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Some Microbiological and Pysico-Chemical Quality of Turkish Sucuk (Sausage)

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Abstract: In this study, a total of 100 Turkish sucuk samples were analyzed for pysico-chemical and microbiological quality. The maximum levels of moisture, fat and pH limited by Turkish food regulation are 40, 40% Fresh Matter (FM) and 5.8, respectively and sucuk should not contain any starch. In these respects, while 51.5 and 32% of the samples was found high for moisture and pH values, respectively, all the samples were almost suitable for fat value. Starch was detected in 66% of the samples. *Lactobacilli* and micrococci/staphylococci were $<10^2$-10^8 and $<10^2$-10^7 cfu g^{-1}, respectively. There were negative correlations between *Lactobacilli* levels and pH values ($p<0.01$). However, at a few sucuk samples, there were no correlation between *Lactobacilli* and pH values. NaCl was detected in above 5% Dry Matter (DM) of 25 the samples. Maximum and minimum collagen and hydroxyproline levels were detected in 3.20-9.68 and 0.40-1.21 μg mg^{-1} (DM), respectively. According to analyzed results, the percentage of moisture, fat, pH, NaCl, starch, collagen and hydroxyproline levels showed variation among the sample analyzed. There is not any official regulation for collagen and hydroxyproline levels of Turkish sucuk. Therefore, this and these kinds of studies may serve as a basis for the preparation of Official Standards of quality for collagen and hydroxyproline content of sucuk in Turkey.

Key words: Sausage (sucuk), pysico-chemical quality, collagen, hydroxyproline, starch, *Lactobacilli*, micrococci/staphylococci

INTRODUCTION

Turkish sausage known as sucuk is a kind of fermented meat product and it is widely appreciated in Turkey. Traditionally sucuk preparation is based on natural fermentation by contamination of the environmental flora of sucuk's raw materials. Nowadays, in brief the main ingredients to make sucuk are ground beef, fat, salt, nitrate/nitrite, sugar, garlic, spices, starter culture and other additives. The mixture is stored at 4°C for 12-24 h and then finely ground before stuffing into natural cattle small intestine cases. After stuffing, the sucuk is incubated overnight at room temperature for fermentation and then pasteurized by heating, until its internal temperature reach to 65°C.

Lactobacilli and other Lactic Acid Bacteria (LAB) possess the main role in the sucuk fermentation. They affect both the technological properties and the microbial stability of the final product through the production of lactic and acetic acids and the consequent pH decrease. LAB also affects flavour, texture and hygienic stability (Ordo Nez *et al*., 1999). Micrococci/staphylococci possess also peroxidase and nitrate reduction ability.

Generally, fat of sucuk acts as a reservoir for flavour compounds and contributes to products texture and juiciness. NaCl in meat products contributes to water holding capacity, fat binding, colour, flavour and texture. Proteins (derived from raw meat) are able to form networks and structures and thus, play an important role in the textural, sensory and nutritional quality of foods (Xiong, 1997). In contrast to myofibriller protein, collagen and other connective tissue proteins play a negligible role in gel formation in sausage batters. They provide also cost savings to the meat processor.

The scientific knowledge of the sucuk variety is limited and the existing information in the scientific literature generally refers to some microbiological aspects of the sausage. There is little information of the physico-chemical quality of sucuk (Akol *et al*., 1985; Aytekin, 1986; Gokalp *et al*., 1988). Actually, there is no

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official standards for collagen and/or hydroxyproline amount. The quality of sucuk is very variable and there is very little uniformity among these products manufactured in Turkey as well as other parts of the world. Therefore, the present study was designed to determine some of the pysico-chemical characteristics, *Lactobacilli*, micrococci\staphylococci levels and to determine relationships between lactobacilli number and pH value of the sucuk samples.

MATERIALS AND METHODS

A total of 100 fermented and heated natural gut casing sucuk samples were randomly purchased from local butcher shops and retail markets in Afyon province. The samples were immediately analyzed for pH, fat, moisture, NaCl, collagen and hydroxyproline contents, lactobacilli and micrococci\staphylococci counts.

Determination of pH values, fat, moisture and NaCl content: pH measurement was made using an electrode of pH meter (WTW, Inolab level 1) inserted directly into sucuk samples. Three independent measurements were made on each sample and mean values were calculated. Fat content was determined by using conventional soxhlet extraction method. Moisture content was determined by using Infra red technology (Sartorious, MA 45 model). NaCl content was determined by using Mohr method.

Determination of collagen and hydroxyproline: The collagen and hydroxyproline concentration of the sucuk samples were determined according to methods described by Neuman and Logan (1950) and Reddy and Enwemeka (1996), respectively. The optic density of colour samples was read using the UV 160 Schimadzu spectrophotometer at 550 nm. The results were given as μg mg^{-1} wet tissue and converted dry matter.

Isolation of *Lactobacilli* and micrococci/staphylococci: Ten grams of sucuk samples were aseptically taken and transferred into sterile plastic bags and diluted with 90 mL sterile peptone water (0.01%) (Oxoid, CM 9, Basingstoke, Hampshire, England) and homogenized for 90-120 s using a Stomacher (Interscience Bag Mixer 400). Serial decimal dilutions were prepared in sterile peptone water (0.01%) and inoculated onto Man, Rogasa and Sharpe (MRS) agar (Oxoid, CM 361, Basingstoke, Hampshire, England) for lactobacilli and Baird Parker agar (Oxoid, CM 0275 Suppl. Potassium tellurite and egg yolk emulsion, Oxoid SR O54, Basingstoke, Hampshire, England) for micrococci/ staphylococci isolation by drop plating technique. Then, MRS petri dishes incubated at 30°C for 72 h in anaerobic conditions, BP agar dishes incubated at 37°C for 24-48 h in aerobic conditions.

Statistical analysis: Regression analysis was used for data and calculating correlation coefficient to determine relationships between lactobacilli number and pH value. All analysis and correlations were executed from SAS statistical software.

RESULTS AND DISCUSSION

Results of the pysico-chemical components of the Turkish sucuk are shown in Table 1. The maximum levels of moisture, fat and pH limited by Turkish Food Regulation are 40, 40% Fresh Matter (FM) and 5.8, respectively and sucuk should not contain any starch. In these respects, while 51.5 and 32% of the samples was found high for moisture and pH values, respectively, all the samples were almost suitable for fat value. Starch was detected in 66% of the samples. *Lactobacilli* and micrococci/staphylococci were $<10^2$-10^8 and $<10^2$-10^7 cfu g^{-1}, respectively. There were negative correlations between lactobacilli levels and pH values ($p<0.01$). NaCl was detected in above 5% Dry Matter (DM) of 25 the samples. Maximum and minimum collagen and hydroxyproline levels were detected in 3.20-9.68 and 0.40-1.21 μg mg^{-1} (DM), respectively.

The present study, a significant negative correlation (-0.42911) was found between pH and *Lactobacilli* counts ($p<0.01$) (Fig. 1). However, at a few sucuk samples, there

Table 1: Psycho-chemical composition of analysed Turkish sucuk samples (n = 100)

Paramaters	Average	Minimum	Maximum
pH	5.49	4.84	6.50
Moisture (g/100)	40.90	29.80	47.60
Fat (FM%)	32.90	29.00	42.00
NaCl (DM%)	4.53	3.70	5.85
Collagen (DM%)	7.17	3.20	9.68
Hydroxyproline (DM%)	0.89	0.40	1.21

*Fresh matter; **Dry matter

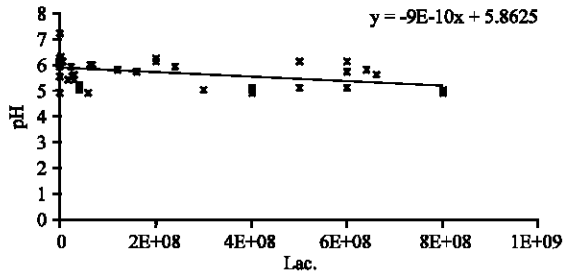

Fig. 1: Correlation between number of *Lactobacilli* levels and pH values of analysed the sucuk samples

Table 2: Number of *Lactobacilli*, micrococci/staphylococci (cfu g^{-1}) and pH values of analysed Turkish sucuk samples

Lactobacilli	Micrococci/ staphylococci	pH	*Lactobacilli*	Micrococci/ staphylococci	pH	*Lactobacilli*	Micrococci/ staphylococci	pH	*Lactobacilli*	Micrococci/ staphylococci	pH
2.0×10^{5}	2.6×10^{4}	6.00	2.0×10^{8}	7.0×10^{6}	5.10	1.4×10^{3}	4.0×10^{4}	6.10	2.0×10^{8}	2.8×10^{4}	5.18
1.6×10^{6}	1.0×10^{4}	6.10	1.1×10^{6}	1.4×10^{4}	5.30	6.0×10^{8}	8.8×10^{6}	5.20	6.0×10^{8}	2.0×10^{6}	5.28
6.0×10^{6}	2.8×10^{6}	6.10	$<2.0\times10^{2}$	2.3×10^{3}	4.90	$<2.0\times10^{2}$	4.0×10^{2}	6.10	2.4×10^{8}	1.6×10^{5}	5.04
1.6×10^{7}	6.0×10^{3}	5.40	3.4×10^{5}	8.0×10^{5}	7.20	$<2.0\times10^{2}$	2.0×10^{3}	6.20	4.0×10^{8}	1.0×10^{4}	5.22
$<2.0\times10^{2}$	1.6×10^{4}	6.00	4.0×10^{8}	3.0×10^{4}	4.90	2.0×10^{6}	1.2×10^{3}	5.30	6.0×10^{7}	8.2×10^{5}	5.44
3.0×10^{8}	1.2×10^{4}	5.00	4.0×10^{7}	8.0×10^{4}	5.10	2.0×10^{7}	2.0×10^{7}	5.20	4.4×10^{7}	2.6×10^{4}	4.76
4.8×10^{6}	8.0×10^{3}	6.10	1.2×10^{8}	1.0×10^{5}	5.80	6.0×10^{4}	2.0×10^{4}	5.90	4.0×10^{8}	1.2×10^{5}	5.34
2.0×10^{4}	1.4×10^{4}	5.90	2.4×10^{8}	1.0×10^{5}	5.90	4.0×10^{8}	1.6×10^{3}	5.30	4.0×10^{8}	5.4×10^{4}	5.30
$<2.0\times10^{2}$	1.3×10^{4}	6.20	3.0×10^{7}	8.0×10^{5}	5.60	7.0×10^{8}	2.0×10^{4}	5.00	8.0×10^{5}	1.0×10^{5}	5.30
8.0×10^{4}	1.6×10^{5}	6.20	6.0×10^{8}	2.0×10^{6}	6.10	8.4×10^{8}	1.4×10^{5}	5.00	3.0×10^{7}	4.0×10^{2}	5.50
4.0×10^{8}	2.6×10^{5}	5.10	6.6×10^{8}	1.8×10^{7}	5.00	$<2.0\times10^{2}$	2.0×10^{3}	6.50	6.8×10^{8}	3.2×10^{4}	4.85
6.0×10^{8}	1.0×10^{6}	5.70	1.6×10^{8}	3.6×10^{6}	5.70	4.0×10^{6}	4.0×10^{3}	5.10	2.0×10^{5}	1.4×10^{3}	5.65
6.0×10^{8}	2.0×10^{6}	5.10	8.0×10^{8}	1.5×10^{5}	5.00	2.8×10^{8}	2.6×10^{4}	5.44	2.0×10^{4}	4.0×10^{2}	6.10
4.0×10^{7}	8.4×10^{4}	5.20	4.0×10^{7}	2.2×10^{6}	5.00	1.0×10^{8}	5.4×10^{7}	5.23	2.4×10^{2}	1.6×10^{4}	5.60
8.0×10^{8}	2.2×10^{5}	4.90	3.4×10^{3}	8.0×10^{2}	6.10	8.0×10^{7}	5.6×10^{4}	5.29	4.4×10^{8}	$<2.0\times10^{2}$	5.45
6.0×10^{8}	3.8×10^{6}	5.10	6.4×10^{8}	4.6×10^{6}	5.80	2.0×10^{7}	5.8×10^{6}	5.20	5.2×10^{6}	4.8×10^{3}	5.77
$<2.0\times10^{2}$	1.2×10^{4}	6.00	2.4×10^{7}	1.4×10^{4}	5.90	$<2.0\times10^{2}$	6.0×10^{2}	6.20	1.0×10^{8}	1.0×10^{4}	5.17
1.1×10^{6}	3.0×10^{5}	5.10	3.4×10^{3}	8.0×10^{3}	6.10	2.2×10^{8}	6.0×10^{2}	4.84	1.4×10^{8}	6.0×10^{2}	5.09
8.0×10^{5}	6.4×10^{6}	5.50	2.0×10^{8}	2.0×10^{6}	5.20	8.0×10^{7}	8.0×10^{6}	5.78	3.2×10^{8}	2.2×10^{4}	4.87
$<2.0\times10^{2}$	8.0×10^{2}	6.00	5.0×10^{8}	3.0×10^{6}	5.10	5.0×10^{7}	2.2×10^{6}	4.90	2.0×10^{6}	2.2×10^{4}	6.16
5.0×10^{8}	4.0×10^{4}	5.10	4.0×10^{8}	1.2×10^{4}	5.00	2.0×10^{8}	4.0×10^{5}	5.13	2.8×10^{6}	2.2×10^{4}	5.93
1.4×10^{3}	2.0×10^{2}	6.10	3.4×10^{5}	1.6×10^{4}	6.00	$<2.0\times10^{2}$	1.8×10^{3}	6.10	1.0×10^{7}	3.2×10^{4}	5.90
2.6×10^{7}	3.8×10^{5}	5.50	3.0×10^{7}	6.0×10^{3}	5.40	9.0×10^{7}	4.4×10^{5}	5.26	2.0×10^{4}	1.0×10^{4}	6.01
6.0×10^{7}	6.4×10^{5}	4.90	6.0×10^{6}	2.8×10^{6}	6.10	6.0×10^{7}	8.0×10^{5}	5.55	3.0×10^{7}	$<2.0\times10^{2}$	5.00
6.8×10^{7}	2.0×10^{4}	5.00	6.4×10^{7}	6.4×10^{7}	5.00	8.0×10^{7}	6.4×10^{5}	4.76	1.4×10^{8}	1.2×10^{5}	5.18

were no correlation between *Lactobacilli* and pH values. As shown in Table 2, although, *Lactobacilli* levels of sucuk samples were $<2.0\times10^{2}$ and 2.4×10^{2} cfu g^{-1}, pH values were measured 4.90 and 5.60, respectively. It was expected that pH values should be higher than these values. It may be depended on using of Glucano dalta Lacton (GdL). We observed that some sucuk producers are using GdL during sucuk production stage to rapid decline of pH. Although, rapid decline of pH gives the sucuk unique lactic acid flavour, it increases firmness, texture and palatability due to the acid denaturation of muscle protein (Mendonca *et al*., 1989). In contrast to this result, few sucuk samples were both higher lactobacilli levels and pH values (Table 2). This non-correlation might firstly, depend on addition of alkali phosphate during sucuk production stage. Secondly, it could be due to the superficial development of molds with a consequent loss of acidifying and deaminase activities (Casaburi *et al*., 2007). Thirdly, it could be micrococci/staphylococci counts. It is known that LAB are increasing in numbers at the very beginning of the fermentations of sausage, producing acids and decrease in the pH, followed in the phases of maturation by the activity of micrococci/ staphylococci that are able to neutralize the acids produced. In the present study, lactobacilli counts were 6.0×10^{6}, 6.0×10^{8}, 6.0×10^{6} and 2.0×10^{6}, micrococci/ staphylococci counts were 2.8×10^{6}, 2.0×10^{6}, 2.8×10^{6} and 2.2×10^{6} and pH values were 6.1, 6.1, 6.1 and 6.1, respectively at the same sucuk samples (Table 2). At the same time, the pH increases during ripening stage also could be production of ammonia, peptides and amino acids by proteolytic micro flora present in the products (Lebert *et al*., 2007).

In this study, pH values ranged from 4.8-6.5, but one sample had a pH of 7.2. These values were in the higher margin of the range described for this parameter in the literature for Spanish traditional raw-cured (Androlla, Botillo and *Chorizo de cebolla*) sausages (Santamaria *et al*., 1992; Lorenzo *et al*., 2000; Salgado *et al*., 2006) and Italy sausages (Comi *et al*., 2005). The present study, the average pH value was 5.49. This value were similar reported by Lorenzo's *et al.* (2000) Androlla and Botillo sausages. The reason, Spanish and Italian traditional and naturally fermented sausages undergo a long drying-ripening than Turkish sucuk. Houben and Hooft (2005) reported their experimental study that pH was measured between 4.51-4.56 in final semi-dry fermented sausage. They used lyophilized starter cultures. So, use of the starter cultures and the controlled ripening rooms affect pH level and textural characteristics of the sausage. The pH values of 4.5-5.0 and the ripening temperature of 15-20°C were optimal for the activity of proteolytic enzymes. It is known that muscle proteinases are activated by the drop of pH and seem primarily responsible for proteolysis during the early fermentation, while bacterial and endogenous enzymes are more important during the latter stages of ripening (Hughes *et al*., 2002).

The amounts of hydroxyproline ranging from 0.4-0.5% were reported for several fermented sausages (Crespo *et al*., 1978; Leon Santamaria *et al*., 1992; Beltran *et al*., 1993). In the present study, 21% of samples are between these values, but 79% of samples are higher than 0.5%. There are also, several studies for hydroxyproline content of Spanish dry-cured sausage. In these studies, hydroxyproline values were reported between 0.195 and 1.99% (DM) and average values between 0.48 and 0.968% (DM) (Lorenzo *et al*., 2000; Salgado *et al*., 2006). For manufacture of these kind of sausages using as ingredients low or secondary quality pork such as diaphragm, inter-rib muscles, jowls, skin, lard, lungs and bacon. In contrast, added of lung, intestine, heart, liver, skin and other kind of edible offal to manufacture of sucuk as ingredients are forbidden according to Turkish food regulation. For manufacture of Turkish sucuk, only beef (80%) and/or buffalo meat (10-20%) or occasionally camel meat has to be used for sucuk production at the same regulation. Compared to Spanish sausage, in the present study, hydroxyproline content of sucuk is higher. It was expected lower. The reason may be results of addition of direct collagen or edible offal, skin or to amount of trimming meat to the mixture of sucuk content. Addition of high percent (≥50%) buffalo meat in the sucuk mixer may be another reason for high hydroxyproline levels. It is known that total collagen content of buffalo meat (7.53 mg g^{-1}) is higher than beef (5.75 mg g^{-1}) (Spanghero *et al*., 2004). Another reason could be high moisture content of sucuk. At last, it depends on added starch instead of meat.

In the present study, the moisture values found in the sucuk were between 29.8 and 47.6% and average value was 40.9%. These results were expected because of the short ripening time for industrial sucuk manufacture instead of traditional Turkish sucuk. Visessanguan *et al*. (2004) reported that an increase in expressible and released water was presumably caused by denaturation of protein during fermentation. It is probably associated with pH changes, since muscle proteins are tented to be denatured as the pH falls, leading to a reduction in their ability to bind water.

There are also several studies for moister content of Spanish and Italian type sausages (Lorenzo *et al*., 2000; Comi *et al*., 2005; Salgado *et al*., 2006). In these studies, they reported that moister contents of sausages were between 18.24 and 62.2% for Spanish (Lorenzo *et al*., 2000; Salgado *et al*., 2006) and 40-46% for Italian sausage (Comi *et al*., 2005). There are several factors affects on moisture content of sausages. The environmental conditions during ripening of sausage and sausage, ingredients such as collagen, starch, alkali polyphosphate and NaCl could affect the moisture content of sausage. There are a positive correlation between the amount of collagen, hydroxyproline, fat and NaCl and the moisture content of products. In the Salgado *et al*. (2006) and the present studies results indicated that the maximum hydroxyproline content as well as the maximum moisture values.They also reported that the losses of moisture were more noticeable in the batches of homemade sausage. The caliber differences of the sausages were identical in both cases, which could be the result of the slight differences in the water retention capacity of the meat, which could obviously be greater in the industrial batches due to their higher content of NaCl. In non-cooked sausages the granulated fat helps to loosen the sausages mixture and this helps the continuous release of moisture from inner layers of the products.

Fat level affects significantly the appearance and consistency of fresh sausages. Papadima and Bloukas (1999) reported that sausages with 20% fat had the highest scores for all sensory attributes. Sausage with 30% fat had the lowest scores for appearance and consistency. They were very light in color and very soft, due to their high fat content. In the present study, fat content of the sucuk samples were found between 29 and 42% (FM). However, sucuk appearance was not bad. They had sensitive colour. The reason may be the use of coloring substances.

The NaCl content of the raw cured sausage studied generally are between 5 and 8% (DM) (Beriain *et al*., 1993; Casiraghi *et al*., 1996; Casaburi *et al*., 2007). The present study results are lower than above studies results. Although, the average content of NaCl is higher than Salgado *et al*. (2006) study results, the maximum content of NaCl is lower than Salgado *et al*. (2006). In addition, higher NaCl values (around 5% DM) have been described in other varieties Spanish sausages (Santamaria *et al*., 1992).

Starch addition is forbidden according to Turkish regulation. It is used emulsified type of sausages. However, it is used many sucuk procedures because it is increasing the moisture binding capacity of sucuk. They provide cost savings to the sucuk processor.

CONCLUSION

The present study showed that there was no uniformity among the analyzed sucuk samples. This will

rebound in an increase of the uniformity of the product, which will favour its diffusion in the markets. It is important to control the major sources of the variation by guidelines for standardization of Turkish sucuk and adequate strategies for labeling Turkish sucuk should be done.

There is no official standards of quality for collagen and hydroxyproline content of Turkish sucuk. The present study may serve as a basis for the preparation of a regulation, although large scale studies should be done.

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