

Hygiene of Smoked Goat Ham Production

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The results presented are a continuation of the research in monitoring the hygiene of facilities and hygienic principles in the production of smoked goat ham. The total number of bacteria, enterobacteria and pathogenic bacteria were examined, wet swabs were taken by non-destructive method from a total of 95 chilled goat hams before salting, after salting and after smoking. Total count of aerobic mesophilic bacteria, the number of *Enterobacteriaceae* and pathogenic bacteria were determined by ISO or other standard methods. Test results show that the average total count of aerobic bacteria in the tested samples after smoking were: for 83 samples within a satisfactory range (d'' 3.5 log cfu/cm²), 10 samples in the acceptable range (3.5 to 5.0 log cfu/cm²) and 2 samples in the unacceptable range (> 5.0 log cfu/cm²). Average values of the total number of *Enterobacteriaceae* in the samples after the smoking process were as follows: 85 samples within the satisfactory range (d'' 1.5 log cfu/cm²) and 10 samples in acceptable range (1.5 to 2.5 log cfu/cm²). Bacteria *Salmonella sp*, *Staphylococcus sp*, *Proteus sp*, *Escherichia coli* and *Listeria monocytogenes* were not isolated in the final product, while *Bacillus sp*, *Streptococcus sp* and *Lactobacillus sp* were isolated in 10.53% of the samples obtained after smoking.

Key words: Goats, Hygiene, Smoked ham, Total number of bacteria, Pathogenic bacteria

In Serbia, goat meat is used as a component for the production of sausages and as a smoked ham which is a specialty. In recent years, the number of farmed goats has increased in Serbia, because goat meat, especially originating from the kids, has increasingly been consumed for its characteristic taste and desired chemical composition and nutritional properties.

On the carcasses of healthy goats the bacteria can reach the surface at the slaughter and during processing of the hull. Skinning and evisceration operations are highly risky for the contamination by microorganisms, particularly pathogenic^[1]. Conventional veterinary examination cannot reveal the presence of these bacteria on the surface of carcasses^[2]. On the slaughter of goats, the most common is contamination of carcasses by pathogenic microorganisms belonging to enterobacteria (*Salmonella spp.*, *Escherichia coli*, *Proteus spp.*). Besides enterobacteria, some Gram-positive bacteria can be found during the work on the hull (*Staphylococcus aureus*, *Streptococcus sp*,

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Listeria monocytogenes, *Lactobacillus* sp, *Bacillus* sp.). If they are present in greater numbers they can cause food-borne illnesses in humans.

Among *Enterobacteriaceae* most importance is given to the genus *Salmonella* sp. In slaughter houses, *Salmonella* is usually transmitted to carcasses of goats from the skin or slaughtering equipment. It is assumed that the prevalence increases as a result of stress during transport of animals and insufficient rest before slaughter^{3,4}.

Escherichia coli is a normal and regular part of the micro flora of the intestinal tract of humans and animals. Only some strains can cause intestinal disease. Various VTEC are present in healthy animals which can be carriers of the bacteria, because occasionally excreted in the faeces⁵.

Like other bacteria of the family *Enterobacteriaceae*, *Proteus* species grow and reproduce in both, aerobic as well as in anaerobic conditions. Their presence in food indicates that it is prepared or stored in unhygienic conditions, or that the contamination arised due to improper treatment of food. These bacteria by its enterotoxins cause alimentary diseases in humans⁶.

Staphylococcus aureus is infectious for humans and animals. Saprophytic micro flora in food is destroyed during processing (cooking) or inhibited (food that contains a high concentration of salt), while *S. aureus* can survive these conditions. If this happens, *S. aureus* will proliferate and lead to recontamination of food^{7,8}.

In addition to *Streptococcus pyogenes* (group A), as the main cause of acute tonsillopharyngitis in humans, infections with streptococci of other serological groups are possible. People are infected by eating contaminated food which was handled by carriers or food which was kept in unsuitable conditions for long time⁹.

Listeria monocytogenes is pathogenic for many different animal species, as well as for humans, with no special specificity for the host. If *Listeria monocytogenes* is found on the working surfaces in abattoirs, it is difficult to remove it, because it can create biofilms and can survive in adverse environmental conditions¹⁰. Because of its subiquity, *Listeria* sp, and especially *L. monocytogenes*, is used as an indicator of hygiene at all stages of food production¹¹.

Lactobacilli are part of the normal flora of the human mouth and gastrointestinal tract¹², and are generally considered safe for use in food¹³. Infections with lactobacilli are rare, but may occur opportunistically, especially in immuno compromised individuals¹².

B. ceareus of all *Bacillus* species is the most often cause of alimentary toxic infections in humans. It is usually present as a contaminant in food of animal and plant origin and, because of its resistant spores, it can survive the various physical and chemical conditions. Some strains produce a toxin which is highly resistant to heat^{9,14}.

The results of smoked goat ham presented in this paper are a continuation of our research in monitoring facility hygiene and hygienic principles at the slaughter and during the food production processes. Total count of bacteria, number of enterobacteria and the presence of certain types of pathogenic bacteria in various stages of production of smoked goat ham were examined in accordance to EU legislation¹⁵.

MATERIALS AND METHODS

Sampling

Wet swabs were taken by non-destructive method¹⁶ from a total of 95 chilled goat hams before salting, after salting and after smoking. The final product intended for sale was sliced and vacuum packed. Swabs were taken from smoked products after slicing and before vacuum packing. A total of 285 swabs were taken from area of 100 cm² per ham. Swabs were transferred to the laboratory in the liquid transport medium (peptone water). At the same time, the sampling for testing of *Salmonella* presence was carried out using a swab method by abrasive sponge according to EU legislation¹⁵.

Bacteriological examination

Total count of aerobic mesophilic bacteria (log cfu/cm²), the number of *Enterobacteriaceae* (log cfu/cm²) and the presence of *Salmonella* spp. were determined in the swabs. Total aerobic mesophilic bacteria count was determined by ISO method¹⁷, number of *Enterobacteriaceae* by ISO method¹⁸ and the presence of *Salmonella* species was analysed in accordance to ISO method¹⁹. Obtained results were interpreted according to the table given in EU legislation¹⁵ (Table 1).

Table 1. Microbiological criteria of the production process hygiene

Microbiological profile of samples	Total count of aerobic mesophylic bacteria (log cfu/cm ²)	<i>Enterobacteriaceae</i> (log cfu/cm ²)	<i>Salmonella</i> spp.
Acceptable	≤ 3,5 log cfu/cm ²	≤ 1,5 log cfu/cm ²	Must not be present in the examined carcass area
Limit value	3,5 - 5,0 log cfu/cm ²	1,5-2,5 log cfu/cm ²	
Unacceptable	> 5,0 log cfu/cm ²	> 2,5 log cfu/cm ²	

From the same samples was carried out isolation and identification of: *Staphylococcus aureus* by ISO method²⁰, *Listeria monocytogenes* by ISO method²¹, *Escherichia coli* by ISO method²², *Streptococcus* sp., *Proteus* sp., *Bacillus* sp. and *Lactobacillus* sp.

The samples for the isolation of *Streptococcus* sp. were streaked onto the agar with 5% sheep blood, Edwards and MacConkey agar. Incubation was done under aerobic conditions at a temperature of 37°C during 24 to 48 h. Edwards' base and the blood agar plates were also incubated under conditions of 10% carbon dioxide. Primary identification of the culture was performed by colony appearance, model of hemolysis on the blood agar (±, ² and non-hemolytic) and based on the microscopic examination by Gram staining. Catalase test was used for the distinguishing of staphylococci and streptococci. Standard biochemical tests were done according to statements of Quinn *et al.*²³. As a positive test BBL Crystal G/P kit (Becton, Dickinson and Company, USA) was used.

Samples for the isolation of *Proteus* sp. were streaked on blood agar, SS (Salmonella-Shigella) agar, XLD (Xylose Lysine dehydrogenase) and MacConkey agar. These plates were incubated under aerobic conditions at a temperature of 37°C during 24 to 48 h. According to an estimation of colony appearance, the suspected enterobacteria were subcultured in an appropriate medium. For final identification was applied API 20E (bioMérieux SA, France).

Identification of bacteria of the genus *Bacillus* was carried out after the cultivation on the agar with 5% sheep blood and Hicrom *Bacillus* agar. The plates were incubated at 37°C under aerobic conditions during 24 to 48 h. Suspect colonies were subcultured to obtain pure cultures. By microscopic examination gram-positive sticks were observed. Catalase reaction test was positive.

The appearance of hemolysis on blood agar, mobility and degradation of gelatin was also tested. For *Lactobacillus* identification streaking was done on blood agar and *Lactobacillus* MRS agar. The plates were incubated under aerobic and anaerobic conditions at a temperature of 37°C for 24 to 48h. Identification of anaerobically grown cultures was carried out by the appearance of colonies in the microscopic preparation, catalase and oxidase reactions and biochemical tests.

Salting, smoking and determining the a_w value

Salting and smoking: processed goat hams with associated bones were dry salted with about 6% nitrite salt (99.5% sodium chloride and 0.5% sodium nitrite). Salted hams were kept in salt for 30 days. During the salting period they were rotated every second day. Desalting was carried out in cold water for 24 h while thereby the water was changed four times. Hams were cold-smoked for 45 days. Smoke temperature did not exceed 20°C. During the first 10 days smoking was carried out every day for 2 hours, but between the tenth and twentieth day it was done every second day for 2 hours. After the smoking period hams were only air dried.

Determination of a_w value: the water activity in the finished product was determined according to the formula (1) which was defined by Gimenez and Dalgaard²⁴:

$$a_w = 1 - 0,0052471 \cdot SVF - 0,00012206 \cdot SVF^2 \quad \dots(1)$$

a_w - the water activity; SVF - the content of salts in the aqueous phase

RESULTS AND DISCUSSION

Results of the total count of aerobic bacteria, *Enterobacteriaceae* and other isolated bacteria in different phases of production of smoked goat ham are shown in Tables 2, 3 and 5.

Table 2 shows the results related to the total number of aerobic bacteria in different stages

Table 2. Total count of aerobic mesophilic bacteria in different phases of production of smoked goat ham (n=95)

Phase	Satisfactory		Acceptable		Unsatisfactory	
	n	%	n	%	n	%
After chilling	85	89.47	5	5.26	5	5.26
After salting	87	91.58	8	8.42	1	1.05
After smoking	83	87.37	10	10.53	2	2.10

of production of smoked ham. The table shows that after salting the percentage of “satisfactory” results (91.58%) was higher than after chilling (89.47%), as well as than after smoking (87.37%). The percentage of “acceptable” results was also higher after salting (8.42%) than after chilling (5.26%), but lower than after smoking (10.53%). The percentage of the “unsatisfactory” results was lower after salting (1.05%) compared to the phase after chilling (5.26%). After smoking, the percentage of “unsatisfactory” results (2.10%) was lower than in the production phase after chilling, but higher after salting (1.05%).

Our results in all three stages of production in the categories “satisfactory” and “acceptable” were not in accordance to the results of Eze and Nwosu²⁵, while the results in all three stages of production in the category “unsatisfactory” were in accordance to the results of the above authors. These authors collected samples of fresh goat meat in the market. Their results of the total count of aerobic bacteria ranged from 5.39 ± 0.04 to 5.48 ± 0.05 log cfu/g. Discrepancy in our results could be explained by subsequent

contamination in the markets which probably occurred. Our findings related to the product after the smoking process may be compared with the results of Losantos *et al.*,²⁶. These authors used in their examination two types of smoked ham: Serrano ham and Iberian ham. They contaminated the samples and compared them with uncontaminated which served as controls. Our results could be compared with the controls. In the first Serrano ham the total count of bacteria was 3.8×10^4 cfug⁻¹, while in the Iberian ham was 8.2×10^3 cfug⁻¹. Our results regarding the final product, which is obtained after smoking, in the category “satisfactory” and “acceptable” were not in accordance to the results of mentioned authors, because significantly lower total aerobic mesophilic bacteria count was detected, while the results from the category “unsatisfactory” were agreed. In Iberian ham ratio is somewhat different. Our results in the category “acceptable” were consistent with the results of mentioned authors, while our results for the final product in the category “satisfactory” showed lower total aerobic mesophilic bacteria count.

Table 3. Number of enterobacteria in different phases of production of smoked goat ham (n=95)

Phase	Satisfactory		Acceptable		Unsatisfactory	
	n	%	n	%	n	%
After chilling	80	84.21	13	13.68	2	2.10
After salting	87	91.58	7	7.37	1	1.05
After smoking	85	89.47	10	10.53	0	0.00

Table 3 shows the results related to the total number of enterobacteria in different stages of production of smoked goat ham. The percentage of “satisfactory” results after chilling (84.21%) was lower than after the salting phase (91.58%) and after smoking (89.47%). Percentage of

enterobacteria in the category “acceptable” was the lowest in the phase after salting (7.37%), but the highest after chilling (13.68%), while in the stage after smoking it was 10.53%. After smoking phase none of the samples was classified as “unsatisfactory” and after chilling in this category

was 2.1% of the samples, while after salting was 1.05%. Total number of enterobacteria which were isolated from the product after smoking can also be comparable with the findings of Losantos et al.^[26]. These authors isolated in Serrano ham < 10 and in Iberian ham < 10 cfug⁻¹ enterobacteria. The

water activity of Serrano ham was 0.909 and 0.888 in the Iberian ham. Our results were consistent with the results for the phase after smoking in the “satisfactory” category, while the phase “acceptable” did not agree.

Table 4. The basic chemical composition of smoked goat ham

Smoked ham	Ash (%)	Proteins (%)	Fat (%)	Moisture (%)	Nitrites (mg/kg)	NaCl %	a _w
	5.6	38.9	16.6	39.9	27.0	4.7	0.8575

Table 4 shows the basic chemical composition of smoked ham with the percentage of salt in the product. This information was necessary for the calculation of water activity in the product, because it influences the survival of many microorganisms. In smoked goat ham water activity (a_w) was 0.8575.

In the EU legislation¹⁵, which is the basis of this research, it is emphasized that if the samples do not completely satisfy stated criteria, additional identification of certain bacteria, which can often

been found in the product ready for consumption and can in a large number cause diseases in humans, should be done. In the literature, little information about the presence of those bacteria in the final product is available. So, in this study determination of the presence of *Staphylococcus aureus*, *Bacillus* sp, *Escherichia coli*, *Salmonella* sp, *Proteus* sp, *Streptococcus* sp., *Listeria monocytogenes* and *Lactobacillus* sp. was chosen (Table 5).

Table 5. Isolated microorganisms by production stages (n=95)

Bacteria (cfu/cm ²)	Chilling		Isolated microorganisms after Salting		Smoking	
	n	%	n	%	n	%
<i>Staphylococcus</i> sp.	8	8.42	3	3.16	0	0.00
<i>Bacillus</i> sp.	50	52.63	10	10.53	10	10.53
<i>Escherichia coli</i>	30	31.58	0	0.00	0	0.00
<i>Salmonella</i> sp.	0	0.00	0	0.00	0	0.00
<i>Proteus</i> sp.	20	21.05	Not found	Not found		
<i>Streptococcus</i> sp.	30	31.58	10	10.53	10	10.53
<i>Listeria monocytogenes</i>	5	5.26	0	0.00	0	0.00
<i>Lactobacillus</i> sp	0	0.00	0	0.00	10	10.53

Staphylococcus aureus was isolated in 8.42% of the samples after the chilling stage, 3.16% of the samples after the salting, while it was not isolated in the sample after the smoking process. A lower a_w, for *S. aureus* growth is particularly important. These microorganisms are resistant to drying and can grow and produce enterotoxin in food with a_w lower than 0.85. For *S. aureus* is of particular importance, ability to grow in the presence of 7 to 10% of NaCl, but it also shows growth when NaCl is present up to 25%^[9]. As it

was not isolated from the final product (after smoking), it is assumed that some of the components of smoke seemed bactericidal to this bacterium. Here obtained results in the phase after chilling were analogous with the results of Eze and Nwosu²⁵. These authors isolated *Staphylococcus aureus* from 10.3% of samples of fresh goat meat taken in the markets, but the results that we obtained after salting (3.16%) and after smoking (0%) did not agreed with the results of the above mentioned authors.

Bacillus sp. in the phase after chilling was isolated in 52.63% of the samples, in the phase after salting and smoking in 10.53% of the samples. Number of cells/g of the tested sample was 10^2 . The ability to produce spores most likely enabled them to survive the manufacturing process. For development of the alimentary toxic infection it is necessary to ingest 10^5 *B. cereus* cells per gram of food^[14]. We isolated *Bacillus sp.* after chilling in the significantly higher percentage of tested samples (52.63%) comparing to authors Eze and Nwosu²⁵ who isolated *Bacillus sp.* in 20.7% of samples. We isolated these bacteria in a significantly lower percentage (10.53%) in the samples tested in stages after salting and after smoking.

Escherichia coli was isolated in 31.58% of the samples after chilling, but in none of the samples after salting and smoking. In this case, nitrite salt, smoking and low water activity ($a_w = 0.8575$) were bactericidal for *E. coli*. Our findings related to the phase after chilling (31.58%) were significantly higher compared to the results of Eze and Nwosu²⁵. They isolated the bacteria in fresh goat meat in 17.2% of samples.

Salmonella sp. was not isolated from the samples at any stage of production. Our results were not in agreement with the results of Lilic et al.²⁷ who detected the presence of *Salmonella sp.* in a total of 5 samples among 100 samples taken from carcasses of heifers. By subsequent serological tests they determined *Salmonella enterica subsp. enterica* serovar Typhimurium (3 isolates), *Salmonella enterica subsp. enterica* serovar Dublin (1 isolate) and *Salmonella enterica subsp. enterica* serovar Infantis (1 isolate). Our results also were not consistent with the results obtained by Nouichi^[2]. This author isolated *Salmonella sp.* in one sample of 120 samples taken from 120 carcasses of sheep, while he isolated it in 7 of 90 samples, taken from 90 carcasses of cattle. By serotypization he found the presence of *S. anatum* (76.9%), *S. arizonae* (15.4%) and *S. abortusovis* (7.7%).

Proteus sp. was isolated only in the phase after chilling (21.05%). In all other stages it was not isolated. Salt and smoke components were bactericidal to these bacteria. Obtained results related to the phase after chilling (21.05%) were in agreement with the results by Eze and Nwosu²⁵

who isolated this bacteria in fresh goat meat in 9.2% of samples.

Streptococcus sp. was isolated in 31.58% of the samples in the phase after chilling. This microorganism was affected by salting and smoking components as in the phases after salting and after smoking it was isolated in lower percentage of the samples (10.53%). According to EU Regulation¹⁵ this organism does not pose a threat to human health, but it can be an indicator of hygiene during processing and handling the product. The percentage of isolated *Streptococcus sp.* in the phase after chilling (31.58%) was significantly higher than the result published by Eze and Nwosu²⁵ which was 16.1%.

Listeria monocytogenes was isolated in 5.26% of samples tested in phase after chilling, but it was not isolated after salting and after smoking. The assumption is that it was not isolated from the final product due to its low water activity ($a_w = 0.8575$). Such results could be compared with those obtained by Kahraman et al.²⁸. These authors investigated the contamination of sheep carcasses at the slaughterhouse in accordance to EU legislation²⁹. Samples were taken from the surface of 1000 carcasses of slaughtered sheep during the period of one year. None of the samples were positive to the presence of *Listeria monocytogenes*. Our results regarding the production phase after chilling were not in agreement with the findings of these authors, because *Listeria monocytogenes* was isolated in 5.26% of tested samples.

Lactobacillus sp. was isolated in a 10.53% of the samples after smoking, while in the previous stages was not isolated. Such result indicates that there was a subsequent product contamination. Comparing our results with the results of Losantos et al.,²⁶ for both types of ham it was concluded that they agree, because the number was the same: $< 10^2$ cfug⁻¹.

Applied technological processes in the production of smoked goat ham significantly reduced the number of bacteria, so it could be concluded that the final product was safe for human consumption and it did not contain pathogenic bacteria. Considering the low a_w value, but depending on the method of storage and packaging, this product is well sustained on the market.

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