BEHAVIOR OF *STAPHYLOCOCCUS AUREUS* IN SUCUK WITH NETTLE (*URTICA DIOICA* L.)

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ABSTRACT

The behavior of Staphylococcus aureus *was studied in sucuk (Turkish dry fermented sausage) with different levels of nettle (*Urtica dioica *L.). Sucuk batters containing different levels (1, 3 and 5%) of nettle were prepared and contaminated with* S. aureus *at approximately* 10^4 *cfu/g. Sucuk batter not inoculated with S. aureus and not containing nettle addition was set as the control lot.* Lactobacillus sakei *Lb 706 was used as a starter culture. During ripening period,* S. aureus, Micrococcus/Staphylococcus *and lactic acid bacteria counts were determined. pH and water activity* (a_w) *values were also analyzed.*

The S. aureus *count was under detectable levels in the control lot, and the pH level dropped below 5.0 after the first 24 h of fermentation. Initial pH values increased gradually in all three lots (1, 3 and 5%). At the end of the first 24 h of fermentation, a significant increase in* S. aureus *counts was determined in all lots of sausage except for the control lot. The pH value of the sucuk sample containing 1% nettle was under 5.0 on the second day. However, pH values for sucuk samples containing 3 and 5% nettle did not drop below 5.0 during the ripening period, including the fermentation phase. Nettle usage had no effect on the lactic acid bacteria count; however, it had significant effects on the* Micrococcus/Staphylococcus *count and* a_w *value.*

PRACTICAL APPLICATIONS

Plants such as *Urtica dioica* L. has increasingly been used in the meat products in recent years. However, there are limited studies on the microbio-

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logical properties of such products. A research on the behavior of *Staphylococcus aureus*, which is a critical foodborne pathogen in fermented meat products, is considered to be important for food safety. This study was designed and conducted to determine the behavior of *S. aureus* in sucuk containing nettle.

INTRODUCTION

Nettle (*Urtica dioica* L.) is a widespread plant frequently suggested for use in the treatment of rheumatism, arthritis and inflammatory diseases (Szabo *et al.* 2006). As reported in the literature, nettle has antioxidant, antimicrobial, antiulcer and analgesic effects (Gülçin *et al.* 2004; Dostbil *et al.* 2005; Szabo *et al.* 2006). This plant is also frequently used in traditional medicine in several countries (Bnouham *et al.* 2003; Guil-Guerrero *et al.* 2003).

There are limited studies in the literature about the use of nettle in sucuk (Turkish dry fermented sausage) (Aksu 2003; Aksu and Kaya 2004; Kaban *et al.* 2006). Aksu (2003) and Aksu and Kaya (2004) have determined the chemical, physical, microbiological and organoleptic characteristics of sausages produced by using *U. dioica* L. Kaban *et al.* (2006) have also studied the effect of *U. dioica* L. on the growth of *Staphylococcus aureus* in traditional sucuk without starter culture and determined that the use of *U. dioica* L. had a positive effect on the growth of *S. aureus* during fermentation.

S. aureus is a major foodborne pathogen responsible for food poisoning incidents in a wide range of food products, including fermented sausage (Smith *et al.* 1983; Bryan 1988; Moore 2004). Given the appropriate conditions, *S. aureus* can multiply and produce toxin during the initial stage of raw meat fermentation (Lücke and Earnshaw 1991; Sameshima *et al.* 1998; Lücke 2000; Kaban and Kaya 2006). To control the growth of *S. aureus*, lactic acid bacteria are widely used as starter cultures in fermented sausage products (Bacus 1984; Marcy *et al.* 1985; Kaban and Kaya 2006). Various factors, such as the initial pH and water activity (a_w), sugar and nitrite, the fermentation temperature, and the rate and extent of acid formation are important in the sausage fermentation (Lücke and Earnshaw 1991).

The aim of this study was to determine the effect of lactic starter culture on the growth of *S. aureus* in sucuk produced by using different levels of nettle.

MATERIALS AND METHODS

Strains and Nettle

S. aureus ATCC 13565 (Oxoid, Hampshire, UK) was used for the inoculation of sucuks except the control lot and cultivated in tryptic soy

broth (TSB, Merck, Darmstadt, Germany) at 37C for 24 h. *Lactobacillus sakei* Lb 706 was obtained from the Federal Meat Research Institute, Klumbach, Germany. This strain was grown in De Man Rogosa Sharpe (MRS) agar (Merck) broth at 30C for 24 h. Dried ground leaves of nettle (*U. dioica* L.) were used after sifting through a 14-in. sieve.

Sausage Formulation and Processing

Sucuk was manufactured according to the following formulation: based on per kilogram of beef and fat (4:1), 25 g salt, 10 g garlic, 4 g saccharose, 7 g red pepper, 5 g black pepper, 9 g cumin, 2.5 g allspice and 0.15 g $NaNO_2$ (Kaya and Gökalp 2004). Sucuk batters were prepared in the cutter (MADO Typ MTK 662, Dornhan/Schwarzwald, Germany). First, the control lot without nettle was prepared. Then, sucuk batters containing 1, 3 and 5% nettle were prepared. All lots of sucuk batter except the control lot were contaminated with *S. aureus* at approximately 10^4 cfu/g level. Starter culture *L. sakei* Lb 706 was inoculated in order to achieve approximately 10^6 cfu/g in all lots of sucuk batter. Prepared batters were stuffed into collagen casings (ø 38 mm, Naturin, Darm, Germany) using a laboratory-type filler (MADO Typ MTK 591, Dornhan/Schwarzwald). Sucuk pieces were hung in a room, in which controlled temperature and humidity could be achieved. The ripening process was carried out under the following conditions: 24C and relative humidity (RH) 90 ± 2% for the first 3 days, 22 ± 1C and RH 85 ± 2% for the following 4 days and 18 ± 1C and RH 80 ± 2% for the last 7 days.

Sampling Procedures

Samples for microbiological analysis and pH and a_w determination were taken from sucuks immediately after stuffing (0 day), and after 1, 2, 3, 5, 7, 11 and 14 days of ripening.

Microbiological Analysis

Twenty-five grams of sucuk sample was aseptically weighed and then homogenized in a stomacher (Lab Stomacher Blander 400-BA 7021, Seward-medical, London, UK) for 1 min with 225 mL of physiological water (NaCI 0.85%). Further decimal dilutions were prepared with the same diluent. The number of *S. aureus* was determined on Baird–Parker's egg yolk telluride agar (BP, Merck) incubated at 37C for 2 days; lactic acid bacteria on MRS agar (Merck) at 30C for 2 days in an anaerobic jar; *Micrococcus/Staphylococcus* on mannitol salt agar (MSA, Merck) at 30C for 2 days.

pH and a_w Analyses

pH. pH was determined in a slurry made of 10 g sample and 100 mL of distilled water after homogenization in an ultra–turrax (IKA Werk Tp 18 – 10 20.000 UpM, Staufen, Germany); the measurements were performed using a pH meter (ATI ORION 420 A, Boston, MA).

a_w. Samples were placed into plastic cups for a_w determination using a Novasina a_w sprint (Novasina Model TH 500, Pfäffikon, Switzerland).

Statistical Analysis

The data values of the sausages with nettle, depending on nettle amount and ripening time, were further analyzed according to a complete randomized design (two replications). All data were subjected to variance analyses, and differences between means were analyzed by Duncan's multiple range test using the SPSS statistic program (version 10.01 for windows, SPSS Inc.) (SPSS 1999).

RESULTS AND DISCUSSION

Microbiological counts, pH and a_w values of the control lot (without nettle addition and *S. aureus* inoculation) depending on the ripening period are given in Table 1. *S. aureus* count was found to be <100 during the ripening period. After 24 h of fermentation (day 1), the initial pH value (5.82 ± 0.01) was below 5 (4.92 ± 0.02). Lactic acid bacteria counts reached to over 1×10^8 cfu/g. *Micrococcus*/*Staphylococcus* counts increased to 0.5-log unit at the first day; however, these counts decreased in the following days. Kaban and Kaya (2006) have also found that once pH drops below 5.0 during the first 24 h of fermentation, *S. aureus* growth could be inhibited by the starter culture addition in sucuk samples.

Nettle (*U. dioica* L.) usage had a significant ($P < 0.01$) effect on *S. aureus* growth. The highest average *S. aureus* counts were observed in sucuks with 5% nettle ($P < 0.05$) (Table 2). Ripening period also had a significant ($P < 0.01$) effect on *S. aureus* growth (Table 3). After 24 h of fermentation, there was an increase of 1.5 log in *S. aureus* counts. The interaction of different levels of nettle × ripening period showed a significant ($P < 0.01$) effect on *S. aureus* counts (Fig. 1). *S. aureus* counts increased more rapidly in sausages containing 3 and 5% nettle in comparison to the samples containing 1% nettle. In the following days of the ripening, *S. aureus* counts of sucuk samples containing 5% nettle were higher than those of sucuk samples containing 1 and

TABLE 1.
ANALYSIS RESULTS OF THE CONTROL LOT (MEAN ± SD)

	Ripening period (days)							
	0	1	2	3	5	7	11	14
pH	5.82 ± 0.01	4.92 ± 0.02	4.71 ± 0.01	4.68 ± 0.01	4.75 ± 0.01	4.80 ± 0.01	4.88 ± 0.01	4.92 ± 0.01
a_w	0.966 ± 0.001	0.965 ± 0.001	0.957 ± 0.001	0.957 ± 0.001	0.947 ± 0.00	0.931 ± 0.001	0.878 ± 0.004	0.857 ± 0.00
Staphylococcus aureus	<100	<100	<100	<100	<100	<100	<100	<100
Micrococcus/ Staphylococcus	4.69 ± 0.27	5.22 ± 0.08	5.11 ± 0.15	5.02 ± 0.03	4.62 ± 0.31	4.48 ± 0.67	4.39 ± 0.01	4.55 ± 0.57
Lactic acid bacteria	6.21 ± 0.13	8.56 ± 0.11	8.16 ± 0.01	8.99 ± 0.15	8.52 ± 0.06	8.88 ± 0.03	9.09 ± 0.16	8.87 ± 0.02

a_w, water activity.

TABLE 2.
THE EFFECTS OF LEVELS OF *URTICA DIOICA* L. ON SOME PROPERTIES OF SUCUK

U. dioica L. (%)	pH	a_w	*Staphylococcus aureus*	*Micrococcus/ Staphylococcus*	Lactic acid bacteria
	*	*	*	*	NS†
1	5.13 ± 0.42c	0.931 ± 0.041b	4.73 ± 0.48c	5.12 ± 0.55b	8.52 ± 0.98a
3	5.42 ± 0.38b	0.931 ± 0.036b	4.88 ± 0.44b	5.20 ± 0.42b	8.79 ± 0.93a
5	5.75 ± 0.32a	0.937 ± 0.032a	5.49 ± 0.70a	5.69 ± 0.59a	8.64 ± 0.97a

* $P < 0.01$.
† $P > 0.05$, ± SD.
a–c Values in a column with the same letter are not significantly different by Duncan's multiple range test ($P > 0.05$).
a_w, water activity; NS, not significant.

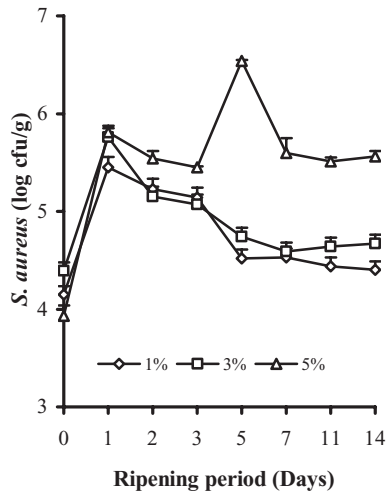

FIG. 1. THE INTERACTION OF DIFFERENT LEVELS OF NETTLE × RIPENING PERIOD ON *STAPHYLOCOCCUS AUREUS* COUNTS

3% nettle. Kaban *et al.* (2006) found that in traditional sucuk (without starter culture) produced by using 1 and 3% nettle, there is an increase of more than 2-log units on the second day of the ripening, while there is an increase of more than 3-log units in sucuk with 5% nettle, and there is no decrease in the count on the following days. In the present study, although lactic starter culture was added, growth of *S. aureus* could not be prevented. This is probably caused by the initial pH values, which were above 6.0 in the lots with nettle.

TABLE 3.
THE EFFECTS OF RIPENING PERIOD ON SOME PROPERTIES OF SUCUK

Ripening period (days)	pH	a_w	*Staphylococcus aureus*	*Micrococcus /Staphylococcus*	Lactic acid bacteria
	*	*	*	*	NS†
0	6.36 ± 0.17a	0.966 ± 0.001a	4.16 ± 0.24d	4.50 ± 0.42d	6.27 ± 0.20d
1	5.33 ± 0.29c	0.962 ± 0.002b	5.67 ± 0.20a	5.82 ± 0.16a	8.78 ± 0.19c
2	5.18 ± 0.28f	0.958 ± 0.001c	5.31 ± 0.22b	5.90 ± 0.18a	8.89 ± 0.09bc
3	5.22 ± 0.31e	0.954 ± 0.001d	5.22 ± 0.22b	5.44 ± 0.30b	9.07 ± 0.24ab
5	5.31 ± 0.35c	0.939 ± 0.007e	5.27 ± 0.99b	5.45 ± 0.75b	8.95 ± 0.38bc
7	5.25 ± 0.26d	0.930 ± 0.007f	4.90 ± 0.57c	5.09 ± 0.39c	9.02 ± 0.17abc
11	5.40 ± 0.34b	0.891 ± 0.007g	4.86 ± 0.52c	5.21 ± 0.39bc	9.03 ± 0.42abc
14	5.40 ± 0.27b	0.862 ± 0.013h	4.87 ± 0.56c	5.27 ± 0.51bc	9.21 ± 0.11a

* $P < 0.01$.
† $P > 0.05$, ± SD.
a–f Values in a column with the same letter are not significantly different by Duncan's multiple range test ($P > 0.05$).
a_w, water activity; NS, not significant.

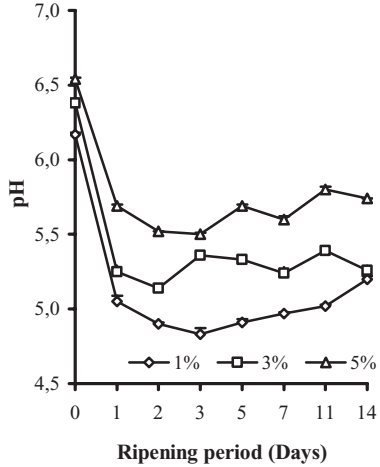

FIG. 2. THE INTERACTION OF DIFFERENT LEVELS OF NETTLE × RIPENING PERIOD ON pH VALUE

Lücke (2000) determined that the initial pH value is an important factor for the inhibition of pathogen bacteria.

The initial pH values of sucuk batters with nettle were found to be much higher than those of the control lots (Tables 1 and 2). This is because of the pH-increasing effect of nettle. According to the results of the variance analysis, both nettle and the ripening period had significant ($P < 0.01$) effects on *S. aureus* growth (Tables 2 and 3). The highest average pH value was determined in lot with 5% nettle (Table 2). The interaction of ripening period × nettle had a significant ($P < 0.01$) effect on the pH value (Fig. 2). The initial pH value dropped below 5.0 in the second day in sucuk samples produced with 1% nettle (Fig. 2). On the other hand, the initial pH dropped below 5.0 in the control group on the first day (Table 1). However, pH value did not decrease below 5.0 in sucuk samples containing 3 and 5% nettle throughout ripening period. Raccach (1981), Stiebing (1996) and Kaban and Kaya (2006) emphasized that a rapid pH decrease was necessary for controlling the growth of *S. aureus*.

The usage of different levels of nettle and ripening period had a significant ($P < 0.01$) effect on the *Micrococcus*/*Staphylococcus* numbers (Table 2 and 3). The highest average value of *Micrococcus*/*Staphylococcus* numbers was observed in sucuk samples with 5% nettle (Table 2). The interaction of levels of nettle and ripening period had a significant ($P < 0.01$) effect on *Micrococcus*/*Staphylococcus* numbers (Fig. 3). This interaction showed a similar trend with *S. aureus* counts.

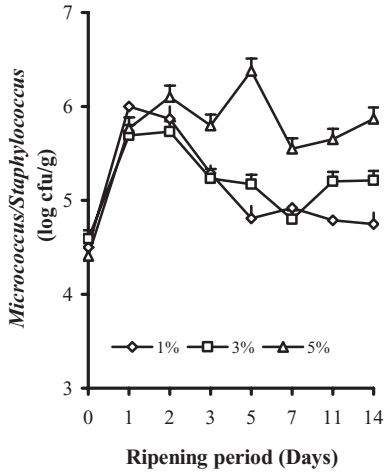

FIG. 3. THE INTERACTION OF DIFFERENT LEVELS OF NETTLE × RIPENING PERIOD ON THE *MICROCOCCUS/STAPHYLOCOCCUS* COUNTS

The use of nettle had no significant ($P > 0.05$) effect on lactic acid bacteria counts (Table 2). On the other hand, ripening period had a significant ($P < 0.01$) effect on the lactic acid bacteria. Lactic acid bacteria counts reached to 10^8–10^9 cfu/g (Table 3). Ripening period × level of nettle interaction was also found to be insignificant ($P > 0.05$). According to these results, nettle has no negative effects on the growth of lactic acid bacteria. However, as it increased the initial pH value, it was difficult to reach a lower pH value during fermentation. Higher pH values during the first days of fermentation caused *S. aureus* to grow easily.

The interaction of different levels of nettle × ripening period had a significant ($P < 0.01$) effect on a_w value (Fig. 4). The lowest a_w value was observed in the lot containing 1% nettle at the end of ripening. Similar results were also found by Kaban *et al.* (2006).

CONCLUSIONS

The use of starter culture in sucuk is necessary for the control of *S. aureus*. However, in case of adding nettle, the use of starter culture alone is not adequate at the initial ripening temperature of 24C. Because of this, nettle should be added in such a proportion that the initial pH value of the batter does not exceed 5.8. On the other hand, the formulation can be modified according to the rate and extent of acid formation, fermentation temperature and the amount of sugar added.

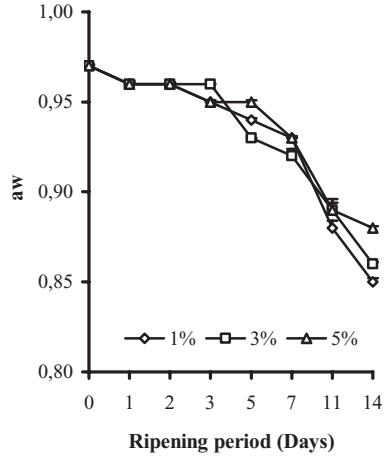

FIG. 4. THE INTERACTION OF DIFFERENT LEVELS OF NETTLE × RIPENING PERIOD ON a_w VALUE

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