MICROBIOLOGICAL QUALITY AND PHYSIOCHEMICAL PARAMETERS OF COLD-SMOKED SAUSAGES DURING RIPENING

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Abstract

Many recent studies in food safety have investigated non-thermal processing of ready-to-eat food products, but there is little information about the survival of food pathogens in different ripening stages of cold smoked sausages. Therefore, the microbiological quality (total aerobic count – TAC, *Staphylococcus aureus*, *E. coli*, *Salmonella spp.*, and *Listeria spp.*), water activity (a_w), and pH were determined in cold-smoked sausages during the ripening time from days 0 to 21. The temperature in smoke camera was 28 °C for the first 3 days of maturation and 15±1 °C during days 4 to 21. As a result the TAC of starter culture bacteria increased from 5.72 lg cfu g^{-1} at the beginning to a maximum of 9.41 lg cfu g^{-1}. The count of *S. aureus* increased from 1.38 to 2.68 lg cfu g^{-1} and *E. coli* decreased from 2.47 to 0.85 lg cfu g^{-1}. *Salmonella* spp. was not detected at any time. *Listeria monocytogenes* was found in one of 5 sausage series, but measured only during the first 5 days when the count decreased from 3.41 to 2.08 lg cfu g^{-1}. The mean value of pH decreased from 5.80 to 4.65 in the first 5 days and stabilized. Water activity (a_w) decreased slowly and generally correlated with air humidity in the ripening camera and the mean value changed from 0.963 to 0.817 a_w. A significantly different correlation between the bacterial count and a_w values was found. The results indicate that the microbiological safety of cold-smoked sausages depend on the initial contamination level with food pathogens. The analysis was done at the Faculty of Veterinary medicine of the Agricultural University of Latvia and at the sausage manufacturer's laboratory.

Key words: food pathogens, cold-smoked sausage, water activity, pH

Introduction

Growing consumer interest to foodstuffs of high nutritional value that guarantee health from food pathogens and proper hygienic conditions has prompted interest in ready-to-eat meat products, partly to cold-smoked (fermented) meat products (Bohaychuk et al., 2006), because the processes used in their production, and specification of content inhibit many pathogenic bacteria. However, when fermentation process is not adequate, there is a potential microbiological risk – some food pathogens may survive and proliferate during ripening (fermentation) (Adams and Mitchell, 2002).

Among the most representative cold-smoked sausages, produced in Latvia, are "Jelgavas Jubilejas", "Kantvurst", and some others, manufactured at Jelgava meat factory. Due to tradition and consumption habits the ready-to-eat cold-smoked sausages are produced and consumed in large amounts. On the other hand, these products due to specific recipes of production, very long shelf-life, special storage conditions, and sometimes inappropriate management at meat warehouses or shops might be unsafe for consumption.

Theoretically most of relevant food pathogen bacteria can be found in cold-smoked sausages (Bohaychuk et al., 2006). The most common pathogens which are present in fermented sausages and therefore keep a health risk are *Salmonella*, *E. coli*, and *Staphylococcus aureus*. In E. Drosinos et al. (2006) study it was shown that *L. monocytogenes* can also survive fermentation. For that reason, bacteria mentioned higher, has been examined and enumerated.

Salmonella spp. is accounted for the most reported food-borne outbreaks in European Union. Eggs, egg-products and meat are the main sources of outbreaks. Positive to salmonella spp. have occasionally been found up to 5% ready-to-eat meat products (EFSA, 2010).

Listeria monocytogenes is a significant foodborne pathogen that is readily present in raw meat products used in the manufacture of processed meats (Sheen, 2008). In ready-to-eat food the occurrence of *L. monocytogenes* in quantities exceeding the Community *Listeria* criteria (100 cfu g^{-1}) remained at low levels in 2008 (EFSA, 2010). Post-processing contamination from a plant environment (equipment, personnel, floors, etc) is the most frequently reason for

its presence in meat or its product surface (Gudbjornsdottir et al., 2004). Fermentation process usually destroys *L. monocytogenes*, but at optimal pH and water activity (a_w) conditions it is capable of surviving (Drosinos et al., 2006). The infective dose of *L. monocytogenes* to human risk groups – children, elderly, immunocompromised people and pregnant women is not known, but the latter opinion is that listeriosis can be caused by *L. monocytogenes* population of 2.0 - 3.0 lg (cfu g^{-1}) (Kendall et al., 2006).
The pathogenic *Escherichia coli*, including the following sub-species, as entero-pathogenic, entero-invasive, entero-toxigenic, and entero-haemorrhagic *E. coli* are most significant for food-borne outbreaks. Entero-haemorrhagic *E. coli* has produced vero-toxins or shiga-toxins and is the most common serotype isolated from the reported cases (EFSA, 2010).
Heat-resistant staphylococcal entero-toxins have high heat tolerance and cannot be destroyed by a normal heat treatment. 291 food-borne outbreaks caused by *Staphylococcus* spp., which constituted 5.5% of the total number of bacterial toxin outbreaks in the EU and two fatal cases in one possible *Staphylococcus* outbreak was reported in 2008 (EFSA, 2010).
According to literature data, many recent studies in food safety have investigated non-thermal processing of ready-to-eat food products, but there is little information about survival of food pathogens found in different ripening stages of cold smoked sausages when water activity and pH value changes affect bacterial growth. Therefore, the aim of the study was to determine the survival limits of most popular food pathogens in manufactured cold smoked sausages depending on water activity (a_w) and pH values.

Materials and Methods

The microbiological tests were done in Agricultural University of Latvia Faculty of Veterinary Medicine and sausage manufacturer laboratory of a real company located in Latvia in 2009– 2010. The five series of cold smoked sausages were investigated (total 105 samples) on presence and count of planed investigated bacteria, and the mean values of lg (cfu g^{-1}) were estimated, in addiction to pH and water activity (a_w) changes at ripening time.
Ingredients of a 100 kg cold-smoked sausage raw material were: pig meat – 30 kg, beef – 10 kg, bacon 35 kg, structural emulsion – 25 kg. Salt and species summary – 3.25 kg and starter culture 'Optistart Plus' – 0.02 kg (control No. L9694599, prepared by Raps GmbH and Co.KG, Germany, sachet composition in lg values: 11.38 *Lactobacillus sake* L 110, 11.38 *Staphylococcus xylosus* M86, and 10.00 *Debaryomyces hansenii* DH3).
For detection and enumeration of bacterial cultures standard microbiology of food and animal feeding stuffs ISO methods, adapted in Latvia were used, namely: LVS EN ISO 6887-2:2004 Preparation of test samples, initial suspension and decimal dilutions for microbiological examination; LVS EN ISO 4833:2003 Horizontal method for the enumeration of microorganisms - Colony-count technique at 30; LVS EN ISO 6579:2003 Method for the detection of *Salmonella* spp.; LVS ISO 7251:2006 Horizontal method for the detection and enumeration of presumptive *Escherichia coli* - Most probable number technique; LVS EN ISO 6888-1:1999 Horizontal method for the enumeration of coagulates-positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird-Parker agar medium, and LVS EN ISO 11290-1:1996 /A1:2005 Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection method, Part 2: Enumeration method.
pH was measured on 0, 1st, 3rd, 5th, 7th, 14th and 21st days of maturation. Three individual pieces of sausages were measured each time, and then mean pH value was calculated. The pH-meter Testo 205 (Testo AG Germany), with automatic temperature compensation, was applied. Calibration was done by means of 2 point method with pH standard solutions 4.01 and 7.00.

Water activity was measured with PawKit (Decagon) simultaneously with pH measurings. Calibration of devices was done with saturated NaCl (sodium chloride) 6.0 molal standard solution (0.760 a_w at 20 °C). Samples for water activity measuring were collected in original polyethylene vessels with caps and measured immediately after collecting.
Statistical analysis. All measurements were reiterated three times, and tests were triplicated. The results represent the mean ± standard deviations. Means were compared by Student's t test. Differences were considered statistically significant when $p<0.05$. Statistical analysis was conducted by means of SPSS 17.0 (SPSS, Chicago, Ill., USA). Tables and chart figures were done by means MS Excel 2007 software.

Results and Discussion
It was observed that bacterial counts – total aerobic count (TAC) and *S. aureus* increased from 5.72 to 9.41 lg and from 1.38 to 2.68 lg respectively. The count of detected *E. coli* decreased approximately by 1.5 logs (from 2.48 to 0.85 lg) during sausage ripening time of 21 days. *L. monocytogenes* was detected in one of five sausages series, but tested only during the first 5 days when the count decreased from 3.41 to 2.08 lg cfu g^{-1}, and was not detected on days 7, 14, and 21of sausage ripening. *Salmonella* spp. was not detected at any time. The mean microbiological test results of 5 manufactured cold-smoked sausages series are summarized and shown in Figure 1.

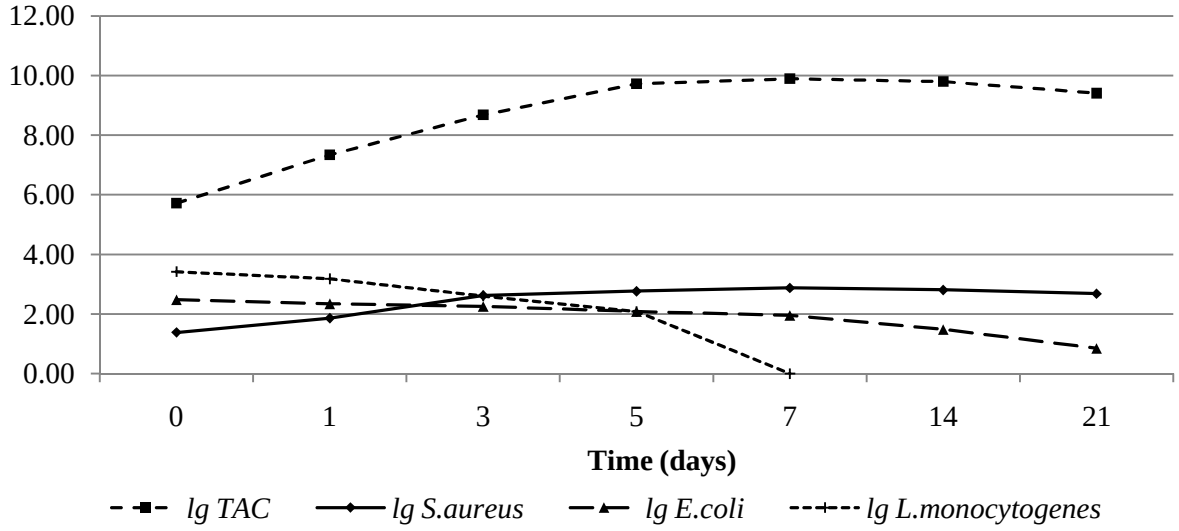


Figure 1.The count lg (cfu g^{-1}) changes of detected bacteria spp. in cold-smoked sausages during 21 day ripening time

The samples of cold smoked sausages had a mean initial pH value of 5.80±0.04, which agrees with the results found by V. Paleari et al. (2003). A rapid decrease in pH was observed during the first three days of fermentation. The final pH of the fermented sausages had a mean value of 4.64±0.05; this drop in pH was due to lactic acid production by the starter culture used for fermentation (Vermeiren and Debevere, 2004). Lactobacilli are the major producers of lactic acid responsible for the decrease in pH and the increase in acidity during fermentation (Schillinger et al., 1991). Lactic and acetic acids are often suggested to be major contributors to the acid aromas and tastes and the development of the texture of fermented sausage (Visessanguan et al., 2005).
The mean value of initial water activity was 0.96 which decreased in the product from 0.963±0.004 to 0.817±0.006 in 21 days. Decreasing trends of a_w and pH are shown in Figure 2.

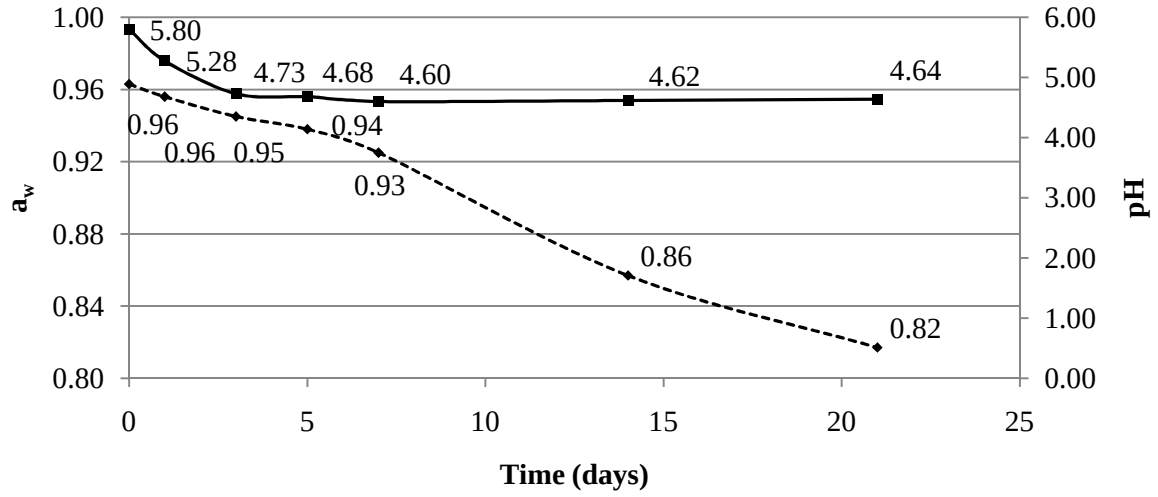
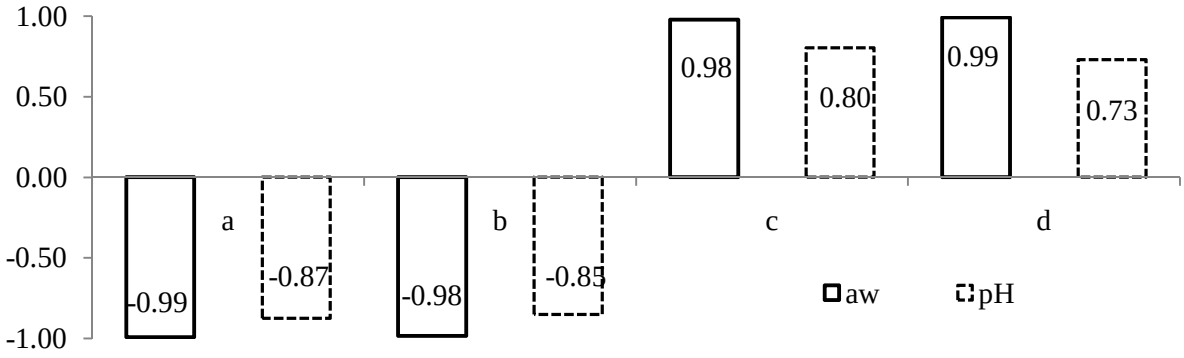


Figure 2. Decreasing trends of a_w and pH values in cold-smoked sausages during 21 day ripening time (pH ------, a_w ·····)

All measured physical and chemical parameters significantly ($p<0.01$) correlate with the decreased *L. monocytogenes*, and *E. coli* count, at the same time with the increased TAC and *S. aureus* count in first 5 days. The correlation coefficient 'r' of values is shown in Figure 3.



(TAC = a, *S. aureus* = b, *E. coli* = c, *L. monocytogenes* = d)

Figure 3. Pearson's correlation coefficient of values between bacteria lg(cfu g^{-1}) and physical and chemical parameters – a_w and pH

In prior study (Siliņš and Liepiņš, 2010) showed that decreasing a_w in cold-smoked sausages during ripening reduced *L. monocytogenes* count to approximately lg 0.44 day^{-1}. In this study the reduction of *L. monocytogenes* count was lg 0.27 day^{-1}, but for *E. coli* count a decrease rate was lg 0.08 day^{-1}. Evaluating correlation coefficients in Figure 3, it is seen that a_w as a factor has more force for degradation of bacteria growth than pH. It is also seen in Figure 3, that a_w and pH value changes did not affect the growth of *S. aureus* in partly ripening time in 15 days, what is recommended for microbiological tests of raw sausage material for detecting initial level of contamination and possibility to forecast final count and toxins level, when a_w decreased to 0.86 and *S. aureus* growing stopped. In this study *S. aureus* growth rate was lg 0.10 every day and could be explaine on the one hand as less sensibility to a_w decreasing process and on the other hand as support of genus kin from added starter culture. It will be possible starter culture quality could affect the *S. aureus* growth rate, and stimulate customers of more attention on starter culture genus structure.

Conclusions

1. A significant Pearson's correlation ($p<0.01$) was established in the decreased count of *L. monocytogenes*, and *E. coli*, increased count of *S. aureus* and aerobic bacteria between decreased water activity and pH values in cold-smoked sausages during ripening.
2. The continual decreased changes of water activity and partly pH diminished possible initial count of some bacterial species, such as *L. monocytogenes* and *E. coli* that allows considering cold-smoked sausages being relatively safe and healthy meat product, under condition that initial contamination with *Staphylococcus aureus* is minimal.

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