Assiut University web-site: www.aun.edu.eg

INFLUENCE OF ESSENTIAL OILS ON THE VIABILITY OF *LISTERIA MONOCYTOGENES*

MARY ISHAQ [1]; WALAA M. ELSHERIF [1]; AND MOHAMMED SAYED [2]
[1]Department of Food Hygiene, Animal Health Research Institute, Agriculture Research Center, Egypt
[2]Department of Food Hygiene, Faculty of Veterinary Medicine, Assiut University, Egypt

Received: 20 December 2021; **Accepted:** 28 January 2022

ABSTRACT

The present investigation was applied to study the influence of cinnamon (C), rosemary (R) and thyme (T) essential oils (EOs) on the viability of *Listeria monocytogenes*, in which, a total of 225 cheese samples (Tallaga, Bramily and Ras cheese, 75 each) were collected from different dairy markets and shops in Assiut city, Egypt for the isolation of *L. monocytogenes*. The isolates were identified and examined for 16S rRNA as positive for *L. monocytogenes*. The obtained results showed that *Listeria spp*. could be detected in 66.2% of the examined samples, while *L. monocytogenes* was in 17.6%. After that, the minimum inhibitory concentration (MIC) of the prepared cinnamon EO (CEO), rosemary EO (REO) and thyme EO (TEO) was detected against the isolated *L. monocytogenes*. Samples of Tallaga cheese were manufactured using MIC of the 3 prepared EOs separately, and the influence of EOs was done by agar well diffusion method and showed the MIC as 1.56% for CEO, 3.125% for REO and TEO. In conclusion, the CEO was the most effective against *L. monocytogenes* after Tallaga cheese manufacture although the unpleasant sensory quality of the manufactured cheese with the 3 oils, in which, the count of *L. monocytogenes* was 6.3 $\log_{10}$ at 0 h and then was 2.7 $\log_{10}$ after 1st week.

Key words: Essential oil; *Listeria monocytogenes*; cinnamon; rosemary; thyme

INTRODUCTION

Cheese can be a vehicle for foodborne pathogens; especially cheese made from raw milk can transmit several pathogens (Brooks *et al.*, 2012; Quero *et al.*, 2014). The microbial contamination cause potential risks for public health as it transmit pathogenic microorganisms to consumers (Gandhi and Chikindas, 2007).

Corresponding author: MARY ISHAQ
E-mail address: dr.marysobhy@gmail.com
Present address: Department of Food Hygiene, Animal Health Research Institute, Agriculture Research Center, Egypt

Several varieties of soft cheese are produced in Egypt; one of these varieties is the cold stored soft cheese known as Tallaga cheese. Tallaga cheese is an Egyptian unripened soft cheese, usually made from heated milk with adding low concentration of salt and stored in the refrigerator until consumption within 2 weeks (Mehanna and Rashed, 1990). Also, Domiati cheese is the most popular soft white pickled cheese in Egypt (Abou-Donia 1986); it is made from buffalo's or cow's milk or mixture of them and consumed fresh or after 3 - 6 months of ripening period in pickling solution (known as Bramily cheese). The salt concentrations used in Domiati cheese manufacture are

affected by some factors such as milk type, ripening time and season (Ismail, 2004). Ras cheese is an Egyptian hard cheese with a dialectic name called Romy; It is made in great amounts under artisan conditions from cows and buffalos milk (Dabiza and El-Deib, 2007; Hattem *et al.*, 2012).

Listeria monocytogenes were detected in many foodborne outbreaks, and these bacteria considered dangerous threats to the safety of fresh and low ripened cheeses (Pimentel-Filho *et al.*, 2014). It is a Gram-positive psychotropic bacterium and has the ability to form biofilms (Gutierrez *et al.*, 2009), and can grow aerobically or anaerobically in a wide range of temperatures (Melo *et al.*, 2015). It causes bacteremia and meningoencephalitis in individuals with impaired cell mediated immunity, including neonates, pregnant women, elderly persons and immunosuppressed recipients of transplants (Lorber *et al.*, 2005).

Essential oils (EOs) were defined by ISO as products obtained from a natural raw material of plant origin, by steam distillation, by mechanical processes, or by dry distillation, after separation of the aqueous phase (Gutiérrez-del-Río *et al.*, 2018). EOs are volatile compounds synthesized in several plant parts rich in terpenoids and phenolic (Ribeiro-Santos *et al.*, 2018). Also, EOs were defined by the FDA as Generally Recognized As Safe (GRAS) ingredients (Liu *et al.*, 2017), and no adverse effect have been recorded for its usage as acceptable daily intake (ADI) (Gouvea *et al.*, 2017).

Various EOs are with multiple effects such as antibacterial, antiviral, antifungal, anti-inflammatory, antioxidant and carcino-preventive. Cinnamon (CEO) is from these EOs belongs to *Lauraceae* family. Transcinnamaldehyde is a major component of cinnamon and responsible for antimicrobial and antioxidant activities (Kim *et al.*, 2018). Also, Rosemary (*Rosmarinus officinalis*) is a herb belongs to *Lamiaceae* family, it has been used in traditional medicine and as a food flavoring agent (Liu *et al.*, 2017). Thyme belongs to *Lamiaceae* family and is a well-known aromatic perennial herb originated from Mediterranean region and used as herbal teas, flavoring agents (condiment and spice) and medicinal plants because of their biological and pharmacological properties (Mahmoudi *et al.*, 2014).

This study was aimed to isolate and identify *L. monocytogenes* from different types of cheeses and studying the effect of EOs (CEO, REO and TEO) on isolated *L. monocytogenes in vitro* and *in vivo*.

MATERIALS AND METHODS

Collection of samples:

A total 225 random samples of Egyptian cheeses (75 samples of each Bramily cheese, Tallaga cheese and Ras cheese) were collected in their retail packages from different dairy shops and supermarkets in Assiut city, Egypt. The samples were transferred directly to the laboratory in an insulated icebox at 4^{O} C with a minimum of delay to be examined physically and bacteriology. The samples were prepared according to APHA (2004).

Isolation and identification of *L. monocytogenes* (Hitchins *et al.*, 2017):

About 25 grams of each prepared sample was simply stomached in 225 ml of buffered Listeria enrichment broth (BLEB) and incubated for 24 h at 30^{O} C. Then a loopful was streaked onto ALOA agar and incubated at 35° C for 24-48 h. Typical colonies were selected from agar media and streaked for purity onto trypticase soy agar with yeast extract and incubated at 37° C for 24-48 h, then subjected to different biochemical identification according to MacFaddin (2000).

Detection of *L. monocytogenes* using PCR:

This part was carried out in the Molecular Biology Research Unit (MBRU), Animal Health Institute, Giza, Egypt. According to Kumar *et al.* (2015), the target gene was 16S rRNA, the primer sequences were

GGA CCG GGG CTA ATA CCG AAT GAT AA
TTC ATG TAG GCG AGT TGC AGC CTA

Studying effect of CEO, REO and TEO on the viability of *L. monocytogenes*:

The essential oils were purchased from National Research Centre, Cairo, Egypt, and were kept at 2 - 8° C in sealed brown vials until used.

1. *In vitro*:

Preparation of the bacterial strains concentration according to Saad *et al.* (2019):

Bacterial suspension adjusted to the point 0.5 of the McFarland standard turbidity growth using McFarland apparatus.

Preparation of the EO dilutions according to Wiegand *et al.* (2008) with slight modifications:

Two-fold serial dilutions method was used. The 1st dilution prepared in BHI supplemented with dimethyl sulfoxide (DMSO) as a fat solvent (2:2:4) as (oil: DMSO: BHI). From this stock dilution, the other dilutions prepared by a 2-fold way using BHI only as 4 ml from it to 4 ml BHI and so on.

A) Agar well diffusion method (Elsherif & Ali, 2019)

0.1 ml of the previously prepared bacterial strains was streaked into Muller Hinton agar plates; then, 80 µl of different concentrations of the essential oils was added in each well. After 24 h incubation, the various levels of inhibition zones were measured.

B) Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) by broth dilution method (Jayana *et al.*, 2010):

Each dilution of the essential oils (100 µl) was inoculated by 100 µl from the previously prepared inoculums broth and then incubated at 37° C for 24 h. Then, after incubation the turbidity of tubes regarded and specific media was inoculated from the previous tubes to detect growth.

2. *In vivo*:

Analysis of the antibacterial activity in cheese model was applied by manufacturing of Tallaga cheese according to Abd El-khalek *et al.* (2016). The prepared CEO, REO and TEO were added during the cheese manufacture according to the obtained results that based on measurement of inhibition zone diameter formed around the well.

Tallaga cheese manufacture:

Firstly, buffalo's milk was obtained and standardized to have 5% fat then pasteurized at 72° C for 15 sec and rapidly cooled to 38-40˚ C. Afterward, calcium chloride (0.02%), sodium chloride (4%) and commercial rennet (0.05%) were added to the standardized milk. At the same time, the bacterial inoculum and the EOs were prepared. The manufactured cheese were grouped as: 1st group as negative control which free from oils and pathogenic bacteria under study; 2nd group as positive control for oils only without any pathogenic bacteria (one concentration from each oil according to the vitro study for organoleptic properties examination); 3rd group as positive control for pathogenic bacteria only; and 4th group for *L. monocytogenes* and the oils (each oil separately).

RESULTS

Table 1: Incidence of *Listeria spp*.in the examined cheese samples.

Samples	No. of the examined samples	The positive samples	
		No.	%
Tallaga cheese	75	46	61.3
Bramily cheese	75	55	73.3
Ras cheese	75	48	64
Total	225	149	66.2

Table 2: Frequency distribution of *Listeria spp*. in the examined cheese samples

Listeria spp.	The examined samples					
	Tallaga cheese		Bramily cheese		Ras cheese	
	No./46	%	No./55	%	No./48	%
L.monocytogenes	4	8.7	7	12.7	6	12.5
L.ivanovii	13	28.3	21	38.2	14	29.2
L.innocua	18	39.1	22	40	21	43.8
L.seeligeri	6	13	4	7.3	4	8.3
L.welshimeri	4	8.7	1	1.8	2	4.2
L.grayi	1	2.2	-	-	1	2.1

Table 3: Incidence of *L. monocytogenes* in the examined cheeses samples.

Samples	No. of the examined samples	The positive samples	
		No.	%
Tallaga cheese	75	4	5.3
Bramily cheese	75	7	9.3
Ras cheese	75	6	8
Total	225	17	7.6

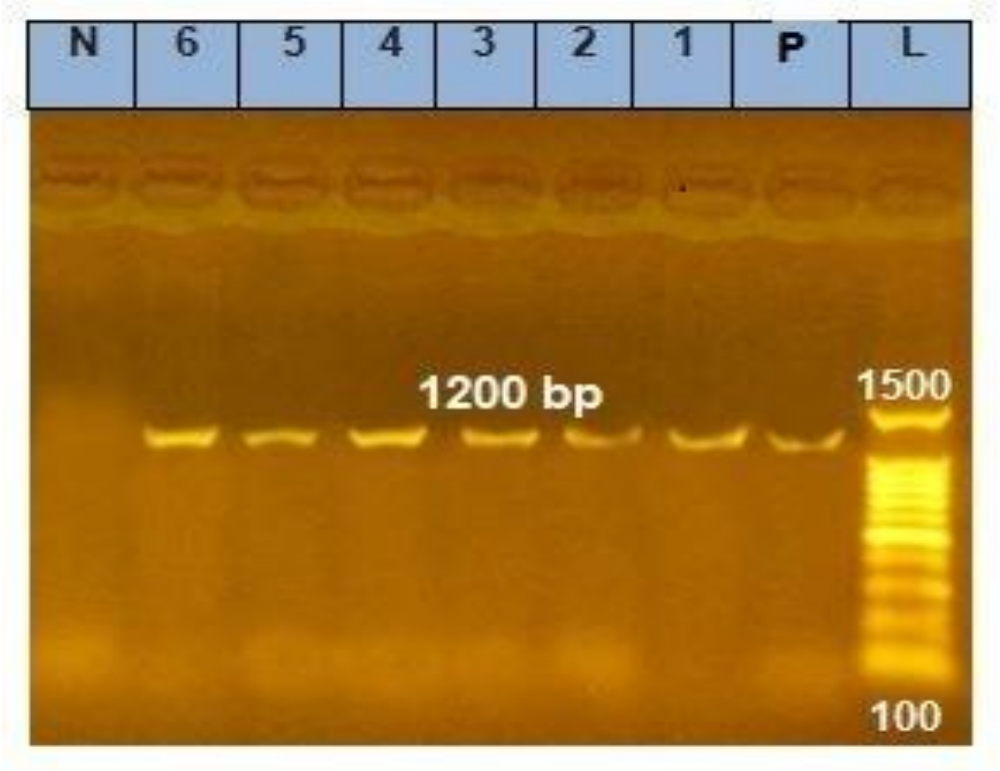

Photo 1: Agrose gel electrophoresis of PCR for 16S rRNA of *L. monocytogenes* isolated from the examined cheese samples

Table 4: MIC of CEO, REO and TEO on *L. monocytogenes* by agar well diffusion method (zone of inhibition) and tube dilution method.

Conc./ well	Zone of inhibition (mm)			Tube dilution		
	cinnamon	rosemary	thyme	cinnamon	rosemary	thyme
100%	36	23	22	-ve	-ve	-ve
50%	33	20	20	-ve	-ve	-ve
25%	30	18	19	-ve	-ve	-ve
12.5%	28	17	18	-ve	-ve	-ve
6.25%	25	15	16	-ve	-ve	-ve
3.125%	24	14	14	-ve	-ve	-ve
1.56%	17	no zone	no zone	-ve	+ve	+ve
0.78%	no zone	no zone	no zone	+ve	+ve	+ve

Table 5: The effect of CEO, REO and TEO on *L. monocytogenes* in Tallaga cheese (log_{10})

Storage period	Negative control	Positive control	Oil concentrations		
			cinnamon 1.56%	rosemary 3.125%	thyme 3.125%
	count	count	count	count	count
0 h	-ve			6.3	
After curdling	-ve	6.4	4.5	4	4.6
Reduction %	-	-	29.7	37.5	28.1
During 1st week	-ve	8.3	2.7	2.8	3.4
Reduction%	-	-	67.5	66.2	59.03

Table 6: Sensory evaluation of Tallaga cheese treated with CEO, REO and TEO.

Treatment	Flavor (aroma & taste)	Body & texture	Color	Over all acceptability (OAA)
Control	7	8	8	8
1.56% Cinnamon	unacceptable	7	7	unacceptable
3.125% Rosemary	unacceptable	7	8	unacceptable
3.125% Thyme	unacceptable	7	8	unacceptable

DISCUSSION

Realizing the data outlined in Table 1, it was evident that 66.2% of the examined cheese samples were contaminated with *Listeria spp*. The obtained result was higher than El-Naenaeey *et al.* (2019) as 19%, Albastami *et al.* (2020) as 20%, Labib (2021) as 22%. The frequency of distribution of the *Listeria spp*. was clear in Table 2. While, the prevalence rate of *L. monocytogenes* was clarified in Table 3, in which, 17 out of the 225 cheese samples were positive for *L. monocytogenes* with a percentage of 7.6%.

It was clear in the current study that the incidence of *L. monocytogenes* was more in the examined Bramily cheese followed by the Ras and Tallaga cheeses; this may be attributed to Bramily cheese is known to make from just warmed milk, in addition bad storage play an important role in the growth and multiplication of *L. monocytogenes*. Zarei *et al.* (2012) paraphrased that soft cheese provide appropriate growth conditions for Listeria because of its psychrotropic and halotolerant nature and its ability to survive in the presence of 1-13% NaCl but also because they are commonly consumed without cooking or heating to decrease contamination during ripening period.

The isolated *L. monocytogenes* was confirmed by PCR (Photo 1), which is a rapid method with high sensitivity and specificity for specific DNA sequences and permits direct detection of the pathogens (Lotfollahi *et al.*, 2011). The extraction of genomic DNA from biochemically-suspected *L. monocytogenes* isolates was carried out using the QIA amp DNA Mini kit (Qiagen GmbH, Germany). The extracted DNA was subjected to PCR using primers specific for 16Sr RNA for the amplification at1200 bp that appeared all tested samples were positive (Kumar *et al.*, 2015).

The antibacterial effect of CEO on *L. monocytogenes* was shown in Table 4, with average zone of inhibitions 36, 33, 30, 28, 25, 24 and 17 mm, for REO were 23, 20, 18, 17, 15 and 14 mm and for TEO were 22, 20, 19, 18, 16 and 14 mm for concentrations 100, 50, 25, 12.5, 6.25, 3.125 and 1.56%, respectively. It was regard the MIC for CEO was 1.56%, while for REO and TEO was 3.125%. This result was higher than that detected by Smith-Palmer *et al.* (2001), as 1% CEO was more effective in reduction of *L. monocytogenes*; also the anti listerial activity was also detected by Shan *et al.* (2011), Tayel *et al.* (2015), Souza *et al.* (2016) for CEO. While for REO, Silva *et al.* (2013) detected higher MIC values. The bacteriostatic and anti-proliferative action of these EOs against these pathogenic bacteria was probably exerted through their bioactive phenolics compound.

When through the light on Table 5, Tallaga cheese was manufactured and inoculated with the detected MIC in vitro as following 1.56% for CEO and 3.125% for REO and TEO. These concentrations revealed good result in reduction the count of *L. monocytogenes* during the 1st week. The more effect of cinnamon is due to their compounds, mainly trans-cinnamaldehyde, have been reported to inhibit bacteria by damaging cell

membrane, altering the lipid profile lead to leakage of small ions, inhibit the enzymes necessary for amino acid biosynthesis (Tiwari *et al.*, 2009). Besides, inhibiting ATPases, decrease in the intracellular ATP and increase extracellular ATP (Negi, 2012), and inhibit the cell division and biofilm formation (Vasconcelos *et al.*, 2018).

For Table 6, the sensory quality of the manufactured cheese with the tested concentrations of the detected MIC revealed unpleasant flavor with unacceptable OAA for the 3 EOs, and that is due to the strong flavor of the EOs, while body, texture and color were of good sensory acceptability.

In conclusion, *L. monocytogenes* was isolated from all the examined cheese samples with high percentage from the Bramily cheese. Furthermore, the count of *L. monocytogenes* was affected by addition of 1.56% CEO and 3.125% for REO and TEO, but the strong flavor of EOs leads to unacceptability sensory quality. Therefore, further studies should be applied to achieve the most acceptable sensory concentration of EOs that also inhibits *L. monocytogenes*.

REFERENCES

Abd El-khalek, A.B.; Mohamed, H.S.S.; Mohamed, T.F. and Kassem, Jihan M. (2016): Tallaga Cheese as a New Functional Dairy Product. American J. Food Technology, 11 (5):182–192.

Abou-Donia, S.A. (1986): Egyptian Domiati soft white pickled cheese, Review. New Zealand J. Dairy Sci. and Tech. 21:167-190.

Albastami, I.; Wajiej, A. and Aburagaegah, S. (2020): Microbiological study on *Listeria* spp. isolated from some food products of animal origin. Damanhour J. Vet. Sci. 4 (1):15-19.

APHA (2004): Standard methods for the examination of dairy products. 17th Ed., American Public Health Association Inc., Washington, DC., USA., ISBN-13: 978-0875530024, Pages: 570.

Brooks, J.C.; Martinez, B.; Stratton, J.; Bianchini, A.; Krokstrom, R. and Hutkins, R. (2012): Survey of raw milk cheeses for microbiological quality and prevalence of foodborne pathogens. Food Microbiol. 31:154-158.

Dabiza, N. and El-Deib, K. (2007): Biochemical evaluation and microbial quality of Ras cheese supplemented with probiotic strains. Pol. J. Food Nutr. Sci. 57:255-300.

El-Naenaeey, El-Sayed, Y.M.; Abdel-Wahab, Ashrasf, M.O.; Abdou, Hadeer, M.A. and Merwad, Abdallah, M.A. (2019): Prevalence of *Listeria spp*. in dairy cows and pregnant women with reference to virulotyping of *L. monocytogenes* in Egypt. Zagazig Vet. J. 47 (3): 248-258.

Elsherif, M.W. and Ali, N.D. (2019): Antibacterial effect of silver nanoparticles on antibiotic resistant *E. coli* O157:H7 isolated from some dairy products. Bulgarian J. Veterinary Medicine, ISSN 1311-1477; DOI: 10.15547/bjvm.2019-0027.

Gandhi, M. and Chikindas, M.L. (2007): Listeria a foodborne pathogen that knows how to survive. Int. J. Food Microbiol. 113:1-15.

Gouvea, F.d.S.; Amauri, R. and Elisa, H.d.R.F. (2017): Plant Extract and Essential Oils Added as Antimicrobials to Cheeses: A Review. Ciência Rural J. 47 (8).

Gutierrez, J.; Barry-Ryan, C. and Bourke, P. (2009): Antimicrobial activity of plant essential oils using food model media: Efficacy, synergistic potential and interactions with food components. Food Microbiol. 26 (2):142–150.

Gutiérrez-del-Río; Ignacio; Javier, F. and Felipe, L. (2018): Plant nutraceuticals as antimicrobial agents in food preservation: Terpenoids,

polyphenols and thiols. International J. Antimicrobial Agents, 52 (3): 309–315.

Hattem, H.E.; Taleb, A.T.; Manal, A.N. and Hanaa, S.S. (2012): Effect of pasteurization and season on milk composition and ripening of Ras cheese. J. Brew Distill. 3:15-22.

Hitchins, A.D.; Jinneman, K. and Chen, Y. (2017): Bacteriological Analytical Manual. 8th ed., Chapter 10: Detection of *L. monocytogenes* in foods and environmental samples and enumeration of L. monocytogenes in foods.

Ismail, M.M. (2004): Evaluation of methods used for examination of Domiati cheese. The 9th Egyptian Conference for Dairy Science & Technology "Milk and Dairy Products for a Healthy Future", Dokki, Cairo, Egypt, 9-11 October 2004.1:6.

Jayana, B.L.; Prasai, T.; Singh, A. and Yami, K.D. (2010): Study of antimicrobial activity of lime juice against *Vibrio Cholerae*. Sci. World, 8:44-46.

Kim, H.; Song, E.B.; So, Y.Y. and Kyung, B.S. (2018): Application of an Antimicrobial Packaging Material from Chicken Bone Gelatine and Cinnamon Bark Oil to Mozzarella Cheese. International J. Food Science and Technology, 53 (3):619–625.

Kumar, A.; Grover, S. and Batish, V.K. (2015): Exploring specific primers targeted against different genes for a multiplex PCR for detection of *Listeria monocytogenes*. Biotech. 5:261–269.

Labib, Mariana I. (2021): Listeria species in raw milk and some locally manufactured milk products, Ph.D. Thesis, Fac. Vet. Med. Assiut Uni. Egypt.

Liu, Q.; Xiao, M.; Ya, L.; Cai, N.Z.; Guo, Y.T. and Hua, B.L. (2017): Antibacterial and Antifungal Activities of Spices. International J. Molecular Sciences, 18 (6):1–62.

Lorber, B.; Mandell, G.L.; Bennett, J.E.; Dolin, R.; Mandell; Douglas and Bennett's (2005): Listeria monocytogenes. In Principles and Practice of Infectious Diseases. 6th ed. Philadelphia: Elsevier, Churchill Living-stone, 2478–83.

Lotfollahi, L.; Nowrouzi, J.; Irajian, G.; Masjedian, F.; Kazemi, B.; Eslamian, L.; Falahat, A. and Ramez, M. (2011): Prevalence and antimicrobial resistance profiles of *L. monocytogenes* in spontaneous abortions in humans. Afr. J. Microbiol. Res. 5 (14): 1990–1993.

MacFaddin, J.F. (2000): Biochemical tests for identification medical bacteria. Warery Press Inc, Baltimore, Md. 21202 USA.

Mahmoudi, R.; Gajarbeygi, P.; Mahmodzadeh, F.; Hassanzadeh, M.; Kiyani, R. and Nadari, M.R.A. (2014): Quality of yogurt blended with thymus kotschyanus essential oil. Malaysian J. Science, 33 (2):176-182.

Mehanna, N.M. and Rashed, M.A. (1990): An attempt to improve the keeping quality of tallaga cheese by using milk treated with carbon dioxide. Egyptian J. Dairy Sci. 18: 377–388.

Melo, A.; Diego, B.O.; Amanda, F.A.; Gustavo, S.F.B.L.; Carla, d.A.L.B.C. and Marcos, H.R. (2015): Antimicrobial effect against different bacterial strains and bacterial adaptation to essential oils used as feed additives. Canadian J. Veterinary Research, 79 (4):285–289.

Negi, P.S. (2012): Plant extracts for the control of bacterial growth: efficacy, stability and safety issues for food application. International J. Food Microbiol. 156 (1): 7–17.

Pimentel-Filho, N.J.; Mantovani, H.C.; Carvalho, A.F.; Dias, R. S. and Vanetti, M.C.D. (2014): Efficacy of bovicin HC5 and nisin combination against *Listeria monocytogenes* and *Staphylococcus aureus* in fresh

cheese. Int. J. Food Sci. Technol. 49:416-422.

Quero, G.M.; Santovito, E.; Visconti, A. and Fusco, V. (2014): Quantitative detection of *Listeria monocytogenes* in raw milk and soft cheeses: culture-independent versus liquid and solid-based culture-dependent real time PCR approaches. LWT Food Sci. Technol. 28:11-20.

Ribeiro-Santos, R.; Ventura, L.A.F.; Santos, C.D.C.; Melo, N.R. and Costa, B.S. (2018): Effects of Oregano, Cinnamon, and Sweet Fennel Essential Oils and Their Blends on Foodborne Microorganisms. International Food Research J. 25 (2):540–544.

Saad, A.H.; Elkosy, O.; Ehab, S.; Ismail, J. and Abd AlGwad, A. (2019): Organoleptic, microbiological, and sanitary evaluation of Tallaga cheese biopreserved by oregano essential oil. 18th Sci. Cong. 2019, Fac. Vet. Med., Assiut Univ., Egypt, page 15.

Shan, B.; Yi-Zhong, C.; John, D.B. and Harold, C. (2011): Potential application of spice and herb extracts as natural preservatives in cheese. J. Medicinal Food, 14 (3): 284–290.

Silva, N.; Sofia, A.; Alexandre, G.; Joana, S.A. and Patrícia, P. (2013): Antimicrobial activity of essential oils from Mediterranean aromatic plants against several foodborne and spoilage bacteria. Food Science and Technology International, 19 (6): 503–510.

Smith-Palmer, A.; Stewart, J. and Fyfe, L. (2001): The potential application of plant essential oils as natural food preservatives in soft cheese. Food Microbiol. 18(4): 463–470.

Souza, G.T.D.; Rayssa, J.D.C.; Jossana, P.D.S.; Josean, F.T.; Donald, S.; Evandro, L.D.S. and Marciane, M. (2016): Effects of the essential oil from *Origanum Vulgare L.* on survival of pathogenic bacteria and starter lactic acid bacteria in semihard cheese broth and slurry. J. Food Prot. 79 (2): 246–252.

Tayel, A.A.; Heba, H.; Noha, M. Sorour and Wael F.E.T. (2015): Foodborne pathogens prevention and sensory attributes enhancement in processed cheese via flavoring with plant extracts. J. Food Science, 80 (12): 886–891.

Tiwari, B.K.; Vasilis, P.V.; Colm, P.O.; Kasiviswanathan, M.; Paula, B. and Cullen, P.J. (2009): Application of natural antimicrobials for food preservation. J. Agricultural and Food Chemistry, 57 (14): 5987–6000.

Vasconcelos, N.G.; Croda, J. and Simionatto, S. (2018): Antibacterial mechanisms of cinnamon and its constituents. A review, Microbial Pathogenesis J. 120: 198–203.

Wiegand, I.; Hilpert, K. and Hancock, R.W.E. (2008): Agar and Broth Dilution Methods to Determine the Minimal Inhibitory Concentration (MIC) of Antimicrobial Substances. Nature Protocols J. 3: 163.

Zarei, M.; Borujeni, P.M. and Khezrzadeh, M. (2012): Comparing the effect of NaCl and KCl on the growth of *L. monocytogenes* with a view to NaCl replacement. Iranian J. Vet. Res. 13(2):147-151.

تأثير الزيوت العطرية على حيوية الليستريا مونوسيتوجينز

ماري إسحق ؛ ولاء محمود الشريف ؛ محمد سيد

Email: dr.marysobhy@gmail.com Assiut University web-site: www.aun.edu.eg

تم تطبيق هذا البحث لدراسة تأثير الزيوت العطرية للقرفة وإكليل الجبل والزعتر على حيوية الليستريا مونوسيتوجينز، حيث تم جمع 225 عينة من الجبن (75 لكل من الجبن البراميلي والجبن الثلاجة والجبن الراس) من الأسواق ومحلات الألبان المختلفة بمدينة أسيوط، مصر، وذلك لعزل الليستريا مونوسيتوجينز. وقد تم التعرف على العزلات وفحصها لـ 16S rRNA وكانت موجبة لليستريا مونوسيتوجينز. وبالنسبة للنتائج التي تم الحصول عليها، فقد أظهرت أن الليستريا قد وجدت في 66.2٪ من العينات المفحوصة بينما الليستريا مونوسيتوجينز كانت في 17.6٪. وبعد ذلك، تم الكشف عن الحد الأدنى للتركيز المثبط (MIC) لزيوت القرفة وإكليل الجبل والزعتر ضد الليستريا مونوسيتوجينز المعزولة. وقد تم تصنيع عينات من الجبن الثلاجة وبإستخدام MIC من الثلاث زيوت العطرية المحضرة وبشكل منفصل لكل زيت، وقد لوحظ تأثير الزيوت العطرية بواسطة طريقة الإنتشار بالأجار وأظهر MIC 1.56٪ لزيت القرفة، 3.125٪ لزيوت إكليل الجبل والزعتر. ونستنتج من الدراسة الحالية أن زيت القرفة هو الأكثر فاعلية ضد الليستريا مونوسيتوجينز بعد تصنيع الجبن الثلاجة على الرغم من الجودة الحسية غير المقبولة للجبن المصنع بالثلاثة زيوت، حيث كان عدد الليستريا مونوسيتوجينز 6.3 لوغاريتم$_{10}$ عند التصنيع ثم كان 2.7 لوغاريتم$_{10}$ بعد الأسبوع الأول.