

## Anti-staphylococcal effect of cinnamaldehyde in milk

Milijana Babic<sup>1†</sup>, Milica Glisic<sup>1†</sup>, Jasna Djordjevic<sup>1</sup>, Nemanja Zdravkovic<sup>2</sup>, Radoslava Savic-Radovanovic<sup>1</sup>, Milan Baltic<sup>1</sup>, Marija Boskovic Cabrol<sup>1‡\*</sup>

<sup>1</sup>Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Belgrade, Bulevar Oslobođenja, Belgrade, Serbia; <sup>2</sup>Department of Bacteriology and Parasitology, Scientific Institute of Veterinary Medicine of Serbia, Vojvode Toze, Belgrade, Serbia

<sup>†</sup>These authors contributed equally to the work.

**\*Corresponding Author:** Marija Boskovic Cabrol, Research Associate, Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Belgrade, Bulevar Oslobođenja 18, 11000 Belgrade, Serbia. Email: [marijaboskovic116@gmail.com](mailto:marijaboskovic116@gmail.com)

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### Abstract

The survival of *Staphylococcus aureus* in inoculated ( $10^5$  colony forming units [CFU]/mL) 3.2% and 0.5% fat ultra-high temperature-pasteurized milk samples containing 0%, 0.05%, or 0.1% cinnamaldehyde stored at 4°C or 10°C was evaluated within 15 days. *S. aureus* populations reached 7.92 (0.5% fat) and 7.95 (3.2% fat) log CFU/mL in control milk samples stored at 10°C, while in milk sample stored at 4°C, *S. aureus* counts remained almost unchanged. At the end of the study, the number of this pathogen decreased by 1.52–4.04 log CFU/mL in milk treated with cinnamaldehyde. The greatest anti-staphylococcal effect was achieved in low-fat milk at 10°C and treated with 0.1% cinnamaldehyde.

**Keywords:** antibacterial activity, cinnamaldehyde, fat, milk safety, *Staphylococcus aureus*

### Introduction

Milk and milk products, being highly nutritious foods, are excellent media for the growth of many spoilage and pathogenic microorganisms (Noël *et al.*, 2016), including *Staphylococcus aureus*. This pathogen commonly exists in dairy production plants (Xing *et al.*, 2016), and it is one of the most important causative infective agents of clinical and subclinical mastitis in dairy cattle (Nam *et al.*, 2011; Basanisi *et al.*, 2017). *S. aureus* presents an important public health burden since it is one of the major pathogens responsible for food intoxication (Jans *et al.*, 2017).

In spite of the fact that pasteurization kills *S. aureus*, it has little effect on thermostable enterotoxins, which

generally preserve their biological activity after exposure to heat (Jablonski and Bohach, 1997; Jørgensen *et al.*, 2005). The presence of *S. aureus* in raw milk before processing is a concern because different physical and chemical production techniques are applied during processing and ripening of milk products to prevent growth of this pathogen and production of enterotoxins. Nevertheless, if one of these limiting factors fails, there is a risk of accumulation of staphylococcal enterotoxins (Jørgensen *et al.*, 2005). Thus, it is important to control growth of *S. aureus* in raw milk and raw milk products.

In order to ensure milk safety and prolong milk's shelf life, while also improving its sensorial characteristics, the dairy industry is developing minimum processing techniques (Cava *et al.*, 2007). It has been suggested

that addition of plant extracts, including cinnamon, can enhance microbiological safety, and it positively affects the sensory attributes of processed dairy products and milk-based desserts such as rice pudding and vanilla cream pudding (Tayel *et al.*, 2015; Lianou *et al.*, 2018). When added to butter, cinnamon (3%) lowered microbial growth during storage and exhibited antioxidant activity, thus retarding the spoilage of butter by positively influencing its sensorial characteristics (Vidanagamage *et al.*, 2016). Thus, cinnamon could be successfully incorporated in butter as a natural preservative instead of synthetic preservatives.

Cinnamon contains 85.3–90.5% cinnamaldehyde (Doyle and Stephens, 2019). Together with eugenol, isoeugenol, vanillin, and safrole, cinnamaldehyde is one of the best studied phenylpropenes (Nazzaro *et al.*, 2013). Trans-cinnamaldehyde exhibits a wide range of beneficial effects, including antibacterial, antifungal, antioxidant, anti-inflammatory, anti-diabetic, neuroprotective, and antitumor (Masghati and Ghoreishi, 2018; Doyle and Stephens, 2019), while cis-cinnamaldehyde, the geometrical isomer of trans-cinnamaldehyde, exhibits antifungal properties (Doyle and Stephens, 2019). Essential oil (EO) of cinnamon has found application in food industry because of its various components, including cinnamaldehyde, a major ingredient of cinnamon bark oil (Masghati and Ghoreishi, 2018). Most essential oils and their components, including trans-cinnamaldehyde, are generally recognized as safe (GRAS) and accepted by consumers (Burt, 2004). Owing to their antibacterial and antioxidant properties, essential oils can be used as potential natural preservatives in different foods, including flavored drinks (Cava *et al.*, 2007). Flavored milk has increased in popularity in recent years; nevertheless, there are few data available in literature about the effect of adding essential oils directly to milk before cheese-making (Licon *et al.*, 2020).

The focus of the present study was to determine whether trans-cinnamaldehyde could be a potential natural antibacterial agent in milk, hence ultra-high temperature (UHT)-pasteurized milk was used as a matrix to eliminate any possible interactions with the microbiota normally present in raw milk. The aims of the study were to: (1) evaluate the anti-staphylococcal effect of different concentrations of cinnamaldehyde (0.05% and 0.1%) on *S. aureus* in milk; and (2) determine the influence of different fat contents (0.5% and 3.2% milk fat) and different storage temperatures on survival of *S. aureus* in milk.

## Materials and Methods

Trans-cinnamaldehyde and *S. aureus* culture, UHT-pasteurized milk samples containing 0.5% and 3.2% fat

were bought from a local supermarket. Cinnamaldehyde (CA) (98% purity) was purchased from Carl Roth, Germany and stored at 4°C prior to use. *S. aureus* was obtained from the American Type Culture Collection (ATCC 25923).

### Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of cinnamaldehyde was determined in a non-milk matrix using sterile U-bottom 96-well microplates. The bacterial inoculum density was set to 0.5 on the McFarland scale, then further diluted 10 times in sterile saline; 5 µL of this suspension was inoculated into 0.1 mL of Cation-Adjusted Mueller–Hinton Broth (CAMHB; Becton, Dickinson and Company, Sparks, USA) to reach a final *S. aureus* ATCC 25923 inoculum of  $5 \times 10^4$  colony forming units (CFU)/well. Cinnamaldehyde was diluted in dimethyl sulfoxide (Serva, Heidelberg, Germany) and added to CAMHB in the levels of 2560–1.25 µg/mL by two-fold dilution in 96-well microtitre plates. After inoculation, plates were incubated for 24 h at 37°C. The MIC was the lowest concentration of cinnamaldehyde that did not show any visual growth of *S. aureus* after macroscopic evaluation, and it was expressed in µg/mL (Clinical and Laboratory Standards Institute [CLSI], 2006). The plates were prepared in triplicate.

### Sample preparation and storage conditions

Milk containing 0.5% or 3.2% fat was analyzed for *S. aureus* to confirm the absence of this pathogen. Approximately 5 log CFU/mL of *S. aureus* was inoculated into *S. aureus*-free milk containing 0.5% or 3.2% milk fat. The concentration of the inoculum was verified by the standard plate count method and determined as 5.55–5.60 log CFU/mL. To study the survival of *S. aureus* in milk, different concentrations of cinnamaldehyde (0.05% and 0.1%) were added to milk samples with 0.5% (reduced fat) and 3.2% (whole milk) milk fat, whereas controls were without cinnamaldehyde but were inoculated with *S. aureus*. The selection of these concentrations of cinnamaldehyde was based on previous sensory evaluations (Babic *et al.*, 2019). After addition of cinnamaldehyde, all milk samples were divided into halves and stored in sterile glass bottles at 4°C and 10°C for 15 days. This temperature of 10°C was selected as an abuse temperature. The milk samples are described in Table 1.

### Microbiological and pH analysis

All milk samples were examined on storage days 0, 3, 6, 9, 12, and 15. For bacterial enumeration, 25 mL of milk

**Table 1. Experimental design.**

Medium	Cinnamaldehyde	Temperature	Milk samples
Milk containing 0.5% fat with <i>S. aureus</i>	0%	4°C	1. Milk containing 0.5% fat with <i>S. aureus</i> and without cinnamaldehyde stored at 4°C.
	0.05%		2. Milk containing 0.5% fat with <i>S. aureus</i> and 0.05% cinnamaldehyde stored at 4°C.
	0.1%		3. Milk containing 0.5% fat with <i>S. aureus</i> and 0.1% cinnamaldehyde stored at 4°C.
	0%	10°C	4. Milk containing 0.5% fat with <i>S. aureus</i> and without cinnamaldehyde stored at 10°C.
	0.05%		5. Milk containing 0.5% fat with <i>S. aureus</i> and 0.05% cinnamaldehyde stored at 10°C.
	0.1%		6. Milk containing 0.5% fat with <i>S. aureus</i> and 0.1% cinnamaldehyde stored at 10°C.
Milk containing 3.2% fat with <i>S. aureus</i>	0%	4°C	7. Milk containing 3.2% fat with <i>S. aureus</i> and without cinnamaldehyde stored at 4°C.
	0.05%		8. Milk containing 3.2% fat with <i>S. aureus</i> and 0.05% cinnamaldehyde stored at 4°C.
	0.1%		9. Milk containing 3.2% fat with <i>S. aureus</i> and 0.1% cinnamaldehyde stored at 4°C.
	0%	10°C	10. Milk containing 3.2% fat with <i>S. aureus</i> and without cinnamaldehyde stored at 10°C.
	0.05%		11. Milk containing 3.2% fat with <i>S. aureus</i> and 0.05% cinnamaldehyde stored at 10°C.
	0.1%		12. Milk containing 3.2% fat with <i>S. aureus</i> and 0.1% cinnamaldehyde stored at 10°C.

was transferred into a sterile Stomacher bag and 225 mL of Buffered Peptone Water (BPW; Merck, Germany) was added. The contents of each bag were homogenized in a Stomacher blender (Stomacher 400 Circulator, Seward, UK) for 2 min. Serial decimal dilutions were prepared and 0.1 mL of appropriately diluted suspension was plated on Baird Parker agar (Oxoid CM 275, Basingstoke, Hampshire, UK) with egg yolk tellurite emulsion (Oxoid CM 275, Basingstoke, Hampshire, UK) and incubated at 37°C for 24 h according to EN ISO 6888-1 (International Organization for Standardization [ISO], 1999). The number of colonies was counted, and results were recorded as colony forming units per milliliter.

The pH of milk samples was measured using a portable pH meter (Testo 205; Testo AG, Lenzkirch, Germany). The pH meter was calibrated with standard buffer solutions of pH 4.0 and 7.0 prior to use.

### Statistical analysis

Six randomized milk samples from each group were analyzed on each examination day. Number of microorganisms were transformed into logarithms (log) before statistical analysis. Statistical analysis of the results was conducted using the SPSS 20.0 software (IBM, Chicago, IL, USA). The *S. aureus* counts were expressed as mean  $\pm$  standard deviation. A three-way ANOVA analysis was used to investigate factor effects (concentrations of cinnamaldehyde, temperature, and fat%) and interactions among them on log-transformed *S. aureus* counts. Statistical differences between examined groups were determined by Tukey's *post hoc* multiple comparisons test.  $P < 0.05$  was considered statistically significant.

## Results and Discussion

### Anti-staphylococcal effect of cinnamaldehyde in milk during storage

The MIC of cinnamaldehyde against *S. aureus* was 160  $\mu\text{g}/\text{mL}$ , showing that cinnamaldehyde was able to inhibit growth of this pathogen at low concentrations in the non-milk matrix used. Alves *et al.* (2016) reported a cinnamaldehyde MIC of 100  $\mu\text{g}/\text{mL}$  against *S. aureus*, in agreement with the result of the present study. Nevertheless, in spite of the good antibacterial effect *in vitro*, hydrophobic essential oil constituents are impaired by interactions with food matrix components, hence higher concentrations are needed to achieve the same antibacterial effect in food (Hyldgaard *et al.*, 2012). Thus, in the present study, approximately 4- and 9-fold higher concentrations (0.05% and 0.1%) of cinnamaldehyde than the obtained MIC were added to milk samples.

Significant ( $P < 0.05$ ) antibacterial activity against *S. aureus* was found in milk samples at the cinnamaldehyde concentrations used (0.05% and 0.1%) when compared with the controls without cinnamaldehyde (Table 2).

Initial *S. aureus* counts ranged from 5.55 to 5.60 log CFU/mL. On day 0, *S. aureus* counts were significantly ( $P < 0.05$ ) higher in controls than in milk samples with cinnamaldehyde at 4°C and at 10°C, indicating the immediate antibacterial effect of cinnamaldehyde. Regardless of fat content, in control milk samples without cinnamaldehyde stored at 4°C, with the exception of a slight decrease observed on day 3, the *S. aureus* populations remained almost unchanged for 15 days compared with the initial populations in milk samples. Nevertheless, at 10°C, *S. aureus* counts increased to approximately 7.92

**Table 2.** *S. aureus* counts (log CFU/mL) in milk with and without added cinnamaldehyde (CA), stored at 4°C and 10°C (mean  $\pm$  SD), and the significance of interactions between cinnamaldehyde, storage temperature, and milk fat.

CA concentration	Temperature	Fat	Days					
			0	3	6	9	12	15
0%	4°C	0.5%	5.56 $\pm$ 0.06 <sup>a</sup>	5.43 $\pm$ 0.10 <sup>ac</sup>	5.46 $\pm$ 0.06 <sup>a</sup>	5.48 $\pm$ 0.04 <sup>a</sup>	5.57 $\pm$ 0.11 <sup>a</sup>	5.56 $\pm$ 0.05 <sup>a</sup>
		3.2%	5.60 $\pm$ 0.07 <sup>a</sup>	5.45 $\pm$ 0.09 <sup>a</sup>	5.49 $\pm$ 0.10 <sup>a</sup>	5.50 $\pm$ 0.06 <sup>a</sup>	5.64 $\pm$ 0.08 <sup>a</sup>	5.63 $\pm$ 0.06 <sup>a</sup>
	10°C	0.5%	5.55 $\pm$ 0.08 <sup>a</sup>	6.98 $\pm$ 0.10 <sup>b</sup>	7.21 $\pm$ 0.05 <sup>b</sup>	7.49 $\pm$ 0.07 <sup>b</sup>	7.47 $\pm$ 0.07 <sup>b</sup>	7.92 $\pm$ 0.07 <sup>b</sup>
		3.2%	5.57 $\pm$ 0.09 <sup>a</sup>	7.00 $\pm$ 0.16 <sup>b</sup>	7.23 $\pm$ 0.15 <sup>b</sup>	7.43 $\pm$ 0.07 <sup>b</sup>	7.45 $\pm$ 0.08 <sup>b</sup>	7.95 $\pm$ 0.10 <sup>b</sup>
0.05%	4°C	0.5%	5.40 $\pm$ 0.06 <sup>b</sup>	5.32 $\pm$ 0.07 <sup>adce</sup>	5.15 $\pm$ 0.07 <sup>c</sup>	4.62 $\pm$ 0.09 <sup>c</sup>	4.44 $\pm$ 0.05 <sup>c</sup>	3.95 $\pm$ 0.09 <sup>c</sup>
		3.2%	5.32 $\pm$ 0.07 <sup>bc</sup>	5.23 $\pm$ 0.04 <sup>def</sup>	5.11 $\pm$ 0.08 <sup>c</sup>	4.89 $\pm$ 0.07 <sup>d</sup>	4.28 $\pm$ 0.06 <sup>d</sup>	4.08 $\pm$ 0.07 <sup>c</sup>
	10°C	0.5%	5.21 $\pm$ 0.05 <sup>cd</sup>	5.26 $\pm$ 0.05 <sup>cdef</sup>	4.51 $\pm$ 0.05 <sup>d</sup>	3.81 $\pm$ 0.05 <sup>c</sup>	3.04 $\pm$ 0.07 <sup>e</sup>	2.45 $\pm$ 0.07 <sup>d</sup>
		3.2%	5.34 $\pm$ 0.07 <sup>bc</sup>	5.30 $\pm$ 0.10 <sup>adef</sup>	4.87 $\pm$ 0.08 <sup>eg</sup>	4.00 $\pm$ 0.13 <sup>f</sup>	3.90 $\pm$ 0.08 <sup>f</sup>	3.23 $\pm$ 0.07 <sup>e</sup>
0.1%	4°C	0.5%	5.28 $\pm$ 0.07 <sup>bc</sup>	5.20 $\pm$ 0.08 <sup>ef</sup>	4.94 $\pm$ 0.06 <sup>g</sup>	4.46 $\pm$ 0.08 <sup>g</sup>	4.10 $\pm$ 0.09 <sup>g</sup>	3.11 $\pm$ 0.07 <sup>c</sup>
		3.2%	5.26 $\pm$ 0.06 <sup>bc</sup>	5.18 $\pm$ 0.05 <sup>ef</sup>	5.04 $\pm$ 0.08 <sup>cg</sup>	4.56 $\pm$ 0.06 <sup>eg</sup>	4.26 $\pm$ 0.08 <sup>d</sup>	3.78 $\pm$ 0.07 <sup>f</sup>
	10°C	0.5%	5.11 $\pm$ 0.08 <sup>d</sup>	5.14 $\pm$ 0.06 <sup>f</sup>	4.08 $\pm$ 0.07 <sup>f</sup>	3.62 $\pm$ 0.08 <sup>h</sup>	2.51 $\pm$ 0.06 <sup>h</sup>	1.51 $\pm$ 0.04 <sup>g</sup>
		3.2%	5.27 $\pm$ 0.06 <sup>bc</sup>	5.25 $\pm$ 0.07 <sup>ef</sup>	4.48 $\pm$ 0.08 <sup>d</sup>	3.18 $\pm$ 0.09 <sup>i</sup>	3.11 $\pm$ 0.07 <sup>e</sup>	2.61 $\pm$ 0.10 <sup>h</sup>
Conc. CA $\times$ *Temp.			NS	**	**	**	**	**
Conc. CA $\times$ Fat%			NS	NS	**	**	**	**
Conc. CA $\times$ Temp. $\times$ Fat%			*	NS	**	**	**	**

<sup>a</sup>-Different superscript letters in the same column, P < 0.05.

NS: Not significant.

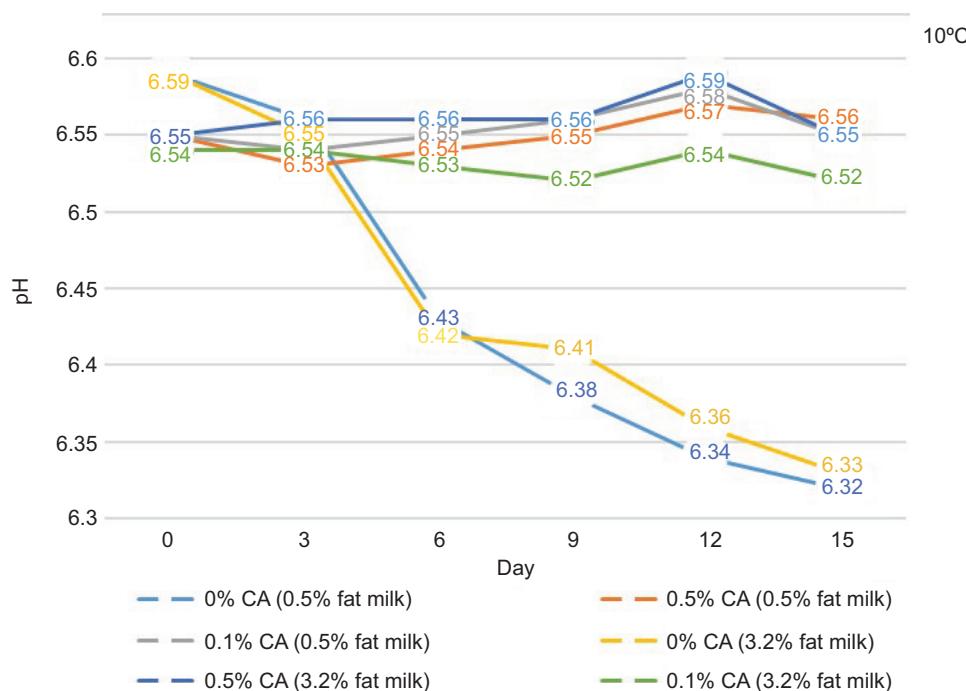
\*P < 0.05; \*\*P < 0.001.

log CFU/mL (0.5% milk fat) and 7.95 log CFU/mL (3.2% milk fat) by the end of storage (day 15) in milk samples without cinnamaldehyde. Growth of *S. aureus* is possible at temperatures above 8°C at optimum pH values ranging between 6.0 and 7.0 (Valero *et al.*, 2009). In all milk groups studied, the pH was within the optimal range (Figure 1) and enabled *S. aureus* to grow and survive at the utilized storage temperatures.

In contrast, *S. aureus* counts decreased during 15 days' storage in all milk samples with added cinnamaldehyde. The decrease was less pronounced during the first 3 days of storage, and during this time no significant differences (P > 0.05) in *S. aureus* numbers were recorded between milk samples stored at 4°C and those stored at 10°C. From day 6 until the end of storage period (day 15), significantly greater *S. aureus* decrease (P < 0.05) was recorded in milk samples with added cinnamaldehyde stored at 10°C than in comparable milk samples stored at 4°C. At the end of the study, in milk samples treated with cinnamaldehyde, *S. aureus* numbers had decreased by 1.61 log CFU/mL (0.5% milk fat with 0.05% cinnamaldehyde at 4°C), 2.45 log CFU/mL (0.5% milk fat with 0.1% cinnamaldehyde at 4°C), 1.52 log CFU/mL (3.2% milk fat with 0.05% cinnamaldehyde at 4°C), 1.82 log CFU/mL (3.2% milk fat with 0.1% cinnamaldehyde at 4°C), 3.1 log CFU/mL (0.5% milk fat with 0.05% cinnamaldehyde at 10°C), 4.04 log CFU/mL (0.5% milk fat with 0.1% cinnamaldehyde at 10°C), 2.34 log CFU/mL

(3.2% milk fat with 0.05% cinnamaldehyde at 10°C), and 2.96 log CFU/mL (3.2% milk fat with 0.1% cinnamaldehyde at 10°C). The anti-staphylococcal effect of cinnamaldehyde found in the present study was in agreement with previous reports. Alves *et al.* (2016) reported that growth of *S. aureus* was inhibited by the combination of nisin and cinnamaldehyde in pasteurized 3% fat milk stored at 4°C for 6 days.

The mechanism of cinnamaldehyde's antibacterial action is known and well described. The antibacterial activity of cinnamaldehyde is attributed to a free hydroxyl group (Nazzaro *et al.*, 2013). Cui *et al.* (2016) reported that after treating *S. aureus* with cinnamon essential oil, cell membrane injury and leakage of intracellular material were observed. Loss of ATP and DNA were detected because of bacterial cell membrane damage. Some reports indicate that cinnamaldehyde inhibits the membrane-bound ATPase activity (Usta *et al.*, 2003; Gill and Holley, 2004). Di Pasqua *et al.* (2006) found that trans-cinnamaldehyde causes changes in the composition of fatty acid and large increase in the proportion of saturated fatty acids in membrane phospholipids. Shen *et al.* (2015) evaluated the effect of cinnamaldehyde on inner membrane permeability of *S. aureus* by measuring  $\beta$ -galactosidase activity. The authors found that  $\beta$ -galactosidase activity increased with increase in cinnamaldehyde concentration, leading to the conclusion that effects on membranes are dose-dependent. In our previous pilot study (Babic



**Figure 1.** pH of milk stored at 10°C.

et al., 2019), we found that the antibacterial effect of cinnamaldehyde was dependent on its concentration in 1.5% fat milk inoculated with  $10^3$  CFU/mL *S. aureus* stored at 4°C for 12 days. The same observation was made in the present study, showing significantly ( $P < 0.05$ ) higher inhibition with 0.1% than 0.05% cinnamaldehyde used, but only for milk samples stored at the same temperature. It is supposed that essential oils are more effective when added at higher concentrations because after interactions with food matrix components (e.g. proteins and fats), more of the essential oil remains to interact with the bacterial cells (Hyldgaard et al., 2012; Boskovic et al., 2017).

One of the most important findings of the present study was the greater bacteriostatic effect of cinnamaldehyde at higher temperature. Significantly greater *S. aureus* decrease ( $P < 0.05$ ) was recorded in milk samples with cinnamaldehyde stored at 10°C than in milk samples with same concentration of cinnamaldehyde stored at 4°C. With the expected exception of day 0, the interactions of storage temperature and cinnamaldehyde concentration ( $P = 0.001$ ; factorial ANOVA) on *S. aureus* counts ( $P = 0.207$ ; factorial ANOVA) were statistically significant. At 4°C, 0.05% cinnamaldehyde decreased the number of *S. aureus* to 3.95 log CFU/mL in low-fat milk and to 4.08 log CFU/mL in whole milk, while at 10°C, this concentration of cinnamaldehyde decreased *S. aureus* counts to 2.45 log CFU/mL in low-fat milk and to 3.23 log CFU/mL in whole milk. When added at higher concentration (0.1%), cinnamaldehyde reduced the initial *S. aureus* population to 3.11 log CFU/mL in low-fat milk

and to 3.78 log CFU/mL in whole milk in samples stored at 4°C, while a significantly lower number ( $P < 0.05$ ) of *S. aureus* was recorded in milk samples treated with the same concentration of cinnamaldehyde and stored at 10°C (1.51 log CFU/mL in low-fat milk and 2.61 log CFU/mL in whole milk).

One possible explanation for this temperature-dependent antibacterial effect of cinnamaldehyde is that bacteria are metabolically more active at higher temperatures. Consequently, growth and death rates are higher at higher temperature (Smith-Palmer et al., 1998; Yuste and Fung, 2003; Guler and Seker, 2009). In addition, the lower growth rate of bacteria at lower temperatures can make them less susceptible to antimicrobials (Martinsen et al., 1992). Also, at lower temperatures, essential oils have lower diffusion rates, and this reduces the efficiency of their antibacterial activity (Wojtys and Jankowski, 2004; Leja et al., 2019). Even a small change in temperature causes significant changes in the efficiency of their action, which is why the doses of essential oils must be significantly higher at lower temperatures (Leja et al., 2019). These effects are in agreement with the results of present study. In milk samples with the same amount of fat, the anti-staphylococcal effect of 0.05% cinnamaldehyde was significantly ( $P < 0.05$ ) more pronounced at 10°C than the effect of 0.1% cinnamaldehyde at 4°C (Table 2). In addition, Smith-Palmer et al. (1998) reported that the target site of cinnamon essential oil can change, and oil penetration to the interior of the cell can be reduced due to alterations in membranes at lower temperatures. Higher antibacterial

activities of essential oils at higher temperatures have been reported previously. Guler and Seker (2009) reported that the effect of cinnamon on *Bacillus cereus* reductions in UHT-pasteurized milk during 28 days was significantly lower when milk samples were stored at 4°C than at 25°C. Yuste and Fung (2003) found that addition of 0.3% cinnamon in apple juice was more effective against *S. aureus* during storage at higher temperatures. The initial contamination was lower (4.34–4.37 log CFU/mL) than in the present study, and counts decreased below detection limits in apple juice stored at 20°C after only 1 day, but it took 7 days of storage at 5°C to obtain the same results. In the present study, the greatest anti-staphylococcal effect was achieved in low-fat milk stored at 10°C and treated with 0.1% cinnamaldehyde, as *S. aureus* numbers were reduced by more than 4 log CFU/mL.

Moreover, the effect of interaction between cinnamaldehyde concentration and fat content in milk on *S. aureus* numbers was significant ( $P < 0.0001$ ) from day 6 until the end of storage, while for the first 3 days no interaction was observed (day 0,  $P = 0.423$ ; day 3,  $P = 0.370$ ; factorial ANOVA). At the end of storage, significant differences ( $P < 0.05$ ) between the *S. aureus* counts in whole milk (3.2%) and low-fat milk (0.5%) were found for the same concentrations of cinnamaldehyde. However, no significant differences ( $P > 0.05$ ) in *S. aureus* counts were found between milk samples stored at the same temperatures without cinnamaldehyde, regardless of content of milk fat. Thus, cinnamaldehyde was more effective in inhibiting the pathogen in low-fat milk than in high-fat milk, which is also consistent with literature. Cava-Roda *et al.* (2012) found significant differences between whole milk (3.9% fat) and skimmed milk (0.3% fat) for inhibiting *Escherichia coli* O157:H7 and *Listeria monocytogenes* using the same concentration of vanillin (which also belongs to a group of phenylpropenes, as does cinnamaldehyde). The authors also reported that there was no effect of content of milk fat on pathogen numbers in control milk samples without vanillin. Therefore, the antibacterial effects of essential oils and other antimicrobial agents are likely to decrease or even limited by the amount of fat in the matrix (Liu and Yang, 2012; Boskovic *et al.*, 2017, 2019). It has been suggested that fats may form a protective layer around bacterial cells and absorb essential oils, leaving the water phase free of antimicrobial agents, and therefore lowering the antibacterial activity (Tassou *et al.*, 1995; Smith-Palmer *et al.*, 2001; Perricone *et al.*, 2015).

### pH of milk during storage

The initial pH of milk samples ranged from 6.54 to 6.59. Cinnamaldehyde initially caused the milk pH to drop very slightly (from 6.59 to around 6.55). Milk pH did not change significantly during storage at 4°C.

However, when milk without cinnamaldehyde was stored at 10°C, decline in pH was observed regardless of the fat content. In fact, on day 3, the pH of milk without cinnamaldehyde stored at 10°C (Figure 1) decreased slightly compared with the initial pH values, but from day 6, significant ( $P < 0.05$ ) pH declines were measured. The pH of this milk kept on decreasing throughout the 15 days' storage, reaching pH values of 5.32 (0.5% milk fat) and 5.33 (3.2% milk fat). Growth of *S. aureus* in these milk samples (Table 2) matched decline in pH. Under aerobic conditions, *S. aureus* can ferment milk sugar and lactose, creating acids responsible for the storage-induced decline in milk pH (Medvedová and Valík, 2012). The pH of milk samples with cinnamaldehyde added and stored at 10 °C did not significantly differ between each other.

### Conclusion

These results indicate that it could be possible to use cinnamaldehyde as a natural anti-staphylococcal agent in milk beverages. *S. aureus* numbers in milk were affected by cinnamaldehyde in a dose-dependent manner. Cinnamaldehyde showed a greater antibacterial effect against *S. aureus* in low-fat milk than in whole milk. Temperature had a strong effect on the anti-staphylococcal effect of cinnamaldehyde; hence, the lower concentration of cinnamaldehyde in milk stored at 10°C tended to have a better anti-staphylococcal effect than the higher concentration of cinnamaldehyde in milk stored at 4°C. Nevertheless, even if the results of our study are promising, and if flavored milk is becoming increasingly popular, further investigations are required to determine the antibacterial effectiveness of cinnamaldehyde in raw milk and dairy products and to conduct sensory analysis of final products.

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### Conflicts of Interest

The authors have no conflicts of interest for this article.

### References

- Alves F.C., Barbosa L.N., Andrade B.F., Albano M., Furtado F.B., Pereira A.F.M., Rall W.L.M. and Júnior A.F. 2016. Inhibitory activities of the lantibioticcinsin combined with phenolic compounds against *Staphylococcus aureus* and *Listeria*

- monocytogenes in cow milk. *J. Dairy Sci.* 99(3):1831–1836. <https://doi.org/10.3168/jds.2015-10025>
- Babic M., Glisic M., Zdravkovic N., Djordjevic J., Velebit B., Ledina T., et al. 2019. Inhibition of *Staphylococcus aureus* by cinnamaldehyde and its effect on sensory properties of milk. IOP Conf Ser Earth Environ Sci. 333:012042. <https://doi.org/10.1088/1755-1315/333/1/012042>
- Basanisi M.G., La Bella G., Nobili G., Franconieri I., and La Salandra G. 2017. Genotyping of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from milk and dairy products in South Italy. *Food Microbiol.* 62:141–146. <https://doi.org/10.1016/j.fm.2016.10.020>
- Boskovic M., Djordjevic J., Glisic M., Cacic J., Janjic J., Zdravkovic N., et al. 2019. The effect of oregano (*Origanum vulgare*) essential oil on four *Salmonella* serovars and shelf life of refrigerated pork meat packaged under vacuum and modified atmosphere. *J. Food Process. Preserv.* 44(1):e14311. <https://doi.org/10.1111/jfpp.14311>
- Boskovic M., Djordjevic J., Ivanovic J., Janjic J., Zdravkovic N., Glisic M., et al. 2017. Inhibition of *Salmonella* by thyme essential oil and its effect on microbiological and sensory properties of minced pork meat packaged under vacuum and modified atmosphere. *Int. J. Food Microbiol.* 258:58–67. <https://doi.org/10.1016/j.ijfoodmicro.2017.07.011>
- Burt S. 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. *Int. J. Food Microbiol.* 94(3):223–253. <https://doi.org/10.1016/j.ijfoodmicro.2004.03.022>
- Cava R., Nowak E., Taboada A. and Marin-Iniesta F. 2007. Antimicrobial activity of clove and cinnamon essential oils against *Listeria monocytogenes* in pasteurized milk. *J. Food Prot.* 70(12):2757–2763. <https://doi.org/10.4315/0362-028X-70.12.2757>
- Cava-Roda R.M., Taboada-Rodríguez A., Valverde-Franco M.T. and Marín-Iniesta F. 2012. Antimicrobial activity of vanillin and mixtures with cinnamon and clove essential oils in controlling *Listeria monocytogenes* and *Escherichia coli* O157: H7 in milk. *Food Bioproc. Tech.* 5(6):2120–2131. <https://doi.org/10.1007/s11947-010-0484-4>
- Clinical and Laboratory Standards Institute (CLSI). 2006. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard, 7th ed. CLSI Publication M7-A7. Clinical and Laboratory Standards Institute, Wayne PA.
- Cui H.Y., Zhou H., Lin L., Zhao C.T., Zhang X.J., Xiao Z.H., et al. 2016. Antibacterial activity and mechanism of cinnamon essential oil and its application in milk. *J. Anim. Plant. Sci.* 26:532–541.
- Di Pasqua R., Hoskins N., Betts G. and Mauriello G. 2006. Changes in membrane fatty acids composition of microbial cells induced by addition of thymol, carvacrol, limonene, cinnamaldehyde, and eugenol in the growing media. *J. Agric. Food Chem.* 54(7):2745–2749. <https://doi.org/10.1021/jf052722l>
- Doyle A.A. and Stephens J.C. 2019. A review of cinnamaldehyde and its derivatives as antibacterial agents. *Fitoterapia.* 139:104405. <https://doi.org/10.1016/j.fitote.2019.104405>
- Friedman M. 2017. Chemistry, antimicrobial mechanisms, and antibiotic activities of cinnamaldehyde against pathogenic bacteria in animal feeds and human foods. *J. Agric. Food Chem.* 65(48):10406–10423. <https://doi.org/10.1021/acs.jafc.7b04344>
- Gill A.O. and Holley R.A. 2004. Mechanisms of bactericidal action of cinnamaldehyde against *Listeria monocytogenes* and of eugenol against *L. monocytogenes* and *Lactobacillus sakei*. *Appl. Environ. Microbiol.* 70(10):5750–5755. <https://doi.org/10.1128/AEM.70.10.5750-5755.2004>
- Guler S. and Seker M. 2009. The effect of cinnamon and guar gum on *Bacillus cereus* population in milk. *J. Food Process. Preserv.* 33(3):415–426. <https://doi.org/10.1111/j.1745-4549.2009.00417.x>
- Hyldgaard M., Mygind T. and Meyer R.L. 2012. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *Front. Microbiol.* 3:12. <https://doi.org/10.3389/fmicb.2012.00012>
- International Organization for Standardization (ISO). 1999. ISO Standard No. 6888-1: Microbiology of food and animal feeding stuffs—horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) part 1: Technique using Baird-Parker agar medium. International Organization for Standardization, Geneva, Switzerland.
- Jablonski L.M. and Bohach G.A. 1997. *Staphylococcus aureus*. In Doyle M.P., Beuchat L.R. and Montville T.J. (ed.). *Food microbiology fundamentals and frontiers*. ASM Press, Washington DC. 353–375.
- Jans C., Merz A., Johler S., Younan M., Tanner S.A., Kaindi D.W.M., et al. 2017. East and west African milk products are reservoirs for human and livestock-associated *Staphylococcus aureus*. *Food Microbiol.* 65:64–73. <https://doi.org/10.1016/j.fm.2017.01.017>
- Jørgensen H.J., Mørk T. and Rørvik L.M. 2005. The occurrence of *Staphylococcus aureus* on a farm with small-scale production of raw milk cheese. *J. Dairy Sci.* 88(11):3810–3817. [https://doi.org/10.3168/jds.S0022-0302\(05\)73066-6](https://doi.org/10.3168/jds.S0022-0302(05)73066-6)
- Leja K., Szudera-Kończal K., Świtała E., Juzwa W., Kowalczewski P.Ł. and Czaczyk K. 2019. The influence of selected plant essential oils on morphological and physiological characteristics in *Pseudomonas orientalis*. *Foods.* 8(7):277. <https://doi.org/10.3390/foods8070277>
- Lianou A., Moschonas G., Nychas G.J.E. and Panagou, E.Z. 2018. Growth of *Listeria monocytogenes* in pasteurized vanilla cream pudding as affected by storage temperature and the presence of cinnamon extract. *Food Res. Int.* 106:1114–1122. <https://doi.org/10.1016/j.foodres.2017.11.027>
- Licon C.C., Moro A., Librán C.M., Molina A.M., Zalacain A., Berruga M.I., et al. 2020. Volatile transference and antimicrobial activity of cheeses made with ewes' milk fortified with essential oils. *Foods.* 9(1):35. <https://doi.org/10.3390/foods9010035>
- Liu T.T. and Yang T.S. 2012. Antimicrobial impact of the components of essential oil of Litseacubeba from Taiwan and antimicrobial activity of the oil in food systems. *Int. J. Food Microbiol.* 156(1):68–75. <https://doi.org/10.1016/j.ijfoodmicro.2012.03.005>
- Martinsen B., Oppegaard H., Wichstrøm R. and Myhr E.G.I.L. 1992. Temperature-dependent in vitro antimicrobial activity of four 4-quinolones and oxytetracycline against bacteria pathogenic to fish. *Antimicrob Agents Chemother.* 36(8):1738–1743. <https://doi.org/10.1128/AAC.36.8.1738>

- Masghati S. and Ghoreishi S.M. 2018. Supercritical CO<sub>2</sub> extraction of cinnamaldehyde and eugenol from cinnamon bark: optimization of operating conditions via response surface methodology. *J Supercrit Fluids.* 140:62–71. <https://doi.org/10.1016/j.supflu.2018.06.002>
- Medvedová A. and Valík L. 2012. *Staphylococcus aureus*: characterisation and quantitative growth description in milk and artisanal raw milk cheese production. In: Eissa A.A. (ed.) *Structure and Function of Food Engineering*. Books on Demand, Intech Open, London, pp. 71–101. <https://doi.org/10.5772/48175>
- Nam H.M., Lee A.L., Jung S.C., Kim M.N., Jang G.C., Wee S.H. and Lim S.K. 2011. Antimicrobial susceptibility of *Staphylococcus aureus* and characterization of methicillin-resistant *Staphylococcus aureus* isolated from bovine mastitis in Korea. *Foodborne Pathog. Dis.* 8(2):231–238. <https://doi.org/10.1089/fpd.2010.0661>
- Nazzaro F., Fratianni F., De Martino L., Coppola R. and De Feo V. 2013. Effect of essential oils on pathogenic bacteria. *Pharmaceuticals.* 6(12):1451–1474. <https://doi.org/10.3390/ph6121451>
- Noël T.S., Kifouli A., Boniface Y., Edwige D.A., Farid B.M. and Fatiou T. 2016. Antimicrobial and physico-chemical effects of essential oils on fermented milk during preservation. *J. Appl. Biosci.* 99:9467–9475. <https://doi.org/10.4314/jab.v99i1.12>
- Perricone M., Arace E., Corbo M.R., Sinigaglia M. and Bevilacqua A. 2015. Bioactivity of essential oils: a review on their interaction with food components. *Front. Microbiol.* 6:76. <https://doi.org/10.3389/fmicb.2015.00076>
- Shen S., Zhang T., Yuan Y., Lin S., Xu J. and Ye H. 2015. Effects of cinnamaldehyde on *Escherichia coli* and *Staphylococcus aureus* membrane. *Food Control.* 47:196–202. <https://doi.org/10.1016/j.foodcont.2014.07.003>
- Smith-Palmer A., Stewart J. and Fyfe L. 1998. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Lett. Appl. Microbiol.* 26(2):118–122. <https://doi.org/10.1046/j.1472-765X.1998.00303.x>
- 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. <https://doi.org/10.1006/fmic.2001.0415>
- Tassou C.C., Drosinos E.H. and Nychas G.J.E. 1995. Effects of essential oil from mint (*Mentha piperita*) on *Salmonella enteritidis* and *Listeria monocytogenes* in model food systems at 4°C and 10°C. *J. Appl. Microbiol.* 78(6):593–600. <https://doi.org/10.1111/j.1365-2672.1995.tb03104.x>
- Tayel A.A., Hussein H., Sorour N.M. and El-Tras W.F. 2015. Foodborne pathogens prevention and sensory attributes enhancement in processed cheese via flavoring with plant extracts. *J. Food Sci.* 80(12):2886–2891. <https://doi.org/10.1111/1750-3841.13138>
- Usta J., Kreydiyyeh S., Barnabe P., Bou-Moughlabay Y. and Nakkash-Chmisse H. 2003. Comparative study on the effect of cinnamon and clove extracts and their main components on different types of ATPases. *Hum. Exp. Toxicol.* 22(7):355–362. <https://doi.org/10.1191/0960327103ht379oa>
- Valero A., Pérez-Rodríguez F., Carrasco E., Fuentes-Alventosa J.M., García-Gimeno R.M. and Zurera G. 2009. Modelling the growth boundaries of *Staphylococcus aureus*: effect of temperature, pH and water activity. *Int. J. Food Microbiol.* 133(1–3):186–194. <https://doi.org/10.1016/j.ijfoodmicro.2009.05.023>
- Vidanagamage S.A., Pathiraje P.M.H.D. and Perera O.D.A.N. 2016. Effects of cinnamon (*Cinnamomum verum*) extract on functional properties of butter. *Procedia Food Sci.* 6:136–142. <https://doi.org/10.1016/j.profoo.2016.02.033>
- Wojtys A. and Jankowski T. 2004. The effect of temperature on the permeation rate of some selected essential oils into baker's yeast cells. *Żywność Nauka Technologia Jakość*, 11(3):77–86.
- Xing X., Zhang Y., Wu Q., Wang X., Ge W. and Wu C. 2016. Prevalence and characterization of *Staphylococcus aureus* isolated from goat milk powder processing plants. *Food Control.* 59:644–650. <https://doi.org/10.1016/j.foodcont.2015.06.042>
- Yuste J. and Fung D.Y.C. 2003. Evaluation of *Salmonella typhimurium*, *Yersinia enterocolitica* and *Staphylococcus aureus* counts in apple juice with cinnamon, by conventional media and thin agar layer method. *Food Microbiol.* 20(3):365–370. [https://doi.org/10.1016/S0740-0020\(02\)00130-2](https://doi.org/10.1016/S0740-0020(02)00130-2)