

Effects of Natural Compounds on Microbial Safety and Sensory Quality of Fior di Latte Cheese, a Typical Italian Cheese

D. Gammariello,* S. Di Giulio,† A. Conte,† and M. A. Del Nobile*†¹

*Istituto per la Ricerca e le Applicazioni Biotecnologiche per la Sicurezza e la Valorizzazione dei Prodotti Tipici e di Qualità, Università degli Studi di Foggia, Via Napoli, 25–71100 Foggia, Italia

†Dipartimento di Scienze degli Alimenti, Università di Foggia, Via Napoli, 25–71100 Foggia, Italia

ABSTRACT

This work presents a preliminary study to assess the efficiency of plant essential oils as natural food preservatives in Fior di Latte cheese. Selected compounds were directly dissolved into Fior di Latte brine. Packaged Fior di Latte samples were stored at 10°C for about 6 d. The cell loads of spoilage and useful microorganisms were monitored to calculate the microbial acceptability limit. Results show that some tested compounds were not acceptable by the panel from a sensorial point of view. Most compounds did not affect the microbial acceptability limit value to a great extent, and only a few such as lemon, sage, and thyme markedly prolonged the microbial acceptability limit of the investigated fresh cheese. Moreover, the above active agents exerted an inhibitory effect on the microorganisms responsible for spoilage without affecting the dairy microflora.

Key words: antimicrobial compound, Fior di Latte cheese, microbial safety, shelf life

INTRODUCTION

Fior di Latte is an unripened “pasta filata” cheese made from cow’s milk, and is similar to Mozzarella cheese, a typical dairy product of Southern Italy. The name “Fior di Latte” is used to distinguish the Mozzarella made from cow’s milk from that made from buffalo’s milk. The manufacture of this cheese has been described in detail in the literature (Parente and Moschetti, 1997; Salvadori del Prato, 2001). It is manufactured according to traditional procedures (raw milk inoculated with natural whey or milk cultures, raw milk ripened under special conditions, without starter addition) or by using pasteurized milk and commercial starter cultures of lactic acid bacteria (Salvadori del Prato, 2001). Although Mozzarella cheese receives heat treatment during curd stretching, post-processing

contamination by microorganisms may occur, causing cheese spoilage, health risks for consumers, and reduction in shelf life (Spano et al., 2003). Few reports are available on methods to prolong the shelf life of fresh cheese. In fact, the main factories use liquid (diluted whey or water from the stretcher-molder) to preserve Fior di Latte cheese. During storage, mass transfer between the product and the liquid occurs and this phenomenon is strictly linked to the composition, microbial growth, moisture degree, liquid type, and storage conditions (Mucchetti and Neviani, 2006).

The application of antimicrobial agents to Fior di Latte packaging could be useful to prevent the growth of spoilage microorganisms and hence, may lead to an extension of shelf life and improved microbial safety of this product. Dairy researchers (Smith-Palmer et al., 2001; Conte et al., 2007a) have found that selected plant essential oils can act as inhibitors of spoilage microorganisms in food products.

The antimicrobial properties of plant essential oils are well known against a wide spectrum of microbes, including bacteria (Deans and Ritchie, 1987; Lis-Balchin and Deans, 1997; Smith-Palmer et al., 1998), yeasts (Lachowicz et al., 1998; Mangena and Muyima, 1999), and fungi (Paster et al., 1990; Mishra and Dubey, 1994). Many scientific reports highlight the antimicrobial effects of these substances by *in vitro* test (Delaquis et al., 2002). Some researchers (Nakatani, 1994; Marino et al., 2001) reported that gram-positive bacteria are more sensitive to essential oils than gram-negative bacteria, but others (Kim et al., 1995; Skandamis et al., 2002) did not observe differences related to this characteristic. The ability of natural compounds to differently inhibit gram-positive or negative bacteria is of considerable importance in the food industry. It has generally been found that a greater concentration of essential oils is needed to achieve the same effect in foods, compared with antibacterial assays *in vitro* (Shelef, 1983; Smid and Gorris, 1999). The great availability of nutrients in foods compared with laboratory media may enable bacteria to repair damaged cells (Gill et al., 2002). Both the intrinsic (fat, protein, water

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¹Corresponding author: ma.delnobile@unifg.it

Table 1. Microbial acceptability limit (MAL; \pm SD) values related to *Pseudomonas* spp. (MAL_P) and coliforms (MAL_C)

Sample	Manufacturer ¹	Concentration (mg/kg)	MAL _P (d)	MAL _C (d)	MAL value ² (d)
Control			1.37 \pm 0.01	1.17 \pm 0.21	1.17 \pm 0.21 ^{a-c}
Lemon	Spencer	10,000	1.80 \pm 0.49	0.68 \pm 0.16	0.68 \pm 0.16 ^{ab}
Lemon	Spencer	5,000	0.76 \pm 0.16	0.58 \pm 0.43	0.58 \pm 0.43 ^a
Citral	Sigma-Aldrich	500	2.04 \pm 0.44	5.00 \pm 2.17	2.04 \pm 0.44 ^{b-e}
Nonanoic acid	Sigma-Aldrich	2,000	1.61 \pm 0.28	NC ³	NC
Grapefruit	Primavera	5,000	1.65 \pm 0.10	>6	1.65 \pm 0.10 ^{a-e}
Lemon	Aboca	5,000	1.60 \pm 0.21	2.40 \pm 0.43	1.60 \pm 0.21 ^{a-e}
Lemon	Agrumigel	5,000	1.41 \pm 0.09	0.82 \pm 0.40	0.82 \pm 0.40 ^{ab}
Lemon	Alkott	1,500	1.39 \pm 0.10	>6	1.39 \pm 0.10 ^{a-d}
Lemon	Alkott	3,000	>6	>6	>6
Lemon	Alkott	6,000	>6	>6	>6
Lemon	Boyajian	1,500	2.51 \pm 0.10	2.46 \pm 0.32	2.46 \pm 0.32 ^{c-e}
Lemon	Boyajian	3,000	>6	>6	>6
Lemon	Boyajian	6,000	2.93 \pm 0.39	>6	2.93 \pm 0.39 ^e
Lemon	Erbavita	5,000	1.17 \pm 2.13	NC	NC
Lemon	Esperis	2,000	3.03 \pm 0.55	0.79 \pm 0.42	0.79 \pm 0.42 ^{ab}
Lemon	Primavera	1,500	1.71 \pm 0.10	>6	1.71 \pm 0.10 ^{a-e}
Limonene	Sigma-Aldrich	500	0.75 \pm 0.09	0.55 \pm 0.12	0.55 \pm 0.12 ^a
Orange	Erbavita	5,000	1.17 \pm 2.02	NC	NC
Propolis	Erbavita	5,000	0.96 \pm 0.86	0.73 \pm 1.57	0.73 \pm 1.57 ^{ab}
Rosemary	OTI	5,000	1.31 \pm 0.23	NC	NC
Sage	Alkott	1,500	2.04 \pm 0.39	>6	2.04 \pm 0.39 ^{b-e}
Sage	Alkott	3,000	>6	>6	>6
Sage	Alkott	6,000	>6	>6	>6
Sour orange	Primavera	5,000	1.72 \pm 0.09	2.69 \pm 0.20	1.72 \pm 0.09 ^{a-e}
Sweet orange	Primavera	5,000	2.58 \pm 1.17	>6	2.58 \pm 1.17 ^{de}
Thyme	Aboca	5,000	3.40 \pm 0.77	2.32 \pm 0.65	2.32 \pm 0.65 ^{c-e}
Thymol	Sigma-Aldrich	1,500	1.25 \pm 1.60	>6	1.25 \pm 1.60 ^{a-d}
Vanilla	Erbavita	5,000	1.3 \pm 0.47	NC	NC

^{a-e}Means within the column with different superscript letters are significantly different ($P < 0.05$).

¹Spencer (Amsterdam, the Netherlands); Sigma-Aldrich (Milan, Italy); Primavera (Lorenzana, Italy); Aboca (Arezzo, Italy); Agrumigel (Pozzo di Gotto, Italy); Alkott (Milan, Italy); Boyajian (Canton, MA); Erbavita (Chiesanuova, Italy); Esperis (Milan, Italy); OTI (Domegge di Cadore, Italy).

²The lowest MAL value among those calculated for each spoilage microorganism was reported.

³NC = not calculated.

content, antioxidants, pH, salt, and other additives) and the extrinsic properties (temperature, packaging in vacuum/gas/air, characteristics of microorganisms) of the food can influence bacterial sensitivity to natural extracts from plants (Shelef, 1983; Tassau et al., 2000).

Because of the potential use of active natural compounds for food preservation and the lack of research on active packaging systems applied to fresh dairy products, we evaluated the effects of different natural compounds on standard hygienic quality of Fior di Latte cheese during storage at 10°C.

MATERIALS AND METHODS

Active Compounds and Media

Salvia officinalis and *Citrus limonum* were purchased from Alkott (Milan, Italy), *Thymus vulgaris* from Aboca (Arezzo, Italy), *Rosmarinus officinalis* from OTI (Domegge di Cadore, Italy), *Citrus aurantium*, *Cit-*

rus sinensis, and *Citrus paradisi macf* from Primavera (Lorenzana, Italy). Moreover, different essential oils from *Citrus limonum* were purchased from Agrumigel (Barcellona, Pozzo di Gotto, Italy), Esperis (Milan, Italy), Aboca, Erbavita (Chiesanuova, Italy), Primavera, Boyajian (Canton, MA), and Spencer (Amsterdam, the Netherlands). Finally, the hydroalcoholic extracts *Melaleuca alternifolia*, *Mentha piperita*, *Propolis*, and *Vanilla planifolia* were purchased from Erbavita and citral 95%, nonanoic acid 96%, thymol, and limonene from Sigma-Aldrich (Milan, Italy). The concentrations of the selected active compounds are listed in Table 1. All media used for microbiological analyses were purchased from Oxoid (Milan, Italy).

Sensory Evaluation

The solutions used for sensorial testing were prepared by diluting each natural compound at different concentrations in brine solutions. All active solutions were prepared 24 h before sensory panel evaluation and

were held at 10°C in an odor-free environment. Samples were equilibrated to room temperature immediately before testing. Sensory evaluation was carried out by 5 panelists, members of the Food Packaging Laboratory of the University of Foggia. They were selected based on their interest in the sensory evaluation of dairy products. Coded portions of each sample (20 mL) were presented to the panelists in random order. The natural extracts were evaluated for odor acceptability using a 5-point scale: 0 = not perceptible, 1 = barely perceptible, 2 = low intensity, 3 = medium intensity, 4 = great intensity, and 5 = extreme intensity. Panelists were also asked to score the defects of the active solution on a panel scale to determine the reasons for the decrease in scores for the discriminated off-odors.

Fior di Latte Cheese Production and Packaging

The Fior di Latte cheese used in this work was manufactured in the Posta la Via dairy plant (Foggia, Italy), according to the following procedure: raw cow's milk was acidified with lactic acid and liquid rennet was added. Curd formation was achieved after approximately 15 to 20 min. When the curd pH reached a value of about 5.80, the whey was removed; the curd was cut, stretched, and molded. The application of heat (82°C) to cheese curd during the manufacturing of Mozzarella is to selectively control the growth of certain types of bacteria and, consequently, to influence the maturation pathway of the cheese. After tempering in cold water, samples of about 50 g were packaged in polypropylene tubs using either brine or active brine. Brine consisted of 2% NaCl solution, whereas active brine was obtained by dissolving each antimicrobial compound at different concentrations into salt solution. The samples were stored at 10°C for 6 d.

Microbiological Analyses

Microbiological analyses were performed before packaging and after 1, 2, 5, and 6 d of storage. Twenty grams of Fior di Latte cheese was diluted in 180 mL of Ringer's solution and homogenized in a blender (Stomacher, International PBI, Milan, Italy). Subsequent serial dilutions were made in Ringer's solution and plated in the following media: plate count agar, incubated at 30°C for 48 h for total mesophilic bacteria; de Man, Rogosa, and Sharpe (MRS) agar, supplemented with cycloheximide (100 mg/L, Sigma), incubated under anaerobiosis (Anaerogen Gas Pack, Oxoid) at 37°C for 48 h for lactic acid bacilli; M17 agar, incubated at 37°C for 48 h for lactococci; yeast peptone dextrose agar, supplemented with chloramphenicol (0.1 g/L) incubated at 30°C for 48 h for yeasts and molds; violet

red bile lactose agar incubated at 37°C for 24h for total coliforms; pseudomonas agar base, with SR103 E selective supplement (Oxoid), incubated at 25°C for 48 h for *Pseudomonas* spp.

pH Determination

Determination of pH was performed at the same sampling time of the microbiological analyses by using a pH meter (Crison 2001, Crison, Barcelona, Spain). Measures were carried out on both liquid and cheese samples.

Calculation of Microbial Acceptability Limit

The microbial acceptability limit (MAL) values of each spoilage microorganism were obtained by fitting the Gompertz equation as reparameterized by Corbo et al. (2006) to the growth cycle data:

$$\log(N(t)) = \log(N_{\max}) - A \cdot \exp \left\{ - \exp \left[\left[\left(\mu_{\max} \cdot 2.71 \right) \cdot \frac{\lambda - MAL}{A} \right] + 1 \right] \right\} + \left[\left(\mu_{\max} \cdot 2.71 \right) \cdot \frac{\lambda - t}{A} \right] + 1 \right\}, \quad [1]$$

where $N(t)$ is the viable cell concentration (cfu/g) at time t , A is related to the difference between the decimal logarithm of maximum bacteria growth attained at the stationary phase and the decimal logarithm of the initial value of viable cell concentration, μ_{\max} is the maximal specific growth rate ($\Delta \log[\text{cfu/g}]/\text{d}$), λ is the lag time (d) and t is the time (d), N_{\max} is the microbial threshold limit (cfu/g), and MAL is the time at which the microbiological threshold limit is reached [d; i.e., the time at which $N(t)$ is equal to N_{\max}].

Statistical Analyses

The MAL values were compared by one-way ANOVA. A Duncan's multiple range test with the option of homogeneous groups ($P < 0.05$) was used to determine significance between treatments. Statistica 7.1 for Windows (Stat-Soft Inc., Tulsa, OK) was used for this purpose.

RESULTS AND DISCUSSION

The effectiveness of different antimicrobial agents on the microbial quality decay of Fior di Latte cheese during storage at 10°C is addressed. Microbial quality

was assessed by monitoring the growth cycle of both *Pseudomonas* spp. and coliforms as representative spoilage microorganisms of the selected dairy product. To assess the effect of different natural compounds on typical microflora, the viable cell concentration of lactic acid bacteria was also monitored. Results on each microbial group are reported separately in the following.

Selection of Natural Compounds

The concentrations of several natural compounds used in this work ranged from 500 to 10,000 mg/kg. This wide range was chosen to guarantee a significant antimicrobial effect. In fact, according to data reported in the literature, these concentrations should range from 0.5 to 1% (Vrinda Menon and Garg, 2001). Moreover, it was also taken into account that the concentration of oil in food should be greater than in laboratory media to achieve the same antimicrobial efficiency because of the complex nature of food (Holley and Patel, 2005).

Several essential oils were screened by a panel test for suitability for dairy applications. Among the selected natural compounds, the panelists selected the following: *Salvia officinalis* (sage), *Thymus vulgaris* (thyme), *Rosmarinus officinalis* (rosemary), *Citrus aurantium* (sour orange), *Citrus sinensis* (sweet orange), *Citrus paradisi macf* (grapefruit), *Citrus limonum* (lemon essential oil), *Propolis*, *Vanilla planifoli* (vanilla), and hydroalcoholic extracts such as citral 95%, thymol, and nonanoic acid 96%. Natural compounds such as *Melaleuca alternifolia* (melaleuca) and *Mentha piperita* (mint) were discarded because the panelists disliked their smell when applied to dairy products. In addition, greater concentrations of natural compounds such as sage, thyme, rosemary, grapefruit, vanilla, and sour or sweet orange dissolved in salt solutions were found acceptable because they were below the threshold of perception.

Pseudomonas Species

Figures 1, 2, and 3 illustrate the evolution of *Pseudomonas* spp. counts plotted as a function of the storage period for the investigated compounds. Data related to the control sample (packaged in brine) shows this trend: a short lag phase, followed by an increase in the viable cell concentration until the stationary phase. Because of the high number of natural compounds, these have been divided into 3 groups based on antimicrobial effect: high, medium, or low. To quantitatively determine the effectiveness of the tested compounds in inhibiting the *Pseudomonas* spp. growth cycle, equation [1] was fitted to the experimental data. The fitting procedure allowed us to determine the MAL values re-

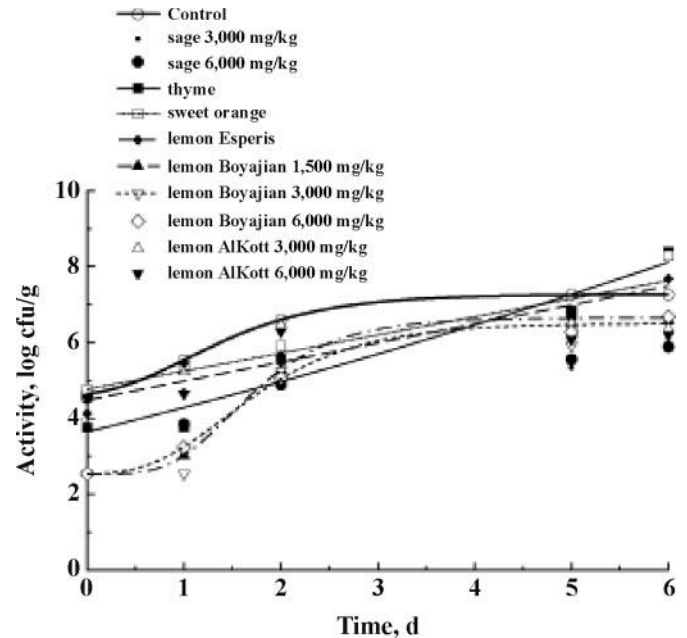


Figure 1. Antimicrobial activity of highly effective compounds on *Pseudomonas* spp. count in Fior di latte cheese during storage at 10°C. The curves shown in the figure are the best fit of the reparameterized Gompertz equation to the experimental data.

lated to *Pseudomonas* (MAL_P) for each natural preservative tested in this investigation (Table 1). The value of log(N_{max}) was set to 6 because at this level of contamination with *Pseudomonas* spp., alterations in the product begin to appear (Bishop and White, 1986). As we can see in Figure 1, sage and lemon (Boyajian and Alkott) do not reach a microbial level (10^6 cfu/g), enabling us to consider the MAL to be >6 d (Table 1). The other substances caused the evolution of *Pseudomonas* spp. count to decrease dramatically during the observation period. Conte et al. (2007a) have also shown that lemon may exert an inhibitory effect on the growth of spoilage microorganisms in Mozzarella cheese. In addition, literature data recorded on different food matrices confirm the pronounced antimicrobial effect of citrus essential oils (Tassou et al., 1996; Caccioni et al., 1998; Dorman and Deans, 2000; Lanciotti et al., 2004).

Figure 2 shows a slight decrease in cell concentration with compounds such as sage (Alkott) at 1,500 mg/kg, lemon 20% (Spencer) at 10,000 mg/kg, lemon (Primavera) at 1,500 mg/kg, lemon (Erbavita) at 5,000 mg/kg, orange, citral, sour orange, and thymol. The values of MAL_P (Table 1) are similar to those of the control.

Natural compounds such as rosemary, lemon 20% (Spencer) at 5,000 mg/kg, propolis, limonene, lemon (Agrumigel) at 5,000 mg/kg, vanilla, lemon (Alkott) at 1,500 mg/kg, grapefruit, lemon (Aboca), and nonanoic acid do not greatly change the *Pseudomonas* spp.

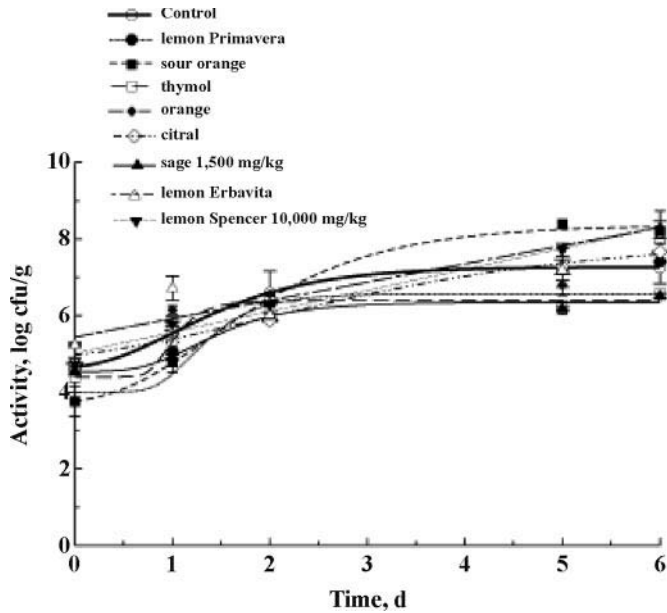


Figure 2. Antimicrobial activity of slowly effective compounds on *Pseudomonas* spp. count in Fior di latte cheese during storage at 10°C. The curves shown in the figure are the best fit of the reparameterized Gompertz equation to the experimental data.

growth cycle compared with the control (Figure 3). Nonanoic acid is known to possess antimicrobial activity particularly on fungi and yeasts (Kiesel, 1913; Novak et al., 1969; Madhu et al., 2005). In the literature, no studies have been recorded on the effects of nonanoic acid on *Pseudomonas* spp. In fact, for these samples, the values of MAL_P (Table 1) are always lower or similar to the traditional packaging. In an earlier work (Farbood et al., 1976), the antibacterial effect of rosemary extract in beef was examined on the growth of total bacterial count and on growth of *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas fluorescens*, *Salmonella typhimurium*, and *Staphylococcus aureus*, and low bactericidal effects were observed. Lemon and orange also possess very little antimicrobial activity (Juven et al., 1994; Skandamis and Nychas, 2000; Suhr and Nielsen, 2003). To summarize, it is notable that essences such as lemon and sage from Alkott and lemon from Boyajian, at 3,000 and 6,000 mg/kg, were the most effective in prolonging the MAL of *Pseudomonas* spp.

Coliforms

Figures 4, 5, and 6 show the evolution of coliform count over a 6-d storage period. As in the previous paragraph, the tested compounds are divided into 3 groups depending on their antimicrobial effect. The control shows the following trend: a short lag phase, followed by a continued increase in the viable cell concentra-

tion until the stationary phase. Conte et al. (2007a,b) also assessed the antimicrobial activity of citrus oil on total coliforms. The coliform count for lemon Boyajian at 3,000 mg/kg was significantly lower than the control during the observation period. Similar trends were observed for sweet orange, lemon (Boyajian at 3,000 and 6,000 mg/kg, Primavera, and Alkott), sage, thymol, citral, and grapefruit seed extract (Figure 4).

Essential oils such as thyme, lemon (Aboca and Boyajian at 1,500 mg/kg), and sour orange brought a slight decrease in coliform growth compared with the control (Figure 5), probably because of the protective effect of the food microorganisms (Hao et al., 1998).

Different behavior was recorded for lemon 20% (Spencer) at 5,000 and 10,000 mg/kg, propolis, limonene, lemon (Agrumigel) at 5,000 mg/kg, and lemon (Esperis) at 2,000 mg/kg (Figure 6). In fact, the evolution of coliform count was slightly greater than the control during the storage period. It is remarkable that natural compounds such as lemon (Ervavita), nonanoic acid, rosemary, vanilla, and orange reached the MAL within 1 or 2 d (Figure 6). To the best of our knowledge, no study on the effects of rosemary, vanilla, and orange extract on coliforms is available in the literature.

The MAL values related to coliforms (MAL_C) were determined according to what is reported above and listed in Table 1. As stated in DPR 54/97 (European Union, 1997), the value of $\log(N_{max})$ was set to 5. In

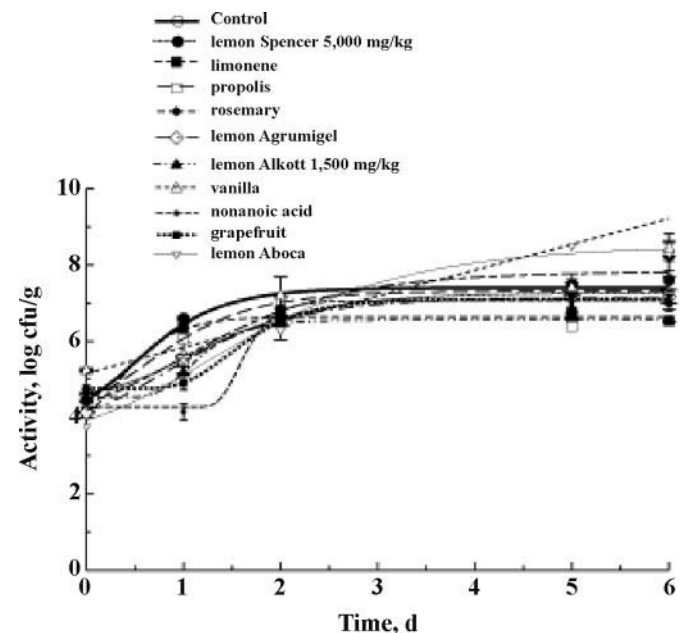


Figure 3. Antimicrobial activity of slowly effective compounds on *Pseudomonas* spp. count in Fior di latte cheese during storage at 10°C. The curves shown in the figure are the best fit of the reparameterized Gompertz equation to the experimental data.

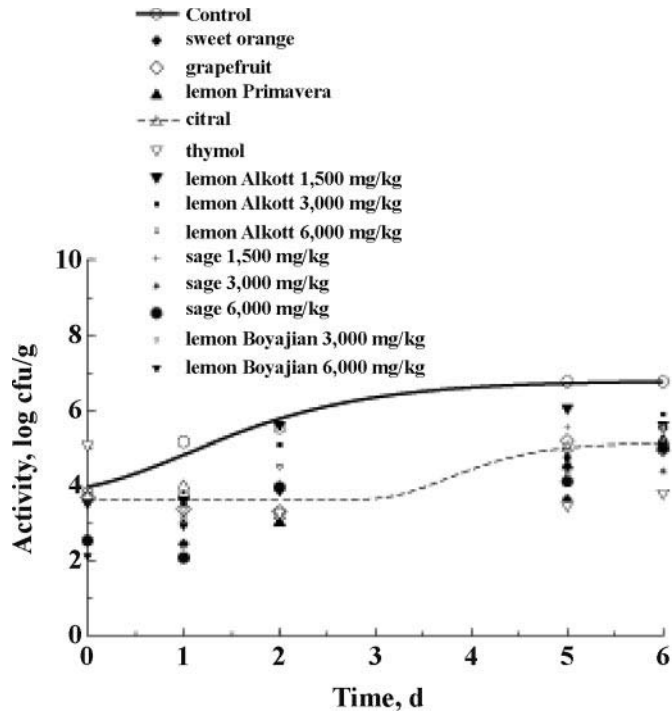


Figure 4. Antimicrobial activity of highly effective compounds on coliform count in Fior di latte cheese during storage at 10°C. The curves shown in the figure are the best fit of the reparameterized Gompertz equation to the experimental data.

summary, sweet orange, sage, thymol, grapefruit, and lemon (Primavera, Alkott, and Boyajian) seemed to be the most effective compounds to prolong MAL_C of coliforms in Fior di Latte cheese.

MAL Values

As also reported in the literature, when the microbial unacceptability of a given commodity is provoked by more than one microorganism, the MAL value of the packaged product is given by the lowest MAL among those calculated for each spoilage microorganism (i.e., MAL_P and MAL_C). In the third column of Table 1, the MAL values of the packaged dairy products are listed. As can be seen, it was not possible to calculate the MAL value for vanilla, lemon (Erbavita), orange (Erbavita), nonanoic acid, and rosemary, because the viable cell concentration of coliforms was above its threshold limit at the beginning of storage. For a few natural compounds, such as lemon 20% (Spencer) at 5,000 and 10,000 mg/kg, propolis, lemon (Agrumigel) at 5,000 mg/kg, lemon (Esperis) at 2,000 mg/kg, and limonene, MAL values lower than the control were observed. Conversely, for thymol, grapefruit, sweet orange, lemon (Alkott) at 1,500 mg/kg, lemon (Boyajian) at 6,000 mg/kg, and lemon (Primavera) at 1,500 mg/

kg, a MAL value slightly greater than the control was found. The MAL value was strongly affected by lemon and sage from Alkott and lemon from Boyajian. In fact, a MAL greater than 6 d was recorded.

Lactic Acid Bacteria

Figures 7 and 8 show the growth kinetics of rods and cocci lactic acid bacteria during storage, as determined at 37°C on MRS and M17 agar media, respectively. Because of the similar results obtained at all tested concentrations, only the experimental data of lemon (Boyajian), thymol, and vanilla were reported. As we can see, the functional microorganisms grew during refrigerated storage. Moreover, there were no marked differences between the control sample and those containing the active compounds. According to the literature, these results suggest that natural compounds do not affect the growth of lactic acid bacteria. Among the generally sensitive gram-positive bacteria, lactic acid bacteria are the most resistant to essential oils (Shelef, 1983; Blaszyk and Holley, 1998; Conte et al., 2007b). This microbial group was already present at high concentrations in the product immediately after packaging because of the technology used in dairy manufacture. The inefficiency of the tested natural oils on useful dairy bacteria is particularly important for a product such as Fior di Latte cheese, which is increasingly advertised as being “preservative-free and rich in viable lactic acid bacteria” (Coppola et al., 1995).

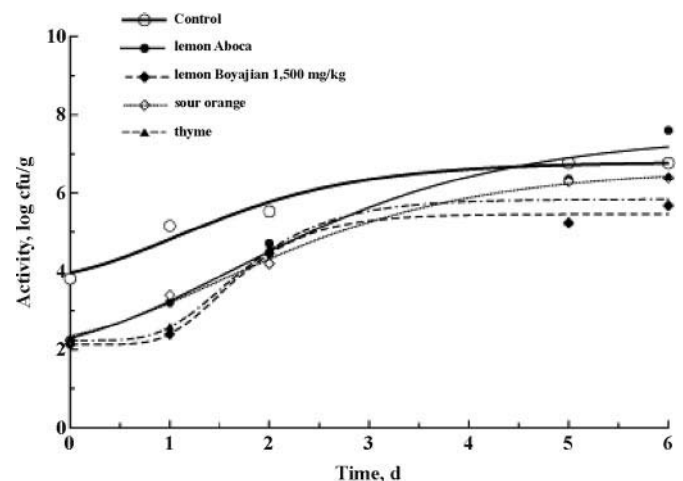


Figure 5. Antimicrobial activity of slowly effective compounds on coliform count in Fior di latte cheese during storage at 10°C. The curves shown in the figure are the best fit of the reparameterized Gompertz equation to the experimental data.

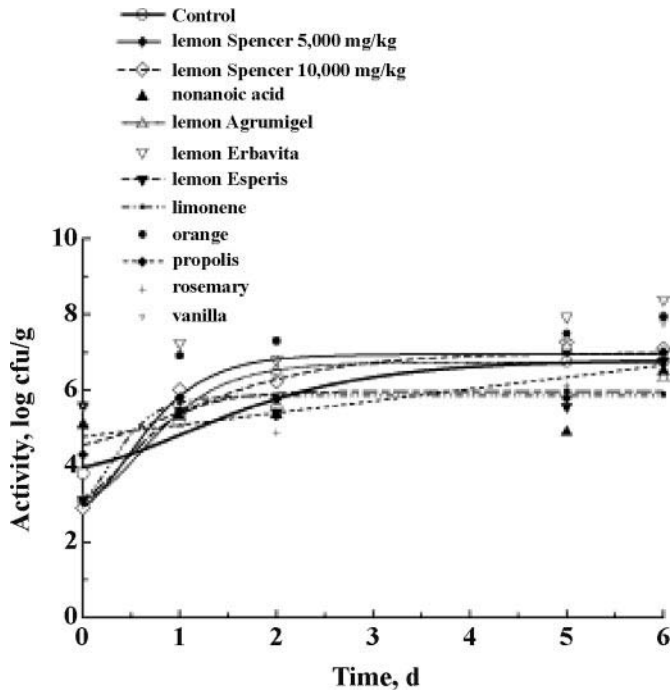


Figure 6. Antimicrobial activity of slowly effective compounds on coliform count in Fior di latte cheese during storage at 10°C. The curves shown in the figure are the best fit of the reparameterized Gompertz equation to the experimental data.

Yeasts and Mesophilic Total Count

The amount of yeasts recorded during the storage period (data not shown), ranging from 2 to 5 log cfu/g, was in agreement with data reported in previous works (Nunez and Dias-Amado, 1981; Coppola et al., 1988). In fact, studies reported in the literature clearly show that yeasts are a significant part of the microflora, be-

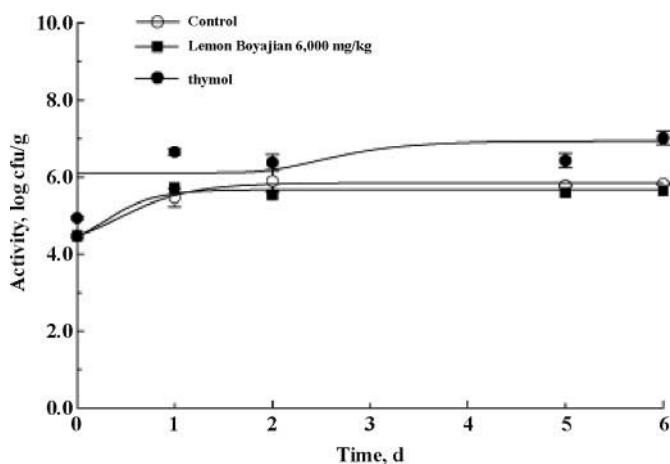


Figure 7. Evolution of lactic acid bacteria rod count in Fior di latte cheese during storage at 10°C. The curves are the best fit of the Gompertz equation to the experimental data.

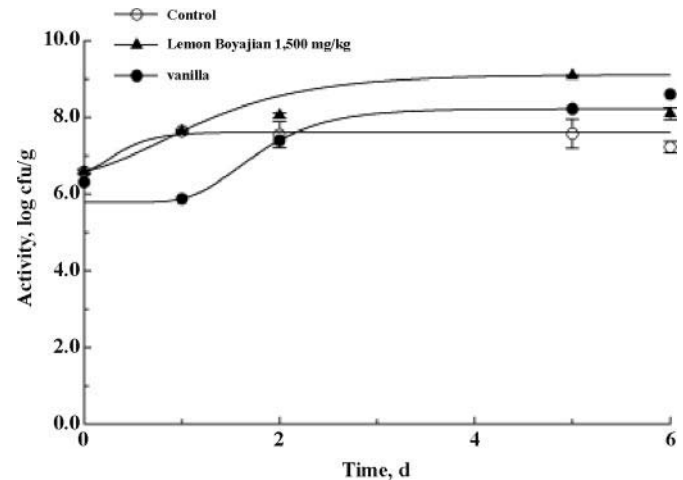


Figure 8. Evolution of lactic acid bacteria cocci count in Fior di latte cheese during storage at 10°C. The curves are the best fit of the Gompertz equation to the experimental data.

ing responsible for the aromatic and sensorial characteristics of Mediterranean cheeses (Morea et al., 1999). The mesophilic total count was similar in all samples (data not shown).

pH

The pH of Fior di Latte cheese was monitored during the entire observation period (data not shown). Results show that it did not change much during storage (ranging between 5.80 and 5.40). Moreover, the pH values were not affected by the presence of active compounds, suggesting that the observed antimicrobial activity has to be exclusively ascribed to the effectiveness of the investigated natural compounds.

CONCLUSIONS

It can be concluded that the use of natural compounds such as lemon, sage, and thyme in Fior di Latte cheese inhibited the growth of microorganisms involved in spoilage. Therefore, a substantial prolongation of the microbial acceptability limit was obtained, without affecting useful microorganisms. It is also evident that not all essential oils act in the same manner. For this reason, in future studies, the use of a central composite design will be considered to assess the individual and interactive effects of some natural compounds on the microbial stability of this fresh dairy product. Moreover, Fior di Latte cheese quality sub-indices other than microbiological stability will be considered for a complete shelf-life evaluation.

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