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Effect of usage *Urtica dioica* L. on microbiological properties of sucuk, a Turkish dry-fermented sausage

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Abstract

The effect of dried-ground *Urtica dioica* L. on microbiological characteristics of Turkish dry-fermented sausage (sucuk) were investigated. Dried-ground *Urtica dioica* L. was added to sucuk batters by the rate of 0%, 1%, 3% and 5%, and microbiological analysis were made on the 0, 3, 7, 10 and 14 days of ripening period.

According to the results, both the level of *Urtica dioica* L. and ripening time had a significant effects on the total aerobic mesophylic bacteria, lactic acid bacteria, *Micrococcus*/*Staphylococcus*, yeast-mould and *Enterobacteriaceae* counts ($p < 0.01$). It was determined that the interactions ripening time × the levels of *Urtica dioica* L. had a significant effects on the counts of total aerobic mesophylic bacteria, lactic acid bacteria, *Micrococcus*/*Staphylococcus*, yeast-mould and *Enterobacteriaceae* ($p < 0.01$). *Enterobacteriaceae* count were also found to be under the detectable level (<100 cfu/g) in sucuks (in day 14 of ripening) containing 3% and 5% *Urtica dioica* L.
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Keywords: *Urtica dioica* L. (stinging nettle); Sucuk (Turkish dry-fermented sausage); Meat product; Microbial flora

1. Introduction

Sucuk is the most popular dry-fermented meat product produced in Turkey. It is produced from beef and water buffalo meat, beef fat, sheep tail fat, salt, sugar, nitrite, nitrate and/or nitrite/nitrate and various spices (Gökalp, Yetim, Kaya, & Ockerman, 1988; Gökalp, Kaya, & Zorba, 1999; Kaya, 1993; Kaya, Gareis, & Kofoth, 1998).

Popular name of *Urtica dioica* L. is stinging nettle and it belong to the family of *Urticaceaea* (Akgül, 1993). In the last few years, *Urtica dioica* L. has been accepted as a healing plant because of its considerable effects on human health both in Turkey and in the other countries all over the world. The seeds and leaves of *Urtica dioica* L. contain vitamins, minerals and amino acids (Baytop, 1999; Wetherilt, 1989). The leaves of the plant have 14.4 mg/100 g α-tocopherol, 0.23 mg/100 g riboflavin, 13 mg/100 g iron, 0.95 mg/100 g zinc, 873 mg/100 g calcium, 75 mg/100 g phosphorus, and 532 mg/100 g potassium (Wetherilt, 1989).

Various researchers have stated that the use of *Urtica dioica* L. (seeds and leaves) with or without others plants have useful effects on curing some illnesses such as diabetes, eczema, hemorrhoid, liver inflammation, anemia, rheumatism, and prostate cancer (Konrad et al., 2000; Leporatti & Corradi, 2001; Miraldi, Ferri, & Mostaghimi, 2001; Petlevski, Hadzija, Slijepcevic, & Juretic, 2001; WHO, 1980).

There has been only one paper published on the use of *Urtica dioica* L. in sucuk product (Aksu, 2003). In that study, the possible use of *Urtica dioica* L. as ingredient in sucuk was investigated. The results showed that the level of *Urtica dioica* L. (0%, 1%, 3% and 5%) and ripening period (0, 3, 7, 10 and 14 days) had significant effect on moisture, pH, residual nitrite, and L^* and $+a^*$ values of sucuk ($p < 0.01$). In addition the use of *Urtica dioica* L. in sucuk (cooked and non-cooked) improved the sensory attributes (especially 1%) compared with control sucuk.

The use of *Urtica dioica* L. in sucuk manufacturing as a new meat ingredient which might improve human health and nutrition. Therefore, in this study, the effect of different levels of *Urtica dioica* L. on

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microbiologic characteristics of sucuk was investigated.

2. Materials and methods

2.1. Preparation of Urtica dioica L.

Urtica dioica L. leaves were dried, grounded and then sifted through 14 in. sieve before use.

2.2. Preparation of sucuk batters and ripening

Sucuk batters were manufactured according to the following formulation (Kaya, 1993; Öz, Kaya, & Aksu, 2002) per kg of beef meat: 800 g/kg beef meat, 100 g/kg beef meat fat, 100 g/kg tallow fat (sheep tail fat), 25 g/kg NaCl, 10 g/kg garlic, 4 g/kg saccarose, 7 g/kg red pepper, 5 g/kg black pepper, 9 g/kg cumin, 2.5 g/kg Pimento and 150 ppm/kg $NaNO_2$. Sucuk batters were divided into four, and in paste each batter (5 kg) different levels of dried-ground *Urtica dioica* L. (1%, 3% and 5%, according to total weight) were added together with a starter culture (*Lactobacillus plantarum* + *Staphylococcus carnosus*, Bactoferm TM T-D-66, CHR HANSEN, Rudolf Müller). As a control, sucuk batter was made without *Urtica dioica* L. All of the sucuk batters were held at 3–4 °C for 12 h to increase the penetration of ingredients into the meat. Then, the mixture was minced (3 mm diameter) and batters were stuffed into natural casings (38 mm diameter, collagen, Naturin Darm, Germany). The ripening programs were as follows: 3 days at 22 ± 1 °C and RH 90 ± 2%, 4 days at 20 ± 1 °C and RH 85 ± 2%, and 7 days at 18 ± 1 °C and RH 80 ± 2%.

2.3. Microbiological analysis

Three samples were taken from each group at several ripening times (0, 3, 7, 10 and 14 days). A 25 g sample was aseptically homogenized in 225 ml physiological water (NaCl 0.85%) in a Stomacher for 1 min. For microbial counts, the following growth media and incubation conditions were used: Plate Count Agar (Merck) for 3 days at 30 °C for aerobic mesophylic bacteria, De Man Rogosa Sharpe Agar (Merck) for 3 days at 30 °C (anaerob) for lactic acid bacteria, Mannitol Salt Phenol-Red Agar (Merck) for 3 days at 30 °C for *Micrococcus*/*Staphylococcus*, Violet Red Bile Dextrose Agar (Merck) for 2 days 30 °C (anaerob) for *Enterobacteriaceae*, and Potato Dextrose Agar (Merck) for 5 days 25 °C for yeast and mould.

2.4. Statistical analysis

In this study, a completely randomized design was employed (three replicate). Results were analyzed using SPSS (1996). Comparison of mean values were made using the Duncan test. The results of statistical analysis were shown as mean values ± standard deviation in tables.

3. Results and discussion

Both amount of *Urtica dioica* L. and ripening time had a significant effect on total aerobic mesophylic bacteria, lactic acid bacteria, *Micrococcus*/*Staphylococcus,* yeast-mould and *Enterobacteriaceae* counts ($p < 0.01$). Duncan comparison test of the average of significant differences in the variance analysis of the values determined at various aging steps of different *Urtica dioica* L. and control Turkish dry-fermented sausages (sucuk) are presented in Tables 1 and 2.

The results of Duncan test showed that the number of total aerobic mesophylic bacteria count was higher in sucuk without *Urtica dioica* L. than with *Urtica dioica* L. (Table 1). The highest total aerobic mesophylic bacteria count was determined on day 3 during the ripening period (Table 2). The interaction the levels of *Urtica dioica* L. × ripening time resulted in a significant effect on total aerobic mesophylic bacteria count ($p < 0.01$). The total aerobic mesophylic bacteria count was increased in both the control group and samples with *Urtica dioica* L. until the third day of ripening. After the third day, the total aerobic mesophylic bacteria count did not change in control samples but it decreased in samples with *Urtica dioica* L. (Fig. 1). However, the sucuks produced with *Urtica dioica* L. had approximately 1 log lower

Table 1
The influence of addition of *Urtica dioica* L. on microbiological status of sucuk (log cfu g^{-1})

The levels of *Urtica dioica* L.	Total aerobic mesophylic bacteria	Lactic acid bacteria	*Micrococcus*/*Staphylococcus*	Yeast-mould	*Enterobacteriaceae*
Control	8.63 ± 1.08^{a}	8.68 ± 1.24^{a}	6.62 ± 0.60^{a}	4.60 ± 0.35^{a}	4.86 ± 0.36^{a}
1%	7.49 ± 0.61^{c}	7.48 ± 0.68^{c}	6.06 ± 0.26^{b}	3.89 ± 0.18^{b}	4.17 ± 0.31^{c}
3%	7.54 ± 0.48^{c}	7.27 ± 0.64^{d}	6.03 ± 0.67^{b}	3.20 ± 0.74^{c}	4.33 ± 0.65^{b}
5%	7.70 ± 0.56^{b}	7.53 ± 0.70^{b}	6.10 ± 0.76^{b}	2.69 ± 0.64^{d}	3.82 ± 1.10^{d}

± Standard deviation.
(a–d) Any two means in the same column having the same letters are not significantly different at ($p < 0.05$).

Table 2
The influence of ripening period on microbiological status of sucuk (log cfu g^{-1})

Ripening period (day)	Total aerobic mesophylic bacteria	Lactic acid bacteria	*Micrococcus/ Staphylococcus*	Yeast-mould	*Enterobacteriaceae*
0	6.64 ±0.18[d]	6.30 ± 0.04[e]	5.76 ± 0.08[d]	3.92 ± 0.49[a]	3.92 ± 0.35[b]
3	8.39 ± 0.38[a]	8.36 ± 0.46[a]	6.87 ± 0.39[a]	3.99 ± 0.56[a]	4.57 ± 0.18[a]
7	8.10 ± 0.62[b]	8.25 ± 0.74[b]	6.54 ± 0.44[b]	3.59 ± 0.98[b]	4.89 ± 0.33[c]
10	8.06 ± 0.77[b,c]	7.86 ± 0.90[d]	6.13 ± 0.73[c]	3.25 ± 1.09[c]	3.81 ± 1.14[d]
14	8.02 ± 0.77[c]	7.92 ± 0.87[c]	5.71 ± 0.44[d]	3.25 ± 1.04[c]	<2.00

± Standard deviation.
(a–d) Any two means in the same column having the same letters are not significantly different at ($p < 0.05$).

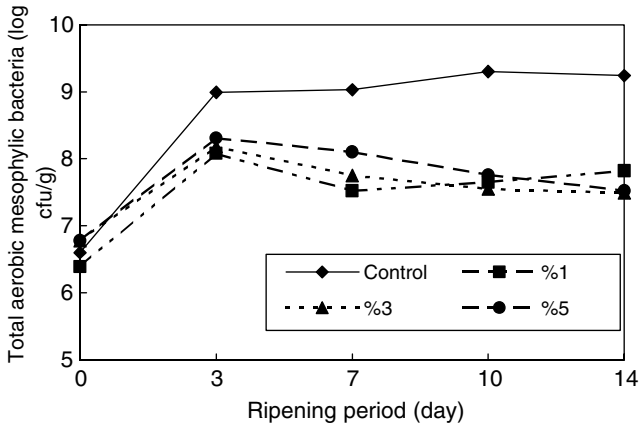

Fig. 1. Effect the interaction between *Urtica dioica* L. × ripening period on total aerobic mesophylic bacteria count.

count of total aerobic mesophylic bacteria than the control sucuk at the end of ripening.

Although *Lactobacillus plantarum* was used in all the sucuk groups, the lactic acid bacteria count was higher in the control group than that of the groups with *Urtica dioica* L. (1%, 3% and 5%) (Table 1). However, the lactic acid bacteria count decreased during ripening time in all groups (Table 2). The interaction of the level *Urtica dioica* L. × ripening time was significant ($p < 0.01$) on the lactic acid bacteria count as indicated in Fig. 2. This count was decreased after the third day of ripening in samples with *Urtica dioica* L. (Fig. 2). Apaydın (2001), Coşansu and Ayhan (2000), Kaya (1993) and Öz et al. (2002) reported similar results in their control sucuks.

The addition of different levels *Urtica dioica* L. in sucuk batters had an effect on *Micrococcus/Staphylococcus* count, and the highest *Micrococcus/Staphylococcus* counts was found in control sucuks (Table 1). The *Micrococcus/Staphylococcus* count started to decrease in *Urtica dioica* L. after 10 days of ripening (Table 2). The interaction of *Urtica dioica* L. × ripening time affected *Micrococcus/Staphylococcus* counts ($p < 0.01$). The maximum *Micrococcus/ Staphylococcus* count was determined at day 7 of ripening in control samples and at the day 3 of ripening in samples with *Urtica dioica* L. (Fig. 3). Apaydın (2001) found the maximum level of *Micrococcus/Staphylococcus* count at day 7 of ripening was reached in Turkish dry-fermented sausage with or without a starter culture.

It has been determined that with increasing the amount of added *Urtica dioica* L. into the sucuk batter, the count of yeast and mold was decreased (Table 1). It was also observed that the count of yeast and mold was the highest in the control group and decreased in all groups during ripening period (Table 2). As seen in Fig. 4 which shows the effect of *Urtica dioica* L. levels × ripening time interactions, the count of yeast and mold in the control samples started to increase compared to

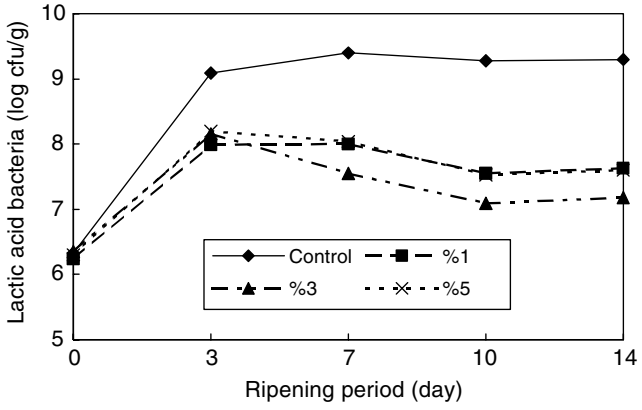

Fig. 2. Effect the interaction between *Urtica dioica* L. × ripening period on lactic acid bacteria count.

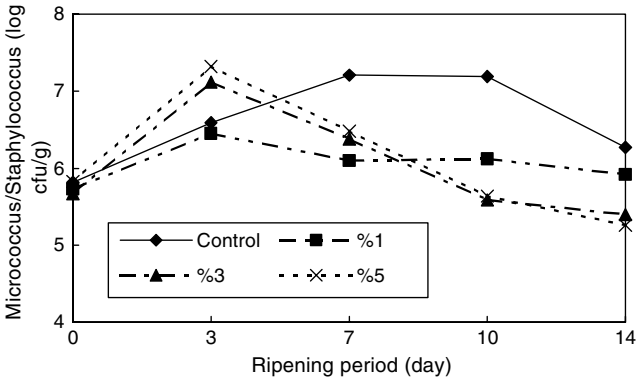

Fig. 3. Effect the interaction between *Urtica dioica* L. × ripening period on *Micrococcus/Staphylococcus* count.

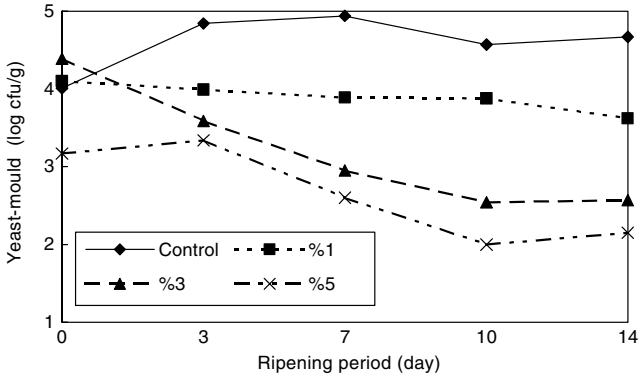

Fig. 4. Effect the interaction between *Urtica dioica* L. × ripening period on yeast-mould count.

other groups on day 3 of ripening time. However, the group with 1% *Urtica dioica* L. did not show a significant change in the count of yeast and mold. The lowest count of yeast and mold was observed in groups containing 3% and 5% *Urtica dioica* L. (Fig. 4). Sokmen, Jones, and Ertürk (1999) reported that seeds of *Urtica dioica* L. did not show any effect on yeast species.

The addition of different levels of *Urtica dioica* L. in sucuk batters had an effect on the *Enterobacteriaceae* count ($p < 0.01$), and the highest *Enterobacteriaceae* was found in the control sucuks (Table 1). The interaction of level of *Urtica dioica* L. × ripening time significantly affected the *Enterobacteriaceae* counts (Fig. 5). The *Enterobacteriaceae* counts were found to be under the detectable level (<100 cfu/g) at day 14 of ripening in sucuks containing 3% and 5% *Urtica dioica* L. and in the sucuks control and containing 1% *Urtica dioica* L. there were 3.69 ± 0.21 and 3.48 ± 0.35 counts, respectively. These results showed that the dried-leaves of *Urtica dioica* L. may have possessed antibacterial properties on these microorganism. However, Sokmen et al. (1999) reported that seeds of *Urtica dioica* L. do not have antibacterial effects on some members of *Enterobacteriaceae*.

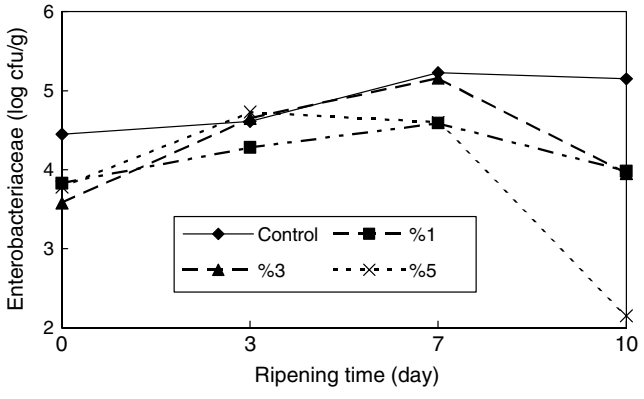

Fig. 5. Effect the interaction between *Urtica dioica* L. × ripening period on *Enterobacteriaceae* count.

Aksu (2003) indicated that pH values and moisture loss of sucuks including 0%, 1%, 3%, 5% *Urtica dioica* L. were 5.16, 5.24, 5.45 and 5.57 and 21.27%, 20.39%, 17.60% and 15.79% respectively. Sensory evaluation showed that addition of *Urtica dioica* L. in sucuk (cooked and non-cooked) improved sensory attributes as compared with control sucuk.

4. Conclusions

It can be concluded that the usage of *Urtica dioica* L. in sucuk manufacturing affected microbial flora of sucuk. Especially, with increasing levels of *Urtica dioica* L. in the formulation of sucuk decreased counts of *Enterobacteriaceae*, and yeast and mold.

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