RESEARCH ARTICLE

Use of thyme essential oil as an antibacterial agent in raw milk intended for the production of farm *cheese*

Yasser Hachana, Bessma Ghandri, Halima Amari and Ismahane Saidi

Received: 02 January 2019 / Accepted: 04 April 2019 / Published online: 22 June 2019
© Indian Dairy Association (India) 2019

Abstract: This study aims to use thyme essential oil (TEO) as an antibacterial agent in raw milk intended for the production of farm cheese and to test its effect on bacteriological and sensory quality. Antibacterial activity of TEO was first tested *in vitro* on four strains of bacteria known for their pathogenicity such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Staphylococcus aureus* and then *in situ* on the total mesophilic aerobic flora (TMAF) in raw milk used to produce farm cheese. All tested bacteria were very sensitive to TEO with a higher sensitivity for *Escherichia coli*, followed by *Pseudomonas aeruginosa*, then *Staphylococcus aureus* and finally *Salmonella typhimurium*. *In situ* study showed that TEO has an effective effect on TMAF of raw milk. Monitoring bacteriological quality of TEO based farm cheese during 6 days of cold storage, revealed the effectiveness of TEO on TMAF, yeasts and moulds. Despite the good bacteriological quality of TEO-based farm cheese, the strong aroma characterizing this essential oil was not well appreciated by some tasters, which had a negative impact on the final score attributed to this cheese. Thyme essential oil can be used as an alternative solution to produce healthy farm cheese without needing heat treatment, while ensuring high nutritional value food product.

Keywords: Antibacterial effect, Farm cheese, Milk quality, Pathogenic bacteria, Thyme essential oil

Introduction

Food security is one of the most important health concerns. According to World Health Organization report (WHO, 2002), 30 % of consumers in industrialized countries suffer from foodborne illness each year. Highly consumed products such as milk, can be an important source of foodborne pathogens for human health (Van Kessel et al. 2004). Human contamination occurs mainly by consuming uncontrolled raw milk (Gravani, 1999). In the United States, some reports link the consumption of contaminated raw milk, inadequately pasteurized milk or adulterated milk products with contaminated raw milk, to many cases of foodborne illness (Evans et al. 1996, Fahey et al. 1995; Fleming et al. 1985). Usually, before its use, raw milk must be subjected to heat treatments in order to destroy bacterial flora that can be harmful to consumers. Pasteurization and sterilization processes are the most commonly used methods by food and dairy industries for microorganism inactivation. However, on farm scale, these processes are quite difficult to install, not only because of their high costs, but also because of high technical skills levels and highly qualified staff requirements. In addition, several studies have reported that in general, heat treatments can cause undesirable changes in taste, color as well as nutrient losses (Balny and Masson 1993; Datta and Deeth 1999). Indeed, a moderate heat treatment can have an adverse effect on both structure and physical properties of proteins, which are first denatured and then damaged by Maillard reactions, altering the physical appearance of the product, as well as its organoleptic and nutritional quality (Mauron, 1981). Recent research has explored other methods of milk processing that have less negative effects on protein quality, such as ultra-high pressure or ultrasonic waves. Using high pressures between 300 and 600 MPa has proven to be an effective method for inactivating microorganisms, including most foodborne infectious pathogens (Antonio et al. 2002). South African and British dairy legislation has stipulated that ultrasound reduces pathogenic food flora to an acceptable level, even when bacterial loads are five times higher than those allowed (Cameron et al. 2009). Nevertheless, the large-scale uses of ultra-high pressure and ultrasound in the dairy industry remain underdeveloped due

Yasser Hachana(✉), Bessma Ghandri, Halima Amari and Ismahane Saidi
Animal Production Department, High Agronomic Institute of Chott-Mariem 4042 Susa, Tunisia
Email: hachana@yahoo.fr

to high cost of equipments. Other future prospects consist in exploiting the natural antibacterial potential of some medicinal and aromatic plants used by humans since antiquity (Jones, 1996). Manju et al. (2017) showed that plant derived antimicrobials trans-cinnamaldehyde or eugenol could be successful approach as biopreservatives to control *L. monocytogenes* to achieve desired inactivation at small amounts in milk. Antimicrobial activity of essential oils and plant extracts has formed the basis for many applications, including preservation of raw and processed foods, pharmaceuticals, alternative medicine and natural therapies (Reynolds, 1996; Lis-Balchin and Deans, 1997). Hydrophobicity of essential oils allows them to spread into cell membrane and mitochondria lipids, rendering them permeable and thus causing a leakage of cellular contents (Helander et al. 1998; Burt, 2004). Active components in essential oil fractions have a wide spectrum of antimicrobial activity against foodborne pathogens and spoilage bacteria (Gutierrez et al. 2009). Several molecules present in essential oils have exhibited antimicrobial properties and in particular phenols, alcohols and aldehydes (Cutter, 2000). Gram-negative bacteria are slightly less sensitive to essential oils than Gram-positive bacteria (Burt, 2004). Antibacterial activity of essential oils was demonstrated *in vitro* studies against *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli*, *Shigella dysenteriae*, *Bacillus cereus* and *Staphylococcus aureus* at levels ranging from 0.2 to 10 µl/mL (Burt, 2004). Using essential oils in lactic acid products has increased their nutritional value, organoleptic qualities and even their hygienic status (Amirdivani and Baba, 2011). Essential oils and plant extracts are widely used in food industry to prevent possible oxidative and/or microbial degradation of food products (Tiwari et al. 2009). However, very few complete studies on the effect of these essential oils on the bacteriological quality of raw milk and dairy products have been performed. In this context, the main objectives of this work are first to evaluate *in vitro* antimicrobial activity of thyme essential oil on four strains of bacteria known for their pathogenicity in raw milk, such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Staphylococcus aureus*, then to evaluate its effectiveness *in vivo* on the total aerobic mesophilic flora of raw milk intended for the production of farm cheese, and finally to test the effect of thyme essential oil on the bacteriological and sensory quality of the produced cheese.

Materials and methods

Thyme essential oil extraction

Raw samples of thyme plant (*Thymus capitatus*) were freshly collected at the flowering stage from the hills of Akouda-Susa Tunisia, during February 2018. Samples were dried for 15 days at room temperature in a well-ventilated dark place. Thyme essential oil extraction was carried out by steam distillation. Thyme essential oil separated from the aromatic hydrolate was dehydrated using sodium sulfate (Na_2SO_4) to remove as much

water as possible. Thyme essential oil samples were quickly stored at 4 °C in dark conditions.

In vitro assessment of the antibacterial potential of thyme essential oil

Bacterial strains

Four bacterial strains from the Higher School of Food Industries of Tunis, Tunisia have been selected for their pathogenicity and their frequent involvement in the contamination of foodstuffs, particularly raw milk. Bacterial strains are composed of three Gram-negative bacteria: *Escherichia coli* NCTC 10418, *Salmonella typhimurium* ATCC 13311 and *Pseudomonas aeruginosa* NCTC 10662 and one Gram positive bacteria: *Staphylococcus aureus* NCTC 6571. Bacteria were kept alive on blood agar. Cultures were prepared by inoculating approximately 2 mL of Mueller Hinton broth with 2 to 3 colonies of each bacterium from the blood agar. Broth was previously incubated at 35°C. Inoculums were prepared by diluting bacterial cultures in saline solution to 10^6 cfu/mL. These solutions were diluted with sterile physiological serum.

Antibacterial activity

Screening for antibacterial activity of thyme essential oil (TEO) was performed by adapting the serial agar dilution method, based on radial diffusion mentioned by Oliveira *et al.* (2013). Plates with 20 mL of nutrient agar were inoculated with 100 µl of different bacterial solutions previously prepared. A 50 µL volume of each dose of TEO, dissolved in ethanol, was placed in 4 mm deep and 6 mm diameter well, dug in the aseptic agar. Each dose was repeated three times. Plates were incubated at 37 ± 2 °C for 18 hours. Inhibition zone diameters were measured with a millimeter ruler with 0.5 mm accuracy. Development of inhibitory zone, identified by a clear halo, indicates the presence of an antibacterial effect of the tested oil (Smania et al. 1999). Based on the work of Oliveira *et al.* (2013) bacterial sensitivity to thyme essential oil has been classified according to the diameters of the inhibition halos measured as follows:

Not sensitive (-) or resistant: diameter < 8 mm.

Sensitive (+): diameter between 9 and 14 mm.

Very sensitive (+++): diameter between 15 and 19 mm.

Extremely sensitive: diameter > 20 mm

In situ assessment of the antibacterial potential of thyme essential oil

Raw milk collection

Raw milk was obtained from the dairy farm of the Superior Agronomic Institute Chott-Meriam Susa Tunisia. Samples were

collected aseptically in sterile containers and carried directly to the animal production laboratory for chemical and bacteriological analysis.

Chemical analysis

Raw milk's chemical composition was evaluated using FT 120 Milkoscan FOSS Denmark. Measured parameters are fat content, protein content and lactose content.

Bacteriological analysis

Before testing the effect of thyme essential oil on bacteriological composition of raw milk, samples were analyzed for total aerobic mesophilic flora according to AFNOR (2013), faecal coliforms and total coliforms according to AFNOR (2009). Thyme essential oil tested doses, were 1, 2 and 3 % respectively. Each raw milk sample treated with thyme essential oil was analyzed in triplicate for all types of bacteria.

Cheese production

Farm cheese was produced in animal production laboratory using a method described by Chandan (2003). First, milk was heated to 35 °C. A dose of 1 % thyme essential oil (TEO) was then added. The mixture milk-TEO was carefully stirred for 2 minutes. Then, a dose of 2.5 % animal rennet was added and stirred for 1 minute. Milk was left to coagulate for 40 minutes. The resulting curd was cut and mixed gently. The curd-serum mixture was filled into moulds and gently pressed by hand to remove as much whey as possible. After draining, cheese was stored at 4 ± 1 °C for 6 days.

Sensory evaluation

Descriptive sensory analysis or scale test was carried out to assess the impact of using thyme essential oil (TEO) on cheese flavor. To this end, 50 voluntary tasters were used. To materialize their judgments, tasters had at their disposal a descriptive scale composed of several factors such as color, smell, texture, mouth texture, taste and aftertaste. For each taster, cheese produced from raw milk treated with TEO is presented simultaneously with a control cheese produced from the same raw milk but without any additives. Spontaneous attributes were used to describe the perceived differences between the two cheeses.

Cheese microbiological monitoring during refrigerated storage

Fresh cheese samples were kept at 4 °C in the refrigerator to monitor bacterial load for 6 days of storage. Microbiological analyses concerning total aerobic mesophilic flora, faecal coliforms, total coliforms, yeasts and moulds, were carried out on the day of cheese manufacture, after 3 and 6 days of storage at 4 °C according to AFNOR (2009) and AFNOR (2013) standard methods.

Statistical analyses

Analysis of variance was performed using the general linear model procedure (GLM SAS 9.3) to study antibacterial effect of thyme essential oil on microbiological composition of raw milk and farm cheese. Different means were compared with Duncan's multiple-range tests; a probability value of $p < 0.05$ was considered significant. All analyses were carried out in triplicate.

Results and discussion

In vitro assessment of the antibacterial potential of thyme essential oil

Antibacterial effect of thyme essential oil (TEO) was tested on four bacteria strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Staphylococcus aureus*. All TEO tested doses showed a very significant effect ($p < 0.05$) on each of the four pathogenic bacteria. As TEO dose increases, inhibition diameter of all bacteria increases (Table 1). Inhibition diameters sizes for all used doses of TEO ranged from 14.7 to 23 mm, clearly indicating that all tested bacteria were very sensitive to TEO with a higher sensitivity of *Escherichia coli*, followed by *Pseudomonas aeruginosa*, then *Staphylococcus aureus* and finally *Salmonella typhimurium* (Table 1). Thyme essential oil is considered to be one of the most active essential oils on bacteria as reported by Cox *et al.* (2000). Indeed, its main compound, thymol, confers a strong antimicrobial activity. Moro *et al.* (2013) reported that TEO has shown satisfactory anti-microbial activity *in vitro* against pathogens and spoilage microorganisms. In addition, Burt (2004) employed doses ranging from 0.02 to 1 % and demonstrated antibacterial activity of TEO against *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli*, *Shigella dysenteriae*, *Bacillus cereus* and *Staphylococcus aureus*. Hammer *et al.* (1999) showed that minimum inhibitory concentration of TEO was approximately 0.12 % for *Escherichia coli*, 0.25 % for *Staphylococcus aureus* and more than 2% for

Table 1 Effect of thyme essential oil doses on bacterial growth (diameter in mm).

Dose %	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Salmonella typhimurium</i>
1	18.2 ^b	16.0 ^b	15.8 ^b	14.7 ^b
2	20.2 ^b	17.8 ^b	18.2 ^a	15.3 ^b
3	23.0 ^a	21.2 ^a	18.7 ^a	17.0 ^a

^{a,b} Column means with different superscripts differ significantly at $p < 0.05$

Fig. 1 Effect of thyme essential oil dose on TAMF of raw milk

Fig. 2 Total aerobic mesophilic flora evolution in cheese during storage at 4°C

^{a,b} Bar means with different superscript differ significantly at $p < 0.05$

Salmonella tiphymirium and *Pseudomonas aeruginosa*. Burt (2004) noted that minimum TEO inhibitory concentrations generally range from 0.005 to 0.5 %. Paster et al. (1990) reported that TEO used at 500 µg/mL inhibited growth of *Staphylococcus aureus* and *Salmonella tiphymirium*, while *Pseudomonas aeruginosa* was not affected even at higher concentrations. Burt and Reinders (2003) and Paster et al. (1990) recorded inhibition diameters for *Escherichia coli* ranging from 24 to 25.7 mm when using diluted TEO. According to Calo et al. (2015) effectiveness of essential oils demonstrated *in vitro* should not be considered strictly true, due to food matrix complexity in which intrinsic and extrinsic factors interfere with activity of these compounds and must be confirmed by an *in vivo* assay.

***In situ* assessment of the antibacterial potential of thyme essential oil**

Thyme essential oil (TEO) was added to raw milk in order to test its effectiveness on total mesophilic aerobic flora (TAMF). Physico-chemical and bacteriological composition of used raw milk is presented in Table 2. Increasing TEO dose in raw milk significantly affected TAMF (Figure 1). Initial total bacterial count in raw milk was about 156×10^3 cfu/mL and then reduced to 36.2, 26.3 and 18×10^3 cfu/mL using doses 1, 2 and 3% respectively (Figure 1). These findings clearly indicate that TEO has proven to be remarkably effective on TAMF in raw milk. According to Ben Jemaa et al. (2016) by incorporating TEO or its nanoemulsion, bacterial growth in various contaminated milks has been positively controlled and physico-chemical and microbiological quality has been improved. In addition, Gammariello et al. (2008) reported that TEO inhibits microorganism's growth responsible for milk deterioration without affecting the dairy microflora.

Fig 3 Yeasts and moulds evolution in cheese during storage at 4°C.

Fig 4 Sensory test results

Table 2 Physico-chemical and bacteriological composition of raw milk

MG(g/l)	MP(g/l)	Lactose (g/l)	pH	TAMF(ufu/mL)*10³	TC(ufu/mL)	FC(ufu/mL)
48±0.05	31.3±0.03	47.8±0.03	6.8±0.1	156	0	0

TAMF: total aerobic mesophilic flora; TC: total coliforms; FC: faecal coliforms.

Assessment of cheese bacteriological quality during cold storage

In vitro and *in situ* results led us to select the dose of 1 % thyme essential oil (TEO) in farm cheese production, since it eradicated all tested pathogenic bacteria, as well as reducing total aerobic mesophilic flora (TAMF) to an acceptable level of about 36.2*10³ ufc/mL. In addition, dose of 1 % TEO was chosen in order to reduce production cost of farm cheese, especially since natural essential oils are expensive. Figure 2 shows that during the first day of production, the TAMF of TEO cheese was significantly lower than that of control cheese (60 and 159*10³ cfu/g respectively). Following 3 days of refrigerated storage, TAMF remained almost stable for cheese produced from TEO, while for control cheese, TAMF increased from 159 to 190*10³ cfu/g. By the 6th day of storage, TAMF had increased to 113*10³ cfu/g for

TEO cheese and 370*10³ cfu/g for control cheese. Therefore, thyme essential oil significantly reduced (p <0.05) TAMF growth in cheese during refrigerated storage for 6 days.

Concerning yeasts and moulds, their count in TEO based farm cheese did not exceed 10*10³ cfu/g after 3 days of storage and 32*10³ cfu/g after 6 days of storage at 4 °C (Figure 3). However, for control cheese, yeasts and moulds have proliferated during storage and their count increased from 34 to 44*10³ cfu/g after 3 days of storage and to 94*10³ cfu/g after 6 days of storage (Figure 3). These results prove the effectiveness of TEO on yeasts and moulds in farm cheese even after six days of cold storage. These findings are in perfect agreement with study of Moro et al. (2013) which demonstrated that TEO has satisfactory antimicrobial

activity against pathogens and spoilage microorganisms associated with cheese contamination. Similarly, Smith-Palmer et al. (2001) reported that TEO used at 1 % concentration in low-fat cheese showed high effectiveness on *L. monocytogenes* and *S. enteritidis* and reduced their counts to harmless levels. Zantar et al. (2013) noted inhibition of unwanted germs, yeasts and moulds in flavored goat cheese using 0.05 % TEO from the first week of production. In the same way, Burt (2004) reported that TEO has high antimicrobial activity in raw milk and produces healthy cheese. It was suggested by Carvalho et al. (2015) that essential oil doses used to control pathogenic bacteria in fermented dairy products, particularly in low-ripened cheeses, may be established with caution, due to potential negative effects on growth and survival of beneficial lactic acid bacteria used in such foods.

Evaluation of sensory cheese quality

Sensory evaluation revealed no difference ($p > 0.05$) between thyme essential oil (TEO) cheese and control cheese in terms of color, appearance and consistency (Figure 4). However, significant differences ($p < 0.05$) in taste, aftertaste and overall appreciation were observed between the two cheeses. If we consider the overall appreciation score, we can notice that the control cheese was more appreciated than the TEO cheese, but the latter was also liked by a large number of tasters. The strong aroma that characterizes TEO has had a negative impact on the final score attributed to TEO cheese by some tasters (Figure 4). Similar results have been reported by Zantar et al. (2013) indicating that goat cheese with 0.05 % TEO was less appreciated by tasters than control cheese. In addition, Burt (2004) pointed out limited use of TEO in food products because of its intense aroma, which negatively affects taste and odor. According to Moro et al. (2015), the strong specific flavor of thyme oil, although used in small concentrations, can significantly affect sensory quality of dairy products and may result in their rejection. Gouvea et al. (2017) stated that acceptance of food products based on essential oils or plant extracts is extremely important, because having high antimicrobial effect is not enough if consumer doesn't approve it in a sensory way. Divergent results have been reported in several studies, including those of Leuschner and Ielsch (2003), Hayaloglu and Fox (2008), Gammariello et al. (2008), who all affirmed that TEO has been successfully incorporated into cheese and have been well accepted by consumers. Tayel et al. (2015) also confirmed that addition of TEO to cheeses resulted in good sensory acceptance. Gouvea et al. (2017) mentioned that general acceptance of cheeses with essential oils and plant extracts can be facilitated if consumers are already familiar with this type of products. To overcome taste problems, Gutierrez et al. (2008) proposed combination of different essential oils and extracts to have good antimicrobial effect without affecting product taste and smell.

Conclusions

Thyme essential oil can be used as an alternative solution for producing healthy farm cheese without needing heat treatment, while ensuring high nutritional value food product. However, the strong aroma characterizing this essential oil was not well appreciated by some tasters, which had a negative impact on the final score attributed to this cheese. This study remains an initiative that should be consolidated by further researches trying to combine other essential oils with thyme oil as a way to attenuate or completely eliminate the negative effects on taste.

References

- AFNOR, (2009). V08-050. Food Microbiology. Coliforms enumeration through colony counting performed at 30°C
- AFNOR (2009). V08-060. Food Microbiology. Thermotolerant coliforms enumeration through colony counting performed at 44°C
- AFNOR (2013). ISO 4833-1. Food Microbiology. Horizontal method for microorganism enumeration - Part 1: Colony counting at 30°C by deep sowing technique. ISO 4833-1
- Amirdivani S, Baba A (2011) Changes in yogurt fermentation characteristics, and antioxidant potential and in vitro inhibition of angiotensin – 1 converting enzyme upon the inclusion of peppermint, dill and basil. LWT – Food Sci Technol 44: 1458-1464
- Antonio JT, Capellas M, Saldo J, Gervilla R, Guamis B (2002). Applications of high-hydrostatic pressure on milk and dairy products: a review. Innov Food Sci Emerg Technol 3: 295–307
- Balny C, Masson P (1993) Effects of high-pressure on proteins. Food Rev Int 9: 611–628
- Ben Jemaa M, Fellah H, Neves MA, Isoda H, Nakajima M, Ksouri R (2016) Quality preservation of deliberately contaminated milk using thyme free and nonemulsified essential oils. Food Chem 217: 726-734
- Burt SA, Reinders RD (2003) Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. Lett Appl Microbiol 36: 162–167
- Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol 94: 223-253
- Calo JR, Crandall PG, O'Bryan CA, Rieke SC (2015) Essential oils as antimicrobials in food systems - A review. Food Control 54: 111-119
- Cameron M, McMaster LD, Britz TJ (2009) Impact of ultrasound on dairy spoilage microbes and milk components. Dairy Sci Technol 89: 83–98
- Carvalho RJ, de Souza GT, Honorio VG, de Sousa JP, da Conceição ML, Maganani M de Souza EL (2015) Comparative inhibitory effects of *Thymus vulgaris* L. essential oil against *Staphylococcus aureus*, *Listeria monocytogenes* and mesophilic starter co-culture in cheese-mimicking models. Food Microbiol 52: 59-65
- Chandan RC (2003) Soft and special varieties. In: Cavellaro B, Trugo LC, Finglas PM (eds). Encyclopedia of Food Sciences and Nutrition Vol 2 New York: Academic Press, pp. 1093–1098
- Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warmington JR, Wyllie SG (2000). The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). J Appl Microbiol 88: 170-175
- Cutter CN (2000) Antimicrobial effect of herb extracts against *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium* associated with beef. J Food Protect 63: 601– 607

- Datta N, Deeth HC (1999). High pressure processing of milk and dairy products. *Aust J Dairy Technol* 54: 41
- Evans MR, Roberts RJ, Ribeiro CD, Gardner D, Kembrey D (1996) A milk-borne campylobacter outbreak following an educational farm visit. *Epidemiol Infect* 117: 457–462
- Fahey T, Morgan D, Gunneburg C, Adak GK, Majid F, Kaczmarek E (1995) An out-break of *Campylobacter jejuni* enteritis associated with failed milk pasteurization. *J Infect* 31: 137–143
- Fleming DW, Cochi SL, MacDonald KL, Brondum J, Hayes PS, Plikaytis BD, Holmes MB, Audurier A, Broome CV, Reingold AL (1985) Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *N Engl J Med* 312: 404–407
- Gammariello D, Di Giulio S, Conte A, Del Nobile MA (2008) Effects of natural compounds on microbial safety and sensory quality of Fior di Latte cheese, a typical Italian cheese. *J Dairy Sci* 91: 4138–4146
- Gouvea FDS, Rosenthal A, Ferreira AREH (2017) Plant extract and essential oils added as antimicrobials to cheeses: a review. *Ciênc Rural* 47: 1–9
- Gravani R (1999) Incidence and control of *Listeria* in food-processing facilities. In: *Listeria listeriosis* and food safety, 2nd ed. Ryser, E.T., and E.H. Marth (ed.), Marcel Dekker, New York, pp. 657–709
- Gutierrez J, Barry-Ryan C, Bourke P (2008) The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *Int J Food Microbiol* 124: 91–97
- Gutierrez J, Barry-Ryan C, Bourke P (2009) Antimicrobial activity of plant essential oils using food model media: efficacy, synergistic potential and interaction with food components. *Food Microbiol* 26: 142–150
- Hammer KA, Carson CF, Riley TV (1999) Antimicrobial activity of essential oils and other plant extracts. *J Appl Microbiol* 86: 985–990
- Hayaloglu AA, Fox PF (2008) Cheeses of Turkey: 3. Varieties containing herbs or spices. *Dairy Sci Technol* 88: 245–256
- Helander IM, Alakomi HL, Kala KL, Sandholm TM, Pol I, Smid EJ, Gorris LGM, Von AW (1998) Characterization of the action of selected essential oil components on Gram-negative bacteria. *J Agri Food Chem* 46: 3590–3595
- Jones FA (1996) Herbs – useful plants. Their role in history and today. *Eur J Gastroenterol Hepatol* 8: 1227–1231
- Leuschner RGK, Ielsch V (2003) Antimicrobial effects of garlic, clove and red hot chilli on *Listeria monocytogenes* in broth model systems and soft cheese. *International Journal of Food Sci Nutr* 54: 127–133
- Lis-Balchin M, Deans SG (1997) Bioactivity of selected plant essential oils against *Listeria monocytogenes*. *J Appl Bact* 82: 759–762
- Manju G, Chand R, Suman R (2017) Combination of trans-cinnamaldehyde and eugenol with nisin for inactivation of *L. monocytogenes*. *Indian J Dairy Sci* 70: 411–420
- Mauron J (1981) The Maillard reaction in food: a critical review from the nutritional standpoint. *Progr Food Nutr Sci* 5: 5–35
- Moro A, Librán CM, Berruga MI, Zalacain A, Carmona M (2013) Mycotoxigenic fungal inhibition by innovative cheese cover with aromatic plants. *J Sci Food Agric* 93: 1112–1118
- Moro A, Librán CM, Berruga MI, Carmona M, Zalacain A (2015) Dairy matrix effect on the transference of rosemary essential oil compounds during cheese making. *J Sci Food Agric* 95: 1507–1513
- Oliveira AA, Segovia JFO, Sousa VYK, Mata ECG, Gonçalves MCA, Bezerra RM, Junior, POM, Kanzaki LIB (2013) Antimicrobial activity of Amazonian medicinal plants. *Springer Plus* 2: 371
- Paster N, Juven BJ, Shaaya E, Menasherov M, Nitzan R, Weisslowicz H, Ravid U (1990) Inhibitory effect of oregano and thyme essential oils on moulds and foodborne bacteria. *Lett Appl Microbiol* 11: 33–37
- Reynolds JEF (1996) Martindale – the Extra Pharmacopoeia 31st edn. London: Royal Pharmaceutical Society of Great Britain
- Smania AJ, Delle Monache F, Smania EFA, Cuneo RS (1999) Antibacterial Activity of Steroidal Compounds Isolated from *Ganoderma applanatum*. *Int J Med Mushrooms* 1: 325–330
- 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: 463–470
- Tayel AA, Hussein H, Sorour NM, El-Tras WF (2015) Foodborne pathogens prevention and sensory attributes enhancement in processed cheese via flavoring with plant extracts. *J Food Sci* 80: 2886–2891
- Tiwari BK, Valdramidis VP, O'Donnell CP, Muthukumarappan K, Bourke P, Cullen PJ (2009). Application of natural antimicrobials for food preservation. *J Agric Food Chem* 57: 5987–6000
- Van Kessel JS, Karns JS, Gorski L, McCluskey BJ, Perdue ML (2004) Prevalence of *Salmonellae*, *Listeria monocytogenes* and fecal coliforms in bulk tank milk on U.S. dairies. *J Dairy Sci* 87: 2822–2830
- WHO (2002) World Health Organization Report. Food safety and foodborne illness. World Health Organization Fact sheet 237, revised January 2002. Geneva
- Zantar S, Zerrouk HM, Zahar M, Said B, Notfia Z, Laglaoui A, Larbi T, Chentouf M (2013) Effect of using essential oils (thyme, rosemary, oregano and myrtle) on physico-chemical, microbiological and sensory properties of fresh and semi-ripened goat's cheese. *Options Méditerranéennes*, 108: 183–190