BACTERIAL EMPIRE
2020, VOL. 3, NO. 1, 5-9

OPEN ACCESS
SCICELL

REGULAR ARTICLE

OCCURRENCE AND GENETIC IDENTIFICATION OF *ARCOBACTER BUTZLERI* IN DIFFERENT CHEESE TYPES

Manal Mohamed Amin[1], *Walaa Mahmoud Elsherif* *[1] *and Usama Ghareeb Awaad*[2]

Address (es):
[1]Animal Health Research Institute, Assiut Branch Egypt.
[2] Army organization, Egypt

*Corresponding author: sch_qana@yahoo.com

ABSTRACT

The objectives of this study were to investigate the presence of foodborne pathogen *Arcobacter butzleri (A. butzleri*) in different types of cheese (Talaga, Mozzarella and Roumy cheese - 30 each) retailed in Assiut dairy shops, Egypt and to test the antimicrobial effect of thyme (*Thymus vulgaris*) and its essential oil (EO) against isolated strains. The highest percentage of *A. butzleri* existence was noticed in Talaga cheese samples and confirmed by PCR using *A. butzleri. 16S rRNA* gene which at the same time, the existence of it in Roumy cheese after detected biochemically. To exam the effect of *Thymus vulgaris* and its EO on *A. butzleri*, were added at 2 and 4% concentratins during cheese manufacturing with addition of *A. butzleri* at 10^5 and the consumer accepatability was studied. The plant could prohibit the growth of organism in cheese nearly at 6^{th} day. These results suggest that 2% of thyme plant have sufficient effect on *A. butzleri* with good consumer satisfaction so, it is recommended that e thyme should be used as natural food additive in dairy product in Egypt factories.

Keywords: *Arcobacter butzleri; Thymus vulgaris;* Talaga; Mozzarella and Roumy cheese

INTRODUCTION

Arcobacter spp. considered as emerging food- and waterborne zoonotic pathogens (**Hänel *et al.*, 2016**), aero-tolerant campylobacters' (**Levican and Figueras, 2013**). It's ability to grow between 15 to 30 °C temperature aerobically and need of microaerophilic condition for primary isolation (**Ferreira *et al.*, 2016**), are nearly similar to *Campylobacter* spp. in biochemical reaction which complicates their phenotypic differentiation. Hence, polymerase chain reaction (PCR)-based methods are more commonly used for specific detection and identification purposes (**Douidah *et al.*, 2010**) and currently *Arcobacter* spp. includes 21 species (**Giacometti *et al.*, 2015**). Among these species, *A. butzleri*, *A. cryaerophilus* (with two subgroups) and *A. skirrowii* which classified as serious hazards to human health by the International Commission on Microbiological Specifications for Foods (ICMSF) (ICMSF, 2002) as it associated with various illnesses such as gastroenteritis, abdominal pain, nausea, vomiting, bacteremia and sepsis in humans, mastitis, diarrhea, abortion, and other reproductive disorders in animals (**Vanderberg *et al.*, 2004; D'Sa and Harrison, 2005 and Girbau *et al.*, 2015)**.

In the Mediterranean countries, *A. butzleri* is a widespread in raw milk cheese production, including, particularly soft cheese (**Serraino and Giacometti, 2014**) and the organism also survived during processing and storage of water buffalo mozzarella cheese, fresh village cheese and sheep ricotta cheese **(Serraino *et al.*, 2013)**. The ability of this microorganism to survive in food products and water strengthened by its resistance to stress created during food storage and processing (**Ferreira *et al.*, 2019**), biofilms formation (**Assanta et al., 2002**) and can survive in pipe and food-processing surfaces (**Douidah *et al.*, 2010**). So, dairy researchers have found that selected plant Essential Oils (EO) can act as inhibitors of spoilage microorganisms in food products (**Smith *et al.*, 2001 and Conte *et al.*, 2007**) Especially with increasing the reports of resistance to current antibiotic employed in treatment of *Arcobacter* related infections there is need to develop new or alternative antimicrobial agents effective against it (**Smith *et al.*, 2003**).

The thyme plants belongs to the family of Labiatae (**Selmi and Sadok, 2008**) and EO or plant extracts originating from common or garden thyme (*Thymus vulgaris*) (**Wiese *et al.*, 2018**) is utilized as a flavor enhancer in a wide variety of foods, beverages and confectionery products (**Boskovic *et al.*, 2013**). It possesses some antiseptic, antispasmodic and antimicrobial properties that make it popular as a medicinal herb and as a preservative for foods (**Cosentino *et al.*, 1999**).

So, in this study focused on isolation of *A. butzleri* from different types of cheeses retailed in Assiut city markets, Egypt, with studying the effect of *Thymus vulgaris* and its EO at different concentrations on isolated *A. butzleri* .

MATERIAL AND METHODS

Sample collection

A total of 90 samples including random samples of different types of cheese soft cheese (Talaga cheese), semisoft (Mozzarella cheese) and hard one (Roumy cheese) 30 each, were collected from different markets and dairy shops in Assiut City, Egypt. The samples were collected in package as marketed to the consumer and sent to the laboratory in an insulated box with a minimum of delay to be examined.

Isolation of A. butzleri

The samples were homogenized by stomacher and prepared for Arcobacter isolation by taking 25 g of these samples and aseptically inoculated in a 1:10 ratio in Arcobacter enrichment broth (oxiod, UK) supplemented with Cefoperazone, Amphotericin B and Teicoplanin (CAT) selective supplement (SR0174, Oxiod, Uk), then incubated at 30°C under microaerophilic condition for 48 h. (Mottola et al., 2016). Then streaked onto Arcobacter selective media supplemented with 5% sheep blood and with CAT (Oxiod, Uk), the agar plates were incubated for 48 h at 30°C and samples of no growth were incubated for another 48h. (Aydin et al., 2007 and Ramees et al., 2014). Subsequently, presumptive Arcobacter colonies (small colourless, translucent, convex with an entire edge) were picked, sub-cultured onto blood agar and incubated at 30°C for 48h. Purified isolates were further confirmed morphologically by Gram staining and biochemical analysis (catalase, oxidase, urease tests and motility, indoxyl acetate hydrolysis, salt tolerance and growth on McConkey agar). The isolates referable at Arcobacter genus (Gram negative, spiral shaped, motile, oxidase and catalase positive, urease negative), were stored in 20% (v/v) nutrient broth glycerol at −80°C, after molecular identification (Ferreira et al., 2016 and Salas-Masso et al., 2016)

PCR confirmation

This part was done in molecular biology department (authorized by EGAC, ISO17025:2017) at Animal Health Research Institute, Dokki, Giza, Egypt.

The isolates were performed using the QIAamp DNA Mini kit (Catalogue no.51304, Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. The spin-column procedure does not require mechanical homogenization, so total hands-on preparation time is only 20 minutes. Primers used were supplied from Metabion (Germany) are listed in table (I).

Table (I): Primers, sequences, target genes, and amplicon sizes

Target gene	Primer sequence (5'-3')	Length of amplified product	Reference
Arcobacter butzleri. 16S rRNA	CGTATTCACCGTAGCATAGC CCTGGACTTGACATAGTAAGAATGA	401 bp	**Lehmann *et al.*, (2015)**

Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 picomole concentrations, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied bio-system 2720 thermal cycler table (II).

Table (II): thermo cycling conditions

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
Arcobacter butzleri. 16S rRNA	94˚C 5 min.	94˚C 30 sec.	61˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.

Analysis of the PCR Products.

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the products was loaded in each gel slot. Gelpilot 100 bp ladder (cat. no. SM0243) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Determination of anti-*A. butzleri* activity of the Thymus vulgaris plants and its extraction

Thymus vulgaris used in this research was obtained from Plant Department, Faculty of Agriculture, Al Azhar University, Assiut branch, Egypt. The plant was washed, dried, and ground in a mortar to be used in Talaga cheese preparation. The alcohol extraction was done by maceration in 70% alcohol for 24 hours. This process was repeated three times. The combined alcoholic EO was filtered and then evaporated under reduced pressure at a temperature not exceeding 50ºC until a semisolid residue was obtained **(Ibraheim and Boulatova, 2002).**

Preparation of *A. butzleri* standard inoculum.

A. butzleri subcultures were first prepared from the stock cultures on Brain Heart Infusion (BHI) agar supplemented with 5% yeasts and 7% sheep blood. BHI agar plates were incubated at 37 ºC in microaerophilic atmosphere (Vandamme et al., 1991). Arcobacter inoculum was prepared by collecting bacterial colonies from BHI agar plates at the exponential growth phase and diluting in 0.85% saline. The resulting bacterial suspension was then standardized by McFarland nephelometry to 10^5 CFU/ml. (**Adesiji *et al.*, 2012**)

Manufacture and treatment of Talaga cheese.

Talaga cheese was prepared from whole raw milk that was pasteurized at 63ºC for 30 minutes. The inoculated milk was salted to a concentration of 5%. Rennet was added, the milk was divided into five equal portions, and each was subjected to the following treatments: two portions for addition of 2% and 4% Thymus vulgaris and another two portions for its EO and 5th portion as a control block (free from *A. butzleri*). The treated milk and the control one were incubated at 30ºC for overnight until coagulation and cheese was obtained. Treated cheese as well as control samples were stored at refrigeration temperature (4±2 ºC). Counts were calculated from the finished cheese after curdling, first, second day and every 3 days for *A. butzleri* count and pH measurement.

Sensory evaluation of Talaga cheese manufactured.

Talaga cheese was prepared as previously mentioned and divided into 5 equal portions; each was subjected to the previous treatments (without adding *A. butzleri*). Samples were stored at refrigeration temperature (4 ± 2 ºC). Twenty-three consumers were selected in teams of different ages, sex and education to taste the samples. The perception of consumers toward cheese with various treatments was studied with respect to three different attributes (flavor, appearance and palatability). The level of agreement was scored as strongly agree (SA), agree (A), disagree (D), and strongly disagree (SD) **(Nelson and Torut, 1981**).

Statistical Analysis

The statistical analysis was performed using programs GraphPadPrism 5.04 (GraphPad, Inc., San Diego, USA) and Statistical 12.0 (Dell, Inc., Tulsa, USA). Least significant differences were used at $p < 0.05$. The data represented by using the Microsoft Excel Spreadsheet.

RESULTS AND DISCUSSION

A.butzleri is considered as one of the most important foodborne pathogen causing sever gastrointestinal disease, with persistent diarrhea in human (**Collado and Figueras, 2011**) and in dairy chain could be isolated from fecal samples of dairy animals (**Shah *et al.*, 2013**), in-line milk filters (**Serraino *et al.*, 2013**), cow and water buffalo milk (**Yesilmen *et al.*, 2014**) and from different localities in dairy industry (**Serraino and Giacometti, 2014**).
In this study soft (Talaga), semisoft (Mozzarella) and hard (Roumy) cheeses examined for existence of *A.butzleri* and could be isolated in percentages of 16.67, 10 and 6.67%, respectively (**Figure 1**). From a previous study, in the same city, *A.butzleri* could be isolated in different percentages from food samples collected from Assiut city, Egypt (6.67%) by **Elsherif and Amin (2012)** and (5%) by **Ammar and AL-Habaty (2015).** Also, confirmed the ability to contaminate the cheese processing plants (**Ferreira *et al.*, 2019**) through, its ability to survival for long time and good growth in milk **(Giacometti *et al.*, 2014),** food surfaces, instruments (**Ferreira *et al.*, 2019**), resistant to several substrates **(D'Sa and Harrison, 2005)** and able to tolerate sodium hypochlorite concentrations close to working solutions used for sanitizing in food processing plants **(Rasmussen *et al*., 2013).**

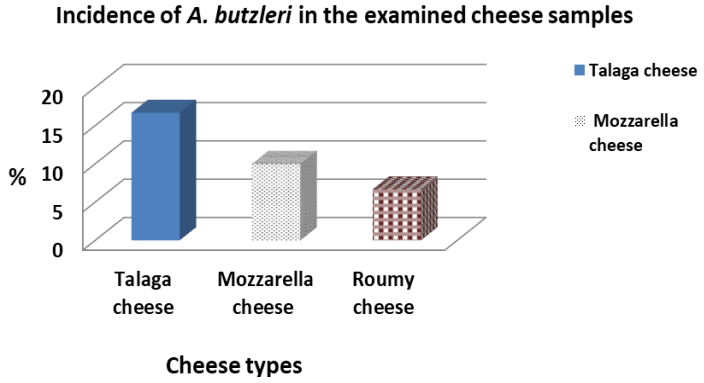


Figure 1 Percentages of isolation of *A. butzleri* from different types of cheese samples (n=30 for each)

The differentiation of *Arcobacter* species from related organisms using laboratory biochemical reaction after selective culturing may be performed the purpose, but these techniques are cumbersome to perform, time-consuming and

highly limited in specificity that because its relatively biochemically inert and morphologically similar to campylobacters, factors that may contribute to incorrect detection and identification of these organisms when relying on agar plating or phenotypic tests **(Prouzet-Mauleon *et al.* 2006)**. In view of culture failure and misidentification, nucleic acid approaches, particularly PCR-based methods, are increasingly being considered for detection, identification, and monitoring of arcobacters in foods (**Prouzet-Maule´on *et al.*, 2006 and Gonza´lez *et al.*, 2007**). So, as shown in **Figure** (2) *A.butzleri* isolates could be confirmed by using *16S rRNA* in Talaga (2 isolates) and Mozzarella (1 isolate) cheese samples. A study included detection using culturing and molecular method in parallel reported that 1.4%of the samples positive by culturing, and 0.7% by molecular detection (**Collado and Figueras, 2011**).

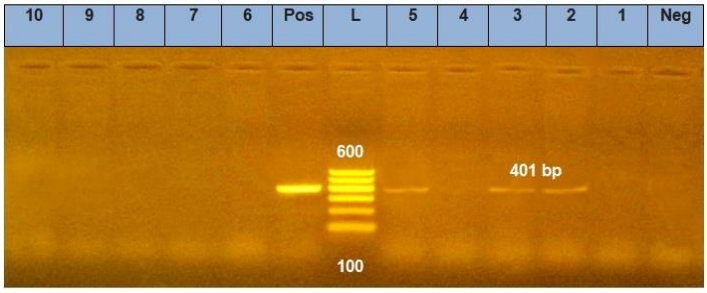


Figure 2 The amplified 16S rRNA gene of *A. butzleri* recovered from different types of cheese samples.
Lane L: Molecular marker; **Lane pos.:** Positive control; **Lane Neg.**: Negative control; **Lanes 1, 4,7-10**: negative isolates; **Lane 2, 3, 5**: positive isolates (Talaga and Mozzarella cheese samples).

The use of herbal and its extracts as alternative medicine, natural therapies (**Adesiji *et al.*, 2012**), food additives and as food preservatives has been documented for ages especially with distribution of antibiotic resistant genes (**Satyanarayana *et al.*, 2004 and Gutierrez *et al.*, 2008**). Thyme oil and *Thymus vulgaris* are widely used as food relish in Egypt nowadays and in an ancient age in embalming (**Beth, 2013**). In this study, the antibacterial effect of *Thymus vulgaris* and its EO against *A.butzleri* was evaluated as showed **Figure** (3, 4), the plant can decrease the count of it throughout the storage time until became couldn't be detected at 10th day specially at concentration 4%, showing significance difference when use the thyme EO at 4% *A.butzleri* undetectable at 8th day. Subsequently, the count stabilized over the remaining period of storage in untreated cheese (positive control samples), a slight decrease in the count of *A.butzleri* was noted toward the end of the 12th day of storage. Thyme belongs to the family of Labiatae and as an aromatic agent is widely used in many cooked dishes, the antimicrobial mechanism of thyme and thyme extract is based on their ability to disintegrate the outer membrane of bacteria, releasing lipopolysaccharides, increasing the permeability of the cytoplasmic membrane to ATP (**Lacroix *et al.*, 1997; Lampert et al., 2001 and Justesen and Knuthsen, 2001**), antioxidants effect based essentially on polyphenolic compounds as flavonoids (**Selmi and Sadok, 2008**). It is also well known that essential oil of this plant is a rich source of thymol and carvacrol which has been reported to possess a high antioxidant activity. Such, essential oils degrade the cell wall, interact with the composition, disrupt cytoplasmic membrane (**Lampert *et al.*, 2001**), damage membrane protein, interfere with membrane-integrated enzymes, cause leakage of cellular components, coagulate cytoplasm, and influence the synthesis of DNA and RNA (**Tannguchi *et al.*, 1988 and Rauha *et al.*, 2000**). Therefore, it is necessary to investigate further to understand the relationship between antibacterial activity and chemical structure of plants.

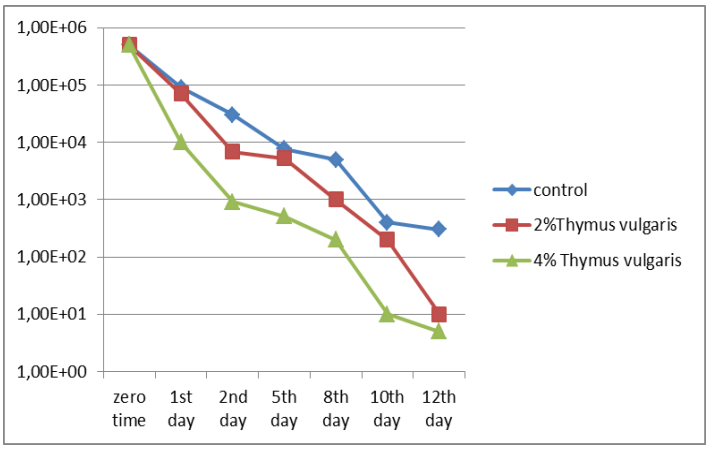


Figure 3 Effect of *Thymus vulgaris* at different concentrations on inoculated *A. butzleri* in manufactured Talaga cheese during refrigeration storage

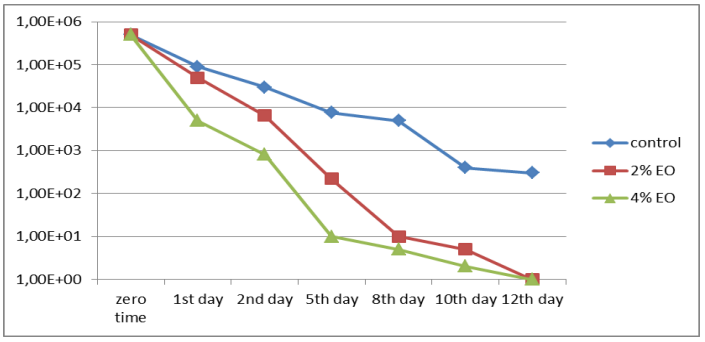
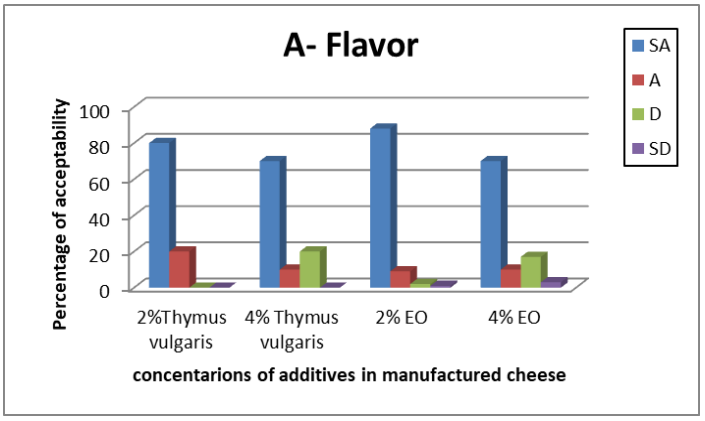
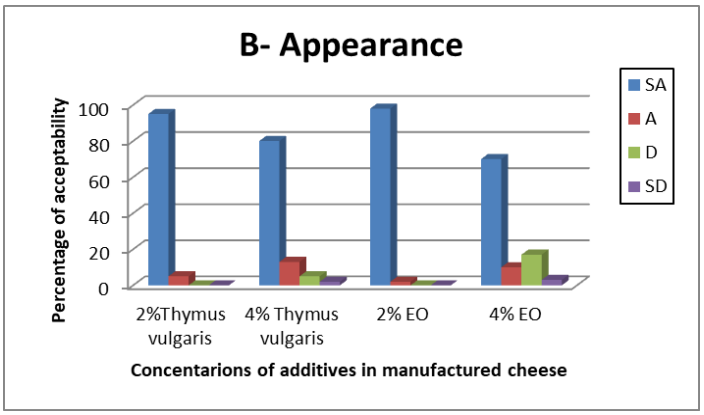


Figure 4 Effect of Thyme EO at different concentrations on inoculated *A. butzleri* in manufactured Talaga cheese during refrigeration storage
*Significance (P<0.05) Sig. difference between 4% EO and control P<0.003

Although the acceptability of consumer to cheese with 0, 2, 4% of thyme plant and its extract investigated based on inner and outer appearance, flavor, palatability and texture also, define the additives (**Figure 5)**. 80% were strongly agree to cheese with 2% *Thymus vulgaris*, 60% accept the palatability at 4% and 77, 65% for 2, 4% extract, respectively with no significant different between trials. These acceptance returned to that thyme plant considered as one of main spices in Egyptian kitchen so, its taste, flavor and palatability considered familiar but the difference in percentages depend on appearance and individual variations.





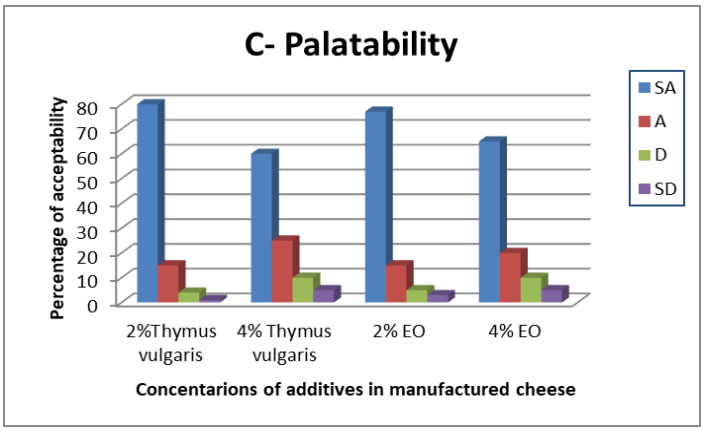


Figure 5 Sensory evaluation of manufactured Talaga cheese with different concentrations of additives according to flavor (A), appearance (B), and palatability (C).

* SA: Strongly Agree A: Agree D: Disagree SD: Strongly Disagree

CONCLUSION

The present study concluded that Thymus vulgaris and its extract have antibacterial activity against *A.butzleri*, which isolated from different types of cheese, in contrast that 4% of plant added to cheese have strongly effect on isolated strains with no really difference with 4% EO and achieved significant antimicrobial effect. Moreover 2% of Thymus vulgaris or its EO were mostly accepted to the consumers and so, it is recommended to add Thymus vulgaris in cheese, to improve the quality of product and increase the benefits from it.

REFERENCES

ADESIJI, Y. O., AKANNI, R. A., ADEFIOYE, O. A. AND TAIWO, S. S. 2012. *In vitro* antimicrobial activity of some plant extracts against *A. butzleri* and *A. cryaerophilus*. Acta Medica Lituanica. 19 (1): 23–29.

AMMAR, M.A.M. AND AL-HABATY, S.H. 2015. *Arcobacter* species and their risks in some meat and fish with a sodium acetate and sodium chloride intervention. Assiut Vet Med J. 61 (146).

ASSANTA, M. A., ROY, D., LEMAY, M. J. AND MONTPETIT, D. 2002. Attachment of *Arcobacter butzleri*, a new waterborne pathogen, to water distribution pipe surfaces. J Food Prot. 65:1240–1247.

AYDIN, F., GUMUSSOY, K. S., ATABAY, H. I., ICA, T. AND ABAY, S. 2007. Prevalence and distribution of *Arcobacter* species in various sources in Turkey and molecular analysis of isolated strains by ERIC-PCR. J Appl Microbiol. 103:27–35.

BETH, D. 2013. A Brief History of Thyme. Hung. Hist. http:// www.history.com /news/ hungry-history /a-brief-history-of-thyme

BOSKOVIC, M., BALTIC, Z. M., IVANOVIC, J., DJURIC, J., LONCINA, J., DOKMANOVIC, M. AND MARKOVIC, R. 2013. Use of essential oils in order to prevent foodborne illnesses caused by pathogens in meat. Tehn mesa. 54:14-20.

COLLADO, L. AND FIGUERAS, M. J. 2011. Taxonomy, epidemiology, and clinical relevance of the Genus *Arcobacter*. Clin Microbiol Rev. 24:174–192.

CONTE, A., SCROCCO, C., SINIGAGLIA, M. AND DEL NOBILE, M. A. 2007. Innovative active packaging system to prolong the shelf life of Mozzarella cheese. J. Dairy Sci. 90:2126–2131.

COSENTINO, S., TUBEERSO, C. I. G., PISANO, B., SATTA, M., MASCIA, V., ARZEDI, E. AND PALMAS, F. 1999. In-Vitro Antimicrobial Activity and Chemical Composition of Sardinian Thymus Essential Oils. Lett Appl Microbiol. 29:130–135.

D'SA, E. M. AND HARRISON, M. A. 2005. Effect of pH, NaCl content, and temperature on growth and survival of *Arcobacter* spp. J Food Prot. 68:18–25.

DOUIDAH, L., ZUTTER, L. D., VANDAMME, P. AND HOUF, K. 2010. Identification of five human and mammal associated Arcobacter species by a novel multiplex-PCR assay. J Microbiol Methods. 80:281–286.

ELSHERIF, WALAA M. A. AND MANAL M. AMIN. 2012. Isolation Of *Arcobacter* spp. - A Potential Food Borne Pathogen From Egg In Assiut City. Zag Vet J. (ISSN.1110-1458) 40 (5): 67-73.

FERREIRA, S., QUEIROZ, J. A., OLEASTRO, M. AND DOMINGUES, F. C. 2016. Insights in the pathogenesis and resistance of *Arcobacter*: a review. Crit Rev Microbiol. 42:364–383.

FERREIRA, S., OLEASTRO, M. AND DOMINGUES, F. 2019. Current insights on *Arcobacter butzleri* in food chain. Current Opinion in Food Science 26: 9-17.

GIACOMETTI, F., SALAS-MASS, N., SERRAINO, A., FIGUERAS, M. J. 2015. Characterization of *Arcobacter* suis isolated from water buffalo (*Bubalus bubalis*) milk. Food Microbiol. 51:186–191.

GIACOMETTI, F., SERRAINO, A., PASQUALI, F., DE CESARE, A., BONERBA, E. AND ROSMINI, R. 2014. Behavior of *Arcobacter butzleri* and *Arcobacter cryaerophilus* in ultrahigh-temperature, pasteurized, and raw cow's milk under different temperature conditions. Foodborne Pathog Dis. 11:15–20. http://dx.doi.org/10.1089/fpd.2013.1597.

GIRBAU, C., GUERRA, C., MARTINEZ-MALAXETXEBARRIA, I., ALONSO, R. AND FERNANDEZ-ASTORGA, A. 2015. Prevalence of ten putative virulence genes in the emerging foodborne pathogen Arcobacter isolated from food products. Food Microbiology. 52: 146-149. PMid:26338128. http://dx.doi.org/10.1016/j.fm.2015.07.015.

GONZA´LEZ, A., BOTELLA, S., MONTES, R. M., MORENO, Y. AND FERRU´S, M. A. 2007. Direct detection and identification of *Arcobacter* species by multiplex PCR in chicken and wastewater samples from Spain. J. Food Prot. 70:341–347.

GUTIERREZ, J., RODRIGUEZ, G., BARRY-RYAN, C. AND BOURKE, P. 2008. Efficacy of plant essential oils against foodborne to eat vegetables: antimicrobial and sensory screening. J Food Prot. 71:1846–1854.

HÄNEL, I., TOMASO, H. AND NEUBAUER, H. 2016. *Arcobacter* - an underestimated zoonotic pathogen? Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 59:789–794.

IBRAHEIM, Z. Z. AND BOULATOVA, N. R. 2002. Studies on the anti-inflamatory properties of parsley, dill and thyme on mice. In: 2002 Assiut University Third Pharmaceutical Science Conference, Assiut, Egypt, pp. 97–102.

ICMSF " International Commission on Microbiological Specifications for Foods". 2002. Microorganisms in Food. Microbiological testing in food safety management. International Commission on Microbiological Specifications for Foods. Kluwer Academic Publ., New York, NY, USA.

JUSTESEN, U. AND KNUTHSEN, P. 2001. Composition of flavonoids in fresh herbs and calculation of flavonoid intake by use of herbs in traditional Danish dishes. Food Chem. 73, 245-250.

LACROIX, M., SMORAGIEWICZ, W., PAZDERNIK, L., KONE, M.I. AND KRZYSTYNIAK K. 1997. Prevention of lipid radiolysis bynatural antioxidants from rosemary (*Rosmarinus officinalis L.*) and thyme (*Thymus vulgaris L.*). Food Res Int. 30, 457-462.

LAMPERT, R. J., SKANDAMIS, P. N., COOTE, P. AND NYCHAS, G. J. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacol. J Appl Microbiol. 91:453–462.

LEHMANN, D., ALTER, T., LEHMANN, L., UHERKOVA, S., SEIDLER, T. AND GÖLZ, G. 2015. Prevalence, virulence gene distribution and genetic diversity of *Arcobacter* in food samples in Germany. Berl Munch Tierarztl Wochenschr. 128(3-4):163-8.

LEVICAN, A. AND FIGUERAS, M. J. 2013. Performance of five molecular methods for monitoring *Arcobacter* spp. BMC Microbiol. 13:220.

MOTTOLA, A., BONERBA, E., BOZZO, G., MARCHETTI, P., CELANO, G. V., COLAO, V., TERIO, V., TANTILLO, G., FIGUERAS, M. J. AND PINTO, A. D.2016. Occurrence of emerging food-borne pathogenic *Arcobacter* spp. isolated from pre-cut (ready-to-eat) vegetables. Int J Food Microbiol. 236:33–37.

NELSON, J. A. AND TORUT, G. M. 1981. Judging Dairy Products, 4th edition revised. Westport, CT: The AVC.

PROUZET-MAULE´ON, V., LABADI, L., BOUGES, N., MENARD, A. AND MEGRAUD, F. 2006. *Arcobacter butzleri*: underestimated enteropathogen. Emerg Infect Dis. 12:307–309.

RAMEES, T. P., RATHORE, R. S., BAGALKOT, P. S., MOHAN, H. V., KUMAR, A., DHAMA, K. 2014. Detection of *Arcobacter butzleri* and *Arcobacter cryaerophilus* in clinical samples of humans and foods of animal origin by cultural and multiplex PCR based methods. Asian J Anim Vet Adv. 9:243–252.

RASMUSSEN, L. H., KJELDGAARD, J., CHRISTENSEN, J. P. AND INGMER, H. 2013. Multilocus sequence typing and biocide tolerance of *Arcobacter butzleri* from Danish broiler carcasses. BMC Res Notes. 6:322

RAUHA, J.-P., REMES, S. AND HEINONEN. M., et al. 2000. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int J Food Microbiol. 56:3–12.

SALAS-MASSO, N., ANDREE, K. B., FURONES, M. D. AND FIGUERAS, M. J. 2016. Enhanced recovery of *Arcobacter* spp. using NaCl in culture media and re-assessment of the traits of *Arcobacter marinus* and *Arcobacter halophilus* isolated from marine water and shellfish. Sci Total Environ. 566–567:1355–1361.

SATYANARAYANA, S., SUSHRUTA, K., SARMA, G. S., SRINIVAS, N. AND SUBBA, GV. 2004. Antioxidant activity of the aqueous extracts of spicy food additives—evaluation and comparison with ascorbic acid in in-vitro systems. Herb Pharmacother. 4:1–10.

SELMI, S. AND SADOK, S. 2008. The effect of natural antioxidant (*Thymus vulgaris Linnaeus*) on flesh quality of tuna (*Thunnus thynnus* (Linnaeus)) during chilled storage. Pan-Amer. J Aquatic Sci. 3 (1), 36-45.

SERRAINO, A. AND GIACOMETTI, F. 2014. Short communication: Occurrence of *Arcobacter* species in industrial dairy plants. J. Dairy Sci. 97 :2061–2065. http://dx.doi.org/ 10.3168/jds.2013-7682.

SERRAINO, A., FLORIO, D., GIACOMETTI, F., PIVA, S., MION, D. AND ZANONI, R. G. 2013. Presence of *Campylobacter* and *Arcobacter* species in in-line milk filters of farms authorized to produce and sell raw milk and of a water buffalo dairy farm in Italy. J. Dairy Sci. 96:2801–2807.

SERRAINO, A., GIACOMETTI, F., DAMINELLI P., ROSMINI R. AND et al. 2013. Survival of Arcobacter butzleri During Production and Storage of Artisan Water Buffalo Mozzarella Cheese. Foodborne Path. Dis. 10(9). DOI: 10.1089/fpd.2013.1485.

SHAH, A. H., SALEHA, A. A., ZUNITA, Z., MURUGAIYAH, M., ALIYU, A. B. AND JAFRI, N.2013. Prevalence, distribution and antibiotic resistance of emergent *Arcobacter spp*. from clinically healthy cattle and goats. Transbound Emerg Dis. 60:9–16.

SMITH, P. A., STEWART, J. AND FYFE, L. 2001. The potential application of plant essential oils as natural food preservatives in soft cheese. Food Microbiol. 18:463–470.

SMITH, S. I., OYEDEJI, K. S., OPERE, B., IWALOKUN, B. A., OMONIGBEHIN, E. A. 2003. The effect of some Nigerian local herbs on *Helicobacter pylori*. Afr J Clin Exper Microbiol. 4: 29–35.

TANNGUCHI, M.,YANO, Y.,TADA, E., IKENISHI, K., HARAGUCHI, H., HASHIMOTO, K. AND KUBO, I. 1988. Mode of action of polygodial, an antifungal sequeterpene dialdehyde. Agric Biol Chem. 52:1409–1414.

VANDAMME, P., FALSEN, E., ROSSAU, R., HOSTE, B., SEGERS, P., TYTGAT, R. AND DE LEY, J. 1991. Revision of *Campylobacter*, *Helicobacter* and *Wolinella* taxonomy: amendation of generic descriptions and proposal of *Arcobacter* gen. nov. Int J Syst Bacteriol. 41: 88–103.

VANDERBERG, O., DEDISTE, A., HOUF, K., IBEKWEM, S., SOUAYAH, H., CADRANEL, S., DOUAT, N., ZISSIS, G., BUTZLER, J. P. AND VANDAMME, P. 2004. *Arcobacter species* in humans. Emerg Infect Dis. 10: 1863–7.

WIESE, N., FISCHER, J., HEIDLER, J., LEWKOWSKI O., DEGENHARDT J. AND ERLER, S. 2018. The terpenes of leaves, pollen, and nectar of thyme (*Thymus vulgaris*) inhibit growth of bee disease associated microbes. Scientific Reports. 8:14634 | DOI:10.1038/s41598-018-32849-6.

YESILMEN, S., VURAL, A., ERKAN, M. E. AND YILDIRIM, I. H. 2014. Prevalence and antimicrobial susceptibility of *Arcobacter* species in cow milk, water buffalo milk and fresh village cheese. Int. J. Food Microbiol. 188:11–14.