Molecular characterization of *Escherichia coli* isolated from cheese and biocontrol of Shiga toxinogenic *E. coli* with essential oils

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Abstract

The current research was carried out to study the incidence of *Escherichia coli* (E. coli) in Egyptian cheese (Kariesh and Ras) and molecular characterization of certain *E. coli* virulence genes (*stx1*, *stx2*, *eaeA*, *hlyA* and *fimH*) using multiplex PCR technique. Biocontrol of *E. coli* with essential oils (clove and thyme oil) was also studied. A total of 150 random samples of Kariesh and Ras cheese (75 each) were collected from various areas in Governorate of Menoufia. According to our results, the frequency of *E. coli* isolated from Kariesh and Ras cheese was 16% and 5.3%, respectively. Serological identification classified the *E. coli* strains into two groups, enterohemorrhagic *E. coli* (EHEC) serogroup (O26: H11, O91: H21, O111: H2 and O103: H2). While the enterotoxigenic *E. coli* (ETEC) serogroup were detected as O125: H21 which is the most prevalent strain. O171: H2, O86 and O119: H6 belonging to enteropathogenic *E. coli* (EPEC). The most prevalent gene detected in *E. coli* strains was *stx1* (87.5%) followed by *stx2* (86%), *fimH* (75%), *hlyA* (50%) and *eaeA* (25%) genes. Concerning the antimicrobial activity with essential oils, thyme oil (1%) is considered as the bactericidal effect against *E. coli* (ATCC35150) with improved the sensory evaluation than clove oil (1%). In conclusion, Kariesh and Ras cheese are extremely tainted with pathogenic *E. coli* strains, which represent a strong hazard on the human health.

Introduction

Food safety is considered one of the most common urgent matters in the food industry worldwide. Spoilage of the food products with foodborne pathogens receive a special concern among food producers, investigators and customers. Consequently, producing a safe food represents one of the most imperative urgencies in the food processing (Friedman et al., 2002; Mohamed et al., 2013). The outbreaks of foodborne illnesses caused by *E. coli* have been studied previously in developing countries after ingestion of milk products such as old-style cheese which is considered the major source of various types of pathogenic bacteria (Elhadidy and Mohammed, 2013). *E. coli* is one of the major significant bacteria, which has a bad effect on both human and animal species. Thus, this type of bacteria can deteriorate the milk particularly raw milk and other milk products as a result of poor hygienic measures (Lara et al., 2016; Garbaj et al., 2016).

*E. coli* is classified into six pathotypes: enterohaemagglutinating, enterohemorrhagic/ Shiga toxin-producing *E. coli* (STEC), enteroinvasive, enteropathogenic, enterotoxigenic, and diffuse adherent (Jafari et al., 2012). lethal STEC named EHEC were also detected (Beutin et al., 2007). Previous studies indicated that STEC represents one of the most significant pathotypes which lead to foodborne illnesses compared with other types *E. coli* (Brett et al., 2003; Kaufmann et al., 2006). In human, the pathogenic effect of STEC nearly due to its ability to produce certain types of cytotoxins for example Shiga toxins (*stx1* and *stx2*), enterohemolysin (*hly*) and intimin (*eae*) virulent genes (Slanec et al., 2009 and Assumpção et al., 2015).

Among the dairy products, cheese is considered one of the major significant bacteria, which contains a higher protein content with a small amount of fats (Hamad, 2015). Nevertheless, cheese is considered a safe food for human being, sometimes its deterioration by various types of foodborne pathogens may take place. *Listeria monocytogenes, Salmonella* and enteropathogenic *E. coli* (EPEC) are considered the most common bacteria isolated from cheese. EHEC such as *E. coli* O157:H7 may also cause high morbidity and mortality rates among young and old people (Kousta et al., 2010).

Essential oils (EO) are known to have antibacterial and antioxidant effects (Yousefi et al., 2017). Numerous researches reported that EO have a potent antibacterial effect against different types of pathogens which indicated their ability to protect the foodstuffs (Burt, 2004; Kotzekidou et al., 2008; Yahyazadeh et al., 2008; Lee et al., 2010; Bajpai et al., 2012 and Jeong et al., 2014). Various EO with multiple effects such as antibacterial, antiviral, antifungal, anti-inflammatory, antioxidant and carcinopreventive effects have been established and about 12°C to be prepared (El-Hofi et al., 2010). Moreover, Kariesh cheese is another prevalent type of cheese which contains a higher protein content with a small amount of fats (Hamad, 2015). Nevertheless, cheese is considered a safe food for human being, sometimes its deterioration by various types of foodborne pathogens may take place. *Listeria monocytogenes, Salmonella* and enteropathogenic *E. coli* (EPEC) are considered the most common bacteria isolated from cheese. EHEC such as *E. coli* O157:H7 may also cause high morbidity and mortality rates among young and old people (Kousta et al., 2010).

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previously by Chorianopoulos et al. (2008) and São Pedro et al. (2013). From the previously revealed data, the current research was achieved to isolate and identify the pathogenic E. coli and recognition of its virulence genes (e.g. stx1, stx2, hlyA eaeA and fimH) using multiplex PCR technique as well as studying the ability and effectiveness of essential oils extracted from thyme, clove and laurel plant on isolated pathogenic E. coli in soft cheese.

Materials and Methods

Sample collection

One hundred fifty samples including Egyptian Kariesh cheese (n=75) and Ras cheese (n=75) were collected randomly from various markets in certain areas in Menoufia Governorate and preserved in the ice box for culturing process within two hours of collection.

Isolation of E. coli

According to the method described by De Boer and Heuvelink (2000), the isolation of E. coli was carried out. In brief, 25 grams of each specimen were moved to sterile tube comprising 225 mL of tryptase soya broth (TSB, Oxoid, UK) and then sterile tube comprising 225 mL of tryptase soya broth (TSB, Oxoid, UK) and then stored in nutrient Broth (NB, Oxoid, UK) at 37°C for 24 h. Peptone Water (Sigma-Aldrich, USA) for preparation the diluents containing 2% of sodium citrate (ISO, standard DIS 6887-5, 2010). One mL of the serial dilutions (ISO, standard DIS 6887-5, 2010). One mL of the serial dilutions was then moved to 10 mL of diluents to culture, color and appearance. Ten grams of cheese homogenate. One mL of main diluents was assessed for flavor, body and texture. Ten grams of cheese samples examination

Cheese specimens were tested after two weeks for sensory evaluation and E. coli counting. All tests were accomplished in three replicates and the mean values were then measured. Sensory evaluation for both control and treated Kariesh cheese specimens was performed based on the method suggested by Clark et al. (2009). The samples were assessed for flavor, body and texture, color and appearance. Ten grams of cheese sample was transferred to 90 mL of diluents containing 2% of sodium citrate (Sigma-Aldrich, USA) for preparation the cheese homogenate. One mL of main dilution was then moved to 10 mL of diluents to get sequential dilutions (ISO, standard DIS 6887-5, 2010). One mL of the serial dilutions was moved onto two plates of Eosin

Molecular characterization of STEC strains

DNA Extraction

QIAGen kits were used for extraction of DNA from E. coli isolates, according to the technique described previously by Hessain et al. (2015).

Primer sequences used for identification of E. coli virulence genes

The Shiga toxins (stx1 & stx2), intimin (eaeA), hemolysin (hlyA) and D-Mannose-specific adhesion “type 1 fimbriae” (fimH) virulent genes of E. coli were amplified according to the technique recommended by Fagan et al. (1999) using designated primers (Pharmacia Biotech) as shown in Table 1. The amplification of fimH gene was carried out according to Pusz et al. (2014)

In vitro susceptibility testing of thyme and clove essential oils on isolated strains of E. coli and ATCC35150

Preparation of bacterial strains

Stock cultures of 16 E. coli strains that obtained from our present study were preserved in nutrient Broth (NB, Oxoid, UK) at 4°C. Microorganism inoculum was fortified in NB at 37°C for 24 h. Peptone Water (Oxoid CM0009) was used to dilute the cell suspension to provide 10⁶ CFU/mL (Celikel and Kavas, 2008). ATCC 35150 (E. coli O157:H7) strain EDL931 genome (GenBank accession no. AWXM00000000.2) was also used in our study.

Preparation of Kariesh cheese

According to El-Khawas and Hassan (2015), cow’s milk was got from the College of Veterinary Medicine, Benha University, Egypt in which the percentage of fat’s milk is 4.2% (AOAC, 2000). Milk was pasteurized at 75°C for 15 seconds, there after cooled to 43°C then inoculated with 3% (v/v) of yoghurt starter culture. All treatments were incubated at 37°C, up to curding. The combination was divided into four 8 pars as: (I); Control (no essential oils or bacterial strains are present), salt at 1% was added between cheese layers and the curd was left to whey drain into small cheese molds at 22-25°C and the mixture was then divided into four 4 parts as follow: (I); Control without antimicrobials or biological agent; (II) E. coli strain with CFU at 10⁹/mL; (III) E. coli with thyme oil 0.5%; (IV) E. coli with clove; (V) E. coli with thyme oil 1%; (VI) E. coli with clove oil 1%. Two parts of clove 1% and thyme oils were set for sensory assessment (1% for each oil), Cheeses from various handlings were kept in firmly locked plastic bottles and enclosed with whey at 6±2°C for two weeks.

Table 1. Primer sequences used for identification of E. coli virulence genes.

| Primer | Oligonucleotide sequence (5′→3′) | Product size (bp) | References |
|--------|---------------------------------|------------------|------------|
| stx1 (F) | ACGTGTGATGGCGCTCAGTGG | 614 | Dhanashree and Mallya (2008) |
| stx1 (R) | CTGAATCCCCCTCATTATG | | |
| stx2 (F) | CCGATCAAGGACGAGCAGTT | 779 | Dhanashree and Mallya (2008) |
| stx2 (R) | CCTGTCAACTGAGCAGCTTTG | | |
| eaeA (F) | GTGCGGCAATCTGGGCGAGACT | 890 | Mazaheri et al. (2014) |
| eaeA (R) | CCCCATCTTTTTTACCCGTCG | | |
| hlyA (F) | ACGATGTGCTTATCTCGQA | 165 | Fratamico et al. (1995) |
| hlyA (R) | CTTCAAGGTGACCATACAT | | |
| fimH (F) | TGCGAAGCGGATAAGCGGTGG | 165 | Chapman et al. (2006) |
| fimH (R) | GCAGTCCTGTCCTCCTGGA | | |
Methylene Blue Agar (Oxoid, UK) for bacterial counting. Subsequently, the plates were kept at 37°C for 24-48 hours. Distinctive *E. coli* colonies were calculated and recorded according to APHA (2004). The analysis of variance (ANOVA) test was carried out to investigate the statistical significance (P≤0.05).

**Results and Discussion**

Dairy products are liable to be contaminated from different sources during production, contamination and their presence in food lead to be unfit for consumption and constitute a public health hazard (Virpari et al., 2013). In our research, the incidence of *E. coli* was 16% and 5.33% in examined Kariesh and Ras cheese samples, respectively from (75 examined samples of each). For Kariesh cheese, higher result (74.5%) was obtained by Farhad et al. (2017). For Ras cheese, higher results (28%) was obtained by Virpari et al. (2013) and Farhad et al. (2017). Lower result (11.54%) for kareish cheese was recorded by Farhad et al. (2017).

The main factors which affect on the quality and composition may be as a result of the clotted skimmed milk, the process of production, the period needed to complete the whey drain, the superiority of the added salt and the practice of management complete cheese (Aldo et al., 2013). The incidence of *E. coli* in in our samples may be as a result of deficiency of appropriate hygiene and lack of sterilization of milk utilized for cheese manufacture.

In our article we identified the EPEC, ETEC and EHEC serogroups in samples of cheese and the EHEC was considered one of the major predominant serogroup (Table 2). In this study EHEC serogroup were detected as O26: H11, O91: H21, O111: H2 and O103: H2. While ETEC serogroup was detected as O125: H21 which is the most prevalent strain. O171: H2, O86 and O119: H6 belonging to EPEC. Ladan and Reza (2006) indicated that O119 represents one of the dominant EPEC serogroup recovered from cheese.

*E. coli* bacteria contain multiple virulence genes that encourage its establishment and attack of the human cells (Ejrnæs, 2011). There are other virulence genes in *E. coli* strains such as toxins which is a secretory virulence factors and the most important of these factors is α hemolysin, this factor encoded by hly gene (Bien et al., 2012). The Multiplex PCR was used for the recognition of *stx1, stx2, eaeA and hlyA* virulence genes in 16 *E. coli* isolates. As shown in

![Figure 1](image1.png)  
Figure 1. Agarose gel electrophoresis of multiplex PCR of *stx1* (614 bp), *stx2* (779 bp), *eaeA* (890 bp), *hlyA* (165 bp). Lane M: 100 bp ladder as molecular size DNA marker; lane C+: Control positive *E. coli* for *stx1, stx2, eaeA* and *hlyA* genes; lane C- : negative control; lanes 1, 2, 4 (O26) and 9 (O111): positive *E. coli* for *stx1, stx2, eaeA* and *hlyA* genes; lanes 6 (O91), 8 (O103) and 10 (O111): positive *E. coli* for *stx1, stx2* and *hlyA* genes; lanes 3 (O26) and 11 (O119): positive *E. coli* for *stx1* and *stx2* genes; lanes 7 (O91): positive *E. coli* for *stx1* and *hlyA* genes; lanes 12, 13, 14 (O125) and 16 (O171): positive *E. coli* for *stx1* gene; lanes 5 (O86) and 15 (O156): positive *E. coli* for *stx2* gene.

![Figure 2](image2.png)  
Figure 2. Agarose gel electrophoresis of PCR of *fimH* (508 bp). Lane M: 100 bp ladder as molecular size DNA marker; lane C+: control positive *E. coli* for *fimH* gene; lane C- : control negative; lanes 1, 2, 4 (O26); 6, 7 (O91); 8 (O103); 9, 10 (O111); 11 (O119); 13, 14 (O125) and 15 (O156): positive *E. coli* for *fimH* gene. Lanes 3(O26); 5 (O86); 12 (O125) & 16 (O127): negative *E. coli* for *fimH* gene.

| Table 2. Serological identification of isolated *E. coli* from the examined samples. |
|---------------------------------------------------------------|
| **Product** | **Serodiagnosis** | **Strain characterization** |
|----------------|------------------|-----------------------------|
| Kareish cheese | 3 O125 : H21     | ETEC                        |
|                | O171 : H2        | EHEC                        |
|                | O86              | EPEC                        |
|                | 3 O26 : H11      | EHEC                        |
|                | O91 : H21        | EHEC                        |
|                | O111 : H2        | EHEC                        |
|                | O156 : H7        | EPEC                        |
|                | O103 : H2        | EHEC                        |
| Ras cheese    | O119 : H6        | EPEC                        |
|                | O111 : H2        | EHEC                        |
|                | O26 : H11        | EHEC                        |
|                | O91 : H21        | EHEC                        |

| Table 3. Incidence of virulence genes of EPEC strains isolated from the examined samples. |
|---------------------------------------------------------------|
| **No. examined isolates** | **stx1** | **%** | **stx2** | **%** | **eaeA** | **%** | **hlyA** | **%** | **fimH** | **%** |
|---------------------------|---------|------|---------|------|---------|------|---------|------|---------|------|
| 16                        |         | 14   | 87.5    |      | 11      | 68   | 4       | 25   | 8       | 50   |

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virulence genes and the majority of hemolytic-uremic disorder in individuals are caused mainly by STEC strains that carry stx2 gene. The eaeA gene is an additional virulence gene for STEC that was important to increase the pathogenicity of STEC. However, the eaeA gene hasn’t been detected in some STEC strains that cause human diseases (Kruger and Lucchesi, 2015). Furthermore, Douellou et al. (2017) showed that the virulence gene profiles of dairy products were similar to human STEC strains.

Biocontrol of E. coli strains (O26) by thyme and clove essential oils was also investigated in our study. The statistics demonstrated in Tables 4 and 5 indicated that the counts of E. coli (O26) were gradually decreased from zero time 3.0×10^6 ± 0.2×10^6, 2.2×10^5 ± 0.1×10^5 and 5.7×10^4 ± 1.0×10^4 in cheese sample with thyme oil 0.5% with reduction % reached to 92.7% at 1st week and 98.1% while in cheese sample with 1% thyme oil the reduction % reach 99.8% at 1st week and disappear at 2nd week of refrigerated storage. Similar results were described by Al Maqtari et al. (2011) who stated that the Staphylococcus aureus and E. coli strains were highly susceptible to the thyme oil and exhibited an imperative antimicrobial effect.

The reduction percentages were in cheese sample with 0.5% clove oil 73.7% and 98.1 at 1st and 2nd week with mean value 7.9×10^5 ± 1.2×10^5 and 2.8×10^5 ± 0.3×10^5, respectively, while in samples with 1% clove oil the reduction % were 92.3 and 98.5 in 1st and 2nd week with mean value 2.3×10^5 ± 0.1×10^5 and 4.5×10^4 ± 0.8×10^4, respectively. While in control group (cheese with E. coli only) the count of E. coli still high from zero time to 2nd week of refrigerated storage with mean 3.0×10^6 ± 0.2×10^6, 2.6×10^6 ± 0.1×10^6 and 2.4×10^5 ± 0.1×10^5, respectively. These results agree with that reported by Ayah and Saad (2016). According to our results, the antimicrobial activity with essential oils, thyme oil (1%) is considered as the bactericidal effect against E. coli (ATCC35150) with improved the sensory evaluation than clove oil (1%). The usage of higher concentration of in vivo EO than in vitro may be as a result of the more complex growth environment in foodstuffs, which play an important role in the microbial cells protection from anti-

### Table 4. The effect of different essential oils (0.5%) on E. coli (O26) count (CFU/g) inoculated into Kariesh cheese.

| Storage time | Strain only | Thyme oil | Clove oil |
|--------------|-------------|-----------|-----------|
| Zero time    | 3.0×10^6 ± 0.2×10^6 | 3.0×10^6 ± 0.2×10^6 | 3.0×10^6 ± 0.2×10^6 |
| 1st week     | 2.6×10^6 ± 0.1×10^6 | 2.2×10^5 ± 0.1×10^5 | 2.8×10^5 ± 0.3×10^5 |
| 2nd weeks    | 2.4×10^5 ± 0.1×10^5 | 5.7×10^4 ± 1.0×10^4 | 2.8×10^3 ± 0.3×10^3 |

R% = Reduction %

### Table 5. The effect of different essential oils (1%) on E. coli count (CFU/g) inoculated into kariesh cheese.

| Storage time | Strain only | Thyme oil | Clove oil |
|--------------|-------------|-----------|-----------|
| Zero time    | 3.0×10^6 ± 0.2×10^6 | 3.0×10^6 ± 0.2×10^6 | 3.0×10^6 ± 0.2×10^6 |
| 1 week       | 2.6×10^6 ± 0.1×10^6 | 3.6×10^5 ± 0.5×10^5 | 2.3×10^5 ± 0.1×10^5 |
| 2 weeks      | 2.3×10^5 ± 0.1×10^5 | ND        | 4.5×10^4 ± 0.8×10^4 |

R% = Reduction %

### Table 6. Sensory evaluation scores of fresh manufactured soft cheese treated with essential oils (1%).

| Group          | Flavor (30) | Texture (60) | Appearance and color (10) | Over all (100) |
|----------------|-------------|--------------|---------------------------|---------------|
| Fresh cheese (zero time) |             |              |                           |               |
| Control        | 22          | 54           | 9                         | 85            |
| Clove oil      | 24          | 53           | 9                         | 86            |
| Thyme oil      | 26          | 55           | 9                         | 90            |
| 1st week       |             |              |                           |               |
| Control        | 22          | 52           | 8                         | 83            |
| Clove oil      | 26          | 53           | 9                         | 88            |
| Thyme oil      | 28          | 55           | 9                         | 92            |
| 2nd week       |             |              |                           |               |
| Control        | S           | S            | S                         | S             |
| Clove oil      | 25          | 50           | 8                         | 83            |
| Thyme oil      | 26          | 54           | 8                         | 88            |

S: Spoiled samples.
Bacteriological agents (Marija and Nevena, 2009). The bacteriological effect of EOs may due to their ability of cellular wall degradation, cell membrane damage, obliteration of membrane proteins and enhanced permeability of the cell membrane leading to escape the different ions and other contents of the bacterial cell (Nazzaro et al., 2013). Thyme oil is recognized to have antibacterial effect against various microorganisms including E. coli isolated from foodstuffs. Smith-Palmer et al. (2001) and Burt and Reinders (2003) indicated that thyme oil has bacteriostatic and bactericidal effects against E. coli O157: H7.

The first impression about food is usually visible, and the most important thing regarding the consumer’s willingness to consume the food is based mainly on its texture (Gambaro et al., 2001). The scores for sensory assessment of fresh kariesh cheese hand-made by different methods are listed in Table 6. At first two weeks of storage, a high flavor score was detected in the thyme oil cheese specimens, whereas a reduced value was detected in clove oil cheese specimens at the 2nd week of refrigerated storage. After adding EOs, no momentous influence on the texture value. The entire value of cheese specimens was significantly (P<0.05) increased at zero day with both thyme and clove oils. A greater sensory value was detected in cheese with thyme oil in the first two weeks of storage compared with the control and clove oil usage, whereas the lowermost score was stated in control samples at the 1st week. Similar results were obtained by Ismail et al. (2006). White cheese treated with essential oils had softer consistency than the control group; because the existence of EOs in cheese can improve the enzymatic action (Mervat et al., 2010).

Conclusions

From the above-mentioned results, it can be clarified the public health importance of pathogenic E. coli and its virulence genes that were determined in our study in milk products (Kariesh and Ras cheese) in Egypt, that might be attributed to contamination which might be explained by improper sanitation, lack of health education and lack awareness about efficient control measures. Furthermore, contamination of milk and milk products as a foodborne zoonosis are remained a constant public concern with various implications in Egypt.

References

Aldo T, Fady AA, Ola AHO, 2013. History, Processing and Quality Enhancement of Traditional Egyptian Kariesh Cheese: A Review. Food Sci Technol 1:1-6.
Al Maqta MAA, Alghalibi SM, Alhamzy EH, 2011. Chemical composition and antimicrobial activity of essential oil of Thymus vulgaris from Yemen. Turk J Biochem 36:342-9.
AOAC, 2000. Official Method of Analysis. 17th Ed., Association of Official Analytical Chemists, Washington, DC., USA.
APHA American Public Health Association, 2004. Standard methods for the examination of dairy products, 17th Ed., 2004. American public health association. Washington D.C.
Assumpção GLH, Cardozo MV, Beraldo LG, Maluta RP, Silva JT, Avila FAD, McIntosh D, Rigobelo EC, 2015. Antimicrobials resistance patterns and the presence of stx1, stx2 and eae in Escherichia coli. Rev Bras Saúde Prod Anim 16:308-16.
Ayah BA, Saad MF, 2016. Influence of selected essential oils on some pathogenic microorganisms in white soft cheese. Int J Chem Tech Res 9:214-20.
Bajpai VK, Baek HK, Kang SC, 2012. Control of Salmonella in foods by using essential oils: A review. Food Res Int 45:722-34.
Beutin L, Miko A, Krause G, Pries K, Haby S, Steege K, Albrecht N, 2007. Identification of human-pathogenic strains of Shiga toxin-producing Escherichia coli from food by a combination of serotyping and molecular typing of Shiga toxin genes. Appl Environ Microbiol 73:4769-75.
Bien J, Sokolova O, Bozko P, 2012. Role of uropathogenic Escherichia coli virulence factors in development of urinary tract infection and kidney damage Int J Nephrol 68:1-15.
Brett KN, Hornitzky MA, Bettelheim KA, Walker MJ, Djordjevic SP, 2003. Bovine non-O157 Shiga toxin 2-containing Escherichia coli isolates commonly possess stx2-EDL933 and/or stx2VHb subtypes. J Clin Microbiol 41:2716-22.
Burt SA, 2004. Essential oils: their抗菌bacterial properties and potential applications in foods: A review. Int J Food Microbiol 94:223-53.
Burt SA, Reinders RD, 2003. Antibacterial activity of selected plant essential oils against Escherichia coli O157:H7. Let Appl Microbiol 36:162-7.
Celikel N, Kavas G, 2008. Antimicrobial properties of some essential oils against some pathogenic microorganisms. Czech J Food Sci 26:174-81.
Chapman T, Wu X, Barchia I, Bettelheim K, Driesen S, Trott D, Wilson M, Chin J, 2006. Comparison of virulence gene profiles of Escherichia coli strains isolated from healthy and diarrheic swine. Appl Environ Microbiol 72:4782-95.
Chorianopoulos NG, Giouarris ED, Skandamis PN, Haroutounian SA, Nycha GJE, 2008. Disinfectant test against monocultures and mixed-culture biofilms composed of technological, spoilage and pathogenic bacteria: bactericidal effect of essential oil and hydrosol of Saturejathymbra and comparison with standard acid-base sanitizers. J Applied Microbiol 104:1586-96.
Clark S, Costello M, Drake M, Bodyfelt F, 2009. The sensory evaluation of dairy products. 2nd ed. Springer. Academic Press, London, 73-134.
De Boer E, Heuvelink AE, 2000. Methods for the detection and isolation of Shiga toxin producing Escherichia coli. J Appl Microbiol 88:133-43.
Dhanashree B, Mallya S, 2008. Detection of shiga-toxigenic Escherichia coli (STEC) in diarrhoeagenic stool and meat samples in Mangalore, India. Indian J Med Res 128:271-7.
Douellou T, Delannoy S, Ganet S, Fach P, Loukiadiis E, Montel MC, Sertgent-Thevenot D, 2017. Molecular characterization of O157:H7, O26:H11 and O03:H2 Shiga toxin-producing Escherichia coli isolated from dairy products. Int J Food Microbiol 253:59-65.
Edwards PR, Ewing WH, 1972. Identification of Enterobacteriaceae. 3rd ed. Burgess Publ. Co., Minneapolis, Minnesota.
Elhadidy M, Mohammed MA, 2013. Shiga toxin-producing Escherichia coli from raw milk cheese in Egypt: Prevalence, molecular characterization and survival to stress conditions. Let Appl Microbiol 56:120-7.
El-Khawas K.M., Hassan H.M. (2015): Control of food poisoning bacteria during manufacturing of acid cheese using some organic acids. Assiut Vet Med J 145:40-6.
Ejmes K, 2011. Bacterial characteristics of importance for recurrent urinary tract infections caused by Escherichia coli. Dan Med Bull 58:1-22.
El Hofi M, Ismail A, AbdRabo F, El-Dieb S, Ibrahim O, 2010. Studies on acceleration of Ras cheese ripening by aminopeptidase enzyme from Dufialove’s pancreas utilization of buffaloe’s pancreas aminopeptidase in acceleration of Ras cheese ripening. New York Sci J 3:9-6.
Fagan P, Hornitzky M, Bettelheim K,
Djordjevic S, 1999. Detection of Shiga-like Toxin (stx1 and stx2), intimin (eaeA), and Enterohemorrhagic Escherichia coli (EHEC) Hemolysin (EHEC hlyA) Genes in Animal Feces by Multiplex PCR. Appl Environ Microbiol 65:868-72.

Farhad SC, Navid MA, Esmat A, Afshin N, Reza SC, 2017. Molecular characterization of Escherichia coli recovered from traditional milk products in Kashan, Iran. Vet World 10:1264-8.

Fratamico P, Sackley S, Wiedmann M, Deng M, 1995. Detection of Escherichia coli O157:H7 by multiplex PCR. J Clin Microbiol 33:2188-91.

Friedman M, Henika PR, Mandrell RE, 2002. International Standard Organization (ISO), Hessain AM, Al-Arfaj AA, Zakri AM, El-Hamad MNF, 2015. Effect of adding rheagenic pathotypes and their role in Escherichia coli Production of flavored labneh with exten-

Gambaro A, Gimenez A, Burgueno J, 2001. Sensory and instrumental evaluation of strawberry yogurt color. J Sensory Stud 16:11-22.

Garbaj AM, Awad EM, Azwai SM, Abolhait SK, Naas HT, Moawad AA, Gammoudi FT, Barbieri I, Eldaghayes IM, 2016. Enterohemorrhagic Escherichia coli O157 in milk and dairy products from Libya: Isolation and molecular identification by partial sequencing of 16S rDNA. Vet World 9:1184-9.

Hamad MNF, 2015. Effect of adding Glucono-Lactone, Different of Starters, Rennet on the Chemical Composition, Yield and economic study of Kariesh cheese. Int J Food Sci Nut Eng 5:130-40.

Hessain AM, Al-Arfaj AA, Zakri AM, El-Jakee JK, Al-Zogibi OG, Henneg HA, Ibrahim IM, 2015. Molecular characterization of Escherichia coli O157:H7 recovered from meat and meat products relevant to human health in Riyadh, Saudi Arabia. Saudi J Biol Sci 22:725-9.

International Standard Organization (ISO), standard DIS 6887-5, 2010. Microbiology of food and animal feeding stuffs preparation of test samples, initial microbiological examination. Part 5: Specific rules for the preparation of milk and milk products.

Ismail AM, Harby SA, Salem S, 2006. Production of flavored labneh with extended shelf life. Egypt J Dairy Sci 34:59-68.

Jafari A, Alani M, Bouzari S, 2012. Escherichia coli: A brief review of diarrheagenic pathotypes and their role in diarrheal diseases in Iran. Int J Manag 4:102-17.

Jeong EJ, Lee NK, Oh J, Jang SE, Lee JS, Bae IH, Jeong YS, 2014. Inhibitory effect of cinnamon essential oils on selected cheese-contaminating fungi (Penicillium spp.) during the cheese-ripening process. Food Sci Biotechnol 23:1193-8.

Koutsa M, Mataragas M, Skandamis P, Drosinos EH, 2010. Prevalence and sources of cheese contamination with pathogens at farm and processing levels. Food Control 21:805-15.

Kotzekidou P, Giannakidis P, Boulamatsis A, Kotzekidou P, Giannakidis P, Boulamatsis A, Kousta M, Mataragas M, Skandamis P, Santurio JM, Alves SH, 2016. Antimicrobial susceptibility of Escherichia coli strains isolated from Aloniuatta spp. feces to essential oils. Evid Based Compl Altern Med 10:30.

Lee NK, Yeo IC, Park JW, Kang BS, Ham YT, 2010. Isolation and characterization of a novel analyte from Bacillus subtilis SC-8 antagonistic to Bacillus cereus. J Biosci Bioeng 110:298-303.

López-Expósito I, Amigo L, Recio I, 2012. A mini-review on health and nutritional aspects of cheese with a focus on bioactive peptides. Dairy Sci Technol 92:419-38.

Marija MS, Nevena TN, 2009. Antimicrobial effects of spices and herbs essential oils. APTEFF 40: 1-220 – Review BIBLIOL 1450-7188, 40: 195–209.

Martin A, Beutin L, 2011. Characteristics of Shiga toxin-producing Escherichia coli from meat and milk products of different origins and association with food producing animals as main contamination sources. Int J Food Microbiol 146-99-104.

Mazaheri S, Ahrabi S, Aslani M, 2014 Shiga Toxin-Producing Escherichia Coli Isolated from Leptostamine in Iran. Iran. Jundishapur J Microbiol 7:e12346.

Mazaheri S, Ahrabi S, Aslani M, 2014. Characteristics of Escherichia coli isolated from different origins and association with food producing animals as main contamination sources. Int J Food Microbiol 146-99-104.

Mazaheri S, Ahrabi S, Aslani M, 2014 Shiga Toxin-Producing Escherichia Coli Isolated from Leptostamine in Iran. Iran. Jundishapur J Microbiol 7:e12346.

Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V, 2013. Effect of essential oils on pathogenic bacteria. Pharma 6:1451-74.

Pusz P, Bok E, Mazurek J, Stosik M, Chudzik K, 2014. Type 1 fimbiae in commensal Escherichia coli derived from healthy humans. Acta Biochem Polon 61:389-92.

São Pedro A, Espírito-Santo I, Silva CV, Detoni C, Albuquerque E, 2013. The use of nanotechnology as an approach for essential oil-based formulations with antimicrobial activity. In “Microbial pathogens and strategies for combating them: science, technology and education”, (A. Méndez-Vilas Ed.). Formatex Research Center, Zurbaran, 06002 Badajoz, Spain, 1364–1374.

Staniec T, Fruth A, Creuzburg K, Schmidt H, 2009. Molecular analysis of virulence profiles and Shiga toxin genes in foodborne Shiga toxin-producing Escherichia coli. Appl Environ Microbiol 75:6187-97.

Smith-Palmer A, Stewart J, Fyfe L, 2001. The potential application of plant essential oils as natural food preservatives in soft cheese. Food Microbiol 18:463-70.

Tandon S, Rane S, 2008. Decoction and hot continuous extraction techniques. In: Hadia, et al., editors. Extraction technologies for medicinal and aromatic plants. Trieste, Italy: ICS-UNIDO, 106.

Virpuri PK, Virpuri JB, Nayak MN, Thaker HC, 2013. Study on isolation, molecular detection of virulence gene and antibiotic sensitivity pattern of Escherichia coli isolated from milk and milk products. Vet World 6:541-5.

Vu-Khac H, Holoda E, Filipcinec E, Blanco M, Blanco JE, Mora A, Dahbi G, López C, González EA, Blanco J, 2006. Serotypes, virulence genes, and PFGE profiles of Escherichia coli isolated from pigs with postweaning diarrhea in Slovakia. BMC Vet Res 2:10.

Yahyazadeh M, Omidsaigi R, Zare R, Taheri H, 2008. Effect of some essential oils on mycelial growth of Penicillium digitatum Sacc. Wild J Microbiol Biotechnol 24:1445-50.

Yousef AM, Khorshidian N, Mortazavian AM, Hossein I, 2017. A review on the impact of herbal extracts and essential oils on viability of probiotics in fermented milks. Curr Nutr Food Sci 13:6-15.