2	1.62×10^6	$7.4 \times 10^4 \pm 5.4 \times 10^4$
Coliform count (MPN/g)	Fresh cream	2.1×10^2	1.1×10^6<	$9.8 \times 10^4 \pm 5.2 \times 10^4$
	Cream filled pastries	40	1.1×10^5 <	$6.1 \times 10^3 \pm 3.7 \times 10^3$
Total Staphylococci count (cfu/g)	Fresh cream	4×10^2	8.04×10^5	$8.3 \times 10^4 \pm 3.1 \times 10^4$
	Cream filled pastries	2×10^2	1.9×10^7	$25 \times 10^4 \pm 7.6 \times 10^4$
Total yeast count (cfu/g)	Fresh cream	4×10^2	4.98×10^7	$2.9 \times 10^6 \pm 1.84 \times 10^6$
	Cream filled pastries	10^3	6×10^5	$4.02 \times 10^4 \pm 2.3 \times 10^4$
Total mold count (cfu/g)	Fresh cream	10^3	4×10^3	$1.7 \times 10^2 \pm 1.4 \times 10^2$
	Cream filled pastries	5×10^3	7×10^3	$4 \times 10^2 \pm 2.8 \times 10^2$

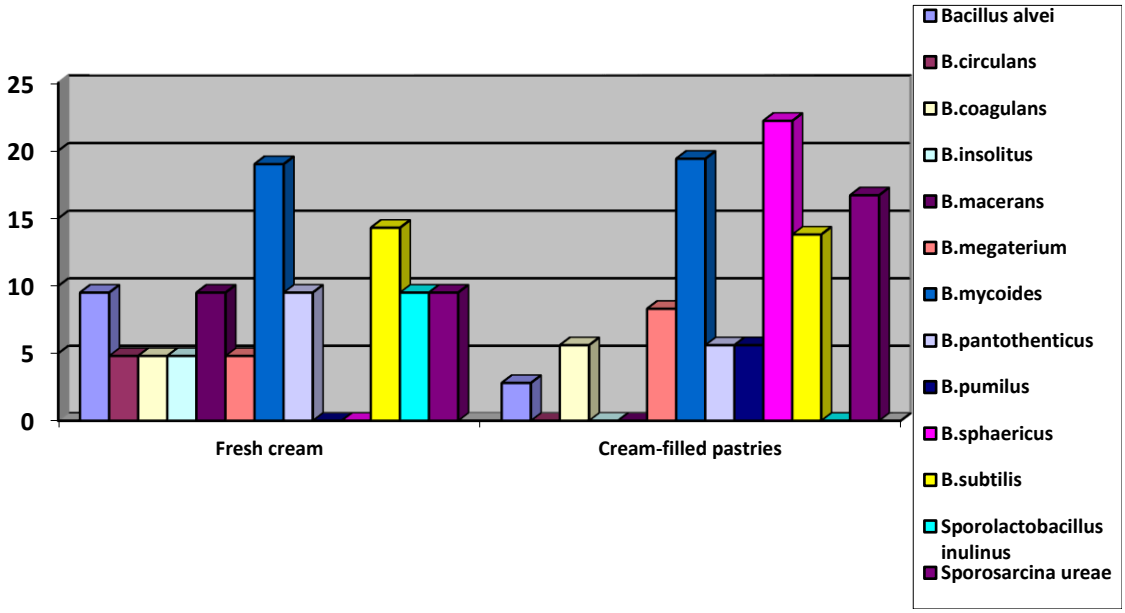

Figure 2: Incidence of the isolated Aerobic spore formers.

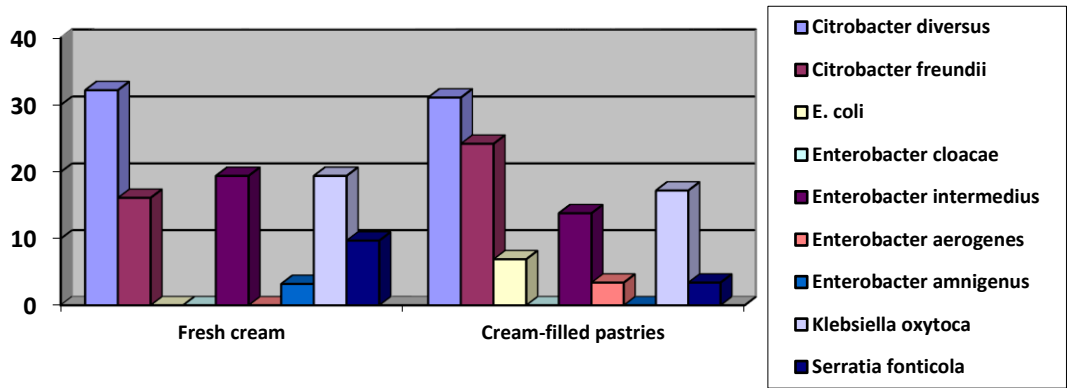

Figure 3: Incidence of isolated coliform from the examined samples.

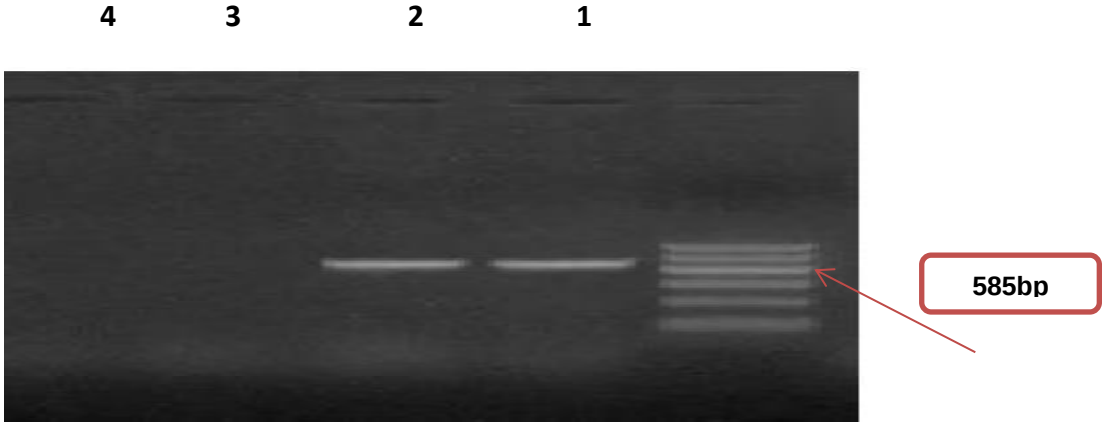

Figure 4: Agarose gel electrophoresises showing posistive amplification for *E.coli* 16s rRNA gene at 585 bp (Lan, 1&2: positive isolates).

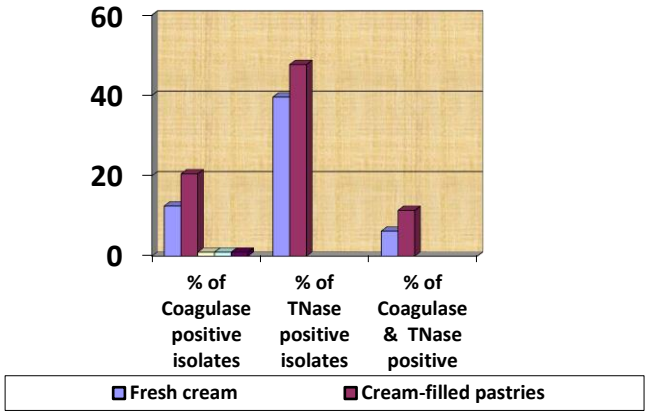

Figure 5: *Staphylococcus aureus* prevalence depending on the outcomes of Coagulase and TNase tests.

The highest incidence depending on both tests was 11.4% in cream filled pastries samples, while 6.25% in fresh cream samples (Fig., 5).

Data depicted in (Table, 2 & Fig., 1) revealed that contaminated yeast and mold could be detected in 36.7& 6.7 and 36.7 & 6.7 % of fresh cream and cream-filled pastries with mean values of 2.9×10^6 & 1.7×10^2 and 4.02×10^4 & 4×10^2 cfu/g, respectively.

DISCUSSION

Dairy product freshness and Bacterial activity in milk, bacterial contamination, and storage temperature are the main factors affecting the acid formation. From the previously mentioned results and according to the Egyptian Standards (Fresh cream 154 - 1 /2005), it can be concluded that 90 and 96.66% of the examined fresh cream and cream filled pastries, respectively were agreed with the Egyptian standard. Higher results of fresh cream samples were reported by Asharf et al. (2015) and Arafa (2013) who studied the bacteriological quality of 38 samples of fresh cream and found that the mean of Titratable acidity % was 0.2. On the other hand, higher results of cream-filled pastries were obtained by Ali et al. *(2016)* who examined 228 cream-filled pastries samples and found that the maximum value was 2.24 with a mean value of 1.52 ± 0.72%.

Cleavage of triglyceride ester bonds by hydrolysis causing accumulation of free fatty acids and subsequent flavor impairment, these free fatty acids can then undergo further auto-oxidation which occurs in several consecutive stages: unsaturated fatty acids react with oxygen forming peroxides, which followed by a series of chain reactions, the final products of these reactions are several volatile substances (aldehydes, ketones, alcohols, inferior acids, acids-alcohols, acids-aldehydes,and acids-ketones) with specific rancid smell (Downey, 1980; Van de Voort et al., 1994; O'Connor & O'Brien, 2006).

All examined samples contained free fatty acids agreed with the Egyptian standard (154 - 5/2005), while only 20% of the examined fresh cream samples agreed for peroxide value. According to EU Regulation No. 1272/2009 which states the following requirements for butter fat: free fatty acid content less than 1.2 mmol per 100 g fat and peroxide value less than 0.3 meq oxygen per kg fat, all examined samples contained free fatty acids agreed with EU standard, while only 3.3% of fresh cream samples agreed with the standard in related to peroxide value. The majority of the examined samples contained oxidative rancidity that affects the organoleptic properties (flavor, color ,and texture), nutritional value (fat soluble vitamins destruction and loss of essential fatty acids) and forms toxic byproduct causing diarrhea, poor growth rate, myopathy, hepatomegaly, hemolytic anemia with involvement in arterial injury, artherosclerotic plaque formation and thrombosis/spasm (Fearon, 2011; Fox & Kelly, 2012). Factors enhancing the occurrence of such a problem are oxygen, light, metals, lack of antioxidants, storage temperature and water activity. The addition of antioxidants with good storage conditions can prevent this problem.

Hossein et al. (2013) showed that the oils used in confectionaries samples were safe in terms of acid value as 100% of the examined samples were accepted, while 85.7% of the oils in confectionaries were usable in terms of peroxide value.

Standard plate count is one of the most common techniques used for assessing the overall quality and safety all over the world. High counts of the examined samples are considered a bad signal indicating low hygiene and poor quality products. The higher initial microbial load of raw milk used, the resistance of spore-forming organisms to the heat treatment, post heat treatment contamination, bad storage and/or bad handling might be the cause of these high counts (Huck et al. 2007; Huck et al. 2008). Whipped cream is more likely to be contaminated by the microorganisms than the cream since the air introduction into cream during the whipping process produces a suitable environment for microbial growth (Wilbey 2002). Products prepared or filled with such cream are more likely to cause outbreaks than cream consumption.

Recorded results of total bacterial count in fresh cream are higher than those obtained by Ashraf et al. (2015) who examined random samples of raw cream and found that the mean value of total bacterial count was $3.01x10^5 \pm 0.7x10^5$ cfu/g, while lower than that recorded by Arafa (2013). On the other hand, results obtained for cream-filled pastries were higher than those reported by Hassan et al. (2018) who analyzed 62 cream-filled pastries samples from the confectioneries microbiologically and found that the mean total bacterial count was 5.33±0.05 log cfu/g., Kotzekidou (2013); Ali et al. (2016) and Mohamadreza et al. (2016).

High level of aerobic spore formers contamination reflects the poor hygiene and sanitation during production and storage of the examined products. Bacillus species can survive the usual milk heat treatment; they largely produce thermo stable extracellular and intracellular hydrolytic enzymes. Besides, some Bacillus species such as *B. cereus* and *B. subtilis* are capable of producing different types of toxins involved in food borne outbreaks (Chen et al., 2004; Svensson et al., 2004).

It is clear from the obtained results of coliform

that 83.33 % of the examined fresh cream contained high numbers of coliform and disagreed with the Egyptian Standards (Fresh cream 154 - 1 /2005), which recommends that coliform count should be less than 10 cells/g in the product. The high incidence of coliforms indicated the neglected sanitary measures, faulty heat processing or post pasteurization contamination by handlers (Ahmed et al. 2009; Asamenew *et al.,* 2012), and such incidence rendered the products of inferior quality and unmarketable during storage or even unfit for human consumption causing economic losses, from the other point of public health importance its implication in gastrointestinal illness such as gastroenteritis, epidemic diarrhea in children and cases of food poisoning (Quinto and Cepeda, 1997). Obtained results of fresh cream were lower than that recorded by Arafa (2013); Ashraf et al. (2015), while higher data of cream filled pastries was reported by Ali et al. (2016) who examined 228 cream-filled pastries samples microbiologically and found that the mean value of coliforms was 2.61 ± 2.23 log cfu/g, Mohamadreza et al. (2016) and Hassan et al. (2018), while lower findings were recorded by Khezri et al. (2007) and Nikniaz (2011).

Fresh cream was free from *E. coli* in contrary to Arafa (2013) who isolated it from 47.7% of fresh cream samples. The presence of *E.coli* in cream filled pastries was an indicator of fecal contamination and suggested that other foodborne pathogens of fecal origin might also be found (Singh and Prakash, 2008). Most strains of *E. coli* are harmless commensals; some cause human gastrointestinal disease with mild to severe symptoms that may progress to long-term squeal or fatal outcomes in high-risk individuals, *E. coli* also is found to be responsible for cases of cystitis, pyelitis, pyelonephritis as well as appendicitis and peritonitis (Nutting et al., 2009; Desmarchelier & Fegan, 2011; Lamiaa & Karima, 2018). Lower results of cream filled pastries were recorded by Al-Jafaeri et al. (2013), while higher findings were reported by Khezri et al. (2007); Kotzekidou (2013), but unlike our results, Mohamadreza et al. (2016) could not isolate *E.coli* from any of the examined samples.

Nearly all samples contained high numbers of Staphylococci*,* which reflected the poor sanitary conditions applied during production, processing, and distribution (Graceleah et al., 2010; Neveen & Lamiaa, 2019). This could be occurred due to the dominance of the genus on parts of the human body such as nose, hands, and skin (Nwagu & Amadi, 2010). Higher results of cream-filled pastries were obtained by Ali et al. (2016).

Coagulase test is used as a diagnostic test for the possibility of enterotoxin production, as all enterotoxigenic strains are coagulase producers but not all of them are TNase producers (Kloos & Bannerman, 1995; Koneman, 1997 and Collins et al., 2010). Garbaj (2004) reported that about 50.0% of the enterotoxigenic *Staphylococcus aureus* isolates were positive for both coagulase and TNase production, and this confirmed by Hill et al. (2012) who reported that in general staphylococci that produce enterotoxins are coagulase and/or thermonuclease positive. Ingestion of food contaminated with staphylococcal enterotoxins caused *S. aureus* food poisoning intoxication, which is emetic, pyrogenic and mitogenic and suppresses the immunoglobulin production that rendered it one of the most common types of foodborne diseases worldwide. S. aureus is a common cause of boils, abscesses and more serious infections including osteomyelitis, endocarditis, enterocolitis, toxic shock and scalded skin syndrome (Stewart et al., 2002 and Zadoks, 2003). Higher results of *staphylococcal aureus* in fresh cream were recorded by Arafa (2013). Nearly similar results of cream-filled pastries were assessed by Kotzekidou (2013), while lower results were obtained by Hassan et al. (2018), whereas higher results were recorded by Nikniaz (2011) who showed that 31.2% of puff pastry samples were contaminated with *Staphylococcus aureus*, Al-Jafaeri et al. (2013); Mohamadreza et al. (2016).

High level of yeast contamination in the examined samples might be attributed to inadequate hygienic measures during production or the use of bad quality raw materials (Varnam & Sutherland, 2009; Aly et al., 2010), it causes economic losses and undesirable changes such as frothy consistency and yeasty flavor. Moreover, some species of yeast constitute public hazards such as gastrointestinal disturbance, endocarditis, and occasionally fatal systemic diseases (Jaquet and Teherani, 1976). Lower findings of fresh cream were reported by Ashraf et al. (2015), Ali et al. (2016) and Mohamadreza et al. (2016) who found that the mean yeast count was 3.31 log cfu/g in puff pastry cream samples. While higher results were recorded by Sonia et al. (2015) who found that 95.8 % of pastries cream samples were contaminated with yeast, Nikniaz (2011); Asadi et al. (2015).

The main causes of mold contamination are warm weather and inadequate refrigeration, besides increased species diversity and

alteration in microbial flora (Moreina et al., 2001). It causes serious economic losses because it is associated with visible signs of spoilage such as off-flavor and discoloration that resulted in product rejection, with the probability of mycotoxins production which implicated in human food poisoning outbreaks (Bullerman, 1980; Robinson, 1990). Higher findings of fresh cream were reported by Ashraf et al. (2015). Whereas lower results of cream-filled pastries were recorded by Sonia et al. (2015) and Mohamadreza et al. (2016).

CONCLUSION

By examining sixty random samples of fresh cream and cream-filled pastries (thirty of each), some biological hazards such as *E.coli & S.aureus* were isolated with the presence of high numbers of contaminated aerobic mesophilic microorganisms, coliforms, aerobic spore formers, staphylococci, yeast, and mold. As well as, the presence of oxidative rancidity were in most of the examined samples. Consequently, public awareness targeting factories and households that produce dairy products and confectionaries should be encouraged and helped to follow strict hygienic control measures with an application of Good Manufacturing Practices (GMP), Hazard Analysis and Critical Control Point (HACCP) system and Food Safety Management System eg. ISO 22000: 2005 to protect the consumers from infection and to save a lot of products from spoilage.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed to all parts of the study.

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