EFFECT OF *URTICA DIOICA* L. ON THE GROWTH OF *STAPHYLOCOCCUS AUREUS* IN TRADITIONAL DRY FERMENTED SAUSAGE ("SUCUK")

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Accepted for Publication May 12, 2007

ABSTRACT

The effect of Urtica dioica *L. on the growth of* Staphylococcus aureus *was investigated in "sucuk," a Turkish dry fermented sausage. The different levels of dried ground* U. dioica *L. (0, 1, 3 and 5%) were added to sucuk batters, and these batters were contaminated with* S. aureus *(10^4 cfu/g). Numbers of* S. aureus, Micrococcus/Staphylococcus *and lactic acid bacteria (LAB) were determined during the ripening period. In addition, values of pH and* a_w *were analyzed.*

The different usages of U. dioica *L. and the ripening period had very significant effects on pH value and on* S. aureus *numbers (*$P < 0.01$*). Because* U. dioica *L. increased the value of pH, initial pH value was determined above 6.0 in groups including* U. dioica *L. During fermentation, the pH did not decrease rapidly in all groups (including the control). Because of this situation, the* S. aureus *number was increased. The usage of* U. dioica *L. has a very significant effect (*$P < 0.01$*) on the number of* Micrococcus/Staphylococcus *but does not have any effect on the numbers of LAB (*$P > 0.05$*). The* a_w *value was affected very significantly (*$P < 0.01$*) by* U. dioica *L. addition.*

PRACTICAL APPLICATIONS

Staphylococcus aureus is an important foodborne pathogen in fermented dry sausage like "sucuk." The behavior of this pathogen has not been investigated in fermented meat products with *Urtica dioica* L. In this research, the

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Journal of Muscle Foods **19** (2008) 399–409. *All Rights Reserved.*
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effects of different levels of *U. dioica* L. on the behavior of *S. aureus* were determined during sucuk production. The use of *U. dioica* L. increased the pH value in sucuk batter. Because of this, *S. aureus* growth was better in sucuks containing *U. dioica* L. than in the control group. In order to control the growth of *S. aureus*, lactic acid bacteria is required to be used as starter culture in sucuk with *U. dioica* L. as well.

INTRODUCTION

"Sucuk," which is a traditional dry fermented sausage made from meat (beef or beef/water buffalo), sheep tail fat, various spices and a small amount of other ingredients, has an important position within meat products in Turkey. Fermentation and drying are the main practices in sucuk production. In spite of the increasing use of starter culture in the production of sucuk day by day, there are still producers who make natural fermentation by the indigenous bacteria (Gökalp *et al.* 2004; Kaya and Gökalp 2004).

Staphylococcus aureus is a significant foodborne pathogen in meat and meat products (Bryan 1988; Kang and Fung 2000; Floret 2001; Ananou *et al.* 2005). It is reported that *S. aureus* causes some food poisoning cases in products such as Genoa salami, dry salami, fermented salami (CDC 1971, 1975, 1980) and dry fermented sausage (Hartog *et al.* 1987). These foodborne pathogen bacteria, which are salt and nitrite tolerant, are able to grow under a wide range of environmental conditions (Gonzàlez-Fandos *et al.* 1999). *S. aureus* can multiply and produce toxin during the initial stage of fermentation (Tatini *et al.* 1976; Marcy *et al.* 1985; Sameshima *et al.* 1998).

Many diseases are known to be treated with herbal remedies throughout the history of mankind (Sökmen *et al.* 1999). *Urtica dioica* L. has been accepted as a healing plant throughout history (Aksu and Kaya 2004). *U. dioica* L. has antioxidant, antimicrobial, analgesic, cholesterol-lowering, cardiovascular, diuretic and antidiabetic effects (Petlevski *et al.* 2001; Testai *et al.* 2002; Gülçin *et al.* 2004; Uzun *et al.* 2004).

In sucuk production, usage possibility of *U. dioica* L. has been researched by Aksu (2003) and by Aksu and Kaya (2004). However, the effect of *U. dioica* L. on the growth of important foodborne pathogen bacteria such as *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *S. aureus* in sucuk has not been searched yet.

The aim of this study was to investigate the effects of different levels of *U. dioica* L. (at 0, 1, 3 and 5% levels) on the growth of *S. aureus* in traditional sucuk (without addition of starter culture).

MATERIALS AND METHODS

The Preparation of *S. aureus* and *U. dioica* L.

S. aureus, American Type Culture Collection (ATCC) 13565 (Oxoid, Hampshire, U.K.), was used. The strain was grown in tryptic soy broth (Merck, Darmstadt, Germany) at 37C for 24 h. Dried ground leaves of *U. dioica* L. were used by sifting through a 14-in. sieve.

Sausage Formulation and Processing

In the production of the sausages, the following formulation was used: per 1 kg of meat and fat (800 g beef + 100 g beef meat fat + 100 g sheep tail fat), 25 g salt, 10 g garlic, 4 g sucrose, 7 g red pepper, 5 g black pepper, 9 g cumin, 2.5 g allspice and 150 ppm $NaNO_2$ (Gökalp *et al.* 2004). Sucuk batters were prepared in a laboratory-type cutter (MADO Typ MTK 662, Dornhan/Schwarzwald, Germany). First, the control group (0% *U. dioica* L.) was prepared, and then the batters including different levels of *U. dioica* L. (0, 1, 3 and 5%) were prepared. All batters were contaminated with 10^4 cfu/g level of *S. aureus* during preparation. Each of the sucuk batters was stuffed into collagen casings (ø38 mm, Naturin Darm, Weinheim, Germany) with a laboratory-type filling machine (MADO Typ MTK 591, Dornhan/Schwarzwald). The ripening programs were as follows: 3 days at 24 ± 1C and 90 ± 2% relative humidity (RH), 4 days at 22 ± 1C and 85 ± 2% RH, 9 days at 18 ± 1C and 80 ± 2% RH. For the analysis, two samples were taken from each group at various stages of the ripening period (0,1, 2, 3, 5, 7, 11, 14 days).

Microbiological Analysis

Twenty-five-gram portions of sucuk were homogenized with 225 mL of physiological salt water (NaCl 0.85%) for 1 min using a stomacher (Lab Stomacher Blander 400 – BA 7021, SewardMedical, London, U.K.). Further decimal dilutions were made with the same diluent. The number of *S. aureus* was enumerated on Baird–Parker's egg yolk tellurite agar (Merck) incubated at 37C for 48 h, lactic acid bacteria (LAB) on de Man–Rogosa–Sharpe agar (Merck) at 30C for 48 h in an anaerobic jar and *Micrococcus*/*Staphylococcus* on mannitol salt agar (Merck) at 30C for 48 h.

Determination of pH

Ten grams of sucuk sample and 100 mL of distilled water were mixed in a Ultra-Turrax (IKA Werk Tp 18 – 10 20.000 UpM). The pH of the homogenate was measured by a pH meter (ATI Orion 420 A, Boston, MA).

Determination of a_w

a_w was determined at 25C, using a Novasina a_w sprint (model TH 500, Novasina, Pfäffikon, Switzerland). The accuracy of measurement was ± 0.003.

Statistical Analysis

The analysis of data was tested by variance analysis (complete randomized design, two replications) and the differences between means were evaluated by Duncan's multiple range test using SPSS statistic programme (SPSS 1999).

RESULTS AND DISCUSSION

The usage of *U. dioica* L. and the ripening period had a very significant effect ($P < 0.01$) on the number of *S. aureus* in sucuk (Table 1). The highest average of *S. aureus* number was observed in sucuk produced with 5% *U. dioica* L., and this average was statistically different from sucuk including 1 and 3% *U. dioica* L. The lowest average of *S. aureus* count was determined in the control group (0% *U. dioica* L.) (Table 1). The ripening period had a very significant effect on the number of *S. aureus* ($P < 0.01$). The number of *S. aureus* increased by approximately 2 log at the end of the first day and approximately 3 log at the end of the second day. The interaction of different levels of *U. dioica* L. × ripening period had a very significant effect ($P < 0.01$) on *S. aureus* counts in sucuk (Fig. 1). As seen in Fig. 1, on the second day of ripening, the increase of *S. aureus* number was >2 log units in the control group and in sucuks with 1 and 3% *U. dioica* L., and was >3 log units in sucuks including 5% *U. dioica* L. on the same day. While the number of *S. aureus* was under 7 log between 2 and 14 days of ripening in the control group, this number was over 10^7 cfu/g in all groups including *U. dioica* L. on the same days. Raccach (1981), Turantaş (1991), Erol and Hildebrandt (1992) and Kaban and Kaya (2006) determined that starter culture must be used for inhibition of *S. aureus*. As a result of this research, *S. aureus* grew in sucuk groups produced without starter culture, and the use of *U. dioica* L. increased the growth rate of *S. aureus*.

In order to control the growth of *S. aureus* in fermented dry sausage, the rapid growth of LAB and the rapid decrease of pH (5.30 and lower) are important (Raccach 1981; Bacus 1984; Kaban and Kaya 2006). The different levels of *U. dioica* L. (1, 3 and 5%) had a very significant effect ($P < 0.01$) on the pH value (Table 1). The pH value increased, depending on the amounts of added *U. dioica* L. (1, 3 and 5%). Similarly, Aksu (2003) reported that the use of *U. dioica* L. raised pH value in Turkish dry fermented sausage. The ripening

TABLE 1.
THE EFFECTS OF LEVELS OF *URTICA DIOICA* L. AND RIPENING PERIOD ON SOME PROPERTIES OF "SUCUK"

Factor	pH	a_w	*Staphylococcus aureus*	*Micrococcus/ Staphylococcus*	Lactic acid bacteria
Level of *Urtica dioica* L. (%)	*	*	*	*	NS
0	5.27 ± 0.34 d	0.934 ± 0.036 b	6.27 ± 0.82 c	6.55 ± 0.72 c	7.21 ± 2.09 a
1	5.53 ± 0.38 c	0.933 ± 0.038 b	6.67 ± 0.94 b	7.04 ± 0.70 a	7.10 ± 1.91 a
3	5.72 ± 0.40 b	0.934 ± 0.035 b	6.58 ± 1.07 b	6.76 ± 1.02 b	7.13 ± 1.86 a
5	6.02 ± 0.38 a	0.936 ± 0.033 a	6.90 ± 1.23 a	6.82 ± 1.00 b	7.05 ± 1.87 a
Ripening period (days)	*	*	*	*	*
0	6.24 ± 0.28 a	0.968 ± 0.001 a	4.17 ± 0.24 e	4.84 ± 0.49 c	2.46 ± 0.26 e
1	6.14 ± 0.30 b	0.964 ± 0.002 b	6.34 ± 0.17 d	6.71 ± 0.33 b	6.35 ± 0.27 d
2	5.82 ± 0.40 c	0.960 ± 0.001 c	7.02 ± 0.32 b	7.34 ± 0.21 a	7.66 ± 0.20 c
3	5.34 ± 0.26 f	0.954 ± 0.001 d	6.79 ± 0.64 c	7.23 ± 0.45 a	8.00 ± 0.50 b
5	5.26 ± 0.25 g	0.942 ± 0.003 e	7.27 ± 0.37 a	6.74 ± 0.71 b	8.35 ± 0.10 a
7	5.33 ± 0.26 f	0.928 ± 0.005 f	7.04 ± 0.43 b	7.17 ± 0.33 a	8.03 ± 0.43 ab
11	5.44 ± 0.34 e	0.893 ± 0.004 g	7.07 ± 0.36 b	7.09 ± 0.32 a	8.13 ± 0.26 ab
14	5.51 ± 0.25 d	0.866 ± 0.004 h	7.11 ± 0.35 b	7.21 ± 0.31 a	7.99 ± 0.24 b
Level × ripening period	*	*	*	*	NS

* $P < 0.01$.
Different letters indicate statistical difference ($P < 0.05$) in each column.
NS, not significant.

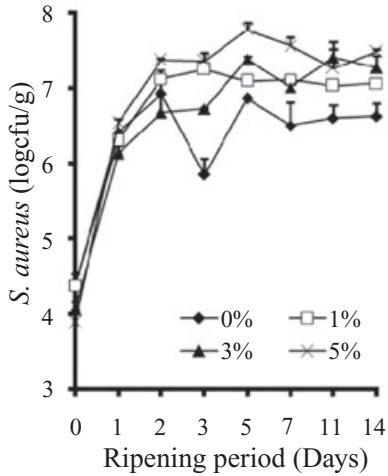

FIG. 1. THE INTERACTION OF DIFFERENT LEVELS OF *URTICA DIOICA* L. × RIPENING PERIOD ON *STAPHYLOCOCCUS AUREUS* COUNTS

period had a very significant effect on the pH value ($P < 0.01$) as well. On the fifth day, pH was 5.26 ± 0.25 (Table 1). According to statistical analysis, the interaction of *U. dioica* L. × ripening period had a very significant effect on pH ($P < 0.01$). Although initial pH value was over 6.0 in groups containing *U. dioica* L., the initial pH value was under 6.00 in the control group. The maximum increase in pH was observed in the group with 5% *U. dioica* L. (Fig. 2). This increase in pH has a positive effect on the growth of *S. aureus* in the first days of ripening. At the fermentation stage, the lowest pH value was determined in the control group. However, the lowest pH value did not obstruct growth of *S. aureus*. Raccach (1981) determined that starter cultures that make pH drop rapidly are required for inhibition of *S. aureus*. In the same fashion, Kaban and Kaya (2006) reported that the rapid drop of pH needs to inhibit the growth of *S. aureus* during the fermentation stage of sucuks.

Both the usage of *U. dioica* L. and the ripening period had very significant effects ($P < 0.01$) on *Micrococcus*/*Staphylococcus* numbers (Table 1). The highest average of *Micrococcus*/*Staphylococcus* numbers was observed in the group with 5% *U. dioica* L. during ripening ($P < 0.05$). Aksu and Kaya (2004) reported similar results in fermented sausage. The interaction of different levels of *U. dioica* L. × ripening period demonstrated a very significant effect ($P < 0.01$) on *Micrococcus*/*Staphylococcus* numbers. As shown in Fig. 3, the highest increase in their number was determined in sucuk with 5% *U. dioica* L. in the first days. These results are related to pH values. Also,

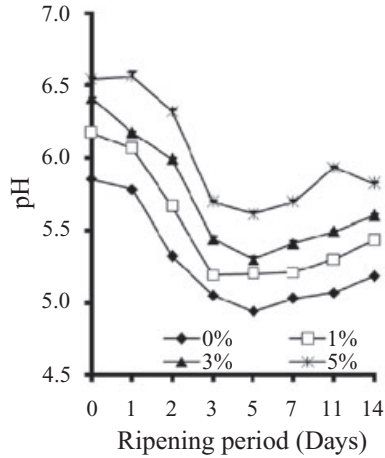

FIG. 2. THE INTERACTION OF DIFFERENT LEVELS OF *URTICA DIOICA* L. × RIPENING PERIOD ON pH

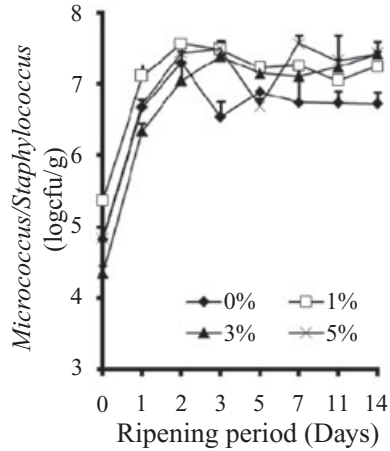

FIG. 3. THE INTERACTION OF DIFFERENT LEVELS OF *URTICA DIOICA* L. × RIPENING PERIOD ON *MICROCOCCUS/STAPHYLOCOCCUS* NUMBERS

Lücke (1985) reported that *Micrococcus/Staphylococcus* grows well with high pH values.

While ripening time had a very significant effect on LAB number ($P < 0.01$), the usage of *U. dioica* L. had no effect on the count of LAB ($P > 0.05$) (Fig. 1). Starter culture had not been used in sucuk production, and

fermentation was initiated by spontaneous LAB. The interaction of different levels of *U. dioica* L. × ripening period had no significant effect on LAB numbers ($P > 0.05$). While LAB count was 10^2 cfu/g in 0 days in all sucuk samples, it increased to 10^8 cfu/g in 3 days (Table 1). There are no significant differences in LAB numbers of sucuks with and without *U. dioica* L. (Table 1). According to these results, LAB had shown the same growth rate both in sucuks containing *U. dioica* L. and in the control group. In the control groups, the initial pH (5.85) decreased to 4.94; this value was the lowest pH value during the process. In sucuk with 5% *U. dioica* L., the initial pH value was 6.54; the lowest pH value was 5.60. According to these results, pH decreased by 0.91 units in the control group and by 0.94 units in sucuk with 5% *U. dioica* L. Schillinger and Lücke (1989) and Lücke (2000) reported that in products fermented at temperatures above 18C, a rapid pH drop to below 5.30 is important for the inhibition of *S. aureus*. In this study, pH dropped very slowly to below 5.30 in the control group and in sucuks containing 1% *U. dioica* L. In sucuks containing 3% and 5% *U. dioica* L., minimum pH values are over 5.30 (Fig. 2).

The usage of *U. dioica* L. and the ripening period had very significant effects on a_w value ($P < 0.01$) (Table 1). The highest average a_w value was observed in sucuk containing 5% *U. dioica* L., and this group was statistically different than the other groups ($P < 0.05$) (Table 1). Aksu (2003) also reported that usage of *U. dioica* L. caused slow drying in sucuk. The interaction of different levels of *U. dioica* L. × ripening period had a very significant effect on a_w value ($P < 0.01$) (Fig. 4).

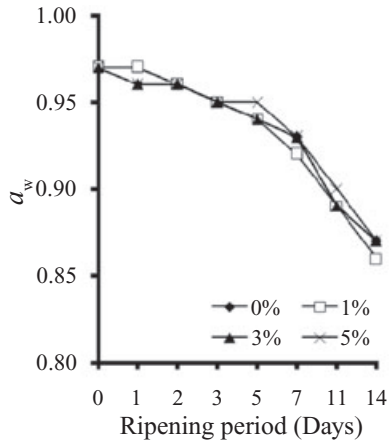

FIG. 4. THE INTERACTION OF DIFFERENT LEVELS OF *URTICA DIOICA* L. × RIPENING PERIOD ON a_w VALUE

CONCLUSIONS

S. aureus grows in sucuk produced without starter culture at the fermentation stage. The growth of *S. aureus* is accelerated by *U. dioica* L. that increases the initial pH value. Because of this, the rapid decrease in pH is very important for the inhibition of the growth of *S. aureus* also in fermented sausages produced by using of *U. dioica* L.

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