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Article

Detection of some microorganisms of public health hazards in cheese

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Abstract

In this study, the microbial quality of Domiati, Kareish, and Ras cheeses was studied. A total of 150 cheese samples were collected, and samples were analyzed for total coliforms, fecal coliforms, *Escherichia coli*, *Staphylococcus aureus*, yeast, and mold counts. The highest distribution of *coliforms* was lied in 10 - < 10^2 (26 %), 10^3 - < 10^4 (60 %) and 10^3- < 10^4 (32 %) in Domiati, Kareish and Ras cheese respectively, 30, 18 and 16% of fecal *coliforms* samples had counts of 10 - < 10^2, while 2, 4 and 16 % had counts of 10^2 - < 10^3, and 2, 30 and 34 % had counts of 10^3 - < 10^4. *E. coli* existed in 28, 28 and 37% of samples, respectively. *Staph. aureus* was detected in 26, 10 and 22 % of samples respectively, with average count of 1.27 x 10^3, 7.5 x 10^4 and 2.15 x 10^3 respectively. Yeasts were detected in 98, 96 and 52 % of samples respectively, with average count of 3.88 x 10^5, 3.26 x 10^5 and 1.04 x 10^4 respectively. Molds were detected in 46, 32 and 35 % of samples with average values of 3.9 x 10^4, 1.2 x 10^4 and 2.61 x 10^4, respectively. The presence of these organisms reflects unhygienic measures, inadequate heat treatment, using bad quality ingredients and improper sanitation during handling and storage.

Keywords
Escherichia coli, Staphylococcus aureus, Yeasts and molds, cheese, Domiati cheese, Kareish cheese, Ras cheese

1. Introduction

Unsafe food is still an important threat in most developing countries, especially in the African region (WHO, 2007). Soft cheese is one of the most appreciated cheeses in Middle Eastern countries. Egypt has a long and rich tradition in cheese making based on the many traditional cheese varieties. Traditionally, Kareish cheese is manufactured from unpasteurized skimmed milk in small family premises with basic equipment. This type of cheese is produced either by enzymatic or acidic coagulation of fresh milk (buffalo's or cow's milk). However, the use of raw milk leads to the possible survival of various pathogens during its manufacture and ripening (Deeb et al., 2004).

Domiati cheese is the most popular soft white cheese in Egypt and makes up about 75% of the cheese produced and consumed in the country (El-Baradei et al., 2007). It is made from buffalo's milk, cow's milk, or a mixture of both. It has been made from pasteurized milk containing different percentages of fat (1–6%) and the addition of different percentages of salt (2–15%). It also has been made with or without the addition of starter cultures to cheese milk (Mehaia, 2002).

Ras cheese, the main traditional hard cheese in Egypt, is manufactured in a high proportion under artisan production, in rural areas and small factories, from raw cow's milk or a mixture of cow and buffalo's without using starter cultures (Awad et al., 2003). In such production, the fermentation occurs by the wild microflora present in raw milk and the surrounding environment. The cheese is usually stored under moist and uncontrolled hygienic conditions, which can be contaminated by molds and yeasts. Therefore, the final flavor and texture will be influenced by the action of the bacterial flora.

Undesirable microbes that can cause spoilage of dairy products include aerobic and anaerobic bacteria, coliforms, yeasts, and molds. In addition, various bacteria of public health concern such as pathogenic strains of Escherichia coli and enterotoxigenic strains of Staphylococcus aureus may also be found in milk and dairy products (Tatini & Kauppi, 2003).

The presence of coliforms indicates environmental or fecal contamination. Amongst the coliforms, E. coli represented the most common contaminant of raw and processed milk (Quinn et al., 2002). Staph. aureus colonizes the nasal passages and skin of approximately 50% of healthy individuals and grows in a wide range of temperatures (from 7 °C to 48.5 °C) and pH (4.2 to 9.3). Staph. aureus can adapt to grow in various foods and is associated with intoxications due to its ability to produce a variety of potent enterotoxins (Le Loir et al., 2003).

The presence of yeasts themselves is not commonly the cause of defects in milk and dairy products unless they ferment lactose. In this case, they can grow rapidly and produce a characteristic yeast or fruity flavor and obvious gas. Molds are important in milk, which is used for the manufacture of dairy products, as they may influence the organoleptic characteristics of the dairy products, they can produce mycotoxins and represent a potential health risk (Wouters et al., 2002).

2. Materials and Methods

2.1. Collection of samples

A total of 150 random samples of Kareish, Domiati, and Ras cheese (50 samples each) were collected in the period from August 2020 to February 2021 from different dairy shops and supermarkets in Assiut city, Egypt. All samples were collected aseptically in sterile plastic containers and were transported immediately under refrigeration conditions (icebox) to the laboratory. Samples were kept in the refrigerator at 4 °C till examined within 12 h.

2.2. Preparation of samples (Roberts and Greenwood, 2003)

Under complete aseptic conditions, 25 grams of cheese sample were weighed and transferred into a sterile homogenizer flask containing 225 ml of sterile sodium citrate solution 2%. The content of the flask was homogenized for 3 minutes at 14000 rpm and then allowed to stand for 5 minutes at room temperature. One ml from the homogenate was transferred into a separate tube containing 9 ml of sterile peptone water 0.1% from which ten-fold serial dilutions were prepared.

2.3. Microbiological examination

2.3.1. Coliform's count "MPN/g" (ISO, 2013)

One ml from each decimal dilution was inoculated into three fermentation tubes containing 5 ml of Lauryl Sulphate Tryptose broth containing 0.5% lactose (LST) and inverted Durham's tubes. The inoculated and control tubes were incubated at 37 °C for 48 hours. The positive tubes showing gas production in Durham, s tubes were recorded. Further, a loopful from each positive tube was transferred into another fermentation tube containing Brilliant Green Bile Lactose Broth (2 %) with inverted Durham, s tubes and incubated at 37 °C for 48 hours. The positive tubes showing gas production were recorded. According to MPN tables for the three tubes dilutions, the results were recorded as the confirmatory MPN of coliforms/g.

2.3.2. Fecal coliforms count "MPN/g" (ISO, 2013)

Loopfuls from the previous positive coliform tubes were inoculated into three fermentation tubes containing 5 ml of EC (Escherichia coli) broth and inverted Durham's tubes. The inoculated and control tubes were incubated at 44±0.5 °C for 24 hours. The tubes showing gas production were recorded as positive tubes.

2.3.3. Escherichia coli count "MPN/g" (ISO, 2013)

Loopfuls from the previous positive EC broth tubes were separately streaked onto Eosin Methylene Blue agar medium (E.M.B.), which was then incubated at 37 °C for 24 hours. Suspected colonies were metallic green in color. Multiplex PCR for the detection of virulence genes of E. coli was done in the Faculty of Veterinary Medicine, Banha University, Egypt. Genomic DNA extraction (Sambrook et al., 1989), using Gene JET Genomic DNA Purification Kit (Fermentas).

2.3.4. Enumeration and Isolation of Staph. aureus (ISO, 2013)

0.1 ml from each of the previously prepared serial dilutions were spread evenly over the Baird Parker agar plate. The inoculated and control plates were incubated at 37 °C for 48 hours, after which they were examined for colony character an appropriate amount from each prepared sample was inoculated into sterile NaCl broth 10%. Inoculated NaCl broth tubes were incubated at 37 °C for 24 hrs. A loopful of the incubated broth was streaked onto a plate of sterile mannitol salt agar. PCR for the detection of enterotoxin genes of Staph. aureus was done in the Faculty of Veterinary Medicine, Banha University, Egypt. Using Gene JET Genomic DNA Purification Kit (Fermentas) (Mehrotra et al., 2000).

2.3.5. Total yeasts and molds count: (ISO, 2008)

From the already prepared serial dilutions, duplicate marked plates of Sabaroud dextrose agar were inoculated with 1 ml from each dilution, and inoculated plates were incubated at 25 °C for 3-5 days before being examined.

2.3.6. Statistical analysis

The statistical program Excel sheet was used for the diagram. Then, described statistics of ANOVA were performed to measure the mean ± standard error (SE).

3. Results and discussion

Table 1. Frequency distribution of the examined cheese samples based on their *coliforms* count (MPN/ g)

Count/g	Cheese					
	Domiati		Kareish		Ras	
	No./50	%	No./50	%	No./50	%
< 3*	16	32	10	20	8	16
3-<10	12	24	4	8	8	16
10 -<10^2	13	26	5	10	10	20
10^2- <10^3	4	8	1	2	8	16
10^3- <10^4	5	10	30	60	16	32
Total	50	100	50	100	50	100

***** Negative samples

Table 2. Frequency distribution of the examined cheese samples based on their *fecal coliforms* count (MPN/ g)

Count/g	Cheese					
	Domiati		Kareish		Ras	
	No./50	%	No./50	%	No./50	%
< 3*	19	38	16	32	11	22
3-<10	14	28	8	16	6	12
10 -<10^2	15	30	9	18	8	16
10^2- <10^3	1	2	2	4	8	16
10^3- <10^4	1	2	15	30	17	34
Total	50	100	50	100	50	100

*Negative samples

Table 3. Frequency distribution of the examined cheese samples based on their *E. coli* count (MPN/ g)

Count/g	Cheese					
	Domiati		Kareish		Ras	
	No./50	%	No./50	%	No./50	%
< 3*	22	44	22	44	13	26
3-<10	16	32	7	14	10	20
10 -<10^2	12	24	15	30	12	24
10 2- <10 3	0	0	1	2	5	10
10 3- <10 4	0	0	5	10	10	20
Total	50	100	50	100	50	100

***Negative tubes**

Table 4. Statistical analytical results of *Staph. aureus* isolated from the examined cheese samples

Examined samples	Positive samples		*Staph. aureus* Count (CFU)/g		
	No./50	%	Min.	Max.	Average
Domiati cheese	13	26	1.0×10^2	7.0 ×10^4	1.27 ×10^3
Kareish cheese	5	10	1.3 ×10^3	2.0 ×10^5	7.5 ×10^4
Ras cheese	11	22	3.0 ×10^2	9.0 ×10^4	2.15 ×10^3

Figure 1. Agarose gel electrophoresis of multiplex PCR of *stx1* (180 bp), *stx2* (255 bp), and *eae A* (384 bp) virulence genes for characterization of the isolated *E. coli*

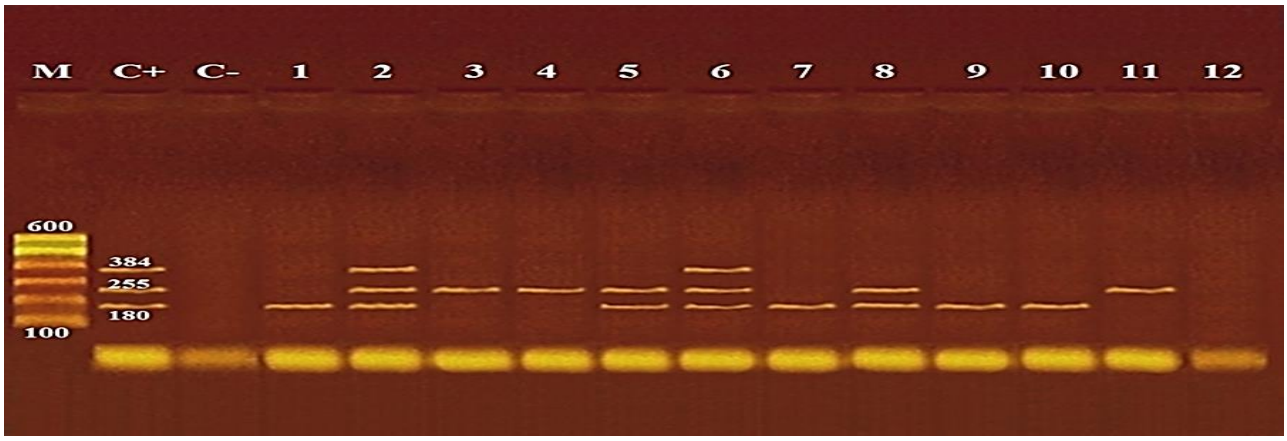

Figure 2. Agarose gel electrophoresis of multiplex PCR of *sea* (120 bp), *seb* (478 bp), *sec* (257 bp) and *sed* (317 bp) as enterotoxin genes for characterization of *Staph. aureus*

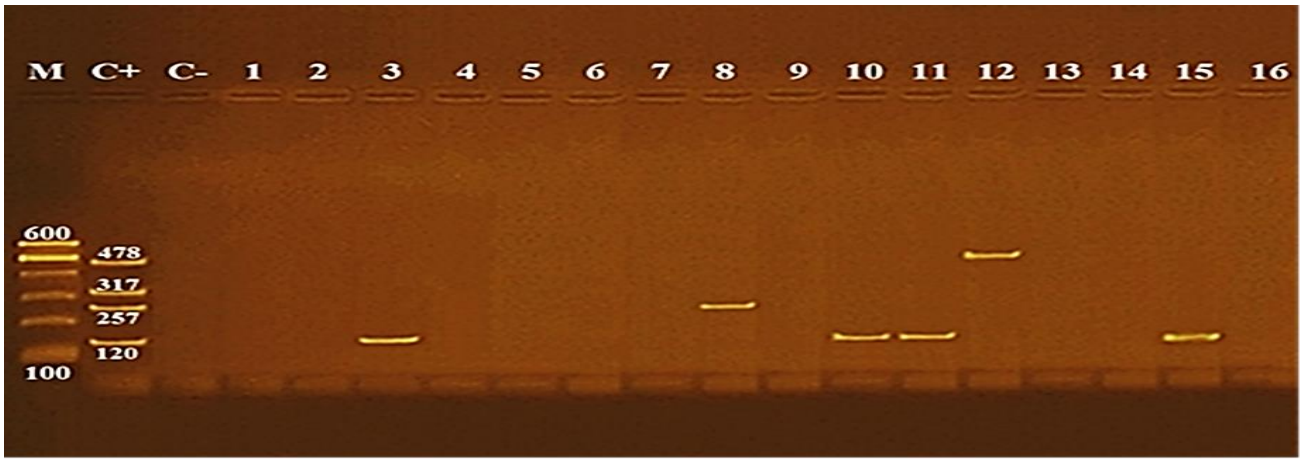

Table 5. Statistical analytical results of *Yeasts* count in the examined samples

Examined samples	Positive samples		Count (CFU)/g		
	No./50	%	Min.	Max.	Average
Domiati cheese	49	98	1.0 ×10^2	5 ×10^6	3.88 ×10^5
Kareish cheese	48	96	1.0 ×10^2	2.0 ×10^6	3.26 ×10^5
Ras cheese	26	52	1.0 ×10^2	2.0 ×10^5	1.04×10^4

Table 6. Statistical analytical results of *Molds* count in the examined samples

Examined samples	Positive samples		Count (CFU)/g		
	No./50	%	Min.	Max.	Average
Domiati cheese	46	92	1.0×10^2	1.8×10^5	3.09×10^4
Kareish cheese	32	64	1.0×10^2	5.0×10^4	1.2×10^4
Ras cheese	35	70	1.0×10^2	4.7×10^5	2.61×10^4

3.1. Coliforms count

From the summarized results presented in Table1., *Coliforms* were not detected (< 3/g) in 32, 20, and 16% of the examined Domiati, Kareish, and Ras cheese samples respectively, while 24, 8 and 16% had a count of 3 -< 10 of the examined cheese samples respectively and 26, 10 and 20 % had a count of 10- < 10^2, and 8, 2 and 16 % had a count of 10^2 - < 10^3, while 10, 60 and 32 % had a count of 10^3 - < 10^4 of the examined cheese samples respectively.

Higher results of coliforms count in Domiati cheese were recorded by Meshref and Hassan (2009), El-Kholy *et al.* (2014), Hassan and Gomaa (2016), and El-Leboudy *et al.* (2017), while nearly similar results were recorded by Abd Ellah (2017). On the other hand, lower counts were obtained by Abo El-Makarem *et al.* (2017) and Kamal *et al.* (2017). Higher results of coliforms count in Kareish cheese were recorded by Hussien *et al.* (2013), Abd Allah (2017), El-Leboudy *et al.* (2017), and Abd El-Halem (2019), while nearly similar results were recorded by Abd Ellah (2017) and Kamal *et al.* (2017). On the other hand, lower counts were obtained by Baraheem *et al.* (2007) and Salem *et al.* (2016). While nearly similar results of coliforms count in Ras cheese were recorded by Hassan *et al.* (2019). On the other hand, lower counts were obtained by Hegab *et al*. (2020) and Samaa *et al*. (2020). According to the limits proposed by Egyptian Standards (ES, 2010), the total coliforms count of the examined Domiati, Kareish, and Ras cheese samples must not exceed 10 CFU/ml milk, 44,72 and 68 % of the examined cheese samples respectively didn't comply with that standard.

3.2. Fecal *Coliforms*

Data presented in Table 2. viewed that, fecal *coliforms* were not detected in 38, 32 and 22 % of the examined Domiati, Kareish, and Ras cheese samples respectively, while 28, 16 and 12 % of samples had counts of 3 - < 10 respectively, and 30, 18 and 16 % of samples had counts of 10 - < 10^2, while 2, 4 and 16% of samples had counts of 10^2 - < 10^3, and 2, 30 and 34 % of samples had counts of 10^3 - < 10^4. It is clear that the highest frequency distribution of fecal coliforms group was detected in the positive cheese samples lied within the range of 10 - <10^2 (30 %), 10^3 - <10^4 (30 %), and 10^3- <10^4 (34 %) in Domiati, Kareish, and Ras cheese respectively. Higher results of fecal coliforms count in Kareish cheese were obtained by Hussien *et al.* (2013), while lower counts were obtained by Salem *et al.* (2016) in Kareish cheese and by El-Kholy *et al.* (2014) in Domiati cheese.

3.3. *Escherichia coli* (*E. coli*) count

As presented in Table 3, *E. coli* existed in 28, 28, and 37 % of Domiati, Kareish, and Ras cheese samples respectively. Their counts using the MPN method showed that 32, 14 and 20 % of samples lay in the interval 3 - < 10 respectively, while 24, 30 and 24 % of samples lay in the interval 10 to < 10^2 respectively, and 2 and 10 % of Kareish and Ras cheese samples lied in the interval 10^2 to < 10^3, while 10 and 20 % of Kareish and Ras cheese samples lied in the interval 10^3 to < 10^4.

The highest frequency distribution of E. coli in the positive cheese samples lay within the range of 3- < 10 (32 %), 10 - <10^2 (30 %), and 10- < 10^2 (26 %) in Domiati, Kareish, and Ras cheese respectively.

Lower findings were recorded by Ibrahim *et al.* (2015), Hassan and Gomaa (2016), and Abo El-Makarem *et al.* (2017) in Kareish and Domiati cheese, while Ombarak *et al*. (2016) showed lower findings in Ras cheese, meanwhile, Nosir *et al.* (2014) showed lower findings in Kareish and Ras cheese, and El-Kholy *et al.* (2014) showed lower findings in Domiati cheese.

However, Abd El-Halem (2019), Abd Allah (2017), Ombarak *et al*. (2016), Hussien *et al.* (2013), and Baraheem *et al.* (2007) showed higher findings in Kareish cheese, while nearly similar results for *E. coli* in Kareish cheese were recorded by Meshref and Hassan (2009) and El-Bessery (2006). Egyptian Standards (ES, 2010) has stated that *E. coli* should be absent in Domiati, Kareish,

and Ras cheese, only 44 %, 44 %, and 26 % of the examined cheese respectively were matching that standard.

The harboring of the detected *E. coli* serotypes for virulence genes (stx1, stx2, and eaeA) was shown in figure 1. PCR technique was used for the detection of *E. coli* via their virulence gene. The results correlated with those obtained by serological methods. This study used a multiplex PCR for the detection of virulence genes in the serologically identified *E. coli*, PCR products of the examined strains gave bands of the expected sizes for stx1 (180 bp), stx2 (255 bp), and eaeA (384 bp). The obtained results were comparable to that stated by Douellou *et al*. (2016).

3.4. *Staphylococcus aureus*

The results represented in Table 4 verified that *Staph. aureus* was detected in 26, 10 and 22% of Domiati, Kareish and Ras cheese respectively, with a minimum count of 1.0 $x10^2$, 1.3 x103 and 3.0 $x10^2$ respectively, and a maximum count of 7.0 $x10^4$, 2.0 $x10^5$ and 9.0 $x10^4$ respectively, and average count of 1.27 $x10^3$, 7.5 $x10^4$ and 2.15 x 10^3 respectively.

Higher findings in Kareish cheese were shown by Bahout and Moustafa (2006), Hassan (2008b), Hussien et al. (2013), Eid and Eltalawy (2014), and higher findings in Domiati cheese were recorded by El-Kholy *et al*. (2014), and in Ras cheese by El-Leboudy *et al.* (2015), Hegab *et al.* (2020) and Samaa *et al*. (2020), while higher findings in Kareish and Domiati cheese were recorded by Ibrahim *et al*. (2015) and Hassan and Gomaa (2016), while lower findings in Kareish cheese were recorded by El-Bessery (2006), and lower finding in Domiati cheese were recorded by Eid and Eltalawy (2014), Abd Allah (2017) and Elshall (2019), while nearly similar results in Domiati cheese were recorded by El-Bessery (2006), Hassan (2008b) and El-Leboudy *et al*. (2017a), and in Kareish cheese by Abd Allah (2017) and Abd El-Halem (2019). Egyptian Standards (ES, 2010) has stated that *Staph. aureus* (coagulase-positive) should be absent in cheese, 26 % of Domiati, 10% of Kareish, and 22% Ras cheese samples were not compatible with that standard.

This study used multiplex PCR for the detection of enterotoxin genes of the isolated *Staph. aureus*. According to the results represented in figure 2., Staph. aureus enterotoxins A, C, and D were detected in the examined Staph aureus strains. PCR products of the examined strains gave bands of the expected sizes sea (120 bp), seb (478 bp), sed (317 bp) Figures 1. and 2.

3.5. Yeasts and molds count

The result given in Table 5 showed that yeasts were detected in 98, 96, and 52% of Domiati, Kareish, and Ras cheese respectively, with a minimum count of 1.0 $x10^2$ for the three types of cheese, and a maximum count of 5.0 × 10^6, 2.0 × 10^6 and 2.0 × 10^5 respectively, and the average count of 3.88 × 10^5, 3.26 × 10^5 and 1.04 × 10^4 respectively.

The data presented in Table 6 revealed that molds were detected in 46, 32, and 35 of Domiati, Kareish, and Ras cheese samples. The maximum total mold counts /g in Domiati, Kareish, and Ras cheese samples were 1.8×10^5, 5×104, and 4.7×10^5 the minimum was 1.0x10^2 for the three cheese types with average values of 3.09×10^4, 1.2×$10^{4,}$ and 2.61×10^4, respectively.

Higher results in positive cheese samples were recorded by El-Bessery (2006), Aly *et al*. (2007), Hassan (2008a), and Abd Ellah (2017) in Domiati cheese, while lower results were recorded by El-Shazly (2007), Ahmad (2012), and Kamal *et al*. (2017) in Domiati cheese, while higher results were recorded by Hassan (2008b), Soliman and Aly (2011) and Abd Ellah (2017) in Kareish cheese, and lower results in Kareish cheese recorded by Ahmad (2012), Hussien *et al*. (2013), Ibrahim *et al*. (2015), Abo El-Makarem *et al*. (2017) and Kamal *et al*. (2017), higher results were recorded by El Bagory *et al*. (2014) and Samaa *et al*. (2020) in Ras cheese, while nearly similar results were recorded by Elramly *et al*. (2019) and Hassan *et al*. (2019) in Ras cheese. Egyptian Standards (ES, 2010) have stated that yeast should not be more than 400 CFU /g cheese and according to this standard 98, 96, and 52% of the examined Domiati, Kareish, and Ras cheese samples, respectively were above the stated standards. Furthermore, 92, 64 and 70 % of the examined Domiati, Kareish, and Ras cheese samples respectively failed to confirm the limit of mold counts (<10 /g).

4. Conclusions

The overall hygienic conditions from production to consumption influence the bacterial load in milk and milk products. Information given by the obtained results allows concluding that the overall hygienic condition was not satisfactory. Unhygienic practices adopted during production, handling, processing, storage, and distribution are responsible for the poor sanitary and bacteriological quality of the examined cheese samples. The presence of some pathogenic microorganisms in the examined samples calls for more restrictions and preventive measures in milk herds, milk production, and dairy factories with respect to quality control, sanitation, and health care.

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الملخص العربي

تأثير المستحلبات النانونية للزيوت الأساسية على بعض الميكروبات في الجبن

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تم جمع 150 عينة عشوائية من الجبن القريش والدمياطي والرومي (50 عينة لكل منهم) في الفترة من أغسطس 2020 إلى فبراير 2021 من مختلف محلات الألبان والسوبر ماركت في مدينة أسيوط، مصر. تم فحص العينات لتحديد الجودة المكروبيولوجية لها. لم يتم الكشف عن الميكروبات القولونية في 32 و20 و16 ٪ من عينات الجبن الدمياطي، القريش والرومي التي تم فحصها على التوالي. تم الكشف عن أعلى توزيع تكراري للميكروبات القولونية في عينات الجبن الموجبة ضمن نطاق 10 - < 10^2 (26 ٪)، 10^3 - < 10^4 (60٪) و 10^3- < 10^4 (32 ٪) في الجبن الدمياطي، القريش والرومي على التوالي. لم يتم الكشف عن القولونيات البرازية في 38 و32 و22 ٪ من عينات الجبن الدمياطي، القريش والرومي التي تم فحصها على التوالي. وجدت الايشيريشيا كولاي في 28، 28، 37 ٪ من عينات الجبن الدمياطي، القريش والرومي على التوالي. كان أعلى توزيع تكراري الايشيريشيا كولاي في عينات الجبن الموجبة في حدود 3- < 10 (32 ٪)، 10 - < 10^2 (30 ٪) و10- < 10^2 (26 ٪) في الجبن الدمياطي، قريش والرومي على التوالي. تم الكشف عن المكورات العنقودية الذهبية في 26 و10 و22٪ من الجبن الدمياطي والقريش والرومي على التوالي بمتوسط عدد 1.27 × 10^3 و7.5 × 10^4 و2.15 × 10^3 على التوالي. كانت معظم العينات الإيجابية 16 و14 ٪ على التوالي لعينات الجبن الدمياطي والرومي تتراوح بين 10^2 إلى <10^3 / جرام، بينما في الجبن القريش 6٪ تقع في النطاق من 10^3 إلى <10^4 / جرام. تم الكشف عن الخمائر في 98 و96 و52٪ من الجبن الدمياطي والقريش والرومي على التوالي، بمتوسط عدد 3.88 × 10^5، 3.26 × 10^5 و1.04 × 10^4 على التوالي. تقع معظم العينات الإيجابية (28 ٪) من عينات الجبن الدمياطي في النطاق من 10^4 إلى <10^5 /جرام، ومعظم العينات الإيجابية (34 ٪) من عينات الجبن القريش في النطاق من 10^5 إلى < 10^6 جرام. (26 ٪) من عينات الجبن الرومي تراوحت بين 10^2 إلى < 10^3 تم الكشف عن العفن في 46 و32 و35٪ من عينات الجبن الدمياطي والقريش والرومي على التوالي. كان متوسط الأعداد 2.61 x 10^4 و1.2x 10^4 و3.9 x 10^4 على التوالي. تقع أعلى نسبة تواجد لتعداد العفن في عينات الجبن الموجبة (36 و44 ٪) ضمن النطاق من 10^2 إلى أقل من 10^3 في الجبن الدمياطي والرومي على التوالي. في حين أن معظم العينات الإيجابية (34 ٪) لعينات الجبن القريش تراوحت بين 10^4 إلى < 10^5.