Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

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Analysis of microbial growth in a fermented sausage and risk evaluation of its production

Mikrobiologisk analys av en fermenterad korv samt en riskanalys av produktionen

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Abstract

Fermentation has been used to enhance safety of foods and prolong its shelf lives for a long time. The fermentation was originally a result of spontaneous fermentation by microbes naturally present in the food, it is currently done by addition of known bacteria in a starter culture.

Outbreaks of foodborne illness have frequently been associated with foods containing meat. To reduce health risks and control the production, implementation of hazard analysis critical control point (HACCP) can be of use.

This project is performed on a fermented sausage called isterband is divided into two parts; 1. The microbiological analysis of the batter and sausage when exposed to different environments and 2. The preventing and preparational steps included in the HACCP. Microbial analyses were performed by measuring pH both at the production site and in the laboratory. Isolation of starter culture, pathogenic indicators and total aerobic count was performed using agar plates followed by confirmation with MALDI-TOF MS.

The used starter culture showed some differences in growth between different batches. Measurements of pH in the sausages and batter gave similar values and decrease in pH, with some deviations. Growth of non-starter bacteria was found in batter but not in any of the sausages indicating a sufficient antimicrobial effect.

Development of HACCP resulted in recommendation for changes in the current HACCP for the company. Removal of the one existing CCP was the main change but also recommendation for change in routines in the production.

Keywords: Fermented sausage, isterband, HACCP, LAB, starter culture

Sammanfattning

Fermentering har länge använts för att förbättra säkerheten hos livsmedel och för att förlänga dess hållbarhet. Fermentering var ursprungligen ett resultat av spontan fermentering av mikrober som naturligt förekom i produkten, numera utförs det genom tillsats av kända bakterier i en startkultur.

Utbrott av livsmedelsburna sjukdomar har ofta associerats med livsmedel som innehåller kött. För att minska riskerna och kontrollera produktionen kan implementering av Riskanalys av kritiska styrpunkter (HACCP) användas.

Detta projekt har utförts på den fermenterade korven isterband och kan delas upp i två delar; 1. Den mikrobiologiska analysen av smeten och korven när den utsätts för olika miljöer och 2. De förebyggande och förberedande stegen som ingår i HACCP. Mikrobiell analys utfördes genom mätning av pH både på produktionsstället och i ett laboratorium. Isolering av startkultur, patogena indikatorer och totalt aerobt antal utfördes med användning av agarplattor och bekräftelse med MALDI-TOF MS.

Den använda startkulturen visade vissa skillnader i tillväxt i olika satser. Mätningar av pH i korv och smet gav liknande värden och sänkning av pH, med vissa avvikelser. Tillväxt av bakterier som inte fanns i starterkulturen hittades i smet men inte i någon av korvarna, vilket indikerar en tillräcklig antimikrobiell effekt.

Utformningen av HACCP resulterade i rekommendation för ändringar i nuvarande HACCP för företaget. Borttagandet av den befintliga CCP var den huvudsakliga förändringen, men också rekommendationer för förändring av rutiner i produktionen.

Nyckelord: fermenterad korv, isterband, HACCP, LAB, starterkultur

Table of contents

List of tables

List of figures

Abbreviations

A_W	Water activity
CCP	Critical control point
E. coli	*Escherichia coli*
HACCP	Hazard Analysis of Critical Control Points
L. monocytogenes	*Listeria monocytogenes*
L. plantarum	*Lactobacillus plantarum*
LAB	Lactic acid bacteria
LTLSB	Lactose Tryptone Lauryl Sulphate Broth
MIC	Minimum inhibitory concentration
NaCl	Sodium chloride
NMKL	Nordic Committee on Food Analysis
oPrP	operational prerequisite programme
P. pentosaceus	*Pediococcus pentosaceus*
PrP	Prerequisite programme
S. xylosus	*Staphylococcus xylosus*
SLV	Swedish National Food Agency
TSA	Tryptone Soya Agar
VRBA	Violet Red Bile Agar
VRBGA	Violet Red Bile Glucose Agar

1 Introduction

The use of fermentation to enhance safety of foods and prolong their shelf lives has been widely used for a long time (Caplice & Fitzgerald, 1999). Originating from spontaneous fermentation by microbes naturally present in the food, it is currently done by adding known bacteria or backslopping (Leroy & De Vuyst, 2004).

Outbreaks of foodborne illness have frequently been associated with foods containing meat. To reduce the risks and control the production, implementation of hazard analysis critical control point (HACCP) can be of use (Tompkin, 1990). In Sweden, 2.000-3.000 cases of food poisoning are reported each year. Despite this, reports indicate up to 500.000 cases of food poisoning as most of them remain unreported (SLV, 2016). According to Roy *et al.* (2006) the most common reason for food poisoning in developed counties (Canada, Australia, United Stated, France, Netherlands, Norway, United Kingdom and Ireland) is improper heating and lack of basic hygiene. There has previously been outbreaks of enterohemorrhagic *Escherichia coli* (EHEC) caused by consumption of cold smoked sausages in Sweden (Sartz *et al.*, 2008) which emphasise the importance of a safe production and handling of meat products. Inhibition of pathogenic bacteria can be carried out using various methods (Leistner, 1992), in this work, a few of the factors involved are revised, such as effect of smoke, temperature and bacteria with bactericidal activity.

A fermented sausage such as isterband is traditionally consumed heated and several pathogens associated with other fermented sausages are therefore less relevant. This product is, however, possible to consume raw and the hurdles involved in the production are meant to eliminate all risks. The starter culture used in the examined product is part of one of the hurdles required to function properly in order to obtain a safe product in all batches. In this work, essential aspects of the production are analysed such as the microbiological activity of the starter culture, *Escherichia coli Enterobacteriaceae*, total aerobic bacteria (TAB) and pH. General streaking on blood agar was performed to find growth of all bacteria. A HACCP-plan addressing the microbiological hazards for the specific line of production was also developed to give an extended insight regarding the possible pitfalls.

Isterband is traditionally cold smoked, which is in temperatures of 20 – 30 °C, (Lindqvist *et al.*, 2003) but the examined products reaches a higher temperature up to 55 °C, although it is not high enough to ensure a safe product. In this work it is also tested if it is possible to obtain a safe product at lower fermentation temperature, in this case 30 °C, however without smoke.

The producer of the isterband included in this project is a small-scale company focusing on meat and processing of meat from local meat producers. The custom base is restaurants and groceries primarily in the county and the deliveries are handled directly without intermediaries. For over 20 years has the company operated with a big variety of meat products.

1.1 Problem description

The process to produce isterband has been developed by testing different approaches by the company. A boiling step was introduced for a short period to further eliminate risks of pathogens but was removed as it was considered unnecessary and had a negative sensory effect. As the restrictions of how to produce the fermented sausage called isterband are few, there is no set procedure of how to accurate produce it. Traditionally the product is cold-smoked. Today the company wish to enhance its knowledge of the microbial activity in the sausage both in the current way of procedure and also when the temperature is lowered to meet the criteria of a cold-smoked sausage. A more energy efficient process, better insight regarding the microbial activity and a possibly tastier sausage could be obtained.

1.2 Aims and purposes

The purpose of this study was to provide an enhanced insight in the production of a fermented sausage produced alongside numerous other products. By continuously testing the microbial growth in the product, an increased insight of the process was obtained. Through this the producer can gain more information about the risks of the production and how to mitigate them and get a better knowledge if there are any steps in the production which can be performed more effectively. It was also tested if the temperature can be lowered at heating and smoking without altering the safety of the product.

To make the findings more applicable, a HACCP-plan was developed for the fermented sausage isterband. The focus was on microbial hazards, thus chemical and physical hazards have been omitted. A plan for recalls of products have also been excluded.

1.3 Specific objectives

- How are the microbes of the starter culture and indicator bacteria such as *E. coli* affected at the different stages of the production?
- Is there any step with an enhanced risk which could jeopardize the production, if so, could the risk be minimized?

2 Literature Background

2.1 Production of isterband

A flow chart of the production is illustrated in *Figure 2*. In the production of isterband, a specific starter culture (2.2.1) is used. The culture is a mix of three different bacteria, each with a different purpose for the development of the sausage. Before it is added to the batter, it is kept at freezing temperature and is almost directly after removal from freezer added to the mixture of components. This mixture cannot be too warm, which it could be due to the barleycorn recently being parboiled. A too warm mixture could kill or harm the bacteria in the starter culture.

Isterband is classified as a semi-dry sausage as the water activity (A_W) of 51.95 % exceeds the limit of 50.00 % to be classified as semi-dry. The meat content in the product is 59.77 % which consists of beef and two kinds of pork, small pieces of pork (< 30 % fat) and shank with rind (30 – 50 % fat). The temperature of pork and beef are checked upon arrival, same with starter culture. The beef arrives in cuts and pork in carcasses. Spices and other components kept in room temperature are not temperature controlled, only checked for package damages. The ice is produced in the facility by an icemaker which uses municipal water.

When the alternative environments were tested, methods described in 3.2.1 were used.

2.1.1 Prior to heat treatment

Beef and pork are the meat products used in the production of isterband. The pork and beef are delivered from a small-scale slaughter house. The beef is quartered before arrival and the pork is delivered in carcasses and quartered at production site.

The blending of the meat is initially performed in a carriage where pieces of beef type R III is put together with pork (small pieces of pork and shank with rind). The mixture is minced together and put on the barleycorn in another carriage. Water is added to the carriage as crushed ice before it is put in a cutting machine and blended.

When the mixture is homogenized, the remaining ingredients are added and mixed; such as spices, ascorbic acid and starter culture. The starter culture (2.2.1) is stored frozen and is removed from the freezer shortly before added to the mixture. Staff at the production site determine when the batter is ready, and all ingredients (Table 1) are mixed until the staff decides it is mixed properly enough. Once the batter is ready, it is stuffed in intestines from pigs using a stuffing machine. The sausages are hung in rows on trolleys which are put in the smokehouse. The initial mixing of ingredients is performed ~ 1 pm and inserted into the smokehouse ~ 3 pm, storage temperature during this time is about 13 °C.

2.1.2 Heat treatment and storage

The smokehouse is set to a fixed programme which includes heating and smoking. The initial step is heating at 35 °C for 11 hours, followed by smoking at 55 °C for 4 hours. Dehumidification for 5 minutes finish the process in the smokehouse. Once the sausages are removed from the oven they are stored in a cold room at 5 °C until it has reached at temperature cold enough to allow packaging in. The sausage must reach a temperature of 1 — 8 °C before transported to the packaging machine. Staff separate the sausages from each other manually with a knife and put the sausages in plastic containers which are being sealed in vacuum.

The factory holds three rooms for smoking but solely one is used. The other two smokehouses have a boiling step included in the end of the programme at 76 °C.

2.2 Ingredients

Isterband consists of several components essential for the development of the specific product. In this section, the ingredients with most effect on the quality are described.

2.2.1 Starter culture

The starter culture used is a combination of three bacteria; *Staphylococcus xylosus*, *Pediococcus pentosaceus* and *Lactobacillus plantarum*. It is stored frozen and is thawed prior to being added to the batter. Two of these, *L. plantarum* and *P. pentosaceus* are bacteria producing lactic acid (LAB) (Engesser & Hammes, 1994). Proportions of the starter culture are *S. xylosus*: 5 x 10^6 cfu /g meat (50% of culture), *P. pentosaceus* 3 x 10^6 cfu /g meat (30% of culture) and *L. plantarum* 2 x 10^6 cfu /g meat (20% of culture).

LAB can have the ability to produce bacteriocins which are low-molecular-mass peptides or proteins. The antibacterial mode of action is by the bacteriocin is restricted to gram-positive bacteria or related. LAB producing these bacteriocins can be applied to food for preservation because of the microbiological, physiological and technological advantages. (Leroy & De Vuyst, 2004).

2.2.1.1 Lactobacillus plantarum

The species *L. plantarum* belongs to the genus *Lactobacillus* (Felis & Dellaglio, 2007). *L. plantarum* has been shown present in both oral and rectal mucosa of healthy persons in western Europe (Ahrné *et al.*, 1998). In an *in vitro* study the bacteria has shown a critical limit at pH 1.5 (presence from pH 1.5 – 2.5) when exposed to hydrochloric acid and cholic acid. It also depicted growth at presence of 10 mM bile salts and a 38 % de-conjunction of bile salt. *L. plantarum* has the ability to hydrolyse primary bile acids which is a reason why it is useful in fermentation of foods (Haller *et al.*, 2001).

L. plantarum has been used as a preservative in fermented olives, in production of nutraceuticals such as low-calorie sugars and for reduction of phytic acid content (Leroy & De Vuyst, 2004). In fermented meat it has proved to lower pH to 4.8 – 4.9 without any flavour deviations and also to act antibacterial (Klingberg *et al.*, 2005). *L. plantarum* produces a bacteriocin, the mode of action for the bacteriocin effect is breakage of cell membrane. The bacteriocin produced by *L. plantarum* has shown ability to kill the LAB *P. pentosaceus* (Andersson *et al*., 1988) and also act preventative against enteric bacteria (Kingamkono *et al.*, 1999). Studies have shown antibacterial activity against pathogens such as *E. coli (Wang et al., 2018; Wang et al., 2017)* and *S. aureus* (Wang *et al.*, 2018).

2.2.1.2 Pediococcus pentosaceus

P. pentosaceus has shown antibacterial characteristics against *S. aureus* and *E. coli*. The MIC value by the cell free supernatant of *P. pentosaceus* was in an interval of 250 – 500 µg/mL and depicted an effect on the membrane of the pathogens causing

a reduction in viability of the cells. It also showed an inhibitory effect on biofilm formation of the given pathogens and an antibacterial effect on both gram positive - and negative bacteria (Bajpai *et al.*, 2016). Wang *et al.* (2018) did a study which depicted a high antibacterial activity against *S. aureus* and another study by Damodharan *et al.* (2015) showed antimicrobial activity against the pathogens: *S. aureus, L. monocytogenes, B. cereus* and strains of *Salmonella* while strains of LAB were unaffected.

P. pentosaceus converts glucose to DL-lactic acid through the Embden-Meyerhof pathway where CO_2 and ethanol won't be produced. The optimal temperature for growth is in the interval 25 – 35 ℃ and at 30 ℃ it has the ability to grow in both aerobic and anaerobic environments. Optimum pH for growth is 6.0 – 6.5 (Dicks & Endo, 2009). It has shown great stability in environments with fluctuating temperatures and pH and the has a bactericidal mode of action (Anastasiadou *et al.*, 2008).

2.2.1.3 Staphylococcus xylosus

A study by Sørensen and Jakobsen (1996) showed that the lipase activity in meat fermentation was very limited. The study also showed an increase in growth of *S. xylosus* when temperature and pH was increased in the interval of 10 – 30 ℃ and pH 5.1 – 6.0, respectively. When *S. xylosus* has been used in starter culture it has shown to produce several fragrant esters to the sausage not found in sausages without the bacteria. The inclusion of the bacteria gives fermented sausages a less rancid taste an also contributes to the stability of the sausage by reducing auto-oxidation (Stahnke, 1994).

2.2.2 Barley

Fermentation of starch from barley provides an effective lactic acid yield when using *Lactobacillus* as fermenting bacteria. Compared to glucose, barley gives a bigger lactic acid yield (Linko & Javanainen, 1996). *L. plantarum* has shown to be more competitive than other strains of *Lactobacillus* and also to ferment barley efficiently (Feng *et al.*, 2005). Liquefaction of barley prior to fermentation enhances the availability of the LAB to ferment the starch (Linko & Javanainen, 1996).

2.2.3 Sodium nitrite

E250 – Sodium nitrite. Nitrite is the only component in isterband specifically added to inhibit growth of bacteria, except the starter culture (Nyman, 2018). Nitrite added to a sausage in an early step can inhibit growth of *Salmonella* and *Listeria*. Addition

of nitrate that convert to nitrite will not result in adequate inhibition of the pathogens due to too slow conversion to nitrite (Christieans *et al.*, 2018). Growth of *Enterobacteriaceae* is inhibited by sausages produced with nitrite (González & Díez, 2002).

2.2.4 Ascorbic acid

E300 – Ascorbic acid. Ascorbic acid is primarily used in the product to prevent it from oxidation. In fermented sausages it has been used to improve colour and stability (Adams & Moss, 2007). Ascorbic acid has been shown to inhibit lipid oxidation when used in combination with other antioxidative substances, such as rosemary extract, borage meal or green tea powder. The combination with rosemary extract also inhibited microbial growth (Martínez *et al.*, 2006).

2.3 Inhibitory factors

A bacteriostatic drug solely inhibits the growth of an organism, the effect is reversible and will lose effect if removed. If a bacteriostatic drug is used, the dose has to be above the minimum inhibitory concentration (MIC) consistently for the drug to be considered as bacteriostatic. Bactericidal drugs on the other hand, cause death of the microbe and are preferred for infections unable to be controlled by host mechanisms (Maddison *et al.*, 2008). In this study, primarily the inhibitory actions of pH and starter culture are analysed. Other factors with potential effect are also described.

2.3.1 pH

pH inhibits growth of pathogens by decreasing the intracellular pH and restricting essential metabolic reactions (Wang *et al.*, 2014). The components of a foodstuff will affect the acid production. A number of different spices such as ginger, red pepper, mustard, mace, cinnamon, cumin, oregano and clove have shown to have positive effect on the acid production of *L. plantarum* (Kivanç *et al.*, 1991; Zaika & Kissinger, 1979).

2.3.2 Smoke

Smoking is not only performed to give the smoky taste, it also contributes with an antioxidative effect. The phenolic compounds produced both gives the characteristic smoke taste and have antioxidative and antimicrobial effect (Adams & Moss, 2007).

Smoking of sausages will result in a reduced oxidation, primarily due to the prevention of lipid peroxidation. This occurs as a result of the phenolic fractions in compounds present in smoke. Metoxyphenols with an alkenyl side chain is a particularly antioxidative compound present in smoke (Kjällstrand & Petersson, 2001).

Inhibitory actions of phenolic acids were tested in combination with essential oil compounds in a study by Meira *et al.* (2017). *P. pentosaceus* and *Staphylocuccus carnosus* were used as starter culture where *E. coli* was the investigated pathogen. The study showed a negative score for all combinations, but sensory parameters were reduced in most cases.

2.4 HACCP

Operators in the food business are obliged to have, implement and maintain a procedure in which the seven principles of HACCP are included (FSAI, 2004). The overall principles are: 1) Identifying hazards, 2) Identifying critical control points, 3) Establish critical limits where critical control points are identified, 4) Monitoring an established and effective process at critical control points, 5) Monitoring corrective actions at critical control point when compromised, 6) Regularly carried out process to ensure step 1 & 5 are functioning effectively, and 7) Documentation and journals in proportion to the given business.

For producers within the European Union (EU) it is not mandatory for operators to have a HACCP-plan. Despite this, operators are obliged to ensure all procedures of production, processing and distribution align with the given requirements carried out by the EU in accordance to (EC) No 852/2004. The requirements regarding hygiene for all parts of the production need to be met as described in this regulation (FSAI, 2004; Regulation, 2004).

It is necessary to be aware of all constituents of the product (Table 1) in order to perform an accurate hazard analysis and HACCP. Different components such as allergens or spices that might affect the safety of the product need to be taken into account. In the HACCP-plan the steps in the production are divided into prerequisite programs (PrP), operational prerequisite programme (oPrP) and critical control point (CCP) (ISO, 2005).

Table 1. *List of ingredients and nutritional values in isterband produced by the company*

Ingredients	Nutritional value (100 g)	
Beef	KJ	1300
Pork	Kcal	310
Shank with rind	Fat	24 g
Barley	of which saturates	10 g
Potato flakes	Carbohydrate	12 g
Salt	of which sugars	1.5 g
Granules of onion		
White pepper	Protein	12 g
Starter culture	Salt	3.1 g
Ascorbic acid		
Water (crushed ice)		
Intestine of pig		

2.4.1 Microbiological hazards

Isterband constitutes of a number of ingredients which can be source of pathogens. The primary risk component is the meat which will not be heat treated enough to kill bacteria in any step of the production. Another ingredient that has shown to be a potential source for *Bacillus cereus* (King *et al.*, 2007; Turner *et al.*, 2006) and *E. coli* (Park & Beuchat, 2000) is potato flakes. However, the distributor of the potato and onion perform tests prior to delivery and these are therefore unlikely sources of pathogens. Some of the pathogens associated with raw meat is presented below.

2.4.1.1 *Enterobacteriaceae*

Bacteria of *Enterobacteriaceae* are; gram negative, straight rods with peritrichous flagella, facultatively anaerobic, oxidase negative and produce acids by fermenting glucose. *Salmonella enterica, Escherichia coli* and *Yersinia enterocolitica* are examples of possibly pathogenic members of the *Enterobacteriaceae*-family with possible presence in meat (Fegan & Jenson, 2018).

2.4.1.2 *E. coli*

Maturation of sausage batter can achieve a satisfactory reduction of *E. coli*. Despite favourable environments for *E. coli*, the inactivation in subsequent processing steps is usually sufficient but a definite safe product is difficult to achieve (Lindqvist & Lindblad, 2009). The bacteria has a facultative anaerobic metabolism (VetBact,

2018) and grows efficiently in temperatures of 23 – 37 ℃ (Herendeen *et al.*, 1979) even if Cebrian *et al.* (2008) showed a growth of *E. coli* at temperatures of 10 – 42 ℃. Colonies grown at the higher temperatures of 37 and 42 ℃ has a higher survival rate after heat treatment at 57.5 ℃ compared to colonies grown at 10, 20 or 30 ℃. At pH adjusted by HCL, *E. coli* can grow at pH as low as 3.5 for 10 days while growth was inactivated at pH 4.5 when adjusted by lactic acid (Glass *et al.*, 1992). There has previously been outbreaks of EHEC caused by consumption of cold smoked sausages in Sweden (Sartz *et al.*, 2008). Teunis *et al.* (2004) showed an attack rate of 16 % for adults and 25 % for children consuming foods contaminated with *E. coli*.

2.4.1.3 *L. monocytogenes*

L. monocytogenes is a gram positive, facultatively anaerobic bacteria. Described micromorphologically the bacteria constitutes of long thin rods in short chains and is motile using flagella (VetBact, 2017). An increasing rate of inactivation of *L. monocytogenes* is correlated with increasing temperature (Lindqvist & Lindblad, 2009). The infectious dose of *L. monocytogenes* has proved to be difficult to define as it differs between hosts (Golnazarian *et al.*, 1989). Microbiological criteria have been developed for *L. monocytogenes* and the accepted limits in foodstuff are: absence in 25 g of food intended for infants and in products before it has left production site and less than 100 CFU/g in ready-to-eat food. The limit of 100 CFU/g is set to both products that are environments friendly and unfriendly for *L. monocytogenes*. Unfriendly environments are pH $\leq$ 4.4 or $a_w \leq 0.92$ and pH $\leq$ 5.0 together with $a_w \leq 0.94$ (Regulation, 2005). Temperature for no growth of *L. monocytogenes* differs depending on the pH and a_w. In 25 ℃, minimum of pH and a_w to permit growth in broth is 4.45 and 0.900 respectively and pH 5.1 and a_w 0.945 in agar according to Koutsoumanis *et al.* (2004). A pH of 4.4 allows growth at 30 and 20 ℃, pH of 5.23 showed growth at 4 ℃ (George *et al.*, 1988).

3 Methods

3.1 Project design

This project was divided into two parts; 1. Microbiological analysis of the batter and sausage when exposed to different heat treatments and 2. Prevention and preparation steps included in the HACCP.
The scientific information and references used in this work were primarily found in the databases Web of Science, Scopus and PubMed. The website www.vetbact.org gave information about some of the pathogens that could be of potential risk in fermented sausages. Information about the production of the isterband was given by the co-supervisor at the company and by observing the production on site. Both the isterband batter and the isterband sausage produced by the company were included in the project.

3.2 Microbiological analysis

3.2.1 Incubation of isterband

To determine presence and quantities of microorganisms and inhibitory factors, primarily pH and bacterial starter culture, under various circumstances (temperature and time) a laboratory study was conducted. Initially a pilot was conducted to determine suitable dilutions for the microbiological analysis. The pilot was made on the batter and two replicates of the sausage from the company using the same incubations as described below. For the analysis, the sausages were incubated as described in Table *2* and the batter to the sausages treated in in the lab were from the

same origin. The temperatures of incubation were chosen as they matched the criteria for cold smoking. All analyses were performed at the Dept of Biomedical Sciences and Veterinary Public Health, SLU.

The batter was analysed the day of production and the sausages the following day. Both at the production site and in the laboratory the incubation of the sausages started late afternoon and was completed early the following morning. Sampling of the sausages were performed when the temperature had decreased to 4-8 °C. The sausages were manufactured from the same batch as the batter was obtained.

Sausages produced by the company were transferred to the laboratory packed separately in sterile plastic bag. When analysing raw batter in intestine, it was transferred to the laboratory as described above and stored at 4 °C before further processing.

The batter in intestine was put in incubation chamber in the set temperature (Table *2*) and moved to a separate chamber when the temperature was to be changed. As the total time for both procedures was 15 hours, the tests were performed simultaneously. At each trial three sausages or batter were analysed as described under 3.3 and 3.4. The trial was repeated three times for the batter, two times for the sausages incubated in the laboratory and one time for the sausage produced entirely by the company.

Table 2. *Conditions under which the microbiological analyse took place*

Temperature (°C)	Time (h)	Note	Location
30	15	Without smoke	Laboratory
35 + 55	11 + 4	Without smoke	Laboratory
35 + 55	11 + 4	4 h smoke	Company

3.3 Measurement of pH

The pH of the sausage was measured both at production site and in the laboratory. At both sites the measuring instruments were calibrated to pH 4 and 7 the same day as the measuring was performed.

3.3.1 At production site

In the production site pH was measured when the batter was properly mixed and put in carriages. The probe of the pH-instrument (Mettler Toledo) was inserted in the batter at three different sites in each carriage. Between each measurement the probe

was whiped with a paper towel and cleaned with soap and water. Before the sausages were put in the smoking house, three sausages were measured by inserting the probe in each end. Directly after smoking and before packaging, when temperature had dropped to < 8 °C, the measurement was repeated. Calibration was performed using testo Buffer pH 4 & 7 Solvent. Environmental temperatures during these steps are described in 2.1.

3.3.2 At laboratory

In the laboratory pH was measured in the first dilution, that is, 10 g of the sausage was extracted, including some of the surrounding intestine, and peptone salt diluent added to a total of 100 g. The solution was homogenised in a stomacher (easy mix by AES Laboratories) for 30 seconds and the pH was measured in this solution using pH meter inoLab by WTW.

3.4 Isolation of bacteria

Samples were collected from the batter, and from the sausage after incubation. Each sample consisted of 10 g and was analysed immediately after collection. All samples were homogenised using a stomacher for 30 seconds and diluted using peptone salt diluent to concentrations varying from 10^{-1} to 10^{-7}. 10.0 g of sausage was used for each series of dilution. The diluted samples were added to the agar within 20 minutes after diluted. In order to prove presence and quantify bacteria in the product a number of different agar were used. The methods used for the bacteriological analyses were based on the methods from Nordic Committee on Food Analysis (NMKL) but modified as all confirmation steps were performed using Bruker MALDI-TOF MS. Bacteria isolated from blood agar or De man, Rogosa and Sharp Oxoid CM0361 (MRS) agar were used directly on Bruker MALDI-TOF MS, bacteria from other selective agar was re-cultured on blood agar before confirmation.

3.4.1 Lactic acid bacteria

0.1 ml dilutions of 10^{-4} to 10^{-6} where surface spread on the selective agar for LAB, MRS, and incubated anaerobically at 25 °C for 5 days.

All colonies where counted as LAB and plates with 15-150 colonies were preferably used. The amounts of LAB were calculated for 1 ml sample. Typical morphology of LAB is 1.5 ± 0.5 mm wide colonies with white or grey colour when grown on MRS-plates.

Isolation was performed in accordance with NMKL No 140, 2th ed. 2007.

3.4.2 Escherichia coli

1.0 ml sample dilutions of 10^{-1} to 10^{-2} were applied to an empty petri dish and 5 ml of Tryptone Soya Agar Oxoid CM0131 (TSA) was added. The sample and agar mixture was gently stirred and pre-incubated at 20 – 25 ℃ for 1-2 hours, 10 ml VRBA was added to the petri dish on top of the solidified TSA. TSA:VRBA-ratio should be at least 1:2. The plates were incubated up-side down at 44.0 ± 0.5 for 24 ± 3 hours.

Preferably plates with 10 – 100 colonies typical for *E. coli* were counted. Typical appearance is dark red colour with a diameter of minimum 0.5 mm surrounded by a red precipitation zone. Plates with more colonies will have smaller individual colonies. Colonies typical for *E. coli* was plated on blood agar and ran in MALDI-TOF MS.

Isolation was performed in accordance with NMKL No 125, 4th ed. 2005.

3.4.3 Enterobacteriaceae

1 ml sample dilutions of 10^{-1} to 10^{-2} were applied to an empty petri dish and 15 ml of Violet Red Bile Glucose Agar DIFCO 218661 (VRB) was added. The dilution and agar were mixed by carefully rotating the petri dish. When the mixture was solid, an additional thin layer of 5 ml agar was added on top. The petri dish was incubated up-side down at 37.0 ± 1.0 ℃ for 24 ± 2 hours.

All plates with 15-150 colonies with pink to red colour were counted. Well-isolated colonies were transfer to blood agar and incubated at 37.0 ± 1.0 ℃ for 24 ± 3 hours. Bruker MALDI-TOF MS was used for confirmation.

Isolation was performed in accordance with NMKL No. 144, 3rd ed. 2005.

3.4.4 Aerobic microorganisms

Inoculation was performed by adding 1 ml of homogenized sample in dilutions of 10^{-4} to 10^{-6} into empty petri dishes. 20 ml of Standard Plate Count Agar CM0463 was poured into each dish. The dishes were mixed by rotating the dishes gently and solidified in room temperature before incubated up-side down in 30.0 ± 1.0 ℃ for 72 ± 6 hours.

The plates with 25 – 250 colonies were read manually using a plate counter. Isolation was performed in accordance with NMKL 86, 5th ed. 2013.

3.5 Development of HACCP-plan

Since the potential bacterial hazards in isterband to a great extent originate from the handling of the raw products and also depend on the efficiency of the inhibitory steps for pathogens, a HACCP-plan for this specific line of production was developed.

The focus on the HACCP in this project was to develop a plan which would be relevant for the fermented sausage produced by the company. Focus was on the microbiological aspects involved in the production and its raw materials, chemical hazards was therefore left out. How the company handles withdrawal of products was also excluded.

Information about how to perform a HACCP-plan was obtained from Swedish National Food Agency (SLV), document from Bergström & Hellqvist about how to understand and interpret ISO 22000, (EC) No 852/2004, - 853/2004 and - 2073/2005 along with documents from the company about how their current plan was developed.

4 Results

4.1 Microbiological growth

4.1.1 Growth of starter culture

As shown in *Table 3*, variations in presence of bacteria from the starter culture were found. Confirmation of LAB was performed from the 10^{-4} dilution in all MRS plates from batter. The 10^{-5} dilution on MRS was used for the sausage produced by the company and both sausages incubated at 30 + 55 ℃ in the laboratory. The 10^{-6} MRS dilution was used for both sausages incubated at 30 ℃ in the laboratory.

For identification of *S. xylosus*, the 10^{-2} dilution was used for all samples. *P. pentosaceus* was found in all blood agar but not *L. plantarum*. *S. xylosus* was identified in all samples but the sausage produced by the company where only *P. pentosaceus* was found on blood agar.

The decrease in pH varied between the different batches as shown in *Figure 1*. pH values in batter were similar in all batches with small deviations. Values for the sausages treated in the laboratory had a drop similar to average of the drop for the sausages produced at production site.

Table 3. *Presence of bacteria from starter culture in batter and sausage. 1,2 and 3 represent the batch from which the batter or sausage was analysed. + represent identified bacteria, - represent no detection. * is detection with low confidence identification*

		Bacteria		
Product	Batch	*L. plantarum*	*P. pentosaceus*	*S. xylosus*
Batter	1	+	+	+
	1	+	+	+
	2	+	+	+
	2	+	+	+
	2	+	+*	+
	3	+*	-	+
	3	+	-	+
	3	+	-*	+
Sausage				
- Company	1	-	+	-
	1	-	+	-
- 35+55 ℃	2	-	+	+
	2	-	+	+
	2	-	+	+
	3	-	+	+
	3	-	+	+
	3	-	+	+
- 30 ℃	2	-	+*	+
	2	-	+*	+
	2	+	-	+
	3	-	+	+
	3	+	+	+
	3	-	+	+

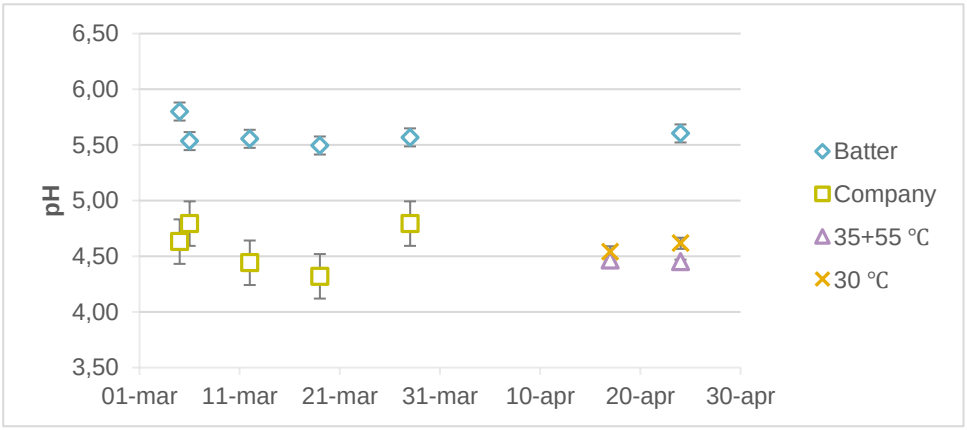

Figure 1. Values of pH-measurements on batter, sausage produced by the company and sausages treated in laboratory at 35+55 ℃ and 30 ℃.

4.1.2 Growth of non-starter bacteria

In batter, several pathogenic bacteria were found on VRB agar. *E. coli*, *E. cloacae*, *Raoultella ornithinolytica*, *Raoultella planticola* were identified but none of them was found in any of the sausages. *Hafnia alvei* was also detected on VRB agar. On blood agar pathogens such as *Kocuria rhizophilia* and *Kocuria Salsicia* with a gene sequence 98.9 % similar to *K. rhizophilia* were identified. *Kocuria varians* and *Staphylococcus carnosus* were also found in batter grown on blood agar. The LAB *Lactobacillus fermentum* was found on MRS agar.

Growth of aerobic bacteria was higher in sausages heated in 30 ℃ compared to the sausages heated in 35 + 55 ℃, with and without smoke (Table *4*). The sausage produced by the company had a higher growth compared to the sausage heated in the laboratory. There was an apparent difference within batches, 2.2 and 2.3 in 35 + 55℃ did for instance differ in one logarithm.

Table 4. *Amount of TAB/ml and SD in each sample. Values are presented in means of the samples*

Product	Batch	TAB/ml	SD
Batter	1	1.24E+07	1.87E+06
	1	8.90E+06	
	2	2.08E+07	3.45E+06
	2	1.30E+07	
	2	1.41E+07	
	3	1.07E+07	4.08E+05
	3	1.12E+07	
	3	1.17E+07	
Sausage			
- *Company*	1.1	4.70E+07	4.22E+07
	1.2	3.20E+06	
	1.3	2.12E+07	
- *35+55 ℃*	2.1	7.30E+07	2.04E+07
	2.2	5.00E+07	
	2.3	1.00E+08	
	3.1	9.90E+07	1.39E+07
	3.2	6.50E+07	
	3.3	8.40E+07	
- *30 ℃*	2.1	5.95E+08	6.01E+07
	2.2	4.61E+08	
	2.3	4.75E+08	
	3.1	4.96E+08	1.40E+08
	3.2	7.84E+08	
	3.3	4.80E+08	

4.2 HACCP

A flow chart for the entire production site for all products has already been developed by the company. However, a more specific flow chart for the fermented sausage has been developed in this project and is presented in *Figure 2*. A qualitative risk assessment for isterband production has also been developed based on an existing one for the entire production. In this project, the risk assessment has been adjusted to better describe the hazards involved the specific line of isterband production.

4.2.1 Qualitative risk assessment

The entire risk assessment is shown in Table *5* where the processing steps, hazards, measurements, assumed risk and results for PrP, oPrP and CCP are included. No CCP was found, five oPrP and the remaining were PrP.

The “probability *consequence” shown in Table *5* is partly based on the table for microbial hazards shown in Appendix 1. The table in the appendix is based on previous analysis carried out by the company.

4.2.2 Flow chart

The flow chart viewed in *Figure 2* describes all steps involved in the production of isterband from reception of raw material to delivery to customer. It is in the chart shown what steps in the production is considered to be PrP, oPrP and CCP.

Current HACCP by the company has a CCP at heating and smoking which in this flow chart is removed.

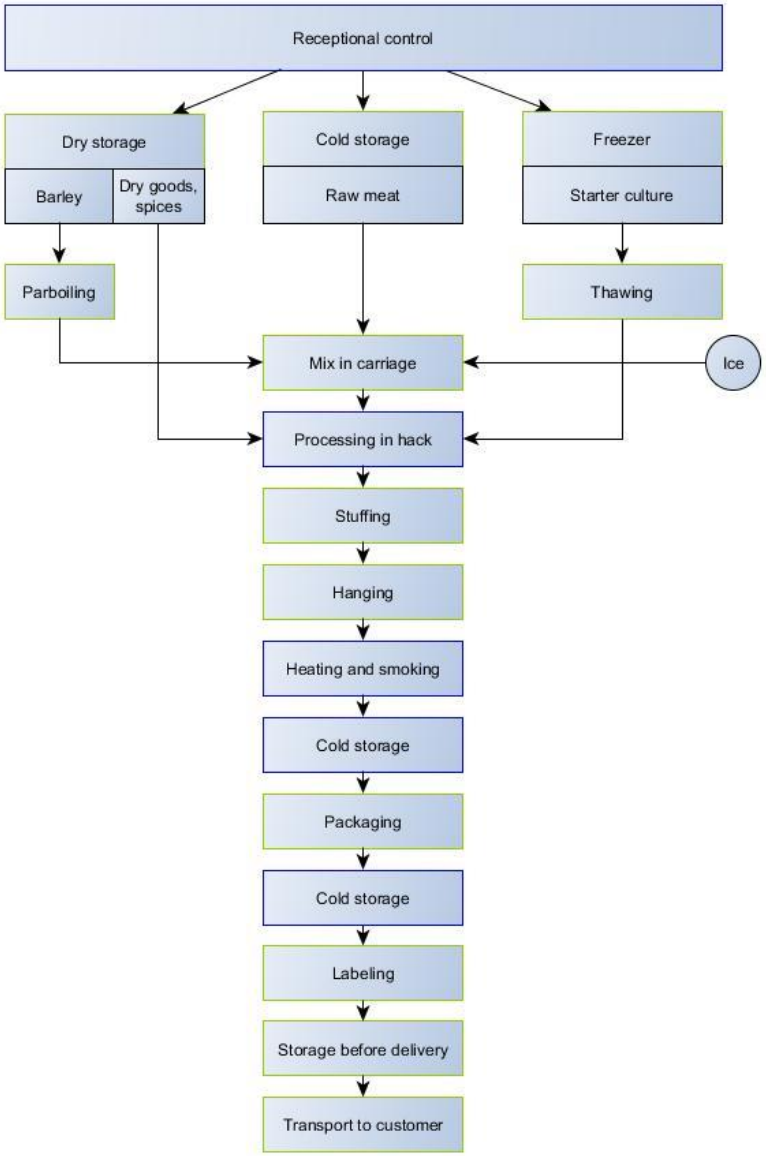

Figure 2. Flow chart showing the steps in production of isterband. Green lines = PrP and blue lines = oPrP.

Table 5. *Risk assessment for production of isterband. M – microbiological risk, A – Allergenic risk*

Step	Hazard	Control Measures	Monitoring process	Risk (Probability * Konsequence) 1-5	RESULT (PrP, oPrP, CCP)
Receiving of carcass	Prevalence of feacal impurities (M)	Control at reception, ocular inspection	Self monitoring	1*3	oPrP
	Growth of microorganisms due to high temperature (M)	Temperature control (< 7°C)	Random sampling, self-monitoring	1*2	oPrP
Recieving of raw material	Growth of microorganisms due to high temperature (M)	Temperature control, contact distributer if deviation	Random sampling, self-monitoring	1*3	PrP
	Contamination through damaged package (M, A)	Control at reception, ocular inspection	Random sampling, self-monitoring	1*2	PrP
Spices	Cross contamination, allergens (A)	Order of production, cleaning between batches, separate equipment	Sampling, allergens	1*4	PrP
Ice	Contaminated ice in sausage paste(M)	Howses for water approved for food production, regional water	Sampling of water	1*3	PrP
Mix in carriage	Incorrect proportions	Reciept for ingredients	Self-monitoring	1*1	PrP
Processing in hack	Insufficient mixing of ingredients and starter culture	Ocular inspection	Self-monitoring	3*4	oPrP
Heating and smoking	Insufficient activation of starter culture. Less bactericidal activity and drop of pH following growth och pathogens	Correct time and temperature	Random sampling	2*4	oPrP
	Insufficient inhibition of microorganisms from smoking	Correct performed smoking	Random sampling	2*2	oPrP

Cold storage	Spread of allergens if stored incorrect (A)	Order of production		1*3	oPrP
	Growth of microorganisms due to high temperature (M)	Temperature control (< 7°C)		2*2	oPrP
Packaging	Inaccurate atmosphere in package (M).	Control of vacuum process		1*2	PrP
	Cross contamination, allergens (A)	Order of production, cleaning between batches	Sampling, allergens	1*3	PrP
	Cross contamination, fresh meat to ready-to-eat sausage (M)	Order of production, cleaning between batches	Sampling for listeria and durability load	2*3	PrP
Labeling	Inacurate labeling of storage temperature and shelf life (M)	Documentation of information on label at each shift of product	Random sampling, self-monitoring	1*4	PrP
	Inaccurate labeling, risk for wrong description of allergens (A)	Extra control of information on label at each shift of product	Random sampling, self-monitoring	1*3	oPrP
Cold storage	Too high temperature, growth of microorganisms (M)	Alarm when temperature is low.	Random sampling, self-monitoring	2*2	PrP
	Cross contamination of of sausage from raw meat (M)	Cover fresh raw meat with bag or lid on carriage. Raw sausage hang separate from ready-to-eat product	Internal audit	1*3	PrP
Distribution	Transportation in too high temperature (M)	Control of temperature on product and transportation before delivery	Temperature control at each delivery	2*2	PrP

5 Discussion

5.1 Microbial analysis

The starter culture composing of *L. plantarum, P. pentosaceus* and *S. xylosus* showed differences in growth in the different batches and also within batches. As seen in *Table 3, L. plantarum* was not identified in most of the sausages but in all of the batters. In 2.2.1.1 it is described how *L. plantarum* produces a bacteriocin which could kill *P. pentosaceus*. The results obtained does not align with that fact as *L. plantarum* was present in the batter but not in the sausages. In un-published data from this project, only the starter culture was grown on MRS plates under identical conditions as the LAB in batter or meat. Both LABs' were then identified which depicts that it is not being inactivated by *P. plantarum* but of another aspect involved in the production of sausage. The heat treatment is an aspect which might have affected the growth of the LAB. According to (Jordan & Cogan, 1999), the z-value increases for two strains of *L. plantarum* when temperatures exceed 50 ℃. This does however not explain why *L. plantarum* was absent in majority of the sausages only heated to 30 ℃.

Insufficient mixing of the batter could have caused the lack of LAB in a few samples. The temperature is a less reliable source of error as the LAB in the starter culture can and should grow in all temperatures tested. A larger amount of data during a longer period of time would likely give a clearer picture of growth/non-growth of starter culture in the batter. During the preparation of the batter, the starter culture is spread over the homogenized batter along with the other spices. According to the supplier of the culture, the starter culture should be dissolved in water prior to mixing with the batter, this could possible give a proper and more efficient distribution of the starter culture in the entire batch. This was not the standard procedure performed by the company during this project.

LAB isolated from MRS plates were collected from the 5th dilution from sausages produced at 35 + 55 ℃ while the LAB was collected from the 6th dilution on MRS by sausages produced at 30 ℃. This indicates a higher growth of LAB when incubated at 30 ℃, which should give a lower pH compared to the other sausages. The pH of sausages produced at 30 ℃ did despite this show a slightly higher pH than the sausages produced at 35 + 55 ℃ in laboratory and similar to those produced by the company.

In general, the measurements of pH within the sausages and batter gave similar results. It is though possible the values are slightly adverse. Since the pH of the sausages were measured on intact sausages at production site and on first dilution at laboratory, the results might have differed. Since the product is constituted of larger pieces of meat it is possible the exact location of the probe that measures the pH will give different results. Two samples were taken in each sausage and three samples in each carriage with batter. When the production site produces the sausage, normally two or three carriages are produced.

According to Glass *et al.* (1992), the use if intrinsic lactic acid or a starter culture in fermented sausage makes no difference in inactivation of *E. coli*. As it was also shown in the same study that the lowering of pH using HCL was less efficient compared to lactic acid, the effect of lactic acid is essential.

In the second batch of batter, *E. cloacea* and two species of *Acinitobacter* were identified. *E. cloacea* was found in all three samples grown on TSA and VRBA. On the blood agar grown from the same batter, *Acinitobacter junii* and *Acinitobater heamolyticus* were identified. *A. junii* has shown to cause septicaemia in neonates (Bernards *et al.*, 1997) and *A. heamolyticus* is a toxin producing bacteria capable of causing bloody diarrhea (Grotiuz *et al.*, 2006). All of these where however only found in the batter and not in any of the sausages produced by the same batch. The absence of *E. cloacea* in sausage is likely due to the inhibitory action of *L. plantarum* and *P. pentosaceus* towards *Entereobacteriaceae.*

Three species of *Kocuria* were found on blood agar from sausage incubated at 30 + 55 ℃ in laboratory. Two of these, *Kocuria rhizophilia* (Becker et al., 2008) and *Kocuria Salsicia* with a gene sequence 98.9 % similar to *K. rhizophilia* (Yun et al., 2011), are potential pathogens. *Kocuria varians* was also found in the batter and has shown to increase the proteolytic activity of *S. xylosus* when they are used in combination with each other (Tremonte *et al.*, 2007).

In the third batch of batter, the potential pathogens *Raoultella ornithinolytica* (Seng et al., 2016) and *Raoultella planticola* (Skelton et al., 2017) were isolated from VRB agar. *E. coli* was also isolated from the same batch, weather the strain was O157 H7 or not was not investigated. None of these were however found in any of the sausages produced by the same batter.

Another bacteria found on blood agar was *Staphylococcus carnosus*. This bacteria can be naturally present in meat and is suitable for fermentation of meat products (Rosenstein *et al*., 2009). *S. carnosus* in combination with *P. pentosaceus* result in production of various flavour compounds depending on level of inoculation by *S. carnosus* (Tjener *et al.,* 2004). The LAB *Lactobacillus fermentum* was also found but on MRS agar.

As the staff in the production is in direct contact with the raw ingredients, both during preparation and during hanging of the sausages on a trolley, the hygiene is essential. This is particular true as no heating step to eliminate pathogens is recommended before consumption. The processing of batter to sausage did in this study indicate a sufficient bactericidal effect as intestinal bacteria such as *E. cloacae* and *E. coli* was found in the batter but not in sausages produced from the same batter.

As presented in 2.2.1.1, *L. plantarum* had shown antibacterial activity against *E. coli* and *Enterobacteriaceae*. This could be the sole reason why *Enterobacteriaceae* was found in the batter but not in any of the processed sausages, that *L. plantarum* has sufficient inhibitory effect on *Enterobacteriaceae* in the batter before its inactivation. *E. cloacae* has been associated with sepsis (Casewell *et al.,* 1981) and has been found in rectal tracts of human (Flynn *et al.,* 1987) and in healthy domestic pigs (Schierack *et al.,* 2007). Depending on the source of origin, it could indicate flaws in hygiene procedure and it emphasises the importance of an active starter culture. It is not proven in this study if the existence of the bacteria is due to lack of hygiene of the staff or cross contamination from carcasses. Insufficient cleaning of carcasses could result in the same bacteria. *E. coli* was found in the batter of one batch, it is however not analysed if the *E. coli* is verotoxin producing or not. Regardless of *E. coli* strain, it is an indication of faecal impurities.

As the conclusions are based on rather few measurements, further studies are necessary. In the cases with no growth of starter culture, the possible causes are several and the measurement basis is insufficient to make definite statements. The reason for this could be insufficient mixing of the batter resulting in no starter culture in the tested sausages, it could also be a result of the environment the sausage was processed in.

The sausages were stored in a cold room (4-8 °C) after smoking and before packaging. The storage time can differ between batches as it is dependent on the amount of products that need to be packed and delivered. The time stored in the cold room can differ up to two days. As the packages are sealed with vacuum and the LAB has the ability to proceed the fermentation anaerobically, the development of the sausage might vary. As not all bacteria have the ability to grow in anaerobic environments, the LAB will more easily compete with other bacteria such as *E. coli* which is facultative anaerobic.

When isolating the bacteria in the starter culture, both LAB had similar appearances. When *L. plantarum* was isolated it was with good certainty but on plates only containing *P. pentosaceus*, the bigger colonies had an appearance similar to *L. plantarum*. It is however unlikely that the lack of identification of *L. plantarum* was a result of analysing the wrong colonies, as the difference between the bacterial colonies were visible when *L. plantarum* was present. The inhibitory action of smoke was to be found in this work. To find those results, the product was required show growth of microbes in the sausage produced without smoke and show absence of the same microbes in the smoked sausage. As the sausages did not show any growth on non-starter bacteria except for *Kucuria* in one batch, conclusions are difficult to make. As described in 2.3.2, smoke possesses antimicrobial properties and could function as microbial hurdle but cannot be concluded from these analyses.

5.2 HACCP

The developed HACCP was based on the HACCP developed by the company. A few changes were made as the existing HACCP was general for all charcuteries while the HACCP developed in this study only focus on the production line for isterband. Control at reception was considered oPrP due to the risk of faecal impurities and growth of microorganisms as result of too high temperature. Processing in hack is considered an oPrP as it is the step in which the batter is mixed and where the spices and starter culture is mixed with meat, ice and barley. Heating and smoking is not an oPrP because of inactivation of microorganisms as result of heat processing but because of the activation of starter culture and inhibitory aspects of smoke. Cold storage after heating and smoking is considered an oPrP as this is an inhibitory factor for growth of pathogens.

Growth of the starter culture is the main inhibitory factor against pathogens but is difficult to measure quickly and therefore not considered a CCP. Many steps are involved in ensuring an adequate growth and focus is therefore on preventing absence of pathogens instead of killing them. As it is difficult to ensure a safe product such as isterband which does not undergo heat treatment but is fermented, recommendation of proper heating before consumption on package is recommended

By removing the existing CCP, the steps required in a HACCP are greatly reduced. Several of the seven principles of HACCP described in 2.4 are only applicable when a CCP is found. This does not result in a less monitored production line, rather the opposite. The other steps, particularly the oPrP:s are necessary to be monitored more carefully. If a recommendation to heat the sausage before consumption is added to the package, the production steps becomes less critical even if they still are obliged to be carried out correct.

6 Conclusion

The sausages produced by the company are with respect to the analysed parameters safe to consume without heating before consumption. Activity of the starter culture varied and therefore also the pH. Due to the variation of LAB activity and potential pathogenic bacteria found in the batter, heating before consumption is recommended.

Sausage produced at 30 °C did not show more bacterial contamination compared with sausages produced at higher temperature. Also, the pH did not vary between sausages produced at the company and under laboratory conditions. Production of cold smoked isterband can possibly be carried out, however this needs to be investigated further.

6.1 Recommendations

- Solubilise the starter culture in water prior to addition to the batter.
- Make sure there is no possibilities for faecal contamination.
- Remove existing CCP in HACCP and add “Heat prior to consumption” on package.

7 Future perspectives

Dynamics of the starter culture would be interesting to investigate further if sausages are to be processed at 30 °C for a longer period of time. The long-term effect on the starter culture at the different temperatures was not tested and could also be relevant to investigate. It might also be that processing at 30 °C would enable LAB to regulate pH more stable during storage compared to when processed in 35 and 55 °C. The higher temperatures indicated a more efficient drop in pH shortly after the processing but not in long term.

It would also be interesting to investigate if the solubilisation of the starter culture prior to addition would result in a more even distribution of the LAB and less variation in pH drop.

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Appendix 1: Microbiological hazards and risk evaluation carried out by the company.

Microorganism	Type	Concerns	Source of origin	Severity (2,4,6,8)	Probability (1,2,3,4)	Risk number (3-12)	Propagating temp. °C (min/max)	Atmosphere	pH (minimum for growth)	Characteristics	Effect on human
Escherichia coli O157:H7 A3:H4A1:H4A3:H4	Bacteria	Cattle, pig	Intestine of human and warm blooded animals. cattle and and sheep in particular.	**8**	**2**	**16**	35-37°C (4-46°C)	Facultative anaerobic; can grown both in presence and absence of oxygen	4, 3	Produce toxin. < 100 Bacteria required to cause sickness of the toxin produced by Bacteria.	Abdominal cramps and diarrhea, nausea, and vomiting may occur. Incubation time of 1-8 days, recovery normally within a week. 5% (mostly young and older) develop Hemolytic-Uremic Syndrome (HUS). HUS means the red blood cells decay and platelets drop in number causing damage to functions of the kidneys. Can be cause of death. Classifies as zoonosis.
Limit in end product concerning E. coli: mean value 50 cfu/g, no value above 500 cfu/g									Source: Commission Regulation (EC) No 2073/2005:		
Salmonella				**6**	**2**	**12**	37°C		4, 1		

	Bacteria	Cattle, pig	Intestine, can spread from both humans and animals. Spread through direct contact				(5-47°C)	Facultative anaerobic		Can survive in dried foods. Dies when heat treated.	Nausea, vomition, stomach cramps, diarrhea, fever and headache which can last for a few days. Incubation time of less than 6 h -- 10 days. Might cause joint inflammation as sequela. Humans are usually vector for 4 -- 6 weeks, Bacteria can be latent. The Bacteria can cause death to Immunocompromised people.
Limit in end product: Not detected in 25 g										Source: Commission Regulation (EC) No 2073/2005:	
Yersinia enterocolitica	Bacteria	Pig	Primairly from intestine and mouth of pork. Can be found in big numbers on toung and tonsils	**6**	**3**	**18**	29°C (-1-44°C)	Facultative anaerobic	4, 5	Unknown infectious dose	Causes yersinos. Can give rise to diarrhea, stomach pain, light fever, nausea, sore throat, vomiting. Symptomes usually las for 1-3 days, incubation time of 3 -- 10 days. > 10 % suffer from joint dissorders as sequela. Latent carriers may occur.
Limit in end product: Not detected										Source: ”Kontrollhandbok för provtagning” del 3	

Listeria monocytogenes	Bacteria	Cattle, pig	Frequently found Bacteria in enviriomen. Excists in soil, water and intestines of mammals.	**6**	**2**	**12**	30 - 35°C (0-45°C)	Facultative anaerobic	4, 6	Form biofilm on equipment, and in facilities. Resistant to salt and can form toxins.	Causes listeriosis for risk groups such as; pregnant people and their fetus and people with impaired immune system. High mortality within risk groups. Symptoms are similar to influenza which migh develop into menignitis och blood poisoning. Incubation time of 1 -- 90 days. 2-6% of humans are symptom free carriers. Classified as zoonos
Limit in end product: <100 cfu/g										Source: Commission Regulation (EC) No 2073/2005:	
Staphylococcus aureus	Bacteria	Cattle, pig	Commonly found in warmbloded animals and humans. Excists on skin, cuticle, hair, ear, nose, mouth and wounds.	**4**	**4**	**16**	37°C (7-48°C)	Facultative anaerobic	4. Small amounts of toxin can be producd at pH < 6. Bacteria can grow in pH 4 -- 10.	Can produce a highly heat-resistant toxin in foods. Tolerant to salt (up to 20 %) and low Aw.	Acute nausea, stomach cramps, vomiting normally followed by diarrhea. Headache exhaustion and cold sweating after 1 -- 7 days. Recovery time of 1 -- 2 days. Unusual cause of death
Limit in end product: <10^5 cfu/g										Source: Commission Regulation (EC) No 2073/2005:	

BSE (Bovine spongiform encephalopathy)	Prion	Cattle	Cattle and sheep carry the disease	**8**	**2**	**16**					
Hepatitus A	Virus	Cattle, pig	Contaminates through infected staff.	**4**	**1**	**4**	Does not proliferate in food. Dies when heated			Infectious dose of only 10 particles.	Causes jaundice. Reduce appetite, gived fever, nausea, vomitingm diarrhea, muscular pain and yelllow skin. Low mortality rate.
Limit in end product: Saknas. Sjuka alt nyligen symptomfria personer ska inte hantera oförpackade livsmedel. Source: ”Kontrollhandbok för provtagning” del 3											
Norovirus	Virus	Cattle, pig	Contaminates through infected staff.	**4**	**1**	**4**	Does not proliferate in food. Dies when heated			Endast 10 viruspartiklar behövs för att bli sjuk	Causes winter rheumatoid arthritis (winter vomiting disease). Nausea, vomiting, diarrhoea, abdominal pain, headache, dizziness, muscular pain and fever for 12 - 48 hours. Infection can spread two days after recovery. Common with relapse.
Limit in end product: None descided. Sick or people recently free from symptoms should not handle unpackaged foodstuff. Source: ”Kontrollhandbok för provtagning” del 3											

Trichinella	Para-site	Pig	Pigs with the para-site.	**6**	**1**	**6**	Dies when heated or frozen			Sampling on all car-casses of pig. Unu-sual pres-ence in bred pigs.	Abdominal pain, fever and nau-sea.Buksmärtor, feber, illamående. If the amount is small, incubation time can be up to a month. Large omunts can cause muscular pain and cause death in rare cases. Classified as zoonosis
Limit in end product: 0,0 Not detected											Source: Commission Regulation (EC) No 2073/2005
Bacillus cereus	Bac-teria	Cattle, pig	Found in environ-ment. Ex-cists in soil and on plats.								

Appendix 2: Popular scientific summary

Analysis of risks in production of isterband

Production of sausages can be performed in many ways and the means to ensure a safe production varies. Isterband has historically been produced by smoking it and allowing profitable bacteria already present in the sausage to kill off or inactivate unwanted bacteria. This is achieved by production of lactic acid by the sought bacteria, so called lactic acid bacteria (LAB). The investigated sausage contained two different LAB, *Pediococcus pentosaceus* and *Lactobacillus plantarum* These bacteria lower the pH in the sausage and functions against bacteria which could give a hazardous product at the same time as it gives the sausage its characteristic taste.

This work has evaluated the risks in the production of isterband produced by a small company. To ensure the product taste the same in every batch, a specific mix of bacteria called starter culture is added instead of using the bacteria already present in the sausage. These bacteria are known to give specific flavours and, more importantly, give a safe product. This type of sausage is not heat treated at a temperature high enough to make sure all bacteria are inactivated. Due to this, it is essential the wanted bacteria in the sausage functions properly. The activity of these bacteria is in this work examined and also the activity of unwanted bacteria. Parallel to this, a qualitative hazard analysis of critical control points (HACCP)- plan is carried out. Everyone who handles foodstuff is required to have a HACCP-plan or a plan based of the HACCP-principles. The company has such a plan which includes several of the products produced there but not for this specific line of production.

The production of the sausage was observed in this work to find potential steps which could be more crucial regarding safety of the product. The HACCP-plan was carried out by revising the existing one with respect to the microbiological analyses performed in this work. The microbiological analyses were performed on the batter, the sausage produced by the company and in two different sausages heat treated in different ways in a laboratory. The sausages heat treated in the laboratory were ether heated to the same temperature as by the company, which is 35 ℃ for 11 hours and then 55 ℃ for 4 hours, or at 30 ℃ for 15 hours. The sausage produced by the company is exposed to smoke during the 4 hours in 55 ℃ while the sausage heat treated in the laboratory is not exposed to smoke. Samples from the batter and the different sausages are diluted and applied to different agar plates, allowing different bacteria

to grow more easily. The bacteria that grew where then identified using a machine able to identify a great number of bacteria.

It was concluded that some unwanted bacteria were found in the batter, however, they were not found in the sausages. This indicates a sufficient inactivation of the unwanted bacteria even if the presence in the batter is alarming. One of the LAB applied to the batter was not found in the sausages and based on this project, it can only be speculated on the cause for this. The presence of the bacteria from the starter culture varied and the pH, even if the variations were rather small. If the starter culture is dissolved in water prior to addition to the batter, a more even distribution of the culture might be achieved. The revised HACCP-plan suggested a change of classification on the heating step from critical control point (CCP) to operational prerequisite programme (oPrP).