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Sodium reduction in dry fermented sausages

Effects on the fermentation process, product quality and taste

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Abstract

Dry fermented sausage is considered a traditional craft containing a high amount of salt. Lactic acid bacteria play an essential role in the production of fermented meat production. The bacteria are essential for lowering pH and producing flavour along with the adding of sodium chloride (NaCl) to ensure food safety. Sodium is an important nutrient for the body, but a high intake can cause several negative health effects. Reducing the content of salt affects the lactic acid bacteria, processing, taste, and food safety *e.g.* and has to be taken into consideration when reducing the content or by replacing with other ingredients. Due to this, there is a great requirement to increase the knowledge about the effect of salt reduction in dry fermented to enable the meat industry to produce more salt reduced products.
The aim of the thesis was to study the effects of different starter cultures in combination with sodium reduction, by reducing the amount of added salt (NaCl) or by partial replacement with potassium chloride (KCl), in dry fermented sausages.

In this thesis it was found that the type of starter culture had more effect on physiochemical and sensorial properties of the dry fermented sausages than the replacement of 14-29 % of the added NaCl with KCl on an equimolar amount.
A partial replacement of NaCl with KCl reduced the NaCl content in the sausage with 14 - 30% compared to the reference sausage made in this study (5.5% salt). These sausages reached a water activity below 0.90 after 16 days of drying, and with no texture defects. Sausages added the lowest concentration of NaCl had a reduction in NaCl of 22-26 % compared to the reference sausage made in this study (5.5% salt) and reached a water activity below 0.90 after 22 days of drying, but some texture defects were observed in those sausages.
Sausages fermented with Bitec LS-25 and added KCl seemed to have lower concentrations lactic acid and acetic acid than sausages added only NaCl. Bitec LS-25 reduced pH to 4.6-4.8 in 24-48 h. A significantly chewier texture and stronger taste of lactic acid/salami were found in the sensorial evaluation compared to Bitec LK-30.
Sausages fermented with Bitec LK-30 showed no obvious trend regarding the added concentrations of salt, and lowered pH to 4.9-5.0, but the starter culture required less dextrose than Bitec LS-25. The starter culture yielded sausages with a lower concentration of lactic acid, and important, a lower weight loss than the sausages fermented with Bitec LS-25.
Bactoferm T-SPX seemed not to thrive in conditions added KCl, while Bitec B Mild & Fast seemed unaffected by the type of salt.
An observation found in the thesis was that the water activity decreased after storage in vacuum packing. The sausages added the lowest concentrations of NaCl (1.5 g NaCl + 1.0 g curing salt/100 g meat mixture) had a higher water activity at start since the added salt was lower, but at the water activity approx. 0.93, all sausages actually more or less had the same weight loss and small amount of water had to evaporate from this point. The observations are important for the industry since it could give the opportunity to produce salt reduced sausages without drying a water activity below 0.90 and therefore reduce the risk of texture defects by vacuum packing the sausages at an earlier stage.

A model system to study the initial fermentation phase (2-5 days) based on fermenting the meat mixture in 50 ml plastic tubes was tested. The system can be used to screen starter cultures in combination with different levels of salts (NaCl, KCl, others) and/or glucose. The results in the successful experiments were in reasonable agreement with the results of the sausage production studies, indicating that this may be a useful screening system. However, further refinement of the method is still required.

Sammendrag

Spekepølse er et tradisjonelt håndverk som inneholder store mengder salt. Melkesyrebakterier spiller en essensiell rolle i tillagningen og er nødvendige for å senke pH og lage smakskomponenter sammen med tilsetning av natriumklorid (NaCl) som også sikrer mattryggheten. Natrium er et viktig næringsstoff for kroppen, men et høyt inntak vil igjen kunne gi negative virkninger på helsen. Redusering av saltinnholdet påvirker bl.a. melkesyrebakteriene, prosessering, smak og mattryggheten, og alle disse aspektene må vurderes når saltinnhold skal reduseres eller erstattes med andre ingredienser. På grunn av dette er det nødvendig å øke kunnskapen om hvordan saltreduksjon påvirker egenskapene til spekepølse, slik at kjøttindustrien i samråd med myndighetene kan produsere flere salt reduserte produkter. Målet for denne oppgaven var å studere effektene av ulike starter kulturer i kombinasjon med salt reduksjon, ved å redusere den tilsatte mengden NaCl eller ved å delvis tilsette kalium klorid (KCl) i spekepølse.

Det ble funnet at typen starterkultur hadde mer å si for de kjemiske, og sensoriske egenskapene til spekepølsene enn erstatningen av 14-29% av NaCl med KCl av lik molar mengde. Erstatning av deler av NaCl med KCl reduserte NaCl-innholdet i spekepølsen med 14-30% sammenlignet med en standard spekepølse produsert i forsøket (5,5% salt). Disse spekepølsene hadde en vannaktivitet på under 0,90 etter 16 dager med tørking, og var uten teksturfeil. Spekepølsene som ble tilsatt laveste konsentrasjon av NaCl hadde en reduksjon av NaCl med 22-26% sammenlignet med en standard spekepølse produsert i forsøket (5,5% salt) og hadde en vannaktivitet under 0,90 etter 22 dager med tørking, men med noen teksturfeil. Spekepølser med en kombinasjon av Bitec LS-25 og KCl så ut til å ha en lavere produksjon av melkesyre og eddiksyre enn i kombinasjon med NaCl. Bitec LS-25 ga lav pH (4,6-4,8) på 24-48 timer. Egenskapen seig/deigete (chewy) ble funnet å være signifikant større samt signifikant mer smak av melkesyre/salami når denne spekepølsen ble sammenlignet sensorisk med en spekepølse inokulert med Bitec LK-30. Spekepølser produsert med Bitec LK-30 hadde ingen åpenbare forskjeller med tanke på saltkonsentrasjoner, senket pH til 4,9-5,0, men startkulturen så ut til å kreve mindre dektrose enn Bitec LS-25. Startkulturen (Bitec LK-30) produserte mindre melkesyre og spekepølsene hadde mindre vekttap enn spekepølsen inokulert med Bitec LS-25. Bactoferm T-SPX så ikke ut til å trives i spekepølser tilsatt KCl, i motsetning til Bitec B Mild & Fast som ikke ble åpenbart påvirket av type tilsatt salt.

Under studiet ble det observert en nedgang i vannaktivitet i spekepølsene lagret i vakuumpakning. Nedgangen indikerte at bare en liten mengde vann måtte diffundere fra spekepølsen etter den hadde nådd et kritisk punkt i tørkingen for at vannaktivitet skulle endre seg mye. Observasjonene er viktig for industrien fordi det kan åpne for muligheten å produsere salt reduserte spekepølser uten å tørke disse til under 0,90 i vannaktivitet og derfor muligens redusere risikoen for teksturfeil hvis pølsene blir vakuum pakket på et tidligere stadiet.
Et modellsystem basert på 50 ml rør ble benyttet for å studere fermenteringsfasen (2-5 dager etter inokulering) i kjøttmikser. Systemet kan benyttes til å se på ulike starterkulturer i kombinasjon med ulike nivåer og typer av salt samt sukker. Resultatene funnet i de innledende forsøkene i modellstudiet var i overenstemmelse med resultatene funnet i hovedstudie som ble utført i spekepølser. Resultatene som ble funnet tyder på at modellsystemet kan være et nyttig verktøy for å undersøke ulike parametere enkelt. Modellsystemet krever noen forbedringer før det fungere optimalt, men det har stort potensiale.

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1. Background

Salt (sodium chloride, NaCl) is one of the most common food additives in the world and a high intake of salt in the public diet is a big health problem. An intake of 1.5 g salt per day is enough to cover one adults need, but the average Norwegian diet contains about 10 g salt per day (Helsedirektoratet, 06/2014a).

It has been estimated that 2.5 million deaths could have been prevented each year if the global salt consumption was reduced to 5 g/day as recommended by (WHO, 2012). A reduction in the intake of salt could also give great benefits on the public health by reducing sickness caused by excessive intake of salt. Several calculations show that a reduction by 10-30 % could have a major positive effect on the public health (WHO, 2012).

Industrially processed products like raw fermented sausages (*e.g*. salami, pepperoni) are products with a high salt content and WHO has estimated that: "about three-quarters of the average salt intake come from industrially processed food and food served at restaurants or catering establishments" (WHO, 2012).

One of the oldest, most popular processed meat products on world basis are dry fermented sausages. Dry fermented sausage production is considered as a traditional craft and sausage makers often use their experience to qualify when the sausage is finished. One of the main criteria to achieve a dry fermented sausage is fermentation. Fermentation of dry fermented sausages was in the oldest days achieved by adding a piece of meat from an old batch into the new batch, a method called *backslopping*. In this way they achieved a fermentation process, but it had many potential sources of contamination and a high risk of failure. Currently, freeze dried starter cultures are mostly used in commercial dry fermented sausage production. *Backslopping* and commercial starter cultures both provide an inoculum of lactic acid bacteria that is responsible for the desired acidification during fermentation. Industrial manufactures like Frutarom Savory Solutions GMBH and Chr.Hansen, provide different mixes of bacteria in their starter cultures that should give advantages regarding what type of sausage it's desired to produce.

Different starter cultures have different properties regarding *e.g*. fermentation speed, optimum temperature and production of fermentation products. Corral et al. (2013) studied how different slow fermenting starter cultures are affected by a salt reduction, while Olesen et al. (2004) studied how starter cultures with *Staphylococcus spp.* were affected. Both examined how the starter cultures affected production of aroma compounds when salt was reduced and agreed that a reduction of salt changed the composition of the aroma components. Several articles have also found that the reduction of salt affects the texture of sausages and the fermentation process with regard to fermentation rate. Many of the articles looked at how salt affected the technological properties of the sausages (*e.g.* texture, sensorial) as well as the microbial effects of reducing salt, but not many articles were found on how the reduction affected the starter cultures ability to ferment regarding properties like production of lactic acid and the decrease in glucose.

Traditional Norwegian dry fermented sausages contain sodium (Na) corresponding to approx. 5.2 % (w/w) salt (NaCl) and most of the salt is added to reduce the risk of growth of

undesired microorganism, as well as giving the traditional salty flavour (Matvaretabellen, 2017). The commercial sausage production process (*e.g*. drying, time, temperature) is today adapted to the current salt level. When reducing the content of salt in dry fermented sausages, it is important to know how the salt reduction affects the growth of the desired lactic acid bacteria, pH development, water loss, texture, overall quality and consumer acceptance of the end products. The economic aspects regarding increased production time and water loss are also important. This thesis will increase the knowledge about how salt reduction affects the fermentation process and might contribute to reaching the goal of a Na content corresponding to less than 5 g NaCl/100 g in dry cured fermented sausage. (Helsedirektoratet, 11/2016b).

2. The aim of the thesis

This thesis is a part of the project ExPreSS (Enery-efficient production of reduced-salt dry-cured meat, NFR No. 269070/E50) that aims to of develop an energy-efficient sustainable production technology for reduced-salt dry-cured meats. The project owner is Grilstad AS.

The aim of the thesis was to study the effects of different starter cultures in combination with sodium reduction, by reducing the amount of added salt (NaCl) or by partial replacement with potassium chloride (KCl), in dry fermented sausages.

The specific goals are to:

1) Investigate the fermentation process in a model system, *e.g.* production of organic acids, glucose consumption, and changes in pH, dry weight and a_W in meat mixtures added different commercial starter cultures and different amounts of NaCl and KCl.
2) Investigate the fermentation process in the model system with respect to the same parameters when the meat mixture was added different commercial starter cultures and different amounts of dextrose (glucose).
3) Investigate the fermentation process, as well as the ripening and drying process during production of dry fermented sausages added different commercial starter cultures and different amounts of NaCl and KCl, with respect to the same parameters as above, as well as the sensorial properties of the produced dry fermented sausages.

3. Theory

3.1. Salt (NaCl)

The common cooking or table salt (NaCl) consists of a positively charged sodium ion (Na^+) and a negatively charged chloride ion (Cl^-). It is commonly referred to as salt, although chemically a salt is any compound composed of related numbers of cations and anions so that the product is electrically neutral. Thus, potassium chloride (KCl) is also a salt. However, in this thesis the term "salt" is used for NaCl unless otherwise stated.
Salt has been important for humans for thousands of years due to its taste, taste enhancing properties and because it can be used to preserve food. The location of salt deposits was important in ancient Rome, Egypt and the Middle East. In Latin the term, “Natron” means divine salt, while the term “Salarium” refers to the amount salt given to a worker as payment. The Vikings (800-1050 A.D.) brought salt from the Middle East up to the Nordic countries (Binkerd and Kolari, 1975, Albarracín et al., 2011).

3.2. Salt and health

For millions of years humans survived on a diet with less than 0.25 g salt per day. When the ability of salt to preserve food was discovered about 5000 years ago, it became of great economic importance, and was for a long time the most taxed and traded product in the world. Later, the deep freezer and the refrigerator reduced the need for salt, but in recent times the consumption has again increased to the same level as in the 1870s that was approx. 10 g per person. (He and MacGregor, 2009, Kloss et al., 2015).

Sodium (Na^+) is essential for the body, as it contributes to blood pressure regulation, transport of intracellular water, regulation of osmotic pressure, and transmission of nerve impulses. (Cruz et al., 2011, Helsedirektoratet, 06/2014a). Salt is the main source of sodium in the diet today. However, a high consumption of salt affects blood pressure and is associated with an increased risk of cardiovascular diseases. (Desmond, 2006, Sacks et al., 2001, Selmer et al., 2000).

The World Health Organization (WHO) recommends that the daily intake of sodium should be no more than 2 g per person, corresponding to 5 g NaCl. The estimated daily intake of salt in Norway is 8-10 g per person, and Norwegian health authorities want to reduce this to below 5 g in accordance with the recommendation from WHO. To achieve this, the authorities have formed a salt partnership with the industry. (Helsedirektoratet, 11/2016b). The goal of the National Salt Partnership is: "The salt intake in Norway shall be reduced by 15 percent by 2018 and by 30 percent by 2025". The goal is based on the WHO goal for salt reduction. (Helsedirektoratet, 06/2014a, Helsedirektoratet, 11/2016b). To reach the goal, industrial manufacturers must work together with the government to develop methods to produce salt reduced products.

3.3. Labelling of low salt products

Salt in meat products constitute 24 % of the dietary intake of salt in Norway (Figure 3.3-1). Dry-fermented sausages are frequently used as topping on sandwiches, by both children and adults at breakfast, lunch and evening meals.

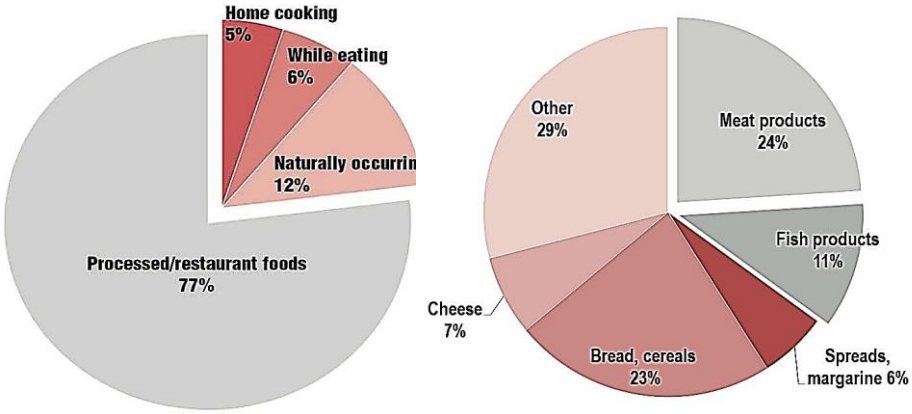

Figure 3.3-1 Left: Sources of salt in the diet. Right: Sources of salt in between food products in Norway. Left: (Mattes and Donnelly, 1991) Right: (Melnæs et al.) Modified by: (Greiff, 2015).

In order for the consumers to easily identify products with less salt, several labelling systems have been designed by the authorities. All products must be labelled with the amount of salt in the nutrient declaration (Lovdata, 2014b). To enable the consumer to easily identify products that are healthier than the regular ones, a voluntary Nordic food labelling system, the "Keyhole", was established. The keyhole label shall signal to the customer that this is a healthier product with less fat, salt and sugar than other products in the same category. The requirements for keyhole labelling of sausages are no more than 2.0 g NaCl and 10 g fat per 100 g (Lovdata, 2015c).

If Norwegian manufacturers want to label their products with claims such as "reducing consumption of sodium contributes to the maintenance of normal blood pressure" they must follow the rules outlined in EU Regulation no. 1924/2006. Norway as adopted this regulation. To label a product with low or reduced sodium can give a competitive advantage in the market by being a different product, but also by signalling a brand with better nutritional value.

Labelling of a product that, "is reduced", require comparison with similar products, and the salt content must be at least 30 % lower than in similar products. For products such as dry-fermented sausages, which have a relatively high salt content and must have so, this is the most likely strategy, but the requirement is a difference of 25 % of sodium. Requirements for other claims such as "low sodium" are also stated in the regulation (EFSA, 2006a, EFSA, 2006b).

3.4. Salt reduction strategies

To reduce sodium, the simplest solution is to add less salt. However, the technological challenges of reducing salt by 30 % as is the goal for 2025 or by 50 % to reach a consumption level of 5 g salt/day, are considerable.
A so large reduction will lead to loss of sensory attributes (less salty foods), change in physiochemical properties, yield, texture, and maybe most important, food safety (Greiff, 2015).

3.5. Salt replacers

Salt is not only taste, but have important functional and preservative properties in food. This is particularly true for dry-fermented sausages. Thus, a significant reduction of salt in such products is likely to require the use of salt replacers. Salt replacers are defined as compounds used to compensate for the reduction of salt (NaCl) in a product. Salt replacers are components that have some salty taste such as potassium chloride (KCl), magnesium chloride ($MgCl_2$), magnesium sulfate ($MgSO_4$) and lactates (Cobcroft et al., 2009). The problem with salt replacers is that they do not necessarily function technologically in the same way as NaCl, and although they have a salty taste, it is not the correct taste (Cobcroft et al., 2009, Collins, 1997, Muguerza et al., 2004). Thus, the selection of salt replacers requires a good understanding of the function of NaCl in that particular product, and the degree of replacement can be critical.

The next sections will look at different aspects regarding dry fermented sausage, and sodium reduction as well as the use of KCl as salt replacer.

3.6. Dry-fermented sausages

One of the oldest, most popular processed meat products on world basis is dry fermented sausage. They are known for the unique flavour and nutritious character and come in many different types. The sausages can be divided into two main types; low acid fermented sausages (pH=5.3-6.0, preserved at low temperature, and added 2.3-3.0 % salt) and high acid fermented sausages (pH=4.8-5.3, added 2.5-3.5 % salt, preserved by fermentation with lactic acid bacteria and drying). (Kumar et al., 2017, Leroy and De Vuyst, 2004).

The term "sausage" derives from the Latin word “salsus” meaning salt, and records of preparation and consumption of sausages date back to 1500 BC in Babylonia and China. Sausages were popular due to their simple preparation, easy transport, easy to vary, reasonable production costs, and high nutritional value (Kumar et al., 2017).

3.7. Ingredients in dry fermented sausages

3.7.1. Meat and back fat

Meat is the main ingredient in dry fermented sausages. It is a complex structure of water, proteins and lipids, as well as minor components such as vitamins and minerals. The composition the meat used in sausages depends on the animal species, the age of the animal and the part(s) of the animal used. In dry fermented sausages the muscles of meat are often used, but the type of meat depends on the product. Pork is the most common meat, but beef or mutton is also used. Often the meat from adult and well-fed animals is preferred, due to its higher myoglobin and fat content. (Vignolo et al., 2010, Toldra et al., 2014).
The functional characteristics of meat, such as composition, pH, salt soluble proteins, water holding capacity, and fat composition are major criteria when selecting meat for production of dry fermented sausages. Meat with pH above 5.9 contains low levels of lactate and sugar levels, and water is tightly retained; resulting in poor binding conditions. Selection of meat that has minimum microbial loads is critical since safety risks and unwanted flavours and texture may be introduced. (Toldra et al., 2014, Vignolo et al., 2010).

Raw fermented salami is mainly added back fat from pork. This fat is composed lipids (mainly triglycerides), water and collagen. The concentration of fatty acids affects the

firmness and the cohesiveness of the tissue and is, among others, determined by its handling "quality". The composition vary depending on factors such as species, age, and thickness of the back fat (Wood et al., 1989).
Fat is often considered unhealthy and has become an unpopular constituent of meat for many customers. In dry fermented sausages, fat is important for aspects such as nutritional value, texture, and flavour.

3.7.2. Salt

Salt affects texture (See section 3.9), flavour and shelf life. European dry fermented sausages are typically added less than 4 % NaCl (w/v), but during drying the concentration increases due to loss of water (Toldra et al., 2014).
Sodium ions cause salty flavour, but salt also enhances other flavours and is therefore added to almost all types of food. The salty flavour depends on the composition of the food. Fat and sugar can mask salt flavour, while acids and some herbs can enchance the salty flavour. (Mattes, 1997, Ofstad R, 2015, O'Mahony, 1979).

The functions of salt in processed food are many. In meat, salt enhance flavour, texture, improve water and- fat binding properties, improve preservation, increase water-holding capacity, increase meat-binding in tumbled products and fat-binding in others. (Ruusunen and Puolanne, 2005, Toldra and Nip, 2008). Salt reduces the water activity (a_W). Electrostatic forces around the salt ions tightly bind water molecules, and this means that less water is available for the microorganisms. With enough salt ions in the solution, bacteria are no longer able to take up water, and can no longer grow. If the salt concentration is further increased, water may be drawn out of the cells and the microorganism may start to die. Microorganisms vary considerably in their ability to grow at low water activity. Halophilic (salt-loving) bacteria can grow in saturated salt solutions ($a_W = 0.75$), but when the water activity is reduced to less than 0.90 most pathogenic bacteria will no longer grow, and below 0.85 no pathogenic bacteria will grow. Pathogens that typically may be found in dry fermented sausages are *Escherichia coli*, (minimum growth a_W=0.95), *Listeria monocytegenes* (minimum growth aw=0.92), *Staphylococcus aureus* (minimum growth a_W=0.83), and *Salmonella spp.* (minimum growth a_W=0.95) (Fontana, 2008, Baird-Parker et al., 2000).
A reduction below 0.80 will inhibit the growth of almost all microorganisms. Fungi are more tolerant of low water activity than bacteria, and a few xerophilic (dry-loving) fungi may grow down to a_W = 0.61-0.62. Below 0.60 no microorganisms will grow. When sausages reaches a_W<0.90, they are considered safe for non-refrigerated storage (Fontana, 2008), this is also a criteria for Norwegian types of dry fermented sausages.

3.7.3. Sugar

Glucose (D-dextrose) and sometimes lactose and saccharose, are the main sugars used in industrial manufacturing of fermented meat products. When sugar is converted by lactic acid bacteria (LAB) (*see section 3.10)* it influences flavour, texture and sometimes colour (Maillard or Caramelization reaction if heat applied) in sausages. The amount of added sugar is chosen to ensure a wanted and adequate initial drop in pH, as well as a suitable texture of the product. (González-Fernández et al., 2006).
González-Fernández et al. (2003) studied the effects of different glucose, lactose and sucrose concentrations in Chorizo sausage fermented with *Lactobacillus casei K29*. Results showed that when adding 0.1, 0.5 and 1.0 % glucose, pH decreased faster with higher added amount of glucose the first four days, but on day four the 0.5 and 1.0 % glucose meat mixtures

became similar in pH (4.84-4.86) and remained so, while the meat mixtures added 0.1 % never went below pH 5.5. They also found that the amount of sugar had a significant impact on the texture, but could not conclude without more research.
Stahnke (1995) tested several different temperatures, the concentration of salt, nitrite and glucose using different starter cultures, and found that the pH-lowering effect of glucose was almost zero when the temperature was low, but it had to be seen in context with the other parameters as the starter culture, since some starter cultures needed more sugar than others.

3.7.4. Bacterial fermentation

Dry fermented sausages are produced by a bacterial fermentation, mainly by lactic acid bacteria (LABs), but the contribution from micrococci is also important, particularly in the ripening phase.

LABs are generally recognized as safe (GRAS). The most frequently used LABs in European sausages are strains of *Lactobacillus*, *Streptococcus, Lactococcus*, *Bifidobacterium*, *Pediococcus* and *Leuconostoc*. In addition, the starter cultures usually contain micrococci such as *Enterococcus* spp. or coagulase-negative cocci (CNC; mostly *Staphylococcus* spp. and *Kocuria* spp.)(Ammor and Mayo, 2007, Kumar et al., 2017, Leroy and De Vuyst, 2004).

Several methods can be used to achieve fermentation of the sausages. The safest and most frequently used method today, is to add a starter culture, but an inoculum from a previous batch (*backslopping*) may also be used. One may also rely on natural LABs in raw meat, but this may result in many failed batches.
An alternative to microorganisms to reduced pH is to add glucono-delta-lactone (GDL).(Toldra et al., 2014).

3.7.5. Nitrite

The colour of the meat is very important when consumers decide to buy a product or not. The colour is seen as an indicator of freshness and quality. The colour of sausages comes from the concentration of heme pigments (myoglobin, haemoglobin) and their chemical reaction with sodium nitrite ($NaNO_2$), and the light scattering properties of meat (Toldra et al., 2014).

To obtain the stable red colour in sausages that the consumer wants, the $NaNO_2$ is usually the material of choice, but sodium nitrate can also be used. It has two important functions in meat products, it inhibits growth and toxin production by the bacterium *Clostridium botulinum* (EAKES et al., 1975), and it promotes a wanted meat colour. According to Feiner (2016) the lethal dose of $NaNO_2$ for humans, is about 1.1 g. Manufacturers often use a mixture of salt and nitrite. The nitrite concentration in the mix varies from 0.5 % -20 %, depending on the national regulations. In Norway, sodium nitrite has the E-number (number to identify an additive) E250, and the maximum addition is 150 mg/kg processed meat (Lovdata, 2011a). One of the reasons that sodium nitrite is not allowed as a food additive in all types of foods, is because it can cause severe food poisoning and cancer. Nitrite can accumulate from oxyhaemoglobin ($MbFe^{2+}O_2$) and further to nitrosamines when interfering with the oxygen transport in the blood and cause severe poisoning. (Burden, 1961, Epley et al., 1992, Toldra et al., 2014).

When sodium nitrite is added to meat, it is reduced to nitric oxide (NO) by an enzyme produced by species of *Micrococcaceae* and coagulase-negative *Staphylococci*. Denaturation of myoglobin occurs, and this expose the heme group which NO binds to and forms nitroso-

myoglobin (Mb Fe^{2+} NO) through a complex reaction including several other reactions that can include *e.g.* ascorbic acid (Figure 3.7-1). pH affects the ferrous state of heme iron in myoglobin and therefore affects the development of colour during fermentation (Toldra et al., 2014). Many forms of nitrite reactions with myoglobin can happen, but nitroso-myoglobin (Mb Fe^{2+} NO) is the desired form in most meat products. In cured meat with low pH, globin denaturizes and resulting in a lost hem-group. This reaction leads to nitrosohemochrome that is a stable pink pigment in sausages. (Brooks, 1937, Ordóñez et al., 1999, Toldra et al., 2014).

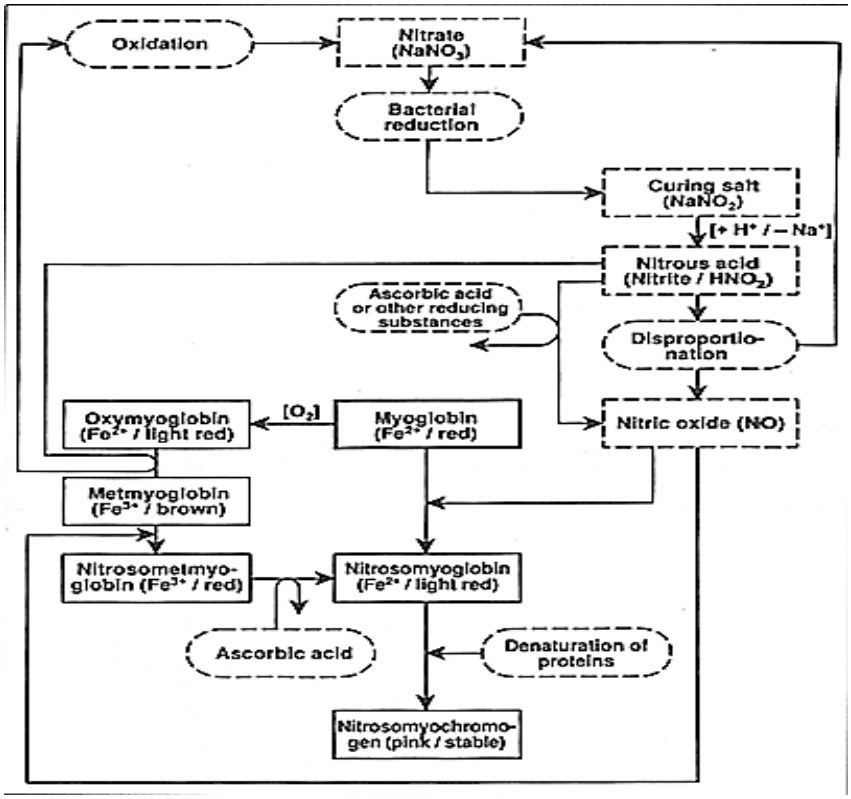

Figure 3.7-1 Reaction between nitrate, sodium nitrite and myoglobin when colour is formed in meat products. Mb Fe^{2+} (myoglobin), Mb Fe^{3+} (met-myoglobin), Mb Fe_2+O_2 (oxy-myoglobin), MbFe^{3+} NO (nitrosomet-myoglobin), MbFe^{2+}NO (nitroso-myoglobin).(Prof. Dr. Herbert J. Buckenhüskes, unknown)

Several other components can be produced in the system that gives the sausages, *e.g.,* grey colour during storage. Most often is the reaction when products are exposed to light and oxygen the reasons for loss of colour. Fe^{2+} in nitrosohemochrome is oxidised to Fe^{3+} during this process and gives a grey/brown colour (Doyle and Buchanan, 2012).

3.7.6. Spices/seasoning

Spices are added to sausages mostly to add flavour, some colour and in some cases a preservative effect. Typical flavours used are garlic, salt, pepper, paprika, whole mustard seed, and coriander. Types of seasoning vary depending on the type of sausage (Viuda-Martos et al., 2010).

Since too much salt is a problem, the industry is looking for natural alternatives to produce sausages with less salt. Viuda-Martos et al. (2010) examined the use of herbs and spices as a substitute for salt and the benefit for health. Hotchkiss (2012) looked at the use of seaweed as

a salt replacer, while Devlieghere et al. (2009) and Minasian (2011) looked at other opportunities. All agreed that a replacement with herbs, spices, and seaweed could lead to advantages such as higher fibre, antioxidants and mineral component, but may also have microbial effects in sausages. The additions can improve the daily intake of such products among customers with low-levels of vegetable consumption, and enhance the "healthy" image of meat products (García-Lomillo et al., 2017).

3.8. Production of dry fermented sausages

The sausages are prepared by mixing chopped meat of the desired composition with pig back fat (animal fat). Then sugar and starter culture (or components to lower pH) are added. Since muscle composition varies between animals and species, the pH and the nature of the raw batter will depend on the quality of the meat. The intramuscular fat may also interfere with the dehydration process, and the microbial flora can vary between different meats. Research also indicates that type and amount of fat can affect microbial processes (Toldra et al., 2014).

Finally, salt, spices and seasoning ingredients are added, and after mixing, the meat paste is stuffed into casing of various diameters, either made from animal intestines, or artificial made of collagen or other polymers. The sausages are hung vertically in a ripening chamber for the desired time. Some smoke their sausages. This is done during the first few days of fermentation to improve flavour and increase shelf life. During fermentation the temperature is usually between 15 and 35 °C to reach the desired level of acidity (pH=4.8-5.3). The temperature is one of the important factors determining microbial performance, and this affects the choice of starter cultures. In Europe, 20-26 °C is often used during fermentation and this implies species that tolerates temperatures below/approx. room temperature (mesophilic strains). After fermentation, air velocity is maintained at a relative high humidity (often 65-90 %) and the sausages typically lose 25-30 % of their weight during drying. The temperature and humidity is important to avoid surface moisture. If the environment is too humid, the sausage can become slimy, and if the climate is to dry, a crust will cover the sausages edges and enable both drying and proper fermentation.(Kumar et al., 2017, Vignolo et al., 2010).

3.9. Textural changes during mincing, fermentation and drying

During mincing, fermentation and drying several changes happen in the sausages. When salt is added to processed meat the solubilisation of functional myofibrillar and sarcoplasmic protein begins. Salt activates proteins to increase hydration and water-binding capacity, and thereafter increasing the mincing properties to improve texture.
WHC is defined as the ability of meat to hold all or part of its water when external forces (*e.g.* heat, additives, and gravity*)* are applied. WHC is important because it reduces cook loss and increases tenderness and juiciness of the meat product In dry fermented sausages a to high water holding capacity is not desired as the water should evaporate. (Honikel, 1987, Desmond, 2006).

The salting in is an effect when the ionic strength of a solution increases the solubility of some solutes such as protein. The effect can be explained by the Debye-Huckel theory where proteins are surrounded by the salt ions of opposite net charge, and when the free energy of the protein decreases, and the activity of the solvent increases, leads to increased solubility. The effect leads to compounds like lactic acid equilibrium in the sausage. The effect also

makes the added salt to have a higher level at the end of the drying process when producing dry fermented sausages. (Paz et al., 2004, Pérez-Juan et al., 2007, Rabe et al., 2003, Offer et al., 1989)

The decrease in pH and the amount of salt is important for the texture of fermented meat products. A protein gel is defined as a structure that is intermediate between a solid and a liquid state, consisting of cross-linked chains that create a continuous network in a flowing medium. During fermentation meat protein (myofibrillar) denatures and coagulates, and a significant increase in hardness is observed.(Toldra et al., 2014, Horita et al., 2014a, Toldrá and M Barat, 2012).
According to Toldra et al. (2014) myosin solubilisation is satisfactory in meat products with 2 % added NaCl, and extremely good when the initial salt content is around 4 %, although this can lead to very salty products.

During ripening the coagulated proteins starts to release water. Drying is an essential part of ripening, as the decrease in water activity is essential to obtain a product with long shelf life. The remaining water is bound more tightly and more proteins denaturize and degrade because of endogenous and microbial enzymes. Water release gives the sausages a denser, chewier, and more firm texture. It is a delicate balance between processes that contribute to hardening and processes that contribute to softening or protein breakdown by proteolytic enzymes. The final product should be a sausage that is stiff and coherent and can be sliced into thin slices without problems (Horita et al., 2014a, Toldra et al., 2014).

Production of sausages is a traditional craft and sausage makers often use gel formation, *i.e.* the increase in hardness, as an estimate for how far the fermentation has proceeded. They simply squeeze the product and feel the change in texture (Toldra et al., 2014).

3.10. Fermentation by starter cultures

Fermentation is one of the oldest traditions in food processing. Fermentation is used to achieve good flavour and aroma and to extend shelf life. Fermentation is defined as an energy-yielding microbial metabolism in which an organic substrate, often a carbohydrate, is incompletely oxidised, and an organic carbohydrate acts as an electron acceptor in the absence of, or in the presence of only low oxygen. In fermented sausages, starter cultures are used to lower pH and give flavour, which together with the added salt extend shelf life and ensure a safe product. (Leroy and De Vuyst, 2004, Kumar et al., 2017).

Lactic acid bacteria (LAB) are the most common food fermenters due to their safe metabolic activity and ability to rapidly produce organic acids (lactic acid, acetic acid and others). LABs are generally recognised as safe (GRAS). The most frequently used LABs in European sausages are strains of *Lactobacillus*, *Streptococcus, Lactococcus*, *Bifidobacterium*, *Pediococcus* and *Leuconostoc*. In addition, the starter cultures usually contain micrococci such as *Enterococcus* spp. or coagulase-negative cocci (CNC; mostly *Staphylococcus* spp. and *Kocuria* spp.)(Ammor and Mayo, 2007, Kumar et al., 2017, Leroy and De Vuyst, 2004).

Lactobacilli spp. such as *L. sakei* generally characterise European starter cultures. They are facultative heterofermentative and may be active down to 4 °C and at high salt concentration (3-9 % NaCl). In addition to the acid production, they may have catalase activity

(decomposition of hydrogen peroxide), contribute to flavour development, metabolize some amino acids, and produce bacteriocins.

Bacteriocins are small peptides produced by some LABs that either inhibits growth of- or kill bacteria closely related to the bacteriocin-producing bacteria. The peptides vary from small (< 3kDa), heavily post-translationally modified peptides to large heat-labile proteins.
The use of bacteriocins is the ability to inhibit undesirable (pathogen, contaminant, spoilage bacteria) organisms to enhance food safety and extend shelf life. Bacteriocin-producing strains can be used directly in food like in starter cultures (*e.g. L.sakei*, and *L.plantarum* has this ability) or added as co-strains. (Toldra et al., 2014)

The homofermentative LABs (typically species of *Pediococcus, Lactobacillus, Lactococcus, Streptococcus*) lower pH by converting sugar into lactic acid through Embden-Meyerhof-Parnas pathway (EMP). One mole of glucose is converted to two moles pyruvate and then reduced to lactic acid. (Figure 3.10-2)
Transport and phosphorylation of sugars occur by free glucose or by the phosphoenolpyruvate phosphotransferase system (PEP: PTS). The system phosphorylates the sugar to for *e.g.* glucose-6-phosphate. Some species of LAB use only the PTS system for transport of galactose; others use the PTS for all sugars (Figure 3.10-1). (Salminen and Von Wright, 2004, Harutoshi, 2013)

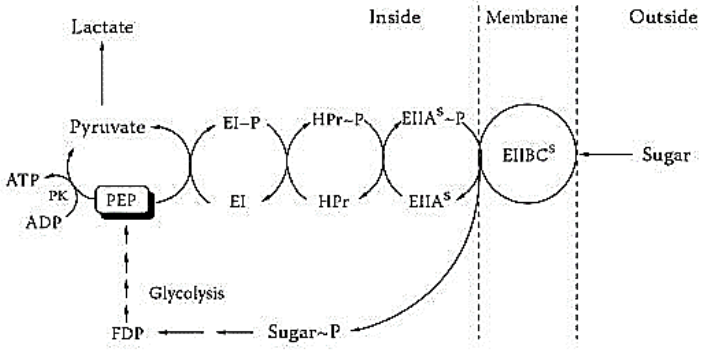

Figure 3.10-1: The pathway for phosphoenolpyruvate phosphotransferase system (PEP: PTS) (Salminen and Von Wright, 2004)

During heterofermentative fermentation (among others *Leuconstoc* spp. and some *Lactobacilli* species) products like acetic acid, ethanol and carbon dioxide are formed via the pentose phosphate pathway (HMP). One mole of glucose-6-phosphate is dehydrogenated to 6-phosphogluconate and decarboxylated to yield one mole of CO_2. Pentose-5-phosphate is cleaved into one mole glyceraldehyde phosphate (GAP), and one mole acetyl phosphate after this lactate metabolises as in homolactic fermentation. (Figure 3.10-2)(Harutoshi, 2013, Salminen and Von Wright, 2004)

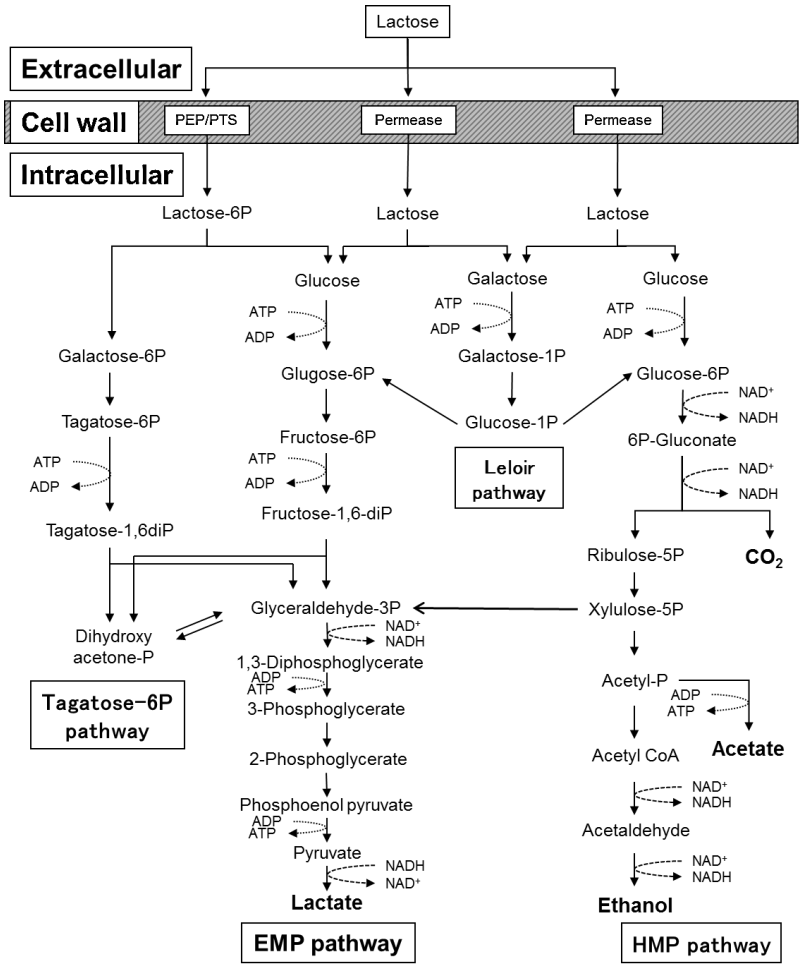

Figure 3.10-2: Pathway for homolactic (EMP) and heterolactic (HMP) acid fermentation in lactic acid bacteria. Different compounds produced gives the sausages its special flavour. (Harutoshi, 2013)

Lactic acid formation in combination with salt, prevent the sausage from spoiling due to unwanted microbial growth. Later various aromatic components are produced during the ripening process, *e.g.* acetic acid is necessary in small amounts for full dry sausage flavour, but in higher concentrations produces a astringent flavour (Leroy et al., 2006). Here species of micrococci also play an essential role. The starter culture give the sausage its characteristic traits such as colour (due to the reaction of nitrate as pH drops) and taste. The compounds produced depend on, among others, the starter culture (*i.e.* type microorganisms added), the raw material, and processing conditions (Kumar et al., 2017, Leroy et al., 2006, Leroy and De Vuyst, 2004).

Fermentation with LABs should yield lactic acid as the main product. Stress conditions such as temperature, salt and additives, can lead to changes in the end products from fermentation. Fermentation like the citric metabolic pathway or pyruvate metabolism are pathways often used by lactic acid bacteria used in the cheese- industry to achieve a buttery/nut and creamy flavour, but is unwanted in meat products.
In the citric acid metabolic pathway citrate is converted (mainly by enzymes that lead to oxidation) into succinate, lactate, acetate, ethanol or acetoin as alternative end products (Figure 3.10-3).

Known bacteria for this pathway include *Leuconostoc spp*. and *Lactococcus lactis*. (Gänzle, 2015, Hugenholtz, 1993).

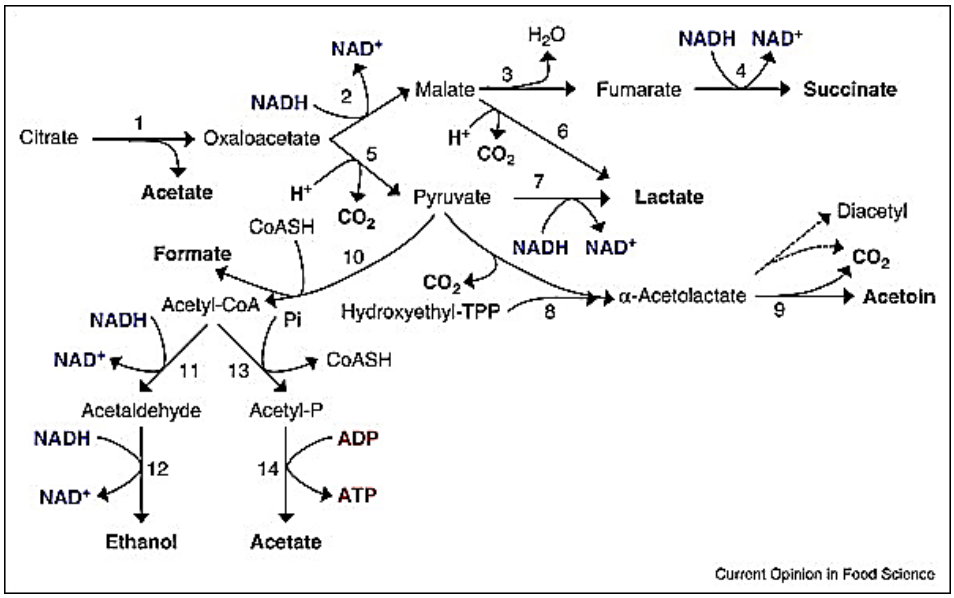

Figure 3.10-3: Alternative fates of citrate in heterofermentative lactic acid bacteria. Citrate is converted to succinate to achieve regeneration of two reduced cofactors, or to acetoin to achieve regeneration of two reduced cofactors. Citrate conversion to lactate or acetate and ethanol combines oxidation of one mole NADH and one carboxylation reaction. Enzymes converting are indicated by numbers: 1, citrate lyase; 2, malate dehydrogenase; 3, fumarate hydratase; 4, succinate dehydrogenase; 5,oxaloacetate synthase; 6, malolactic enzyme; 7, lactate dehydrogenase; 8, acetolactate synthase; 9, acetolactate decarboxylase; 10, pyruvate formate lyase; 11, acetaldehyde dehydrogenase; 12, alcohol dehydrogenase; 13, phosphotransacetylase; 14, acetate kinase. (Gänzle, 2015)

The pyruvate pathway (pyruvic acid pathway) has several alternative pathways for metabolism of pyruvate (Figure 3.10-4). Specific LABs (*e.g. Lactobacillus casei*, *Lactococcus lactis*), have under substrate limitations, an activated pyruvate-formate lyase system, resulting in a mixed acid fermentation between homo- and heterolactic fermentation. The end products of the pathway are lactate, acetate, formate, diacetyl, 2.3-butanediol and ethanol. (Axelsson and Ahrné, 2000, Salminen and Von Wright, 2004).

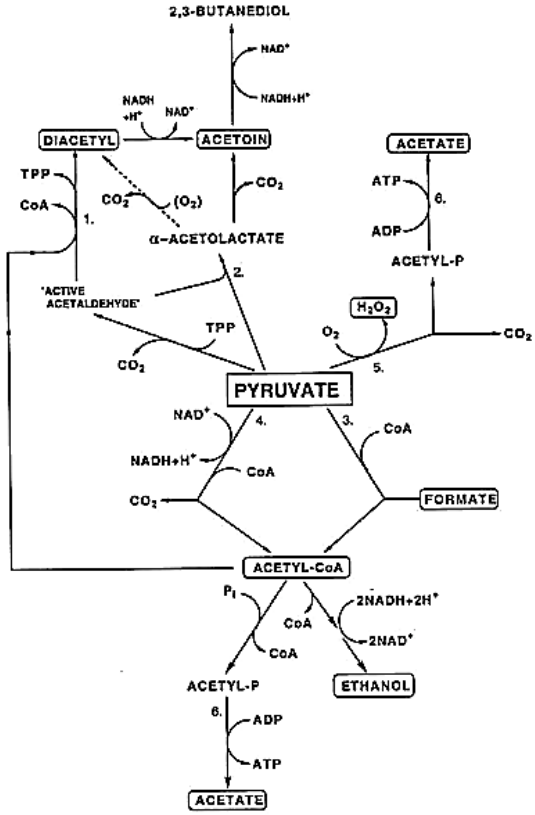

Figure 3.10-4: The figure shows pathways for the alternative ways fates of pyruvate. Enzymatic reactions are numbered: 1, diacetyl synthase; 2, acetolactate synthase; 3, pyruvate-formate lyase; 4, pyruvate dehydrogenase; 5, pyruvate oxidase; 6, acetate kinase. (Salminen and Von Wright, 2004) Figure: (Axelsson and Ahrné, 2000)

3.10.1. Different compounds produced by starter cultures

Coagulase-negative cocci and *Kocuria* species often participate in desirable reactions during ripening such as colour stabilisation, decomposition of peroxides, proteolysis, and lipolysis. Bacteria can further convert components produced during lipolysis and proteolysis to aroma components. (Leroy et al., 2006).
Leroy et al. (2006) and Montel et al. (1996) both stated that microbial proteolytic activity of meat proteins is low during ripening of fermented sausages, but some peptides are generated by proteolysis. In the same article, lipolysis was described, where release of free fatty acids by lipolysis mainly by tissue lipases, generated many aroma components.

Lactobacilli, Enterococci, and catalase-positive cocci as *Kocuria salsicia*, often contribute metabolic activities such as proteolytic and lipolytic activity. In fast ripening sausages, a higher inoculum level of *Staphylococci sp.* may increase production of methyl-branched aldehyde components like Leu (3-methylbutanal), Ile (2-methylbutanal) and Val (2-methylpropanal). These are key aroma compounds of fermented sausages. Species of *Micrococcaceae* plays an essential role in the development of aldehydes.

Many articles have been published on how different starter cultures affect flavour in sausages,
El Adab et al. (2015) found that the composition of the starter culture had a significant impact on pH and the hygienic quality. The results showed that coagulase-negative *Staphylococci*

and LAB were the dominant bacteria and that acidifying activity outgrew more or less *Enterobacteriaceae*. The decrease in pH differed between different starter cultures, but the textural differences were not significant for the tested starter cultures.
Essid and Hassouna (2013) researched *Staphylococcus xylosus* and *Lactobacillus plantarum* as a starter culture and tested various biochemical, microbial and sensorial tests. The bacteria were found to improve sensorial characteristics of fermented sausages. The same article stated that they found high concentrations of the free amino acid tyrosine that contributes to sweet and bitter taste, followed by glutamic acid and aspartic acid associated with fresh taste and alanine that can be associated with sweet taste. *Staphylococcus carnosus* and *Lactobacillus sakei* (found in starter culture LS-25 and LK-30) were tested in another article Mejri et al. (2017) found a lot of the free amino acid alanine (sweet taste) and glutamine (often related to umami taste) at the end of ripening.
Well-selected strains generate different aroma component, and selection of strains should be after the type of sausage desired. (Ammor and Mayo, 2007)

3.11. Dry fermented sausages with reduced salt content

3.11.1. Food safety when salt is reduced

Food with high water content will spoil during storage due to microbial growth and may also become dangerous to consume due to growth of pathogenic bacteria. All species of micro-organisms have a minimum water activity under which they are unable to grow (See section 3.7.2). In dry fermented sausages, addition of NaCl and the drying process are the main factors for lowering water activity (Taormina, 2010).

In addition to a low water activity, good hygiene during production, low pH, and packaging method (*e.g.* modified atmosphere, vacuum) are important factors to ensure a safe product with long shelf life. Additives such as spices may also contribute. Dry fermented sausages are considered safe when the water activity is below 0.90 since most undesired bacteria can't grow under this limit.

Because water activity is such a critical factor for dry fermented sausages, reduced salt addition is a challenge. Important factors when reducing salt is the drying conditions, hygiene, and additives (Desmond, 2006).

3.11.2. Technological and sensory changes when reducing salt

Salt reduction affects not only the microbiological aspects, but also flavour and texture. Salt is one of the primary tastes by humans. The only cations that have primarily salty taste are sodium and lithium. Potassium and calcium have some components of saltiness in their taste, but they have another flavour, often described as bitter or metallic. When the size of the anion associated with sodium increases, the perceived saltiness decreases. (Doyle and Glass, 2010, Desmond, 2006).

The preference for salty food is adaptive. Food with high concentrations of salt is often preferred by people used to eating more salt, while people used to eat less salt prefer food with low concentrations of salt. Consumers can get used to food with less salt, but the change must be gradual. A person used to eat products with lots of salt will often find products with less salt tasteless. (Mattes, 1997, Ofstad R, 2015, O'Mahony, 1979).

NaCl and monosodium glutamate (MSG) are known as taste enhancers. They enhance other flavours in food than themselves. Salt is also known for its ability to suppress or mask bitter flavours. In products containing KCl, which is known for its bitter/metallic taste, this may be masked by adding some NaCl (Saint-Eve et al., 2009).

Not everyone tastes bitter compounds as intense as others. About 25 % of the population of the world are non-tasters and have a low sensitivity to bitter compounds (Doyle and Glass, 2010, Cummings and Starr, 2003). Because of this, reducing salt may affect one-fourth of the population, by making the food almost tasteless. Another fourth of the population may not notice the change.

Salt affects texture and processing in foods (See section 3.9). Ruusunen and Puolanne (2005) state that the possibility to produce sausages with low salt depends on the formulation of the meat mixture and the used conditions, but down to 2 % added salt was possible regarding gel strength.
Zanardi et al. (2010) found that 40 % (3.5-5.2 g/100 g initially) reduction of NaCl in Cacciatore (Italian salami) was possible regarding pH, a_w, free fatty acid composition and sensory attributes when adding some amount of salt replacers like KCl.
Corral et al. (2013) reduced salt (NaCl) down to 2.26 % in slow fermented sausages, but this affected aroma, taste, juiciness and overall quality of the sausages.
Aaslyng et al. (2014) studied how 45-50 % reduction of salt (to a measured NaCl level of 1.9 %) in salami affected sensorial and microbial properties. They found that salami is very sensitive to salt reduction and simply reducing the NaCl content without any substitutes, affected the sensory properties as well as the microbial counts.

3.11.3. Potassium chloride (KCl) as a salt replacer

K^+ (atom weight = 39.1) is a larger ion than Na^+ (atom weight = 23.0) and replacing NaCl with an equal amount by weight of KCl will lead to a reduced number of dissolved ions (colligative units) per volume and thus an increased water activity in the product. To maintain the same number of dissolved ions per volume, NaCl must be replaced by KCl on a molar basis, *i.e.* one mol NaCl must be replaced by one mol KCl, or by weight 1.00 g NaCl must be replaced by 1.28 g KCl (Greiff, 2015).

KCl addition is regulated by EC No. 1333/2008 by the European Parliament, and can be added "*quantum satis*" to food, *i.e.* in the necessary amount for its function, but not higher (EU Commission, 2011). KCl has been shown to be a good replacer for NaCl because of its similarities to NaCl with respect to antimicrobial effect on pathogenic bacteria (Toldrá and M Barat, 2012).
Gelabert et al. (2003) studied the partial substitution of NaCl with KCl and other salt replacers, and found that KCl did not affect microbial flora more than addition of NaCl. They found major flavour defects, a bitter taste, when exceeding a replacement of 30 %.

Guàrdia et al. (2008) and Corral et al. (2013) both studied how the taste was affected when different amounts of KCl partially replaced salt in sausages. They found that replacement of up to 30 and 50 % of NaCl with KCl was possible. In sensorial tests, the meat mixtures with KCl were rated as almost similar to the controls with NaCl.

Aaslyng et al. (2014) found that replacement of NaCl with a small part of KCl was not crucial for product acceptance, but they did not know how it would affect the microbial growth.

3.11.4. Health aspects of replacing NaCl with KCl

Several studies (Desmond, 2006, Ruusunen and Puolanne, 2005, Gelabert et al., 2003, Corral et al., 2013, Campagnol et al., 2011) have shown that KCl has many of the same properties as NaCl, but technical aspects regarding water activity and taste is a problem. A replacement of KCl is possible, and a reduced intake of sodium even by increasing the intake of potassium has been shown to could reduce blood pressure for individuals with hypertension and thus the risk of stroke. In Norway, approx. 13 000 (2014) individuals suffered from acute stroke each year. (Steffensen et al., 2018, Frølich et al., 2014).

The Norwegian Scientific Committee for Food Safety (VKM) did a risk assessment of KCl regarding public health in 2014. For healthy people addition of KCl to food in the levels assessed was regarded as safe. For vulnerable groups (people with different kinds of kidney, and, heart problems, infants below one year of age, and elderly people) the intake of potassium was considered as a higher risk. In 2013, the number of people aged 85 years and older was 113 700, and the number is expected to increase in the coming years (Frølich et al., 2014, Steffensen et al., 2018).
VKM concluded in 2014 that it was reasonable to anticipate that the percentage of persons likely to face an increased risk was far greater than the percentage of persons likely to benefit from this measure. (Frølich et al., 2014).

Use of KCl is allowed in Norway, but since VKM not has agreed to its safety, most manufacturers do not use it yet. England's scientific advisory committee on nutrition (sacn) was until recently negative to the use of KCl in food due to the increased health risk for vulnerable groups. However, in 2017 the committee did a risk assessment where they weighted the increased risk of health problems for people with kidney problems, with the reduction in health-related risks for the general population due to a lower sodium intake. The new assessment recommended that the government should encourage food companies to explore the use of potassium-based sodium replacers so that the sodium levels in food could be reduced (Scientific Advisory commitee on nutrition, 2017).

4. Materials and Methods

4.1. Starter cultures

Four commercial freeze-dried starter cultures with different properties (Table 4.1-1) were used in the studies.

Table 4.1-1: Overview of the starter cultures used during the studies.

Starter culture	Recom-mended addition (g/100 kg meat)	Bacteria	Properties according to the producer	Producer, LOT no.
Bitec LS-25	25	Lactobacillus sakei Staphylococcus carnosus	Fast raw sausage fermentation. Fast pH-drop. Highly competitive.	Frutarom, Bitec, 247158
Bitec Mild & Fast[A]	25	Uknown	Mild and fast acidification, for mild and harmonious fermentation aroma even in lower pH-levels	Frutarom, Bitec, T1150930T0000
Bactoferm T-SPX	25	Pediococcus pentosaceus *Staphylococcus xylosus*	Mild acidification and good flavour and colour development.	Chr.Hansen, Bactoferm, 501095
Bitec LK-30	25	L. sakei S. carnosus *Kocuria salsicia*	Slow raw sausage fermentation. Harmonic pH-drop. Highly competitive.	Frutarom, Bitec, 483815100.001

A The starter culture got new name in 2018: Old name Bitec MSC 10271 and this is the one tested in this thesis, but the new name was used.

In this thesis the starter cultures are named by Bitec LK-30=LK-30, Bitec LS-25=LS-25, Bactoferm T-SPX=T-SPX, and Bitec B Mild & Fast= Mild & Fast from now if deemed necessary.

The four starter cultures tested in this thesis were recommended for use in dry fermented sausages. It was desired to have one slow fermenting starter culture that might produce more aroma compounds and a fast fermenting starter culture lowered pH fast. In this study the focus was to study the effect on the starter cultures when salt was reduced on behalf of taste compounds, fermentation time *e.g.*

In Preliminary study 1, the added amount of starter was according to the manufacturer's recommendations. The starter cultures were dispersed in 10 ml of ion-free water held at 30 ° C. In preliminary study 2 and 3, the starter cultures were added in the same way, but the temperature of the water was 21.0 ° C. In preliminary study 4, the LS-25 was added as recommended by the manufacturer, but dispersed in 30 ml of ion-free water at 21.0 °C. However, starter culture in the same study LK-30 had behaved somewhat variable in the previous trials with variable growth rate and slower than expected. It was therefore added to a reactivation medium. Reactivation medium was prepared according to Figure 4.1-1.

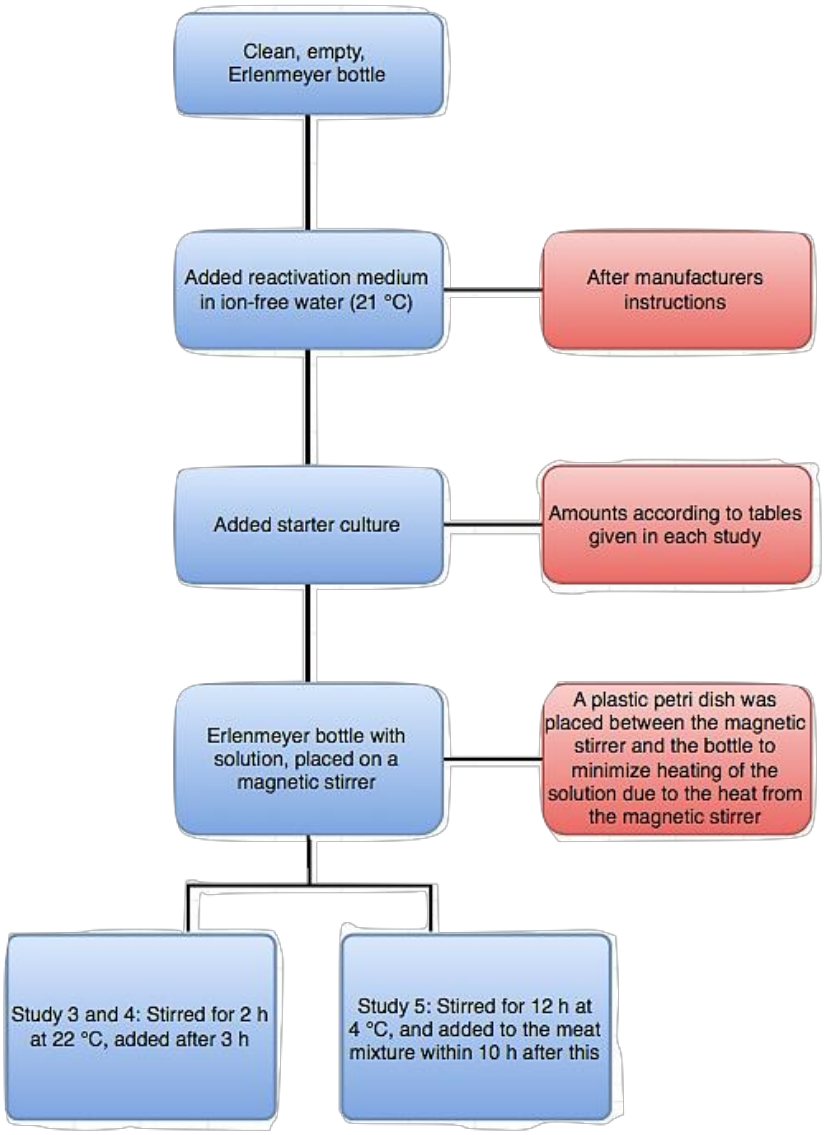

Figure 4.1-1: Flow chart for the use of reactivation medium when inoculating starter cultures.

4.2. Media, buffers and solutions

4.2.1. Sodium nitrite solution

The nitrite solution contained 0.95 g sodium nitrite in 100 ml ion-free water. Per 100 g chopped meat, 1.0 ml was added.

4.2.2. Diluted acids

4.2.2.1. Hydroclorid acid

Hydrochloric acid was used to test the buffer capacity of the meat. Hydrochloric acid (12.1 M) was diluted with ion-free water to 0.500 M.

4.2.2.2. Percloric acid

Perchloric acid (70%) was diluted with ion-free water to 0.6 M.

4.2.2.3. Saline solution (0,9 %)

Saline solution (0.9 %) was made by dissolving 9.0 g NaCl in 1000 ml ion-free water and sterilized at 121.0 °C for 15 minutes.

4.2.2.4. Microbiological media

The media used, de Man Rogosa Sharpe Agar pH 5.5 (MRS agar), Tryptic Soy Agar (TSA), and de Man Rogosa Sharpe Broth (MRS broth) were prepared according to manufactures instructions.

4.3. Chemicals, producers of all used Equipment

Producer of all ingredients, chemicals, and equipment expect analysis equipment is mentioned in Table 4.3-1.

Table 4.3-1: Producer and lot-number for ingredients, chemicals and one-time use equipment in the study.

Starter cultures	***Producer, LOT no.***
Bitec LS-25	Frutarom, Bitec, 247158
Bitec MSC 10217 [A]	Frutarom, Bitec, T1150930T0000
Bactoferm T-SPX	Chr.Hansen, Bactoferm, 501095
Bitec LK-30	Frutarom, Bitec, 483815100.001
Chemicals	***Producer, LOT no.***
NaCl	Merck, 1.06404.1000
KCl	Sigma-Aldrich, 31248
Dextrose	Arne B. Corneliussen, 5154342
Sodium nitrite	Fluka, 71759
Curing salt	GC Rieber, Part number: 14101
Reactivation medium	Gewürzmüller, 227187
Hypochlorite cleaning solution	Hach, Renovo.X, 16077
Perchloric acid 70 %	Riedel-deHaën, 30755
Hydrochloric acid 12.1 M	Honeywell, Fluka, 52BG2610H
De man, Rogosa, Sharpe agar (MRS agar)	Oxoid CM0361, 1885141
Tryptic Soy Agar (TSA)	Difco 236950, 3164358
De man, Rogosa, Sharpe Broth (MRS broth)	Oxoid CM0359, 1823477

L (+) Lactic acid (2-hydroxypropionic acid, Lithium salt) 97 %	Sigma, 33H5706
Sodium acetate ≥99.0 %	Sigma-aldrich, BCBK9019V
Buffer solutions for pH calibration (4, 7 and 10)	Merck, pH 4: 1.09435.1000, pH 7:1.09439.1000, pH 10: 1,09438.1000
D-glucose	Amresco, 0188, 3075C3111
Na-acetate	Amresco, S8750, 078K0157
Na-citrate	Amresco,S32320, SBZA0450
Lactic acid (Li-lactate salt)	Amresco, L2250, 33H5706
Na-pyruvate	Applichem, A3512, AP001784
Na-succinate	Applichem, S2378, 028K0668
Sodium ionic strength adjustor (ISA)	Thermo Fisher Scientific, Waltham, MA, USA, 841111
Equipment	***Producer, lot no.***
2.0 ml microtube	Sarstedt, 7080611
50 ml centrifugal tubes (Cellstar tubes 50 ml)	VWR, Greater bio one, E17123GR
120 ml tube	Sarstedt, 7459211
Acrodisc 13mm, .,2 µm GHP membrane	Pall corporation, FC5548
1.5 ml amber glass (1.5 ml short tread vial amber glass)	Matriks, 887040363315
50 mm sterile needle (Sterican hypodermic needle 1.10 * 50 mm)	B.braun melsungen,16082338
Nitrogen 6.0	Aga 6.0, UNI1066
Aerocult A	Merck, 1.13829.0001
Nunc Cryotube vials 1.8 ml	Thermo scientific, 8243612
Casing 61 mm	Fibrous, Reg N58X25

A The starter culture got new name in 2018: Old name Mild & Fast and this is the one tested in this thesis.

4.4. Preparation of meat mixtures and sausages

4.4.1. Meat mixtures in the model for observing fermentation in the preliminary study 1-4

Preparation of meat mixtures was prepared according to the flow diagram showed in Figure 4.4-1.

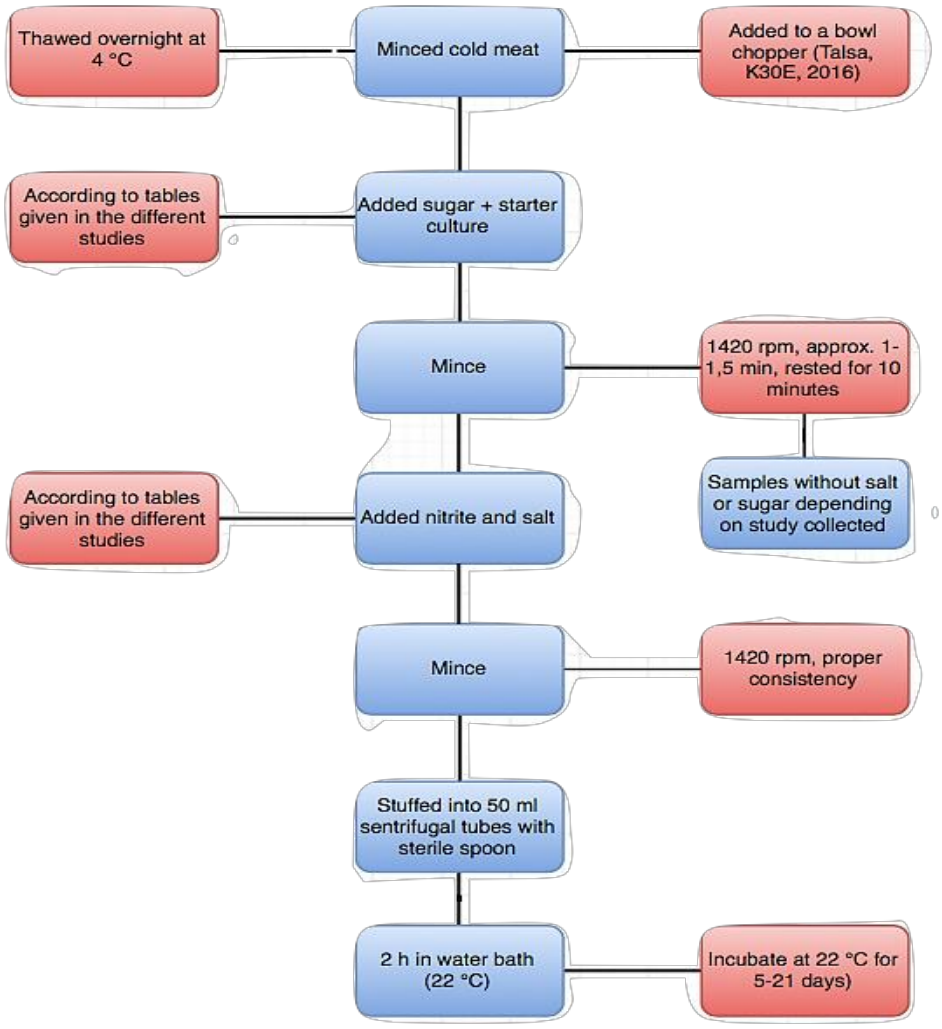

Figure 4.4-1: Flow diagram of how meat mixture was produced in preliminary study 1-4.

After the first experiment, the temperature in the water bath was lowered from 23.0 °C to 21.0 °C. The incubator temperature was set at 23.0 °C, but a temperature check with a thermometer indicated that the actual temperature was 21.5-22.0 °C.
Between different starter cultures, the equipment was thoroughly washed and disinfected. Three tubes (parallels) from each treatment were analysed for water activity (A_W) and dry weight. Meat mixtures for analysis of organic acids and sugar (HPLC) was collected from one tube from each treatment. pH was measured in the same three tubes (parallels) several times but in different places. Colour was observed every day in all tubes until achievement of a solid colour.

4.4.2. Preliminary study 1

Four different starter cultures were combined with six different salt concentrations (plus one control without salt) in this study. The procedure is described in section 4.4.1. Chopped meat was obtained from a local producer. Ingredients added as listed in
Table 4.4-1 .

Table 4.4-1: Ingredients used in the preliminary study one

	Ingredient added / 100 g meat mixture					Meat composition		
Sample id	Dextrose (g)	Starter culture (mg)[AB]	Nitrite solution (ml)[D]	NaCl (g)	KCl (g)	Beef frozen 5 % fat (kg)	Pork frozen 6 % fat (kg)	Back fat (kg)
3.00 g NaCl +1.28 g KCl	0.2	25	1.0	3.0	1.28	4.30	4.24	0.75
4.0 g NaCl	0.2	25	1.0	4.0		4.30	4.24	0.75
3.00 g NaCl[C]	0.2	25	1.0	3.0		4.30	4.24	0.75
3.83 g KCl [C]	0.2	25	1.0		3.83	4.30	4.24	0.75
2.0 g NaCl	0.2	25	1.0	2.0		4.30	4.24	0.75
2.56 g KCl	0.2	25	1.0		2.56	4.30	4.24	0.75
0 g added salt	0.2	25	1.0	0	0	4.30	4.24	0.75

A: Three parallels meat mixtures were made containing the exact content.

B: The tested starter cultures were: LS-25, LK-30, Mild & Fast, T-SPX

C: Starter culture was added by suspending 25 mg in 10 ml water

D: Concentration of the sodium nitrite solution was 9.5 mg $NaNO_2$ solved in 100 ml water.

Three tubes were filled with meat mixtures containing 3.00 g NaCl/100 g minced meat and 3.83 g KCl/100 g minced meat, while for the other salt concentrations, only one tube was filled with each sample. A total of 240 tubes were filled. Meat mixtures for analysis taken after two hours in the water bath were defined as the starting point (0 hours). In later studies, the first meat mixtures (0 hours) were taken *before* incubation in the water bath to minimize fermentation changes due to the added starter cultures.

4.4.3. Preliminary study 2

In this study, starter culture LS-25 was tested with different amounts of dextrose. The preparation procedure was the same as above (section 4.4.1). A total of 4 tubes were filled. Chopped meat mixture from a local producer (composition given in section 4.4.2) was thawed over night at -4.0 °C, and ingredients were added according to Table 4.4-2.

Table 4.4-2: Ingredients used in the preliminary study two

Added ingredient / 100 g meat mixture				Meat composition		
Added dextrose (g)[A]	Starter culture [BC]	Sodium nitrite solution (ml) [D]	NaCl (g)	Beef frozen 5 % fat (kg)	Pork frozen 6 % fat (kg)	Back fat (kg)
0	25	1.0	3.00	4.30	4.24	0.75
0.2	25	1.0	3.00	4.30	4.24	0.75
0.3	25	1.0	3.00	4.30	4.24	0.75
0.4	25	1.0	3.00	4.30	4.24	0.75

A: It was made one tube of each concentration of dextrose

B: Starter culture was added by suspending 25 mg in 10 ml water

C: The starter culture inoculated was LS-25

D: Concentration of sodium nitrite solution was 0.5 mg $NaNO_2$ solved in 100 ml water

4.4.4. Preliminary study 3

Two starter cultures were combined with different amounts of dextrose in this study. The procedure from this point onward was the same as described above (section 4.4.1). Composition was confidential, but had many similarities as the meat mixtures used in the other preliminary studies. Ingredients added to the meat mixtures (except sugar) are listed in Table 4.4-3. A total of 90 tubes was filled.

Table 4.4-3: Ingredients added in preliminary study three.

Added ingredient / 100 g meat mixture			
Added dextrose (g)[A]	Starter culture (mg)[BC]	Sodium nitrite solution (ml)[C]	NaCl (g)
0	25	1.0	3.00
0.2	25	1.0	3.00
0.4	25	1.0	3.00
0.6	25	1.0	3.00
0.8	25	1.0	3.00

A: Three tubes containing the exact same content was made, as well as three tubes of each for withdrawal (3x3) for each concentration of dextrose.

B: Starter culture was added by suspending 25 mg starter culture in 10 ml water

C: The inoculated starter cultures was LS-25 and LK-30

D: Concentrations of the sodium nitrite solution was 0.5 mg $NaNO_2$ disolved in 100 ml water

Preliminary study three was repeated the same way twice except for that the meat was collected from two different batches (the same meat composition). pH was tested for a longer period in the first repeat than the second.
During both repeats, some meat mixtures produced gas during the fermentation (212 hours repeat 1, 36 hours repeat 2) and a sterile 50 mm needle was used to puncture the lid of the tubes to let gas out.

4.4.5. Test of reactivation medium

In the same study (preliminary study 3) was LK-30 (three parallel meat mixtures) cultivated with a reactivation medium as described in section 4.1. Three parallel meat mixtures were also cultivated by adding the starter culture directly to the meat mixture as well as 3 tubes of each for withdrawal. A total of 18 tubes was filled. Ingredients are given in Table 4.4-4. The procedure was the same as describes in section 4.4.1. Only meat mixtures from the second replicate was analysed for organic acids and sugar by HPLC.

Table 4.4-4: Ingredients used for test of reactivation medium in study three.

	LK-30 (mg)	Dextrose (g)	Sodium nitrite solution (ml)[D]	NaCl (g)	KCl (g)
Inoculated with reactivation medium[AB]	25	0.2	0.1	1.5	1.27
Inoculated without reactivation medium[AC]	25	0.2	0.1	1.5	1.27

A: Three parallels meat mixtures, three replicates

B: 25 mg starter cultures was suspended in reactivation medium

C: 25 mg starter culture was suspended in 10 ml water

D: Nitrite solution was made by 9.5 mg NaNO2 solved in 100 ml water

4.4.6. Preliminary study 4

Concentrations of salt, relevant for the dry fermenting sausage industry were tested with two commercial starter cultures.
The meat mixture used was confidential but had many similarities to meat mixtures used in this thesis. Three tubes for each concentrations of salt was made as well as three tubes for each replicates, a total of 72 tubes. The procedure was the same as described above (section 4.4.1). Ingredients were added as listed in Table 4.4-5.
Some gas was produced during the fermentation (LS-25 after 36 hours, LK-30 after 48 hours) and a sterile 50 mm needle was used to puncture the lid of the tubes to let the gas out.

Table 4.4-5: Ingredients used in study the preliminary study four

LS-25	**Ingredient added /10 kg meat**				
Sample id	**LS-25 (g)[A]**	**Dextrose (g)**	**Curing salt (g)[C]**	**NaCl (g)**	**KCl (g)**
Low NaCl	2.5	25	10	150	0
High NaCl	2.5	25	10	250	0
High KCl	2.5	25	10	150	127.6
Medium KCl	2.5	25	10	200	63.8
LK-30	**Ingredient added /10 kg meat**				
Sample id	**LK-30 (g)[B]**	**Dextrose (g)**	**Curing salt (g)[C]**	**NaCl (g)**	**KCl (g)**
Low NaCl	2.5	25	10	150	0
High NaCl	2.5	25	10	250	0
High KCl	2.5	25	10	150	127.6
Medium KCl	2.5	25	10	200	63.8

A: The starter culture was added by suspending 2.5 g in 10 ml water

B: The starter culture was added by suspending 2.5 g starter culture in 1.6 g reactivation medium in 50 ml water

C: Curing salt is NaCl containing 0.54-0.60 % $NaNO_2$

4.4.7. Test of reactivation medium repeat two

In the same study (preliminary study 4) was LK-30 (three parallel meat mixtures) cultivated with a reactivation medium as described in section 4.1. Three parallel meat mixtures with three replicates were also cultivated by adding the starter culture directly to the meat mixture, a total of nine tubes were filled. Ingredients are given in Table 4.4-6. The procedure was the same as describes in section 4.4.1.

Table 4.4-6: Ingredients used for test of reactivation medium in study four.

	LK-30 (mg) [B]	**Dextrose (g)**	**Sodium nitrite solution (ml) [C]**	**NaCl (g)**	**KCl (g)**
Inoculated with reactivation medium[A]	25	0.2	0.1	1.50	1.27

A: 3 x 3 tubes were filled with the same content for parallels and replicates during fermentation

B: 25 mg starter culture was suspended in the reactivation medium

C: Sodium nitrite solution was made by 9.5 $NaNO_2$ solved in 100 ml water

4.4.8. Main study 5

In this study, dry fermented sausages containing different amount of NaCl and KCl concentrations and two different starter cultures were produced at a local factory (Grilstad AS, Trondheim).

Frozen thawed beef 6% fat, pork 5% fat and back fat, 3.8, 3.8 and 2.1 kg, respectively was mixed in a bowl chopper (Ramon cutter AS-40 VAR, 2017, 3000 rpm. Next, sugar and starter culture were added to the chopper and mixed before salt and curing salt were added. Ingredients are listed in Table 4.4-7. Finally, the mixture was chopped to appropriate consistency

Table 4.4-7: Ingredients used in main study five

	Ingredient added /10 kg meat					Predicted salt content	
Sample id	**LS-25 (g)[A]**	**Dextrose (g)**	**Curing salt (g)[C]**	**NaCl (g)**	**KCl (g)**	**Predicted weight loss (%)**	**Predicted NaCl (%)[D]**
Low NaCl	3.0	40	10	150	0	40	4.2
High NaCl	3.0	40	10	250	0	30	5.0
High KCl	3.0	40	10	150	127.6	35	3.9
Medium KCl	3.0	40	10	200	63.8	35	4.6
	Ingredient added /10 kg meat					**Predicted salt content**	
Sample id	**LK-30 (g)[B]**	**Dextrose (g)**	**Curing salt (g)[C]**	**NaCl (g)**	**KCl (g)**	**Predicted weight loss (%)**	**Predicted NaCl (%)[D]**
Low NaCl	3.0	40	10	150	0	40	4.2
High NaCl	3.0	40	10	250	0	30	5.0
High KCl	3.0	40	10	150	127.6	35	3.9
Medium KCl	3.0	40	10	200	63.8	35	4.6

A: The starter culture was added by suspending 2.5 g in 10 ml water

B: The starter culture was added by suspending 2.5 g starter culture in 1.6 g reactivation medium in 50 ml water

C: Curing salt is NaCl containing 0.54-0.60 % $NaNO_2$

D: The salt content was predicted in final sausages using the program PROSIM (ver. 2016.3)

To minimize transfer of salts from one batch to the next, the mixtures were prepared in the sequence low NaCl- medium KCl. Batches added starter culture LK-30 were made first. Thereafter batches added LS-25 was prepared. To prevent contamination between the two starter cultures, all equipment was washed and disinfected between each culture.

Meat mixture with all ingredients was stuffed (Vemag sausage-linker 171, 2017) into casings (Fibrous Reg N58X25, 61mm) with a length of approx. 20-25 cm. tied and hung for fermentation. Sausages were fermented in a smoking chamber before decreasing the temperature and relative humidity. The sausages were dried in a drying chamber to the sausages reached $a_w < 0.90$. (The exact conditions during smoking and drying are confidential because this process was done in the facilities of the producer).

To follow the temperature-development, a temperature logger (Signtrol, SL52T) was inserted in different sausages, and hung at different places on the racks. Also, moisture and temperature were logged in the room using loggers (EasyLog, EL-USB-2-LSD+). The loggers showed that the measured values and the conditions set in the different chambers matched.

Meat mixtures for analysis were taken at start and at intervals during the fermentation and ripening.
At each sampling day, one sausage per treatment was taken out for analysis (except for the one sausage used to measure weight loss, the same sausage was weighted each time).
The experiment terminated when the water activity in the sausages reached $a_w < 0.90$.
Sausages were vacuum packed and stored in a climate room for substance to equalize for two weeks, before analysis for water activity, sensorial test, salt content and MS-analysis were taken.

4.5. Analytical procedures

4.5.1. Dry weight

Frozen meat mixtures were thawed overnight at -4.0 °C (Preliminary study 1-4), while in the main study five they were analysed immediately after sampling after their given time of fermentation/drying. Three parallels meat mixtures, each approx. 10.0 g, were collected from the middle of the tube/sausage and divided into smaller pieces to facilitate even and rapid drying. The meat mixtures were dried at 105 ± 5 °C for 23 ± 1 hours and allowed to cool in a desiccator for 1 hour before weighing. Some meat mixtures that didn't seem dry or that gave variations larger than 2.0 % between parallels were dried for another 23 ± 1 hours at 105 ± 5 °C and re-weighted.

4.5.2. pH measurements

The pH of the meat mixtures (preliminary study 1-4) was at the start (0 hours) determined with an HQ40d Portable pH meter with a probe for semisolid to solid material (Hach company, probe: PHC108). Performing of other measurements was with a PHM 210 pH meter with a probe fit for semisolid material (Radiometer, Copenhagen, Denmark, probe: PHC3359-8 11294-F10). In the main study five the HQ40d portable pH meter with a probe for semisolid to solid material (Hack company, probe: PHC108) was used for all meat mixtures. The pH meters were calibrated with three standards (pH 4.0, 7.0 and 10.0,) immediately before use.

The pH-probe were rinsed with ion-free water and inserted in the centre of the centrifuge tube or sausage. The probe was washed with a fat-dissolving dish wash solution and a hypochlorite solution, whenever it was deemed necessary during the measurements to ensure correct readings. The pH-probe was with ion-free water rinsed between every measurement

and pH was always measured in three parallel tubes. A total of 12 tubes were measured in the first test to see if pH varied depending on the location of the probe inside the tube, but the variations were small, and it was decided to measure pH in the centre and approx. half way down the tube. In the sausages (main study five) the same procedure was used but measurements of pH were at three sites in the sausage (Figure 4.5-1) rather than in three different sausages.

Figure 4.5-1: The crosses indicate where pH was measured approx. in the centre of the sausage. The length of the sausage is indicated on a sausage after drying.

4.5.3. Organic acids by high-performance liquid chromatography (HPLC)

A sample (5 ± 0.5 g) was collected from the core of the tube, transferred to a sterile tube, and added two parts ion-free water. *E.g.* was 5.0 g of sample added 10.0 g of water to a total volume of 15.0 g. The sample and water were mixed for 20 minutes by rotation (45 rpm). The meat mixtures were centrifuged (3220 RCF, 10 min, 21 °C, Eppendorf 5810 R), 800 µl of the water phase transferred to an Eppendorf tube containing 200 µl 0.6M perchloric acid, and stored cool (4 °C) for 24 hours. The Eppendorf tubes were then centrifuged (20817 RCF, 10 min., 4 °C, Eppendorf 5417 R) and an aliquot of the supernatant sterile filtered (0.2 µm) into a 1.5 ml short tread vial amber glass for HPLC analysis.

The meat mixtures were analysed on an Agilent LC1260 with an Aminex-HPX-87H (300*7.8mm) column at 45 °C using 5mM H_2SO_4 (0.6 ml/min) as eluent. A multiple wavelength detector (MWD) set at 210 nm (UV), and a refractive index (RI) detector were mounted in series. Meat mixtures were run for 40 minutes. Two standards were employed (Table 4.5-1). Both standards were placed first, in the middle and at the end of each run. Figure 4.5-2 shows how the standard was detected during a run with Refractive Index detector.

Table 4.5-1: Standards used for analysis for organic acid by HPLC

Standard 1	Molecular weight (g/mol)	Concentration (g/l)
Li-lactate	96.01	9.35
Standard 2		
Na-citrate	294.1	3.53
D-glucose	180.16	4.01
Na-pyruvate	110.05	3.76
Na-succinate	270.1	4.02
Li-lactate	96.01	3.67
Na-acetate	82.03	4.97

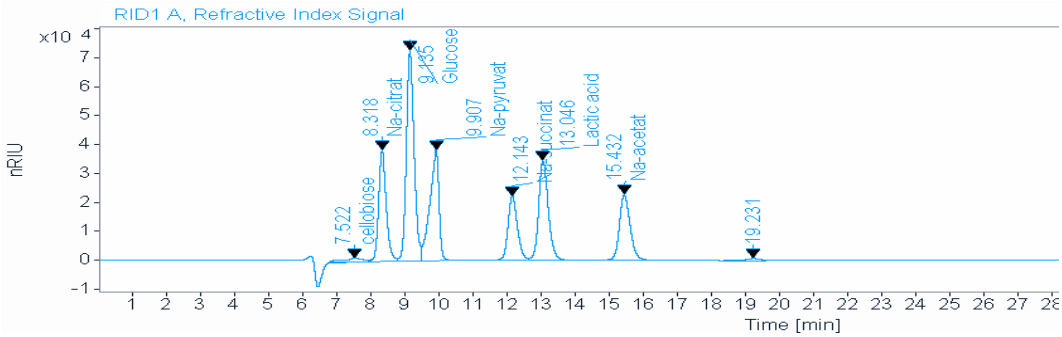

Figure 4.5-2: Example of standard two used for analysis for sugar and acids on HPLC. Figure shows na-citrate, glucose, na-pyruvate, na-succinate, lactic acid and na-acetate detected at different retention times (minutes). Some other peaks were (7.5 and 19.2 min) detected but in so small amounts that they are ignored.

4.5.4. HPLC extraction method test

Three meat mixtures of minced meat (composition not given, but the composition had similarities with meat mixtures used in this thesis) without any additions and three meat mixtures added known amounts of lactic acid (70 µmol/g meat) and Na acetate (10 µmol/g meat) were extracted and analysed by HPLC as described above, but only with the RI detector.

4.5.5. Water activity (a_W)

Water activity was determined with a LabMaster-Aw (Novasina, Lachen, Switzerland,CH 8853), after calibration with two standards (SAL-T 97 (97.3 ± 0.3 % rh) and SAL-T 11 (11.3 ± 0.3 % rh)). The analytical procedure was according to manufacturer's instructions. A sample was filled in small beaker (provided by the manufacturer), and placed in the measuring chamber of the aW-meter. The sample was incubated until the aW-measurement stabilized.

In Preliminary study 1-4, the frozen meat mixtures (-40 ° C) were thawed at room temperature (21 ° C). Approx. 1.0 g sample was collected from the tube, as far as possible from the middle (Figure 4.5-3), and chopped well before a_W measurement. Three parallels were analysed for each sample.

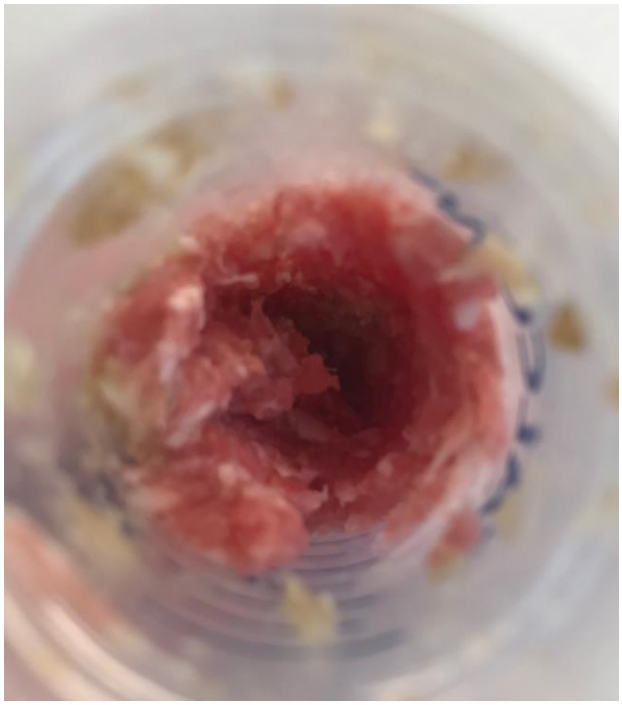

Figure 4.5-3: Centrifuge tube (50 ml) with meat mixture seen from above. The top layer was removed from the tube and only the middle of the sample was used for testing.

In the main study five, two meat mixtures inoculated with LS-25 added medium KCl, and LK-30 added medium KCl was tested to see if it were any differences in water activity inside the sausage. Figure 4.5-4 shows were meat mixtures were taken.

Figure 4.5-4: Sausage tested different places to see if water activity varieties inside. Cross 1: centre; Cross 2: centre toward the end; Cross 3: edge.

In the main study five the meat mixtures were collected by cutting into the middle of the sausage and take a sample (approx. 1.0 g). The meat mixtures were chopped into small pieces and analysed as described above. In Figure 4.5-5 the procedure is described further.

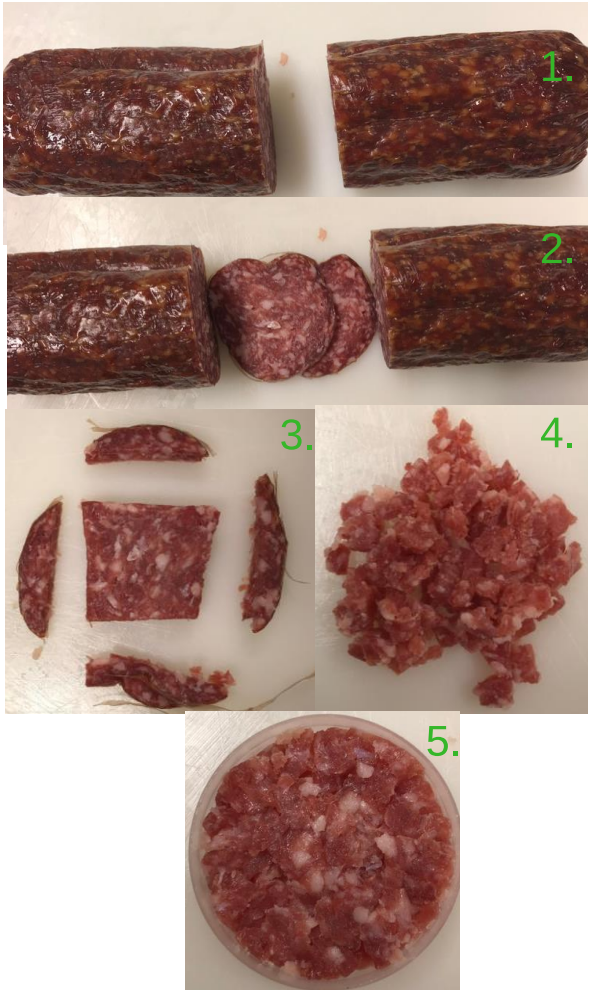

Figure 4.5-5: The picture shows the procedure for preparation of sausage for measurement of a_w. Step one: sausage was separated in half. Step two: a slice was taken from the middle. Step 3: only the centre of the slice was cut out. Step 4: the centre of the slice was chopped into small pieces. Step five: the sausage was placed in the measurement-beaker. The beaker was then placed in the measurement-chamber (not shown).

4.5.6. Wet weight of sausages during fermentation and drying

All sausages were weighed immediately after preparation and the weight of each sausage recorded. Whenever a sausage was taken out during fermentation and drying, it was re-weighted and the weight recorded. A selected sausage (the so called "weighing sausage") was weighed regularly and hung back again to record weight loss during the drying process.

4.5.7. Colour

Colour in meat mixtures was assessed by comparing the meat mixtures with a colour scale from 1-6 (Figure 4.5-6). The colour scale developed in an initial experiment was a tool to assess the colour development visually.
In the preliminary study 2-4 and the main study 5 the colour scale was not used, but the colour was observed.

Figure 4.5-6: Colour scale 1-6 that the meat mixtures were compared to. Degree 1 start of fermentation 0 h, degree 6 - stable and strong pink/red colour.

4.5.8. Microbiological detection of bacteria

Meat mixtures containing Bactoferm T-SPX with salt mix four (two parallel meat mixtures), and one sample containing salt mix two were serially diluted in 0.9% saline solution, and appropriate dilutions plated in triplicate on two different media; MRS Agar, pH 5.5, for detection of lactic acid bacteria, and TSA for detection of bacteria that do not thrive on MRS. The media were made according to the manufacturer's instructions. Three agar plates of MRS agar and three of TSA with the appropriate dilutions were incubated both aerobically and anaerobically for four days at 23 °C
When incubating anaerobically the anaerobic jar with media was flushed with nitrogen for approximately 10 seconds to remove oxygen. An Aerocult (added 35 ml water to start package) was added to remove oxygen that was produced during incubation.

Selected colonies from MRS agar were plated over to new medium of TSA, while selected colonies on TSA were plated over to new medium of MRS agar. Both were incubated for four days at 23 °C. Colonies was cultivated (purified) to ensure clean colonies, so colonies

that grew on MRS agar was grown over two rounds on MRS agar, and colonies that grew on TSA was grown over two rounds on TSA.
All media was incubated for four days at 23 °C in each round.

Selected colonies were purified and transferred to 5 ml of MRS Broth and incubated for four days at 23 °C. Meat mixtures (500 µl) of MRS broth-culture was then vortexed vigorously together with 500 µl of 50% glycerol before being frozen in a cryotube at -80 °C.

4.5.9. Buffer capacity in the chopped meat

The buffer capacity of the minced meat was measured three times (three repeats) by adding 1.5-5.0 ml HCl (0.800 M) to 50.00 g minced meat and measure pH at intervals until it stabilized (up to 4 hours). In the first experiment pH was measured also after 6 and 8 hours, but pH after these time periods was the same as after 4 hours.

4.5.10. Salt analysis

Sodium content in the sausages was measured in an extract of each sample using a Dual Star™ pH/ISE Meter (Thermo Fisher Scientific, Waltham, MA USA) with a Na-selective electrode (Ross® Sodium Ion Selective Electrode, Thermo Fisher Scientific, Waltham, MA USA). The sample preparation method is developed by (Kivikari, 1996) and modified by SINTEF Ocean.
A calibration curve was made using three standards of NaCl and sodium ionic strength adjustor (ISA, Thermo Fisher Scientific, Waltham, MA, USA, 841111) was added to all solutions to ensure that meat mixtures and standards had similar ionic strength. Standards solutions were prepared using dried NaCl (analytical standard). The 1000 ppm Na^+ standard solution was prepared as follow: NaCl (2.5408 g) was transferred to a graduated flask (1L) and added deionized water during thoroughly stirring. To obtain the standard-solution of 100, 10, and 1 ppm Na^+, the 1000 ppm Na^+ standard was diluted by transferring 10 ml (1000 ppm Na^+) to a graduated 100 ml flask and diluted with deionized water and thoroughly stirred. The same procedure was conducted to achieve 10 and 1 ppm Na^+, respectively.

The measurements give the amounts of sodium in the extract, and the sodium in the sample can then be calculated as mg Na^+ / 100 g or % Na^+ of the sample.
One larger piece of the middle (2-3 cm slice) of the sausage was chopped to small pieces in a small food processor. 7.5 g of the sample was homogenised thoroughly with an Ultra-Turax® disperser at low to intermediate speed for 20-30 seconds in 250 ml water and heated for 30 minutes at 90 °C. Meat mixtures were cooled down to room temperature before filtrated through a cellulose filter paper (Whatman no. 4, Wathman International Ltd., Maidsone, UK). Three meat mixtures were taken from each sample and added ISA and analysed as described above. Between each measurement the electrode was rinsed with an Electrode Rinse Solution (ISA diluted 1:100 in distilled water).

4.5.11. Sensory descriptive testing

4.5.11.1. Preparation of sensory evaluation meat mixtures

The finished sausages (eight types) (vacuum-packed for two to three weeks, and stored at 4 °C), were sliced in 2-mm-thick slices and stored in closed bags at 4 °C two hours before evaluation. The sausages were temperate, in room temperature approx. twenty minutes before sensory evaluation. Meat mixtures were served on paper-plates marked with a random three-digit number (one for each sample) and served four and four meat mixtures (randomly order).

4.5.11.2. Sensory evaluation

Volunteers were recruited mainly from the staff at the factory Grilstad AS, Trondheim who are used to evaluate dry fermented products. The panellist also had volunteers from SINTEF Ocean and SINTEF Industry, and a student from NTNU. The panellist had five men and three women within the age 21 to 62 years old.

Panel leader and three others from the panel tasted through all meat mixtures and described the similarities and differences concerning appearance, texture and taste, and made a description of the most fitting attributes for the meat mixtures (Appendix A). Some of the attributes like *e.g.* grainy is an attribute often used by the local manufacturer to describe sausages. The attributes were chosen based on that the panellist should understand them easily and already had used them in earlier tests if it was possible.

Two references that represented the differences of the meat mixtures in the scale was taken out and served to the panellist. Meat mixtures were evaluated during one session and ranked according to colour, odour, taste, and consistency.
A hedonic test using a 9-point scale (1-low intensity; 9- high intensity) was used to evaluate the sample (Figure 4.5-7).
The evaluation form was explained, and the difference between sour and lactic acid/salami was *e.g.* explained. All panellists tasted the reference meat mixtures with no knowledge that this was some of the meat mixtures that they would taste later. The panellist was then informed that this meat mixtures represented a scale 9 *e.g.* on chewiness/rubber feeling, and a scale one on sour. They were also informed that there was a line for comments in the evaluation form so if they tasted, smelled or saw something they didn't could fit anywhere in the form, they could write it there.

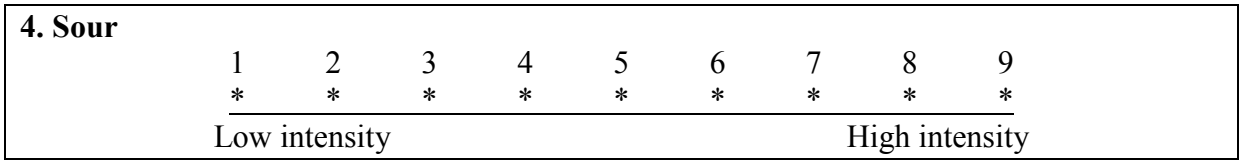

Figure 4.5-7: Example of how the odour sour was evaluated on a scale 1 (low intensity)-9. (high intensity).

While evaluation meat mixtures panellists could taste as much sample as they wanted and they could go back and forward as many times as they wanted. Between meat mixtures the panellist was asked to drink some room temperature water and eat some non-salt flatbread to rinse their palate.

4.5.12. Statistical analysis

For all analyses performed with three parallels average and standard deviation was calculated. A two way ANOVA test was carried out in order to determine significant differences among sausages depending on the starter culture and the concentrations and type of salt. The significance level was set to 0.05 (confidence interval of 95 %) and the data analysis tool in excel was used.

4.5.13. Statistical analysis for sensory evaluation

Panel performance was assessed using profile plots in PanelCheck (version 1.4.0, Nofima, Ås, Norway), statistical analysis was performed using a two-way analysis of variance (ANOVA).

5. Results

The aim of the thesis was to study the effects of sodium reduction on the production and properties of dry fermented sausages, either by reducing the amount of added salt (NaCl) or by partial replacement with potassium chloride (KCl). Four different commercial starter cultures were tested.

In dry fermented sausages, the fermentation process normally occurs the first days after the meat mixture has been stuffed into a casing and hung in a ripening chamber. This is a time consuming and complicated procedure to perform in the laboratory. Thus, a model system was developed where the meat mixture after mixing with the starter culture, was transferred to a 50-ml plastic tube with a screw cap, and incubated at the desired temperature for a few days. The idea was to simulate the first fermentation phase, but the following ripening and drying phase could of course not be studied in this almost closed system. A small hole was drilled in the screw cap to prevent a build-up of overpressure due to CO_2 produced during the fermentation.

In the preliminary studies 1-4, this model was used to assess the fermentation phase with the four different starter cultures using meat mixtures with different concentrations of salt and KCl, and added different amounts of dextrose (glucose) from start.

Study five was the main study and designed to compare a fast and a slow fermenting starter culture in sausage production when the addition of salt was reduced and/or partially replaced with KCl.

5.1. Some control experiments

5.1.1. Buffer capacity in meat mixtures

A key feature during fermentation of sausages is the pH reduction due to production of lactic acid and other organic acids by the added starter culture. The pH reduction is a function of the concentration and type of acids produced and the buffer capacity of the meat meat mixture. To quantify the buffer capacity of the meat mixture, meat mixtures were titrated with hydrochloric acid (HCl) (Figure 5.1-1). The pH took some time to stabilize, but four hours after the acid was added, pH was constant. Approx. 50 µmol HCl per g meat mixture was required to reduce pH from 5.70 to 4.85. This corresponds to approx. 4.5 mg lactic acid/g if we do not consider the pKa value of lactic acid (pKa = 3.86), which means that slightly more lactic acid (approx. 10 %) is required. The pH change as a function of the amount of added acid was almost linear between pH 5.70 and pH 4.85. Using a linear approach, the buffer capacity of the meat mixture can be estimated to approx. 60 mmol acid per kg meat mixture per pH unit in the relevant range.

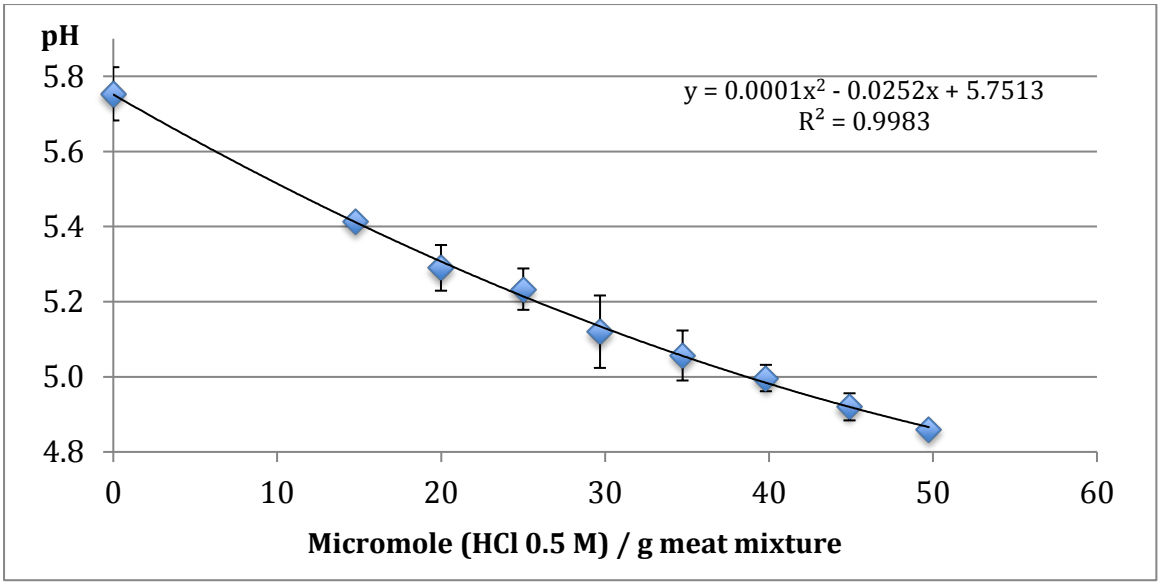

Figure 5.1-1 pH in meat mixture as a function of the concentration of 0.5 M HCl added. The standard deviation (n=3) is indicated.

5.1.2. Test of extraction method of organic acids and sugars for HPLC

The concentration of organic acids and glucose in the meat mixtures and sausages were quantified on HPLC after extraction with water. To test the efficiency of the extraction method, meat mixture was added a known amount of lactic and acetic acid, and the concentration of acids and glucose in the meat mixture determined both before and after addition of the acids using the established extraction protocol.

The results show good agreement between the expected and the measured concentration of lactic acid after addition of acids, while the measured concentration of acetic acid was slightly higher than expected (Table 5.1-1). Glucose and citric acid was not added and was slightly diluted by the addition of lactic and acetic acids, but the measured values were in good agreement with the predicted dilution factor.

Table 5.1-1: Test of the efficiency of the extraction protocol for organic acids and sugars. Three meat mixtures were extracted as they were, while three meat mixtures were added a solution (0.1 ml/g meat) of lactic and acetic acid and mixed well before extraction. The expected concentrations after addition of the acids are calculated considering both the dilution of the sample and the concentration of acids added.

Compound	Measured concentration in meat without additions (µmol/g meat)		Measured dry weight (g/g meat)	Conc. (µmol/g meat dry matter)	Added acid (µmol/g meat dry matter)	Added water (ml/g meat)	Calculated dry weight meat after addition (g/g meat)	Expected conc. after addition (µmol/g meat)	Measured concentration in meat with additions (µmol/g)		Measured as fraction of expected (%)
	Average	stdev							Average	stdev	
Citric acid	14.9	0.2	0.353	42.1	0.0	0.10	0.321	13.5	14.0	0.2	103.5
Glucose	6.2	0.1	0.353	17.5	0.0	0.10	0.321	5.6	5.8	0.1	103.4
Lactic acid	81.7	1.3	0.353	231.4	192.3	0.10	0.321	136.0	137.2	2.4	100.9
Acetic acid	0.0		0.353	0.0	29.0	0.10	0.321	9.3	10.6	0.3	114.3

5.2. Preliminary study 1

The purpose of preliminary study 1 was to evaluate the chosen starter cultures (some slow - and some fast fermenting) with respect to their properties during fermentation such as the amount and types of acids produced, colour development, and how different concentrations and types salt affected them. The meat mixtures were added 0-4 % NaCl and 0-3.83 % KCl and in addition one meat mixture was added a mixture of NaCl and KCl. This experiment was the first real test of the whole process of preparing meat mixtures and transferring them into 50 ml tubes, as well the planned analytical protocols. Before this study, an initial test set of meat mixtures had been produced and tested to reveal any weaknesses in preparation and analysis protocol. These results are not shown.

5.2.1. Development of pH

The pH decrease during fermentation of meat mixture, depend upon the starter culture, the concentration of added glucose and salt, and the temperature. The temperature in preliminary study 1 (23 °C) was perhaps a bit high, and as mentioned in section 4.4.1, was lowered after this study due to an observed long lag phase. The starter cultures were prepared at a somewhat lower temperature (21 °C) than the incubation temperature used in preliminary study one.

The results for the fast fermenting starter culture LS-25 and the slow-fermenting LK-30 are shown in Figure 5.2-1. The starter culture Mild & Fast (results in Appendix B) was a fast-fermenting starter culture comparable to LS-25, while T-SPX, for which we had no previous information about fermentation rate, turned out to be a medium fermentation rate starter culture (see Appendix B).

The meat mixtures fermented with T-SPX added 2.56 and 3.83 g KCl / 100 g meat mixture developed a bad smell at the end of the fermentation despite pH < 5.0 and microbiological analyses were performed to see if the meat had been contaminated with an unwanted bacterium (see later)

The general trend was that all starter cultures decreased pH faster when less salt was added (Figure 5.2-1,Figure 5.2-2). In terms of concentration of added salt ions, and thus expected effect on water activity, 3.0 g NaCl + 1.28 g KCl = 4.0 g NaCl, while 3.83 g KCl = 3.0 g NaCl, and 2.56 g KCl = 2.0 g NaCl. Still, LK-30 used shorter time to reach pH 5.2 in meat mixture added a mix of NaCl and KCl compared to those added pure NaCl. Those findings were the same when pure KCl (2.56 g KCl) was added instead of 2.0 g NaCl. In the three starter cultures addition of NaCl to the meat mixture tended to give a more rapid pH decrease the corresponding addition of KCl. However, mostly the differences were not large, and the assessment of the result is also complicated by the fact that the type and concentration of salt added to the meat mixture also affected the start pH (see later).

The control meat mixtures without any added salt had the highest start pH and the most rapid pH drop during fermentation, but after a few days pH started to increase again, presumably because, the meat started to rot. The pH drop alone was, as expected, not enough to prevent meat degrading bacteria from growing. This observation was typical for all tested starter cultures (graphs shown in Appendix B)

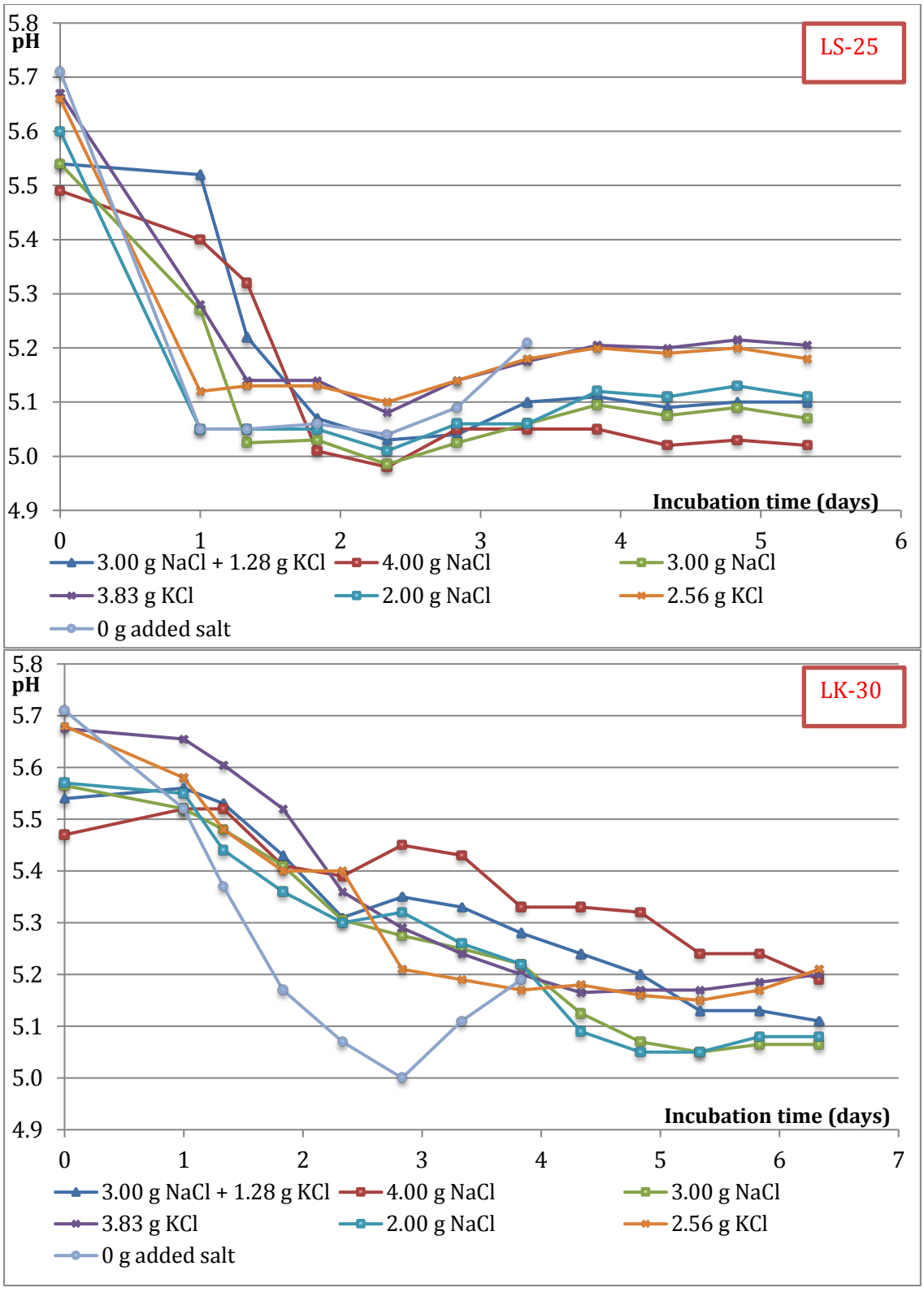

Figure 5.2-1: pH as a function of time when meat mixtures were fermented in 50 ml tubes, with the starter culture LS-25 and LK-30 and different concentrations and types of salts added.

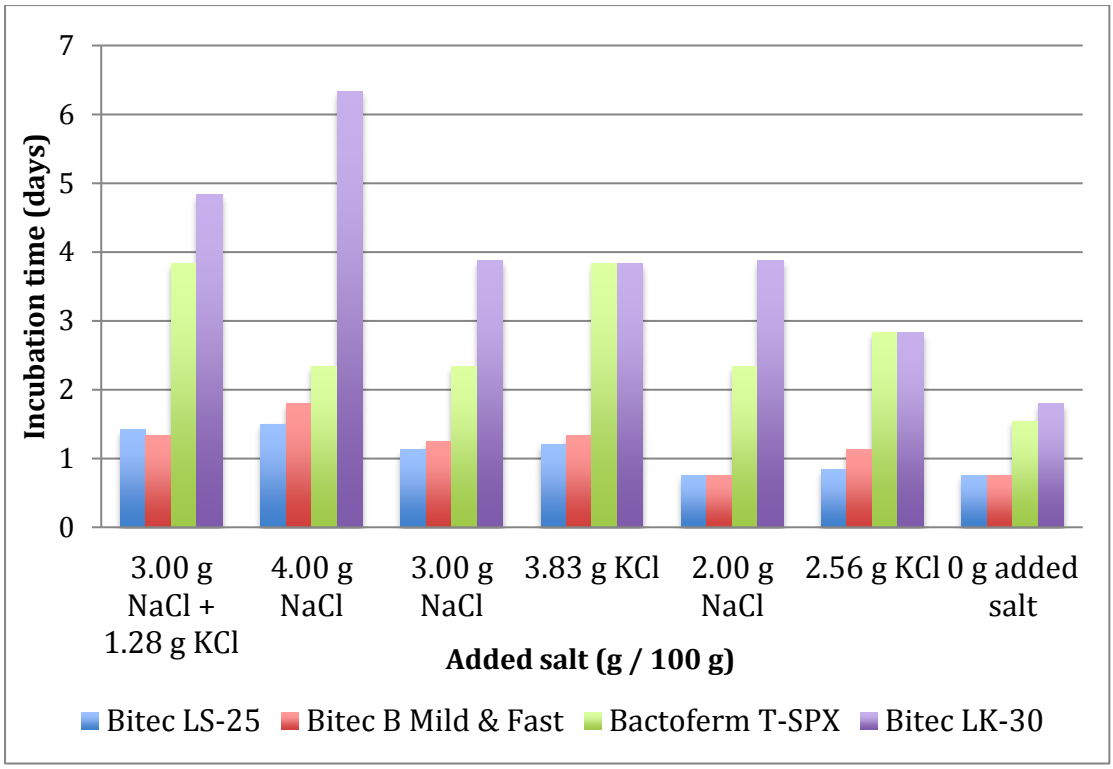

Figure 5.2-2: Time as a function of the added concentration and type of salt for the starter cultures to reach pH =5.2, when meat mixture was inoculated with the four different starter cultures and different types and concentrations of salt.

pH development for starter cultures Mild & Fast and T-SPX are shown in Appendix B

5.2.2. Development of colour

The formation of colour is mostly dependent on the lowering of pH and the enzymes produced by *Micrococceae*. All meat mixtures seemed to reach colour degree six in approx. the same time. The starter culture Mild & Fast could seem to be inhibited by the concentration of NaCl (4.00 g NaCl), while the concentrations of KCl seemed to inhibit colour formation in the meat mixtures. The starter culture Mild & Fast seemed to be induced by 0 g added salt, as well as the mix of NaCl and KCl. Meat mixtures inoculated with LS-25 added KCl seemed to be induced by KCl. (Figure 5.2-3).
The results indicated that the colour developed more rapid when less salt was added, but the type of salt had no effect (Figure 5.2-4).

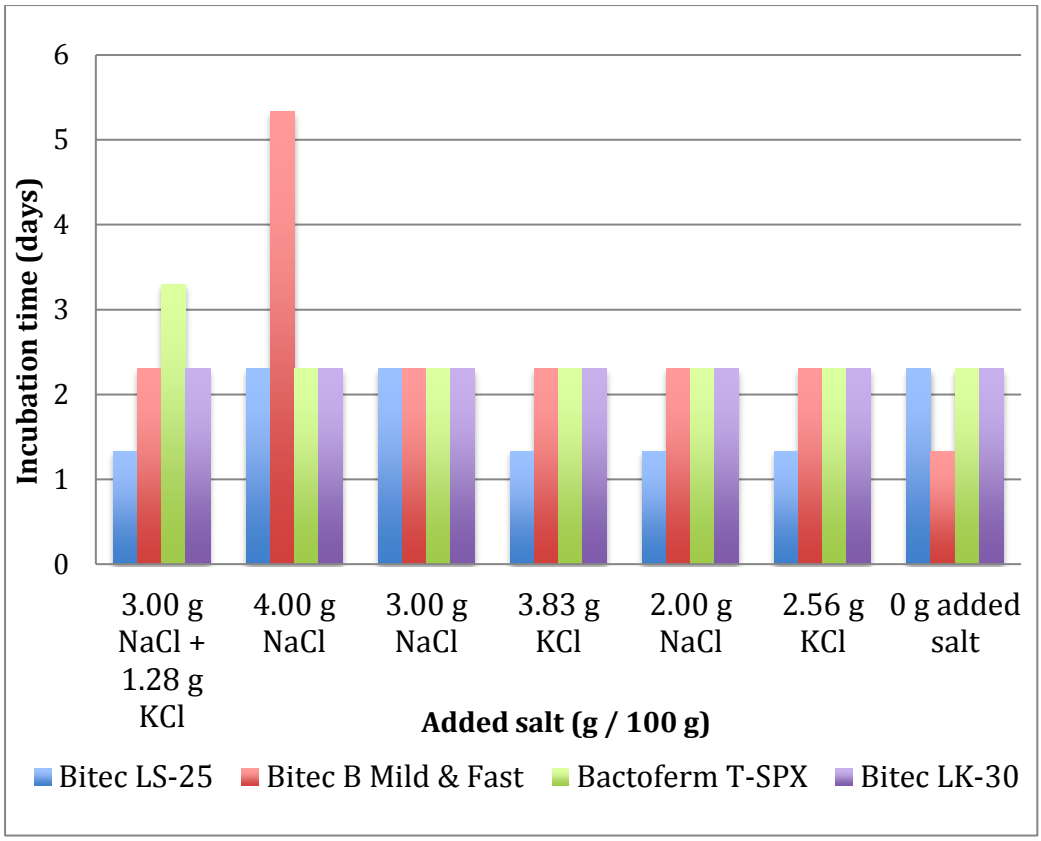

Figure 5.2-3: Time until meat mixture reached colour degree six as a function of the starter culture and the type and concentration of salts added.

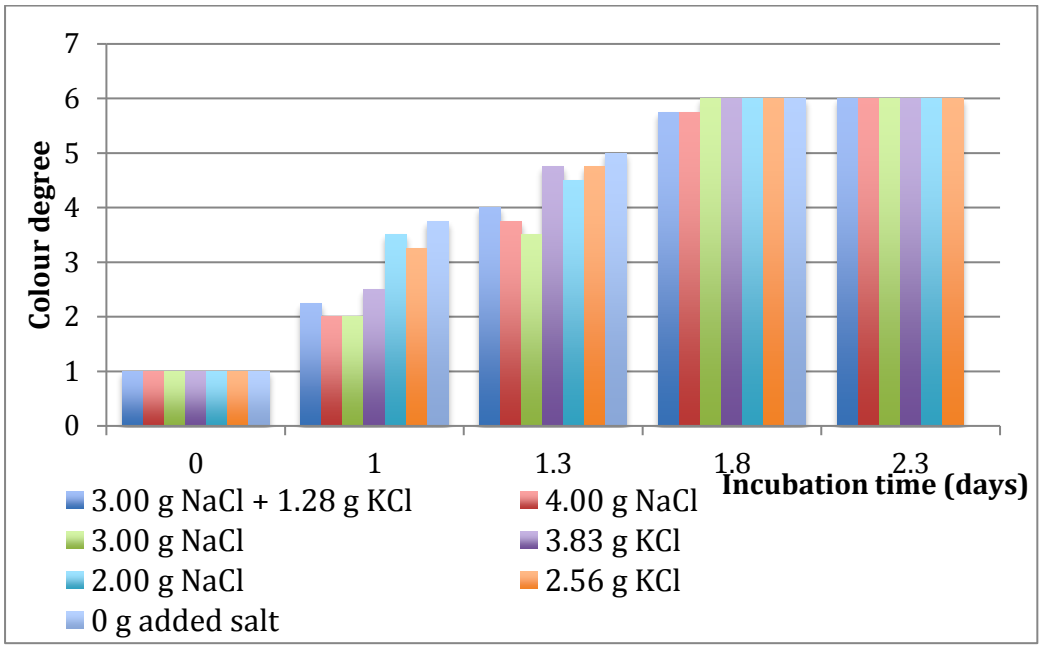

Figure 5.2-4: Colour degree as a function of time and added concentration and type of salt, independent of used starter cultures.

Formation of colour as a function of time for each starter cultures can be seen in Appendix B.

5.2.3. Microbial analysis

T-SPX developed at the end of fermentation a bad smell in the both meat mixtures added 2.56 and, 3.83 g KCl/100 g meat mixture, respectively. The meat mixtures also changed from a strong pink colour to form several brown/grey patches (Figure 5.2-5).

The meat mixtures fermented with T-SPX and added 3.83 g KCl/100 g meat mixture, was analysed for the presence of contaminating bacteria. For comparison, the meat mixture added 4.00 g NaCl/100 g meat mixture was also analysed. Meat mixtures were plated on MRS agar and incubated both aerobically and anaerobically. They showed small white colonies and medium-sized beige colonies. The same meat mixtures were also plated on TSA agar, and incubated both aerobically and anaerobically. The meat mixtures incubated anaerobically on TSA agar, showed white medium sized colonies. The colonies grown on TSA aerobically showed weak growth of small white sized colonies.
On the agar plates with MRS agar and incubated aerobically, it was detected two small yellow colonies in the sample from meat mixture added KCl, but the colonies would not reproduce when transferred to a new MRS agar media for cultivation. All detected colonies were purified to ensure only one colony was frozen for later analysis if desired. No obvious trend was seen regarding the microbial analysis so no conclusion was taken.

Figure 5.2-5: Tubes with meat mixture fermented with starter culture T-SPX for 5.3 days. The tubes containing KCl show the brown-grey patches that developed in these tubes.

5.2.4. Dry weight

For the dry weigh to change, water must evaporate. Since the model system had very little evaporation, no changes in dry weight during the study were observed, but the meat mixtures added Mild & Fast had a significantly lower dry weight than the other meat mixtures (Table 5.2-1).
Assumed that the added starter cultures did not contribute significantly to the dry weight, the dry weigh should reflect the amount of added salt. The dry weigh of the control sample was approx. 4 % points lower than the sample containing 4.00 g NaCl/100 g meat mixture (see section 6.1.1 in discussion).

Table 5.2-1: Average of dry weight (%) for meat mixtures added salt and starter culture taken over time. Values are given as an average independent of time ± standard deviation (n=5).

Additive	**Added salt (g / 100 g)**						
NaCl	3.00	4.00	3.00	0	2.00	0	0
KCl	1.28	0	0	3.83	0	2.56	0
	Dry weight (%)						
LS-25	43.0 ± 0.2	42.2 ± 0.9	42.0 ± 0.3	41.6 ± 0.7	42.6 ± 0.2	42.0 ± 0.5	40.2 ± 2.2
Mild & Fast	40.7 ± 0.1	40.3 ± 0.1	39.9 ± 0.3	40.2 ± 0.4	40.7 ± 0.6	40.1 ± 0.6	34.9 ± 1.9
T-SPX	42.1 ± 0.9	42.2 ± 0.4	41.4 ± 0.4	40.7 ± 0.4	41.7 ± 0.4	40.9 ± 0.3	39.6 ± 1.2
LK-30	41.8 ± 0.5	41.5 ± 0.3	41.3 ± 0.6	40.3 ± 0.9	41.0 ± 0.6	40.7 ± 0.6	38.7 ± 1.4

Figures for dry weight (%) as a function of time during for the tested started cultures can be seen in Appendix B.

5.2.5. Water activity

For the water activity to change as a function of time, water must evaporate. Since the model system was almost closed, no changes were observed in water activity over time. Addition of salt to the meat mixture lowered the water activity, and on a molar basis the effect of NaCl and KCl was more or less the same. The meat mixtures without added salt, but inoculated with Mild & Fast, were significantly lower than the other meat mixtures (Figure 5.2-6). The meat mixtures (controls) without added salt were only analysed at the start and the end, and this may explain the higher standard deviation for these meat mixtures.
The type of salt do not seem to affect the water activity since the water activity had the same effect when added on equal molar weight basis, but a lower concentrations of salt gave a lower water activity.

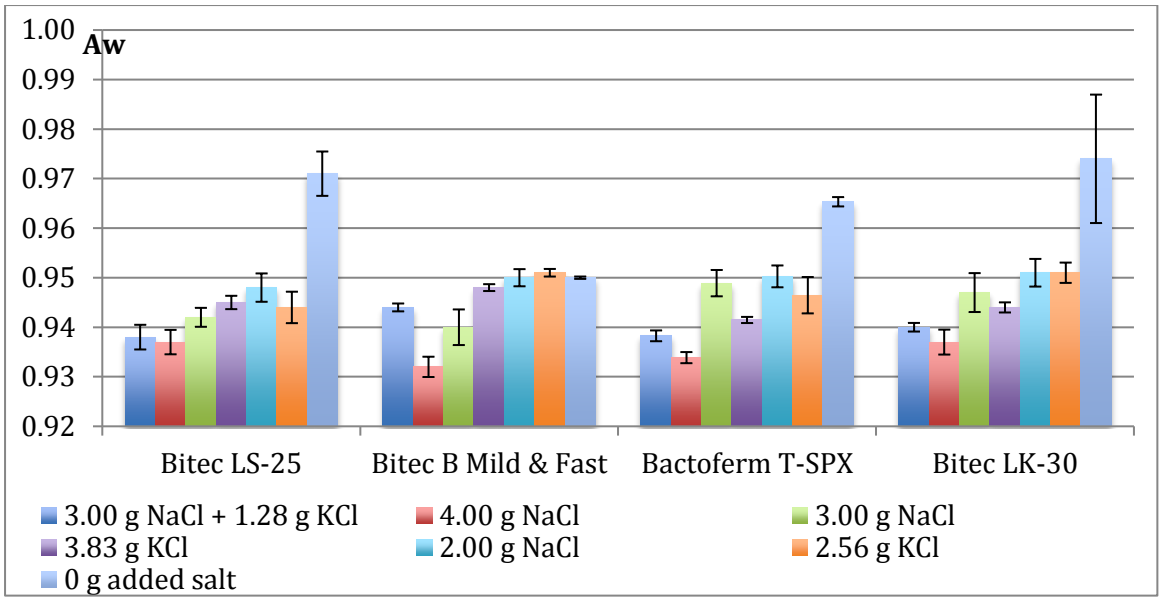

Figure 5.2-6: Water activity as a function of the average of all meat mixtures added salt and starter culture independent of time. Values are given in average independent of time ± standard deviation (n=5).

Figures for water activity as a function of time during for the tested started cultures can be seen in Appendix B.

5.2.6. Determination of organic acids and sugar

All meat mixtures at start of fermentation in this study, were analysed two hours after the starter culture had been added and the meat mixtures incubated at 22 °C in a water bath. After this study the meat mixtures at start of fermentation were taken out immediately after addition of the starter culture in the studies to ensure that the fermentation not had begun. There were problems with the calibration of the HPLC and many analyses had to be performed several times, and this could have caused meat mixtures to not have been analysed correctly. The meat mixture without added salt were only analysed at the start and the end of the study.

5.2.6.1. Glucose

Glucose is the main C-source of lactic acid bacteria. The starter cultures decreased the concentration of glucose during the fermentation (Figure 5.2-7).
The meat mixtures added the fast fermenting starter cultures (LS-25 and Mild & Fast) had a very low concentration of glucose already in the start meat mixtures, indicating that the fermentation might have begun before the meat mixtures were analysed the first time. The meat mixtures with the highest concentrations of added salt contained most glucose at this point.

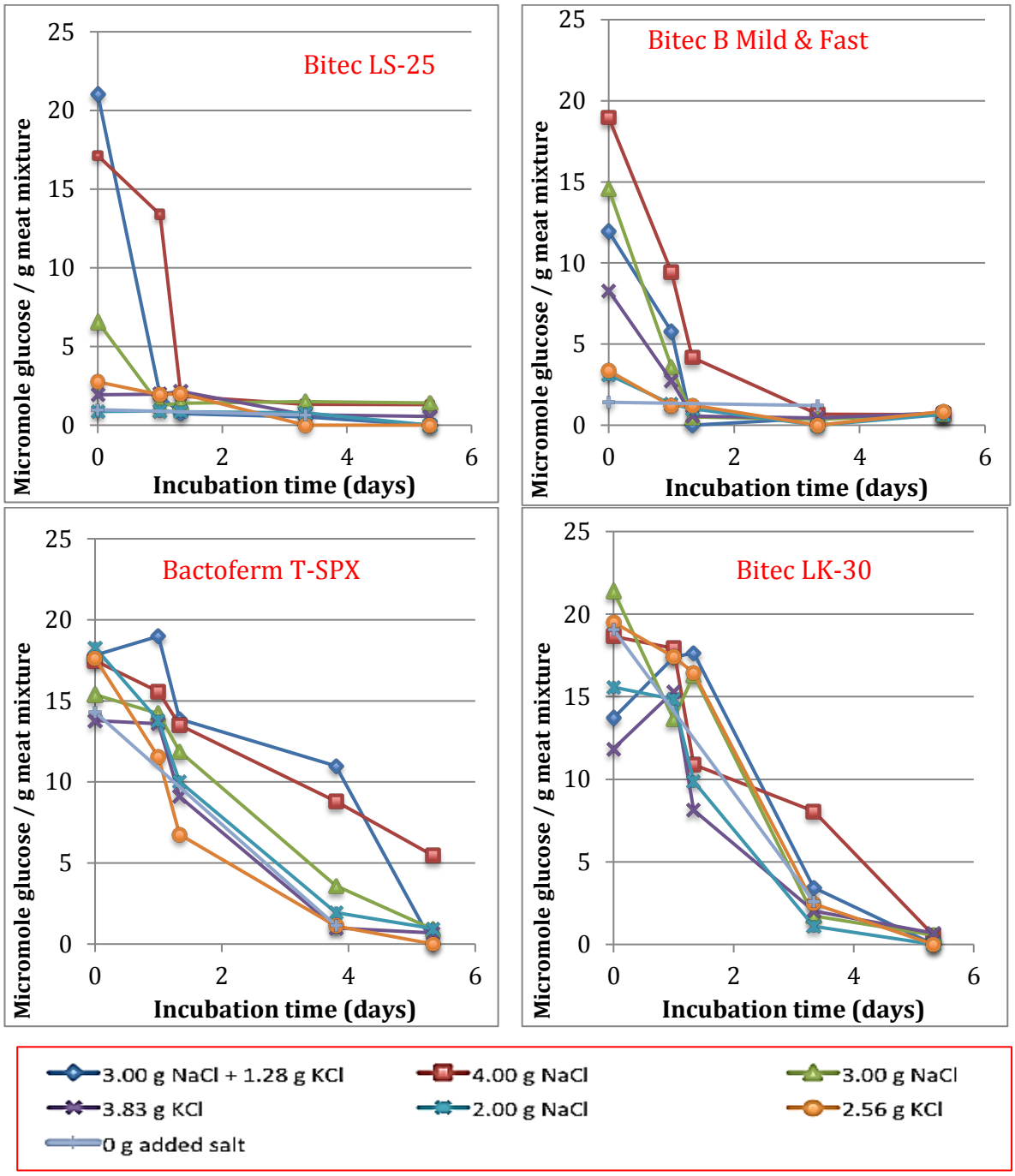

Figure 5.2-7 Concentrations of glucose as a function of time in meat mixtures fermented with the four starter cultures and added different amounts and types of salt per 100 g.

5.2.6.2. Lactic acid

Lactic acid is expected to be the main organic acid produced during the fermentation. However, lactic acid will also be present in the meat mixture before the fermentation starts due metabolism in the meat *post mortem*. The concentration of lactic acid as a function of time varied considerably for all meat mixtures and the expected increase in lactic acid as a function of time was not apparent in the results (Figure 5.2-8).

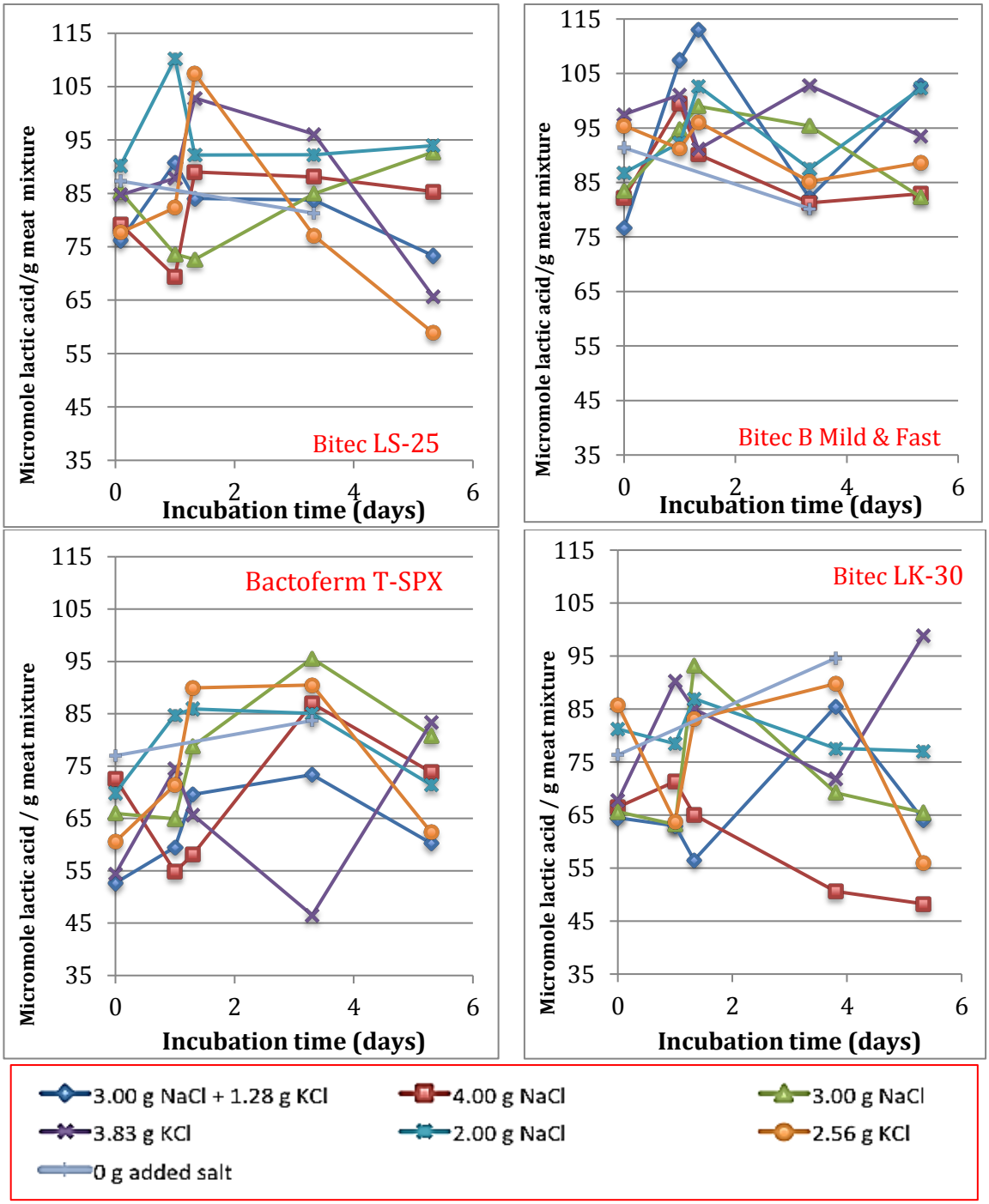

Figure 5.2-8: Concentrations of lactic acid as a function of time for the four starter cultures were used to ferment meat mixture added different concentrations and types of salt.

5.2.6.3. Acetic acid

The starter cultures produced approx. the same concentrations of acetic acid during fermentation, but the concentrations and type of salt seemed affect some of the starter cultures. LS-25 seemed to produce the lowest concentrations of acetic acid in the meat mixtures added KCl, while in the meat mixtures added NaCl and no added salt the concentrations of acetic acid was highest. T-SPX produced the highest concentrations of acetic acid in the meat mixtures added higher concentrations of salt, independent of type, except that the sample added a mixture of NaCl and KCl that had the lowest concentrations. Mild & Fast did not seem to be affected by the different concentrations of added salt, but the sample added the sample added a mix of NaCl and KCl produced the highest concentration of acetic acid. LK-30 seemed to produce higher concentrations of acetic acid in meat mixture added NaCl, and the concentration of acetic acid increased with decreased addition of NaCl. The meat mixtures with LK-30 and added KCl increased until approx. day four, but then the meat mixtures added 3.83 g KCl/100 g meat mixture and the mix of NaCl and KCl decreased (Figure 5.2-9).

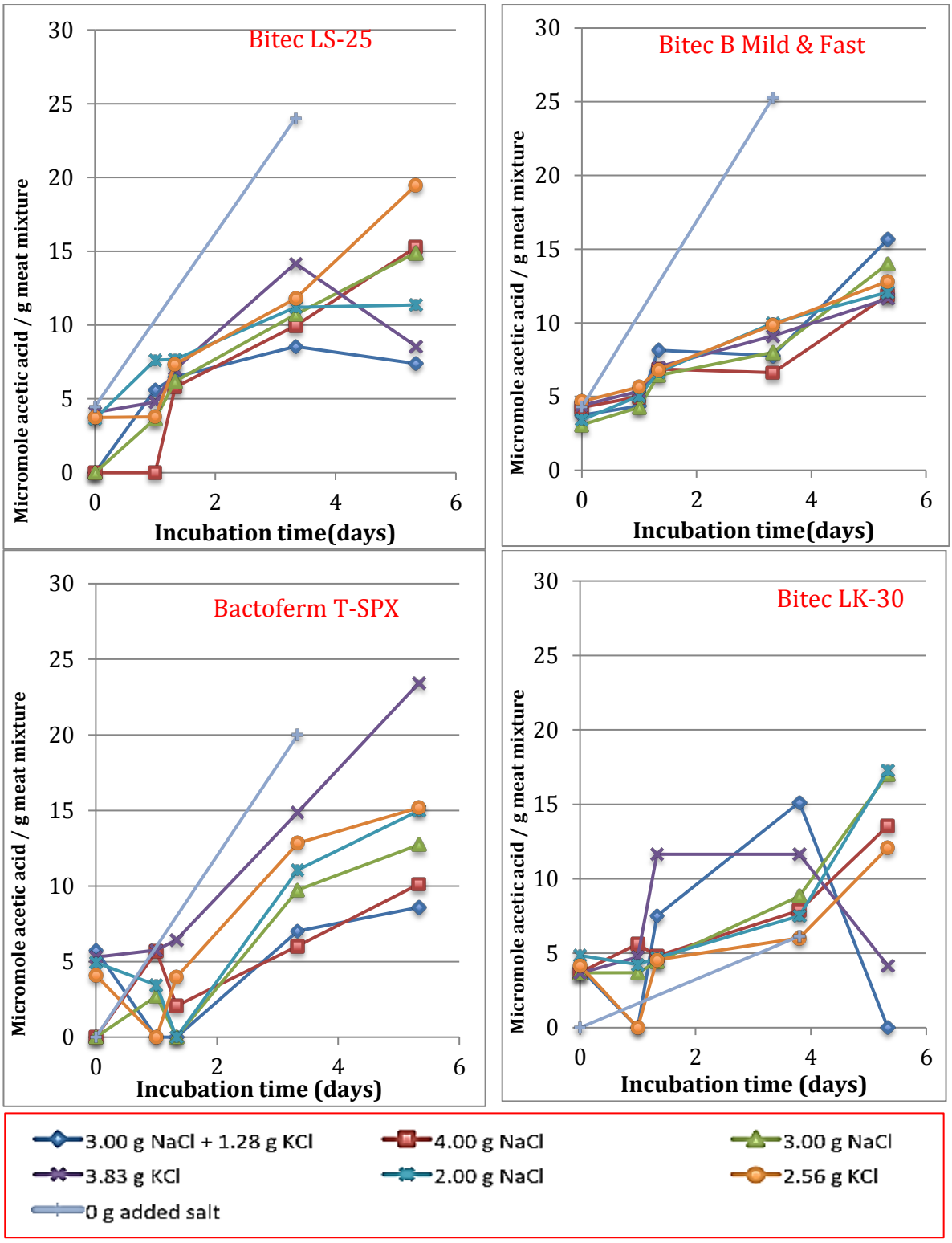

Figure 5.2-9: Concentrations of acetic acid as a function of time in meat mixtures fermented with the four starter cultures and added different amounts and types of salt per 100 g.

5.2.6.4. Citric acid

The concentrations of citric acid in the meat mixtures varied between 10 and 17 µmol/g meat mixture throughout the fermentation no obvious trend (Figure 5.2-10). Citric acid is present in meat from start and is not normally produced during the fermentation. Apparently, it is not consumed either.

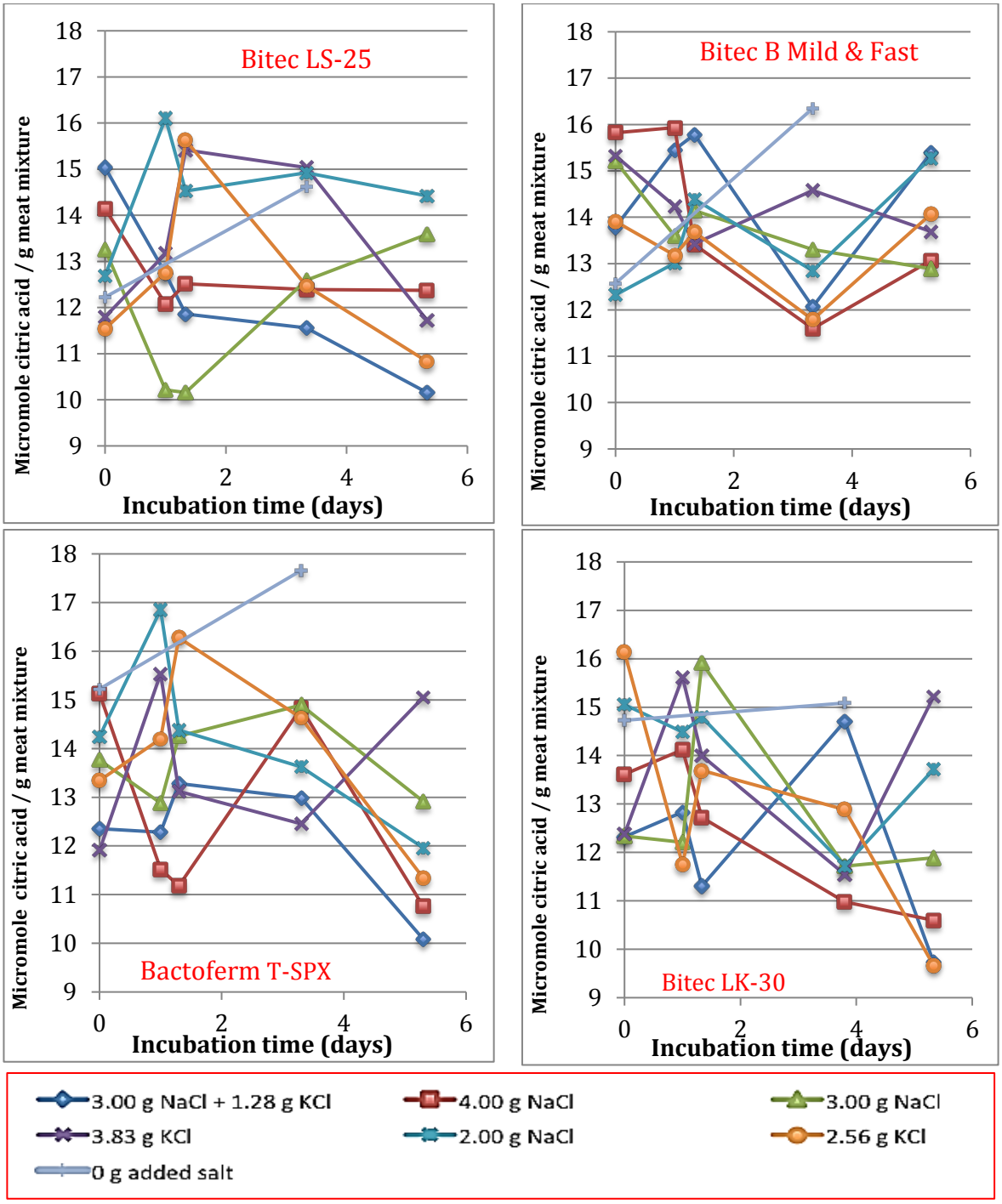

Figure 5.2-10: Concentrations of citric acid as a function of time for the four starter cultures were used to ferment meat mixture added different concentrations and types of salt.

5.2.6.5. Pyruvic acid

Pyruvic acid was present in small amounts from start, and was consumed by all starter cultures except Mild & Fast during the fermentation (Figure 5.2-11).

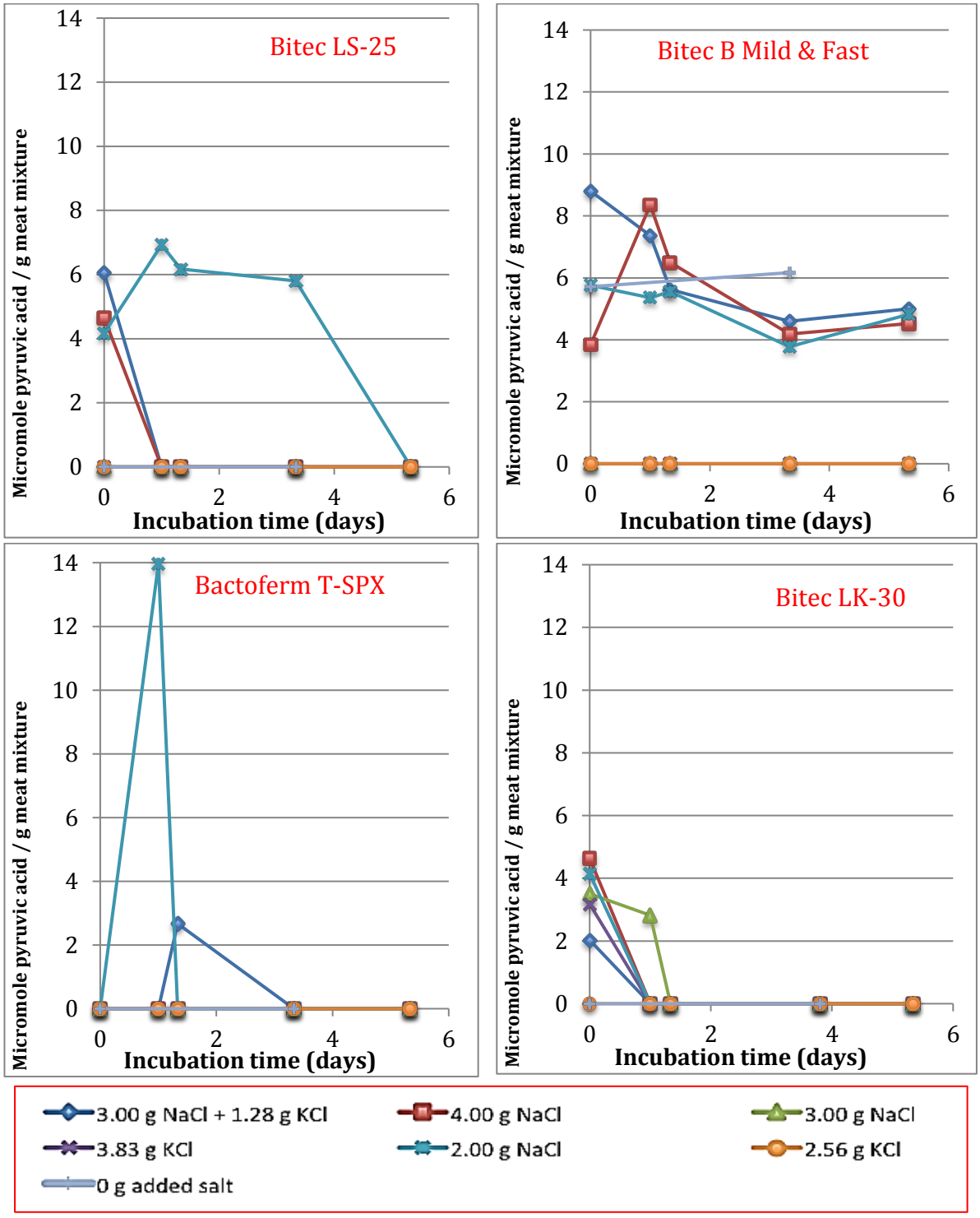

Figure 5.2-11: Concentrations of pyruvic acid as a function of time for the four starter cultures were used to ferment meat mixture added different concentrations and types of salt.

5.2.6.6. Succinic acid

Succinic acid was present in small amounts from start, but were mostly not or only slowly consumed during the fermentation (Figure 5.2-12).

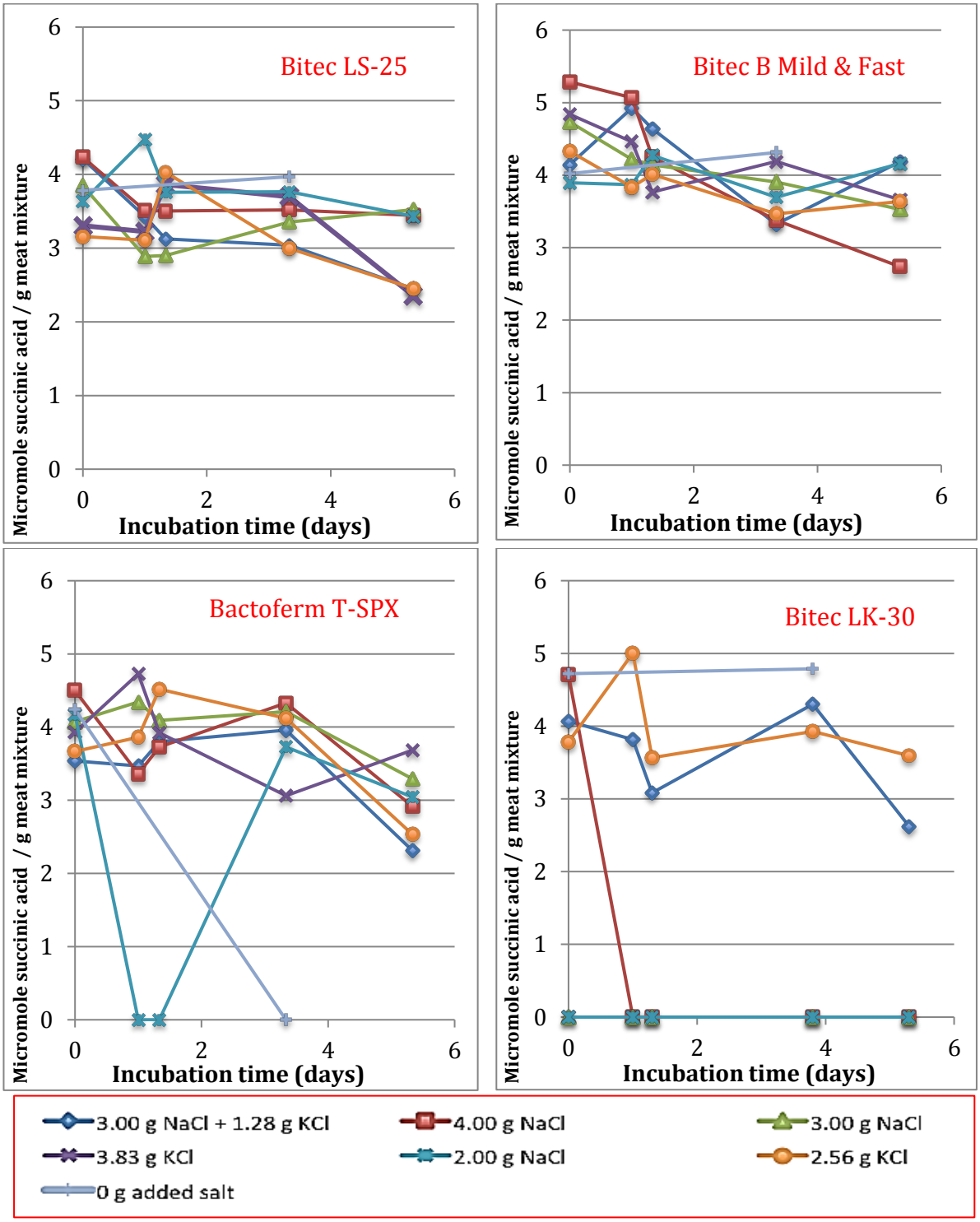

Figure 5.2-12: Concentrations of succinic acid as a function of time for the four starter cultures were used to ferment meat mixture added different concentrations and types of salt.

5.3. Preliminary study 2

Since analysis of sugar in the first study showed small concentrations of glucose left at the end of fermentation, Preliminary study 2 was aimed at determine the amount of dextrose necessary for the starter culture during the fermentation. The usual addition of glucose is around 0.2 g/100 g meat mixture. Here 0 – 0.4 g dextrose/100 g meat mixture was added. The salt addition was 3.00 g/100 g mixture, which is a traditional level used when producing dry fermented sausages in Norway (Hannisdal, 2015). Starter culture LS-25 was used in this study.

In meat mixtures fermented with LS-25 not added dextrose, pH did decreases as expected because of sugar present in the meat, but the pH drop was not as low as desired (Figure 5.3-1). In meat mixtures added 0.2-0.4 g dextrose per 100g, pH decreased below 5.0 after 1.5 days, and there were no large differences as a function of the amount of added dextrose.

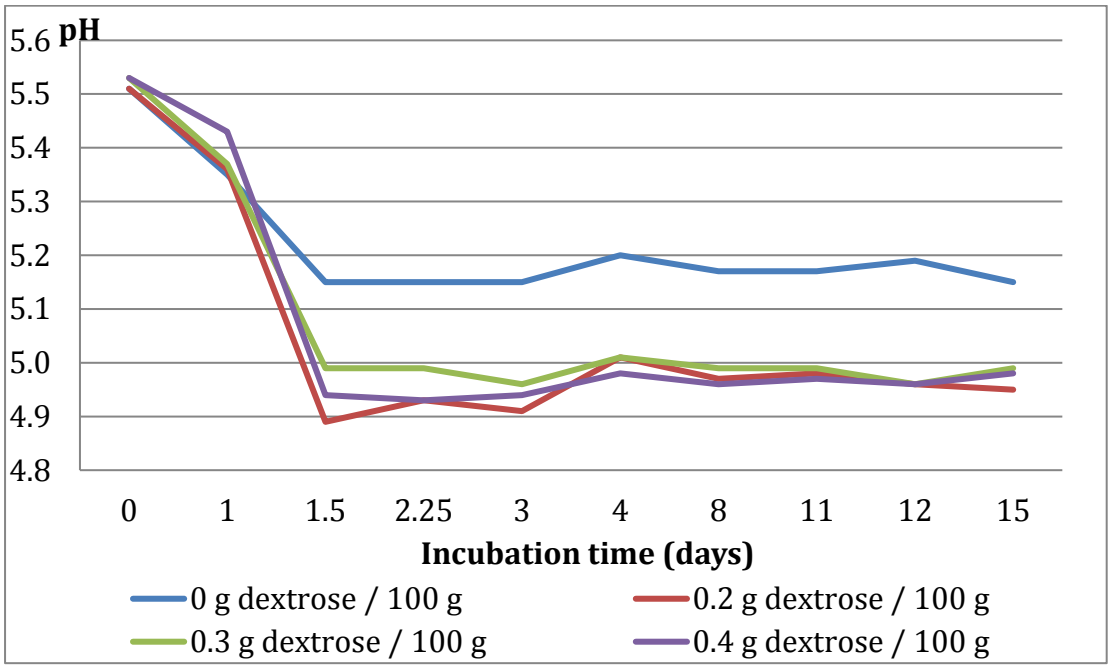

Figure 5.3-1: pH as a function of time in meat mixtures added 0-0.4 g dextrose/100 g meat mixture, 3.00 g NaCl/100 g meat mixture, and fermented with the starter culture LS-25.

The meat mixture without added dextrose contained less lactic acid on day six than the mixtures added dextrose, but, somewhat surprising, a higher concentration of acetic acid (Table 5.3-1). It should also be noted that the meat mixture added most dextrose, had a significant amount of glucose left on day six. Water activity and dry weight was not expected to be affected by the small changes in concentrations of sugar. However, the results showed differences in both water activity and dried weight. A possible explanation is that the inhomogeneity of the meat mixtures, which contained fat particles, affected the result.

Table 5.3-1: Organic acids and sugar in meat mixtures added 0-0.4 g dextrose/100 g, 3.00 g NaCl/100 g, and fermented with the starter culture LS-25 for six days.

	Added concentration dextrose			
Glucose (g/100g)	0	0.2	0.3	0.4
Glucose (µmol/g)	0	11.2	16.7	22.2
Determined concentrations of acids and sugar (µmol/g meat mixture)				
Lactic acid	50.0	95.7	105.2	97.2
Glucose	0.0	1.5	1.5	14.6
Acetic acid	56.0	29.6	30.4	29.5
Citric acid	7.7	7.2	7.5	7.0
Pyruvic acid	0.0	0.0	0.0	0.0
Succinic acid	6.0	5.5	5.8	5.4
Other properties				
Dry weight (%) ± stdev[A]	37.6 ± 0.2	36.5 ± 0.3	38.6 ± 0.2	37.5 ± 0.4
Water activity ± stdev[A]	0.933 ± 0.001	0.942 ± 0.001	0.936 ± 0.001	0.937 ± 0.001

A: Standard deviation is based on n=3.

5.4. Preliminary study 3

Based on the results in Preliminary study 2, it was decided to test the effect of the amount of added dextrose also on the starter culture LK-30 in addition to a repeated study of LS-25. The meat mixtures were added 0-0.8 g dextrose/100 g and 3.00 g NaCl/ 100 g meat mixture. The study was repeated twice.

5.4.1. Development of pH with different concentrations dextrose

For repeat one, the meat mixture inoculated with LS-25 added 0.2 g dextrose / 100 g had no differences from the meat mixture added 0.4 g dextrose / 100 g. In repeat two, the meat mixture added 0.2 g dextrose / 100 g was found significantly higher in analysis on pH than the other meat mixtures (Figure 5.4-1). After the fermentation, the meat mixtures in repeat one added LK-30 seemed to less stable when added less dextrose, since the concentration of 0.2 g dextrose / 100 g meat mixture had a higher pH the other meat mixtures and 0.8 g dextrose / 100 g meat mixture had the lowest pH. The meat mixtures inoculated with LS-25 showed unstable measurements but no obvious trend was seen regarding how the concentrations of dextrose affected the starter cultures after the fermentation was finished (Figure 5.4-1).

The start pH was different in the two studies; pH 5.4-5.5 in study A and pH 5.7-5.8 in study B, possibly reflecting differences in the meat used in the two studies. As expected, in both studies the fast fermenting starter culture LS-25 lowered pH much faster than the slow-fermenting LK-30. For both strains, pH did not differ much as a function of the amount of added dextrose. (Figure 5.4-1). When no dextrose was added the pH decreased not as desired since not enough sugar was present.

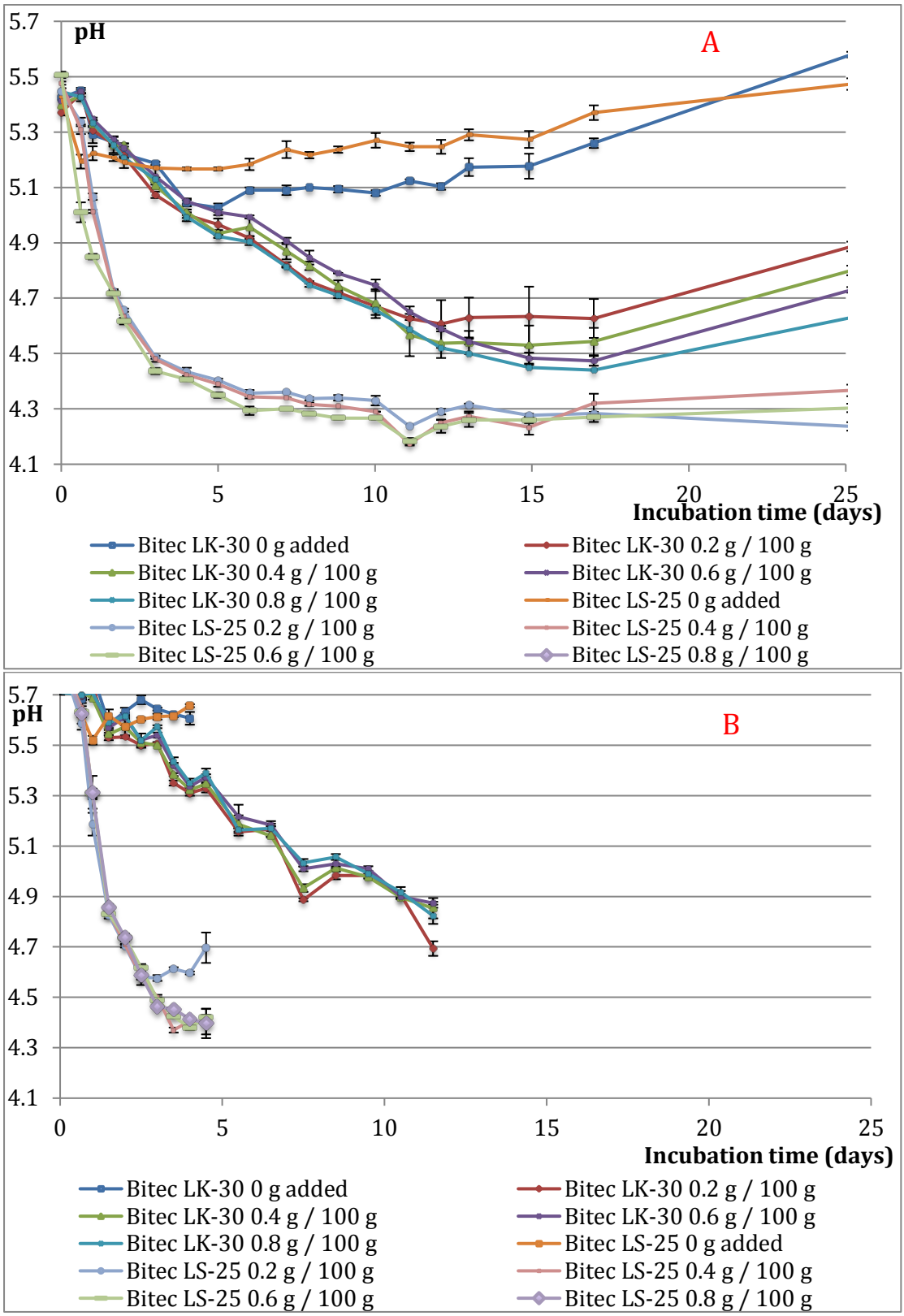

Figure 5.4-1: pH as a function of time in meat mixtures inoculated with the starter cultures LK-30 and LS-25 and incubated in 50 ml tubes at 22 °C. The meat mixtures were added 0.2-0.8 g dextrose and 3.0 g NaCl per 100 g. Standard deviation is indicated (n=3). The study was repeated twice; A is repeat one, B is repeat two.

5.4.2. Water activity and dry weight

Since the model system is almost closed and the amount of added salt was the same for all meat mixtures, no large differences in dry weight or water activity were expected as a function of time or between the two cultures. This was also observed (Table 5.4-1).

Table 5.4-1: The average dry weight (%) and water activity in meat mixtures added 0-0.8 g dextrose/100 g, 3.0 g NaCl/100 g, and fermented with the starter cultures LK-30 (11.5 days) or LS-25 (4.5. days) in 50 ml tubes.

	LK-30	LS-25
Dry weight (%) ± stdev	37.6 ± 0.3	38.9 ± 0.6
Water activity ± stdev	0.944 ± 0.001	0.946 ± 0.001

Figures with dry weight (%) and dry weight as a function of time can be seen in appendix B.

5.4.3. Organic acids and sugar during the fermentations

5.4.3.1. Glucose

Since the pH as a function of time showed no big differences regarding the added concentrations of dextrose except for the sample containing 0 g added dextrose, it was expected that the concentration of dextrose was decreased approx. the same concentration for the meat mixtures added the various concentrations of dextrose.
The starter culture LS-25 and LK-30 added 0 and 0.2 g dextrose / 100 g meat mixture decreased the concentration of glucose to a level approx. below zero in the final meat mixtures.
The starter cultures LS-25 decreased the concentration glucose in a shorter time and seemed to decline a higher concentration of glucose than the starter cultures LK-30. The starter culture LS-25 added 0.8 g dextrose / 100 g meat mixture had a higher start point than expected (Figure 5.4-2).
The high concentration of dextrose indicated that the amount added had to be approx. 5 times as high as initial planned, indicating that the adding of dextrose was approx. 1 g and not 0.2 g / 100 g.

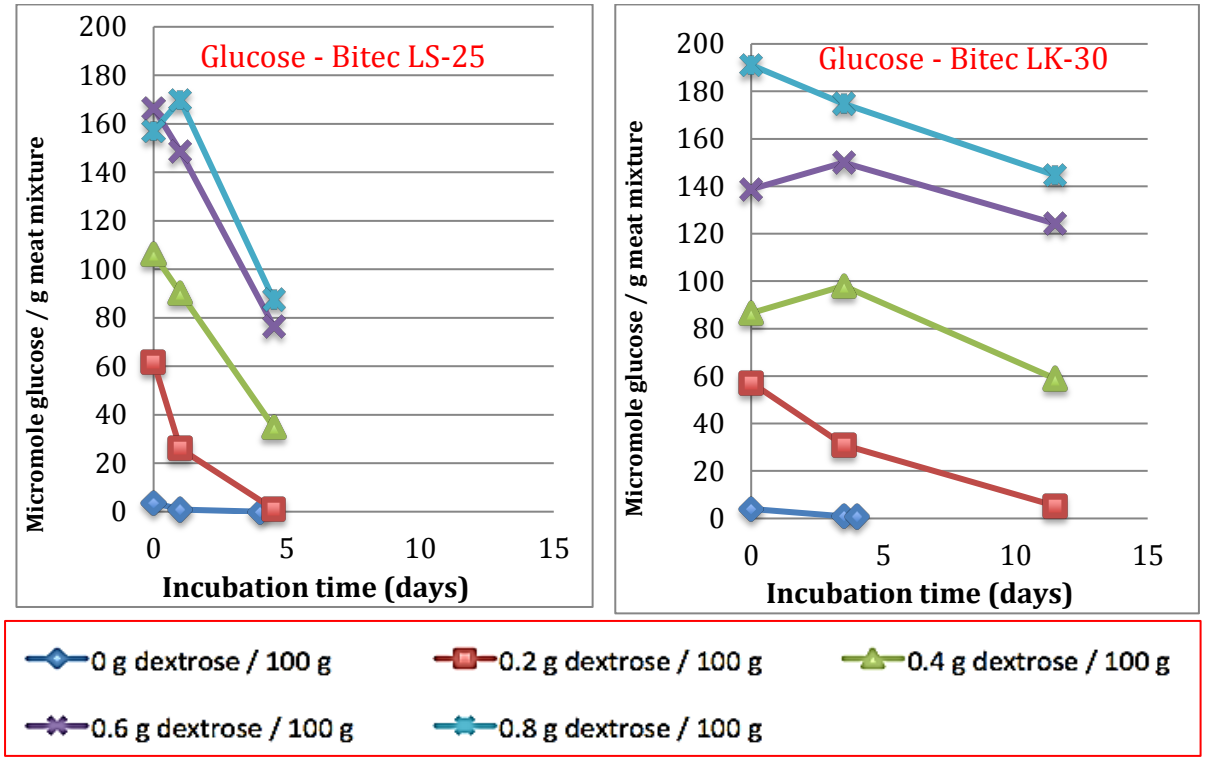

Figure 5.4-2: Concentrations of glucose as a function of time for the two starter cultures LS-25 and LK-30 tested with concentrations of dextrose from 0-0.8 g / 100 g meat mixture and 3.00 g NaCl.

5.4.3.2. Lactic acid

Lactic acid is important in the acidification process as mentioned earlier. During the earlier studies, the fast fermenting starter cultures produced more lactic acid than the slow fermenting starter cultures, and it was desired to see if the concentrations of sugar could affect the concentrations of lactic acid. Even the high concentrations of added dextrose by a mistake, the concentration of lactic acid is not higher than expected.
The fast fermenting starter cultures LS-25 produced more lactic acid than the slow fermenting starter culture LK-30 as expected based on the previous studies. For both starter cultures did the sample containing zero added dextrose produce the lowest concentrations of lactic acid.
The meat mixtures containing LS-25, seemed to have the lowest concentration of lactic acid in the sample containing 0.2 g dextrose / 100 g meat mixture, while the sample added 0.4 g dextrose / 100 g meat mixture had the highest concentration. The starter culture LK-30 added 0.6 g dextrose / 100 g meat mixture had the lowest concentration of lactic acid, while the sample added 0.2 g dextrose / 100 g meat mixture had the highest concentration of lactic acid (Figure 5.4-3).

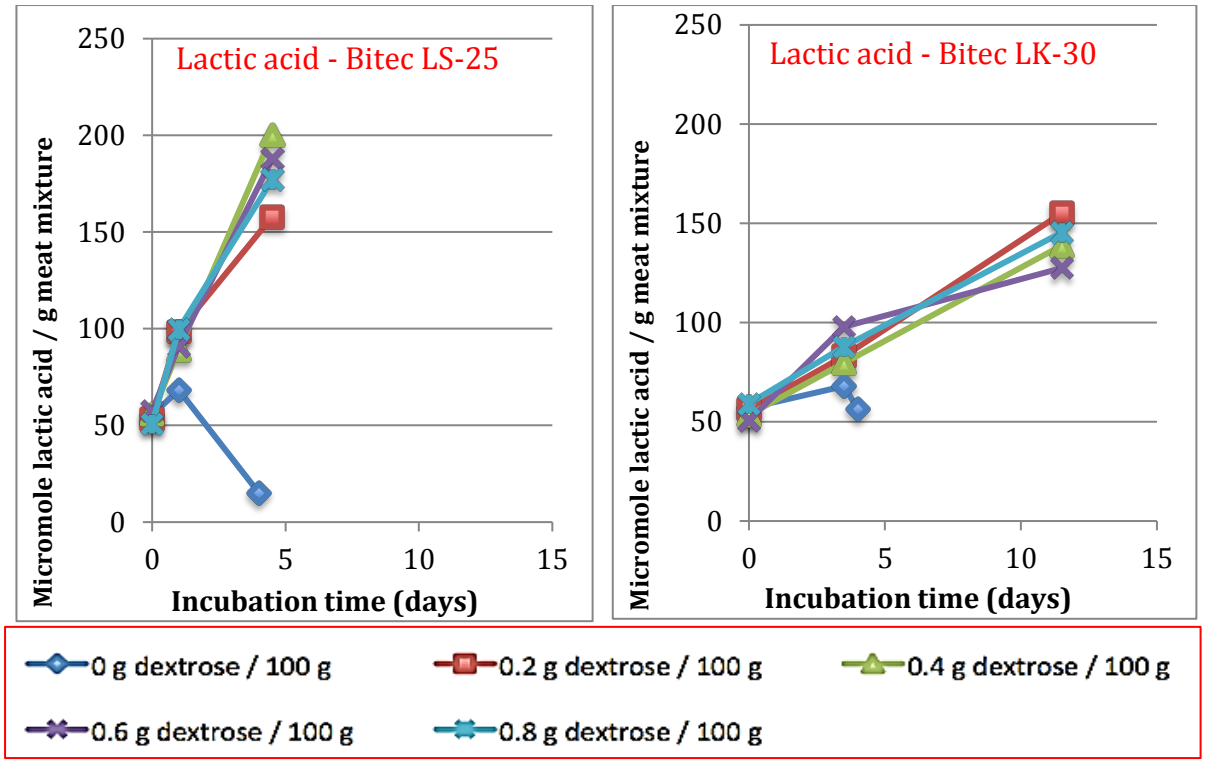

Figure 5.4-3: Concentrations of lactic acid as a function of time for the two starter cultures LS-25 and LK-30 tested with concentrations of dextrose from 0-0.8 g / 100 g meat mixture and 3.00 g NaCl.

5.4.3.3. Acetic acid

The previous preliminary study two, showed no differences regarding the concentrations of acetic acid, but when the concentrations of dextrose were increased further the starter cultures might have enough sugar available to ferment other products than lactic acid in higher concentrations.

The starter culture LK-30 produced higher concentration of acetic acid than LS-25 in the absent of dextrose. LS-25 seemed to had a higher concentration of acetic acid in the sample added 0.8 g dextrose / 100 g meat mixture than the other meat mixtures, the sample added LK-30 and 0.2 g dextrose / 100 g meat mixture had a high concentration of acetic acid at the start (Figure 5.4-4). The high concentration at the start could be a analysis fault since non of the other meat mixtures from the same meat mixture a concentration of acetic acid from the beginning. Since the conclusion of the high concentrations of glucose in the meat mixtures was so high, is could seem like the concentration of acetic acid do not seem to become higher even with a higher added amount.

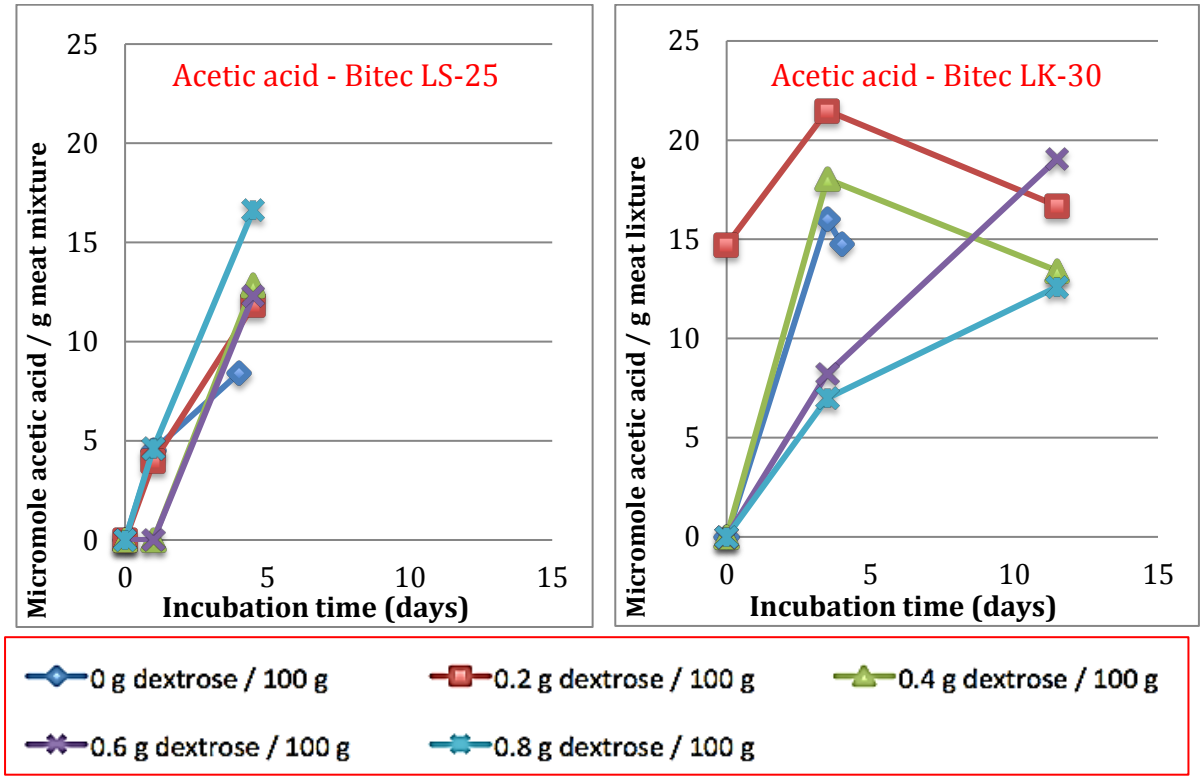

Figure 5.4-4: Concentrations of acetic acid as a function of time for the two starter cultures LS-25 and LK-30 tested with concentrations of dextrose from 0-0.8 g / 100 g meat mixture and 3.00 g NaCl.

5.4.3.4. Citric acid

In the preliminary study two, we looked on how concentrations of sugar affected the starter culture LS-25 and no differences regarding the concentrations of citric acid were found. In this study the starter culture LS-25 and LK-30 were tested.

Both starter cultures added 0 g dextrose / 100 g meat mixture had the highest concentration of citric acid.

The meat mixtures inoculated with LS-25 added 0.8 g dextrose / 100 g meat mixture, had the highest increase of citric acid at day 1.0, but increased most at day 4.5. The starter culture LS-25 seemed to have lower concentrations of citric acid the higher concentrations added dextrose after 4.5 days of fermentation.

The starter culture LK-30 showed the highest concentration after 11.5 days of fermentation in the meat mixtures added 0.2 g and 0.8 g dextrose / 100 g meat mixture. The sample added 0.6 g dextrose / 100 g meat mixture had the highest concentration at day 3.5, but the lowest at day 11.5 of fermentation. The sample added 0 g dextrose / 100 g meat mixture showed the highest concentration at day 3.5, but at day 4.0 the concentration had decreased to almost the equal level as the start sample (Figure 5.4-5).

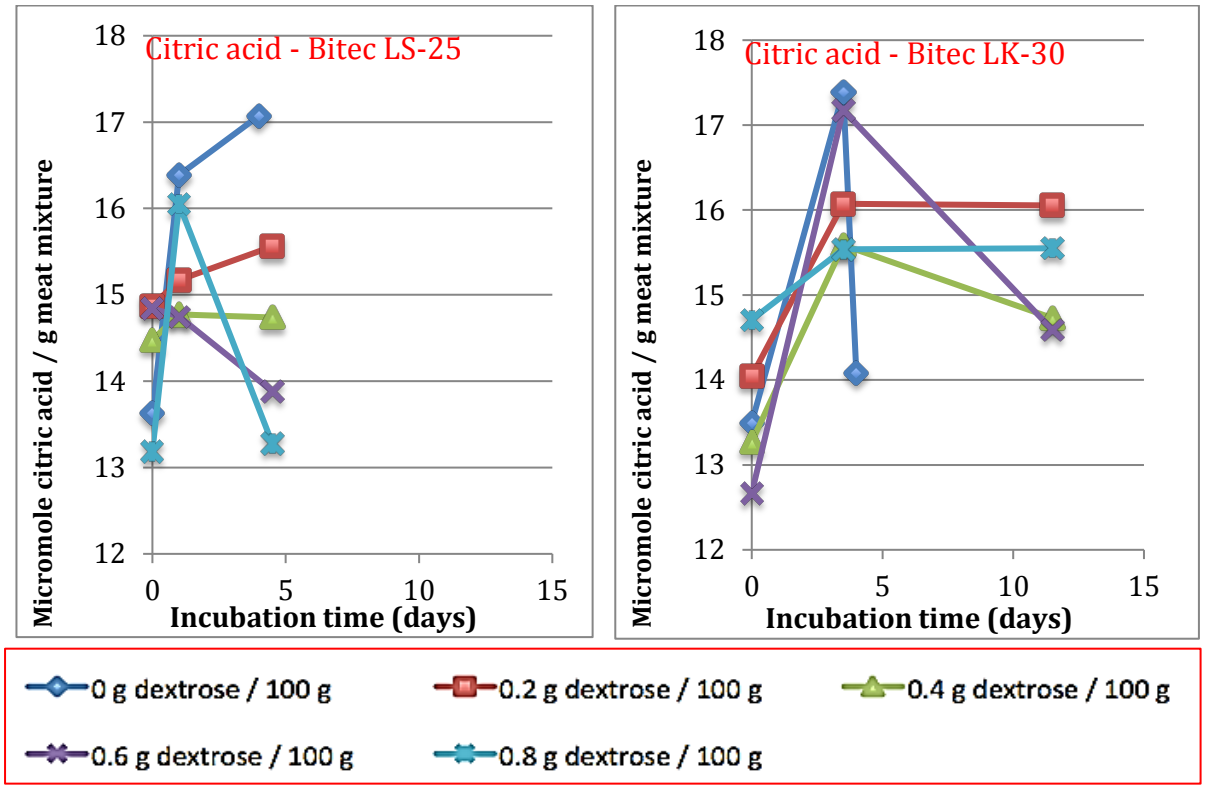

Figure 5.4-5: Concentrations of citric acid as a function of time for the two starter cultures LS-25 and LK-30 tested with concentrations of dextrose from 0-0.8 g / 100 g meat mixture and 3.00 g NaCl.

5.4.3.5. Pyruvic acid

Pyruvic acid was found in higher concentrations in the meat mixtures added 0 and 0.2 g dextrose / 100 g meat mixture for both tested starter cultures. At the start of fermentation had the meat mixtures added 0 g dextrose / 100 g meat mixture a higher concentrations of pyruvic acid than the other meat mixtures added higher concentrations of dextrose.
The starter culture LS-25 added 0.2 and 0.4 g dextrose / 100 g meat mixture had a similar increase of pyruvic acid, but the sample added 0.4 g dextrose / 100 g meat mixture increased later in the fermentation.
The starter culture LK-30 added 0.2 g dextrose / 100 g meat mixture had an increase until day 3.5 before it started to decrease. The other meat mixtures inoculated with LK-30 and higher concentrations that 0.2 g dextrose / 100 g meat mixture showed until day 3.5 a decrease of pyruvic acid and was not detected after this point (Figure 5.4-6).

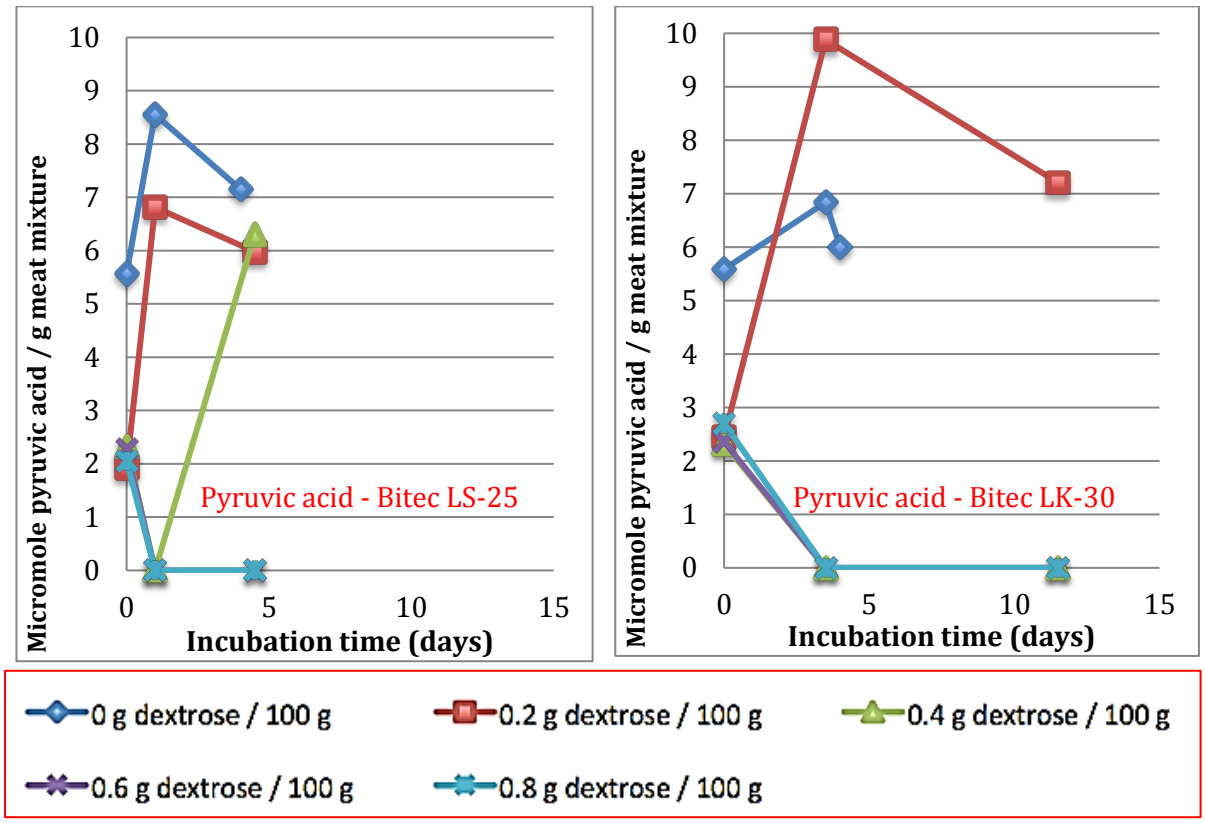

Figure 5.4-6: Concentrations of pyruvic acid as a function of time for the two starter cultures LS-25 and LK-30 tested with concentrations of dextrose from 0-0.8 g / 100 g meat mixture and 3.00 g NaCl.

5.4.3.6. Succinic acid

The starter culture LS-25 had in the final meat mixtures, a higher concentration of succinic acid, as the lower concentrations of dextrose that was added. LK-30 showed a higher increase and decrease in the meat mixtures added 0.6 g and 0.8 g dextrose than the other meat mixtures added lower concentrations of dextrose, but in the final meat mixtures, the concentrations of succinic acid seemed to not have been affected by the different concentrations of added dextrose (Figure 5.4-7).

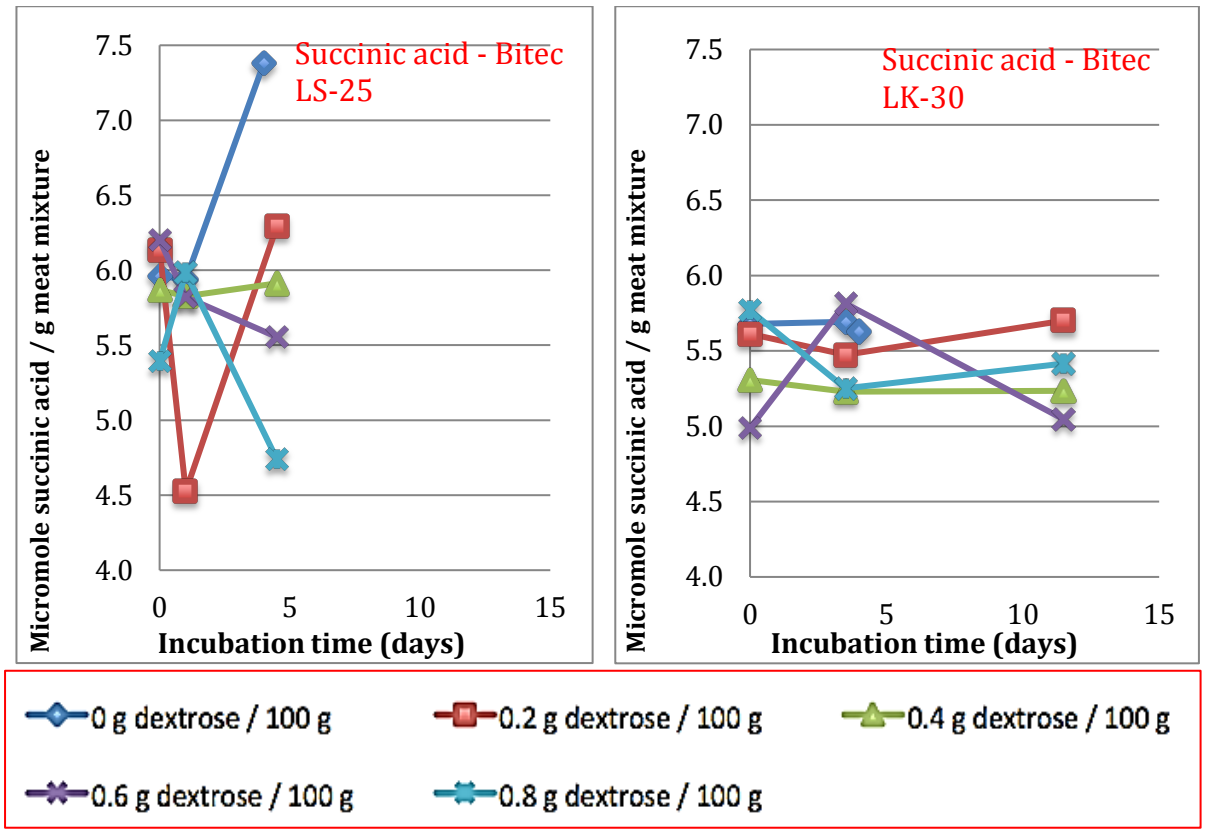

Figure 5.4-7: Concentrations of succinic acid as a function of time for the two starter cultures LS-25 and LK-30 tested with concentrations of dextrose from 0-0.8 g / 100 g meat mixture and 3.00 g NaCl.

5.4.4. Inoculation of starter culture with reactivation medium

The starter culture LK-30 showed a prolonged fermentation in the initial studies than desired as a to long fermentation period could give other microorganism (spoilage *e.g.*) the possibility to grow. A reactivation medium was tested in order to give the starter culture a "head start" by initiating growth before addition to the meat mixture.

The initial pH was lower in the meat mixtures inoculated with the reactivated culture, but during fermentation the meat mixtures inoculated with the reactivated culture had a bit higher pH than the meat mixtures inoculated directly into meat (Figure 5.4-8).

The use of reactivated cells promoted colour formation and gave a deeper pink colour than in meat mixture inoculated directly with LK-30 (Figure 5.4-9).

Another replicate of the study was conducted in preliminary study four to see after the same development.

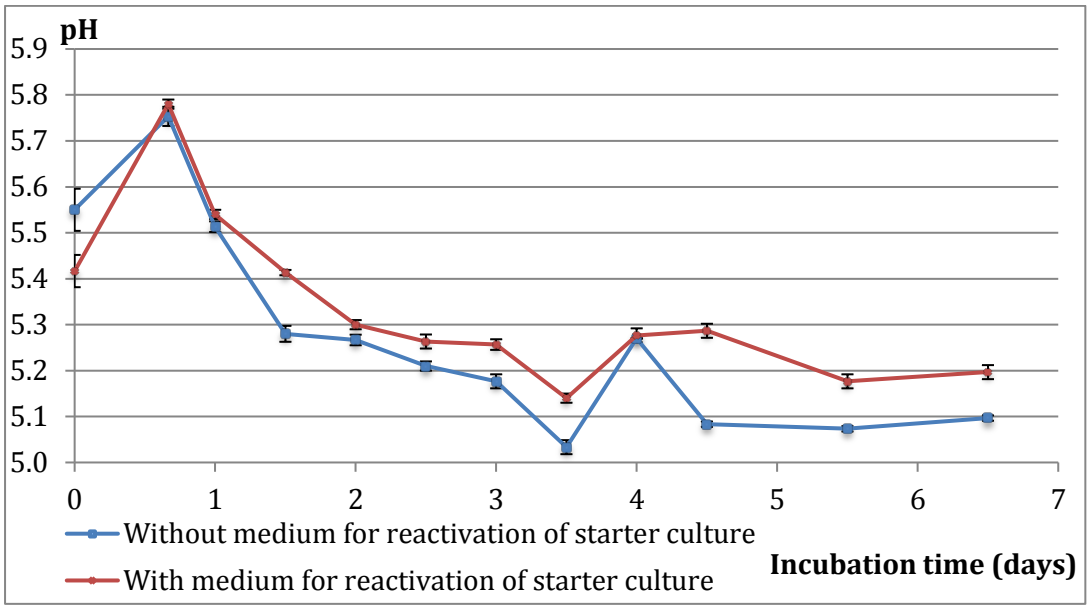

Figure 5.4-8: pH as a function of time in meat mixtures added the starter culture LK-30 either directly into the meat mixture or after pre-cultivation in reactivation medium. The meat mixtures were added 1.5 g NaCl + 1.28 g KCl/100 g. Standard deviation (n=3) is indicated.

Figure 5.4-9: Colour of the meat mixture after 6.5 days fermentation with the starter culture LK-30 either added directly to the mixture or after pre-cultivation in reactivation medium.

5.5. Preliminary study 4

The salt concentrations in this study were selected based on an assumption about what could work in dry fermented sausage production without having large effects on taste, texture, and processing, but also represent the edges of a low concentrations of NaCl and high concentrations of KCl. It was desired to find out how the different concentrations of salt affected the two selected starter cultures LS-25 and LK-30 and the meat mixture based on the technological properties as pH, water activity, organic acids and sugar.

In all meat mixtures added LS-25, pH increased from start to day 2, when it should have decreased, and the final pH was higher than expected. The meat mixtures added LK-30 also yielded a higher final pH than expected (Figure 5.5-1).
A hairy white mycelium and a few black mycelia was observed in the meat mixtures on day 6.0 and 6.5. The meat mixtures also smelled bad. The degree of mould-growth was highest in the meat mixtures added least salt. Some of the meat mixtures were inoculated with reactivated starter culture LK-30. These mixtures appeared to have less mould growth than the comparable mixtures added the starter culture directly into the mixture. As also observed in the previous study, the reactivation medium promoted a deeper and redder colour. No further analyses were conducted in this study, but a change was suggested to the preparation of meat mixture for the model used for observing the fermentation.

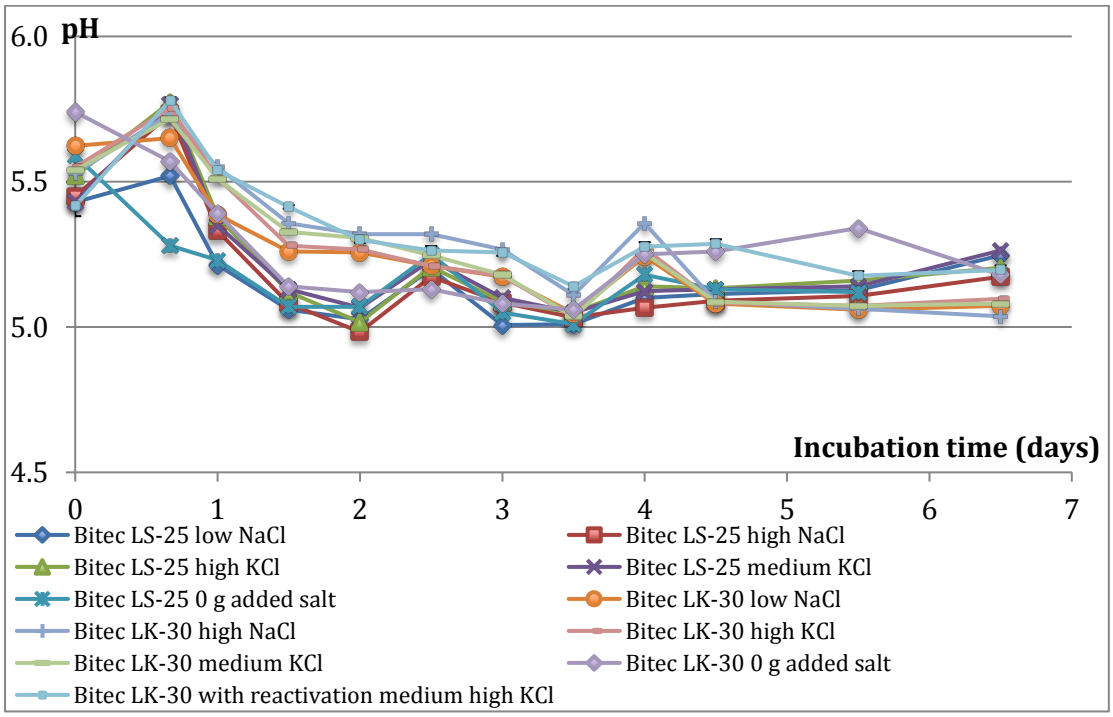

Figure 5.5-1: pH as a function of time for meat mixture fermented with the starter cultures LK-30 and LS-25 and added various concentrations and types of salt (low NaCl=1.5 g NaCl + 1.0 g curing salt, high NaCl=2.5 g NaCl + 1.0 g curing salt, high KCl= 1.5 g NaCl + 1.28 g KCl + 1.0 g curing salt, medium KCl = 2.0 g NaCl + 0.64 g KCl + 1.0 g curing salt / 100 g meat mixture). Meat mixture was added the starter culture LK-30 added 3.83 g KCl / 100 g, the starter culture LK-30 was inoculated with the use of a reactivation medium. The standard deviation (n=3) is indicated.

5.6. Study 5 – main study (dry fermented sausage production)

Study five was the main study and designed to compare a fast and a slow fermenting starter culture in sausage production when the addition of salt was reduced and/or partially replaced with KCl.

5.6.1. Development of pH

pH as a function of time is a good indicator of the fermentation rate. Based on the initial studies, we had an approximate pH curve for both starter cultures and knew that LS-25 used less time to reduce pH than LK-30. This was also verified in this study (Figure 5.6-1).

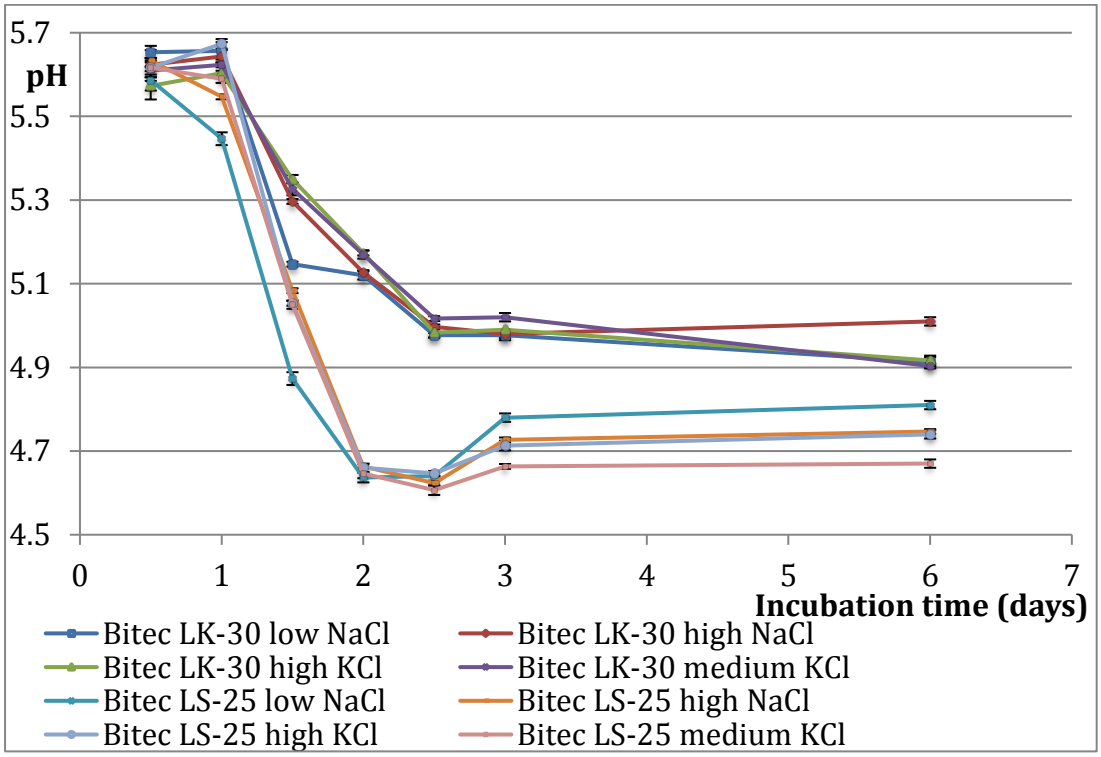

Figure 5.6-1: pH as a function of time from 0.5 to 6 days after start, for sausages inoculated with the starter cultures LK-30, or LS-25, and added different amounts and types of salt. pH measurements for day zero and after day six is left out of figure. The standard deviation (n=3) is indicated

pH-measurements as a function of time with the measurements for day 0 and from day 6 are shown in appendix D.

Both starter cultures initially reduced pH fastest in the meat mixture added the lowest amount of NaCl, but the difference was not large and pH on day six was within 0.1 pH unit independent of the amount and type of salts added. The sausages fermented with LS-25 had a slightly lower pH than the sausages fermented with LK-30 on day 6. The statistical analysis showed that there were with 95 % certainty no interaction between the salt concentrations and the starter culture (p=0.277) when calculated from day 0 to day 16.

In Figure 5.6-1, pH as a function is plotted from day 0.5 to day 6. The determination of pH at start was hampered by problems with the pH-meter leading to unstable measurements. After day six, pH was approx. constant.

5.6.2. Dry weight

Meat mixtures from the different batches collected before addition of salt, had an average dry weight of 35.6 ± 0.4 % (for details, see appendix D)

At start, the dry weight mostly varied from 41-43 % and remained at this level for the first few days. Then it started to increase, presumably due to the drying of the sausages (Figure 5.6-2).
The endpoint for the drying process is defined by a water activity below 0.90, and not by the dry weight in this study.
Sausages fermented with LK-30 and added the lowest amount of NaCl ended up with a significantly higher dry weigh than the other sausages fermented with this starter culture. In sausages fermented with LS-25, sausages added low NaCl and sausages added medium KCl, both ended up at a significantly higher dry weight than the others, while the sausages inoculated with LS-25 high NaCl also had a high dry weight at the end even the shorter incubation time. The statistical analysis showed that there were with 95 % certainty significant differences between the salt concentrations ($p=0.000$) and the starter cultures ($p=0.000$) when calculated from day 0 to day 16.

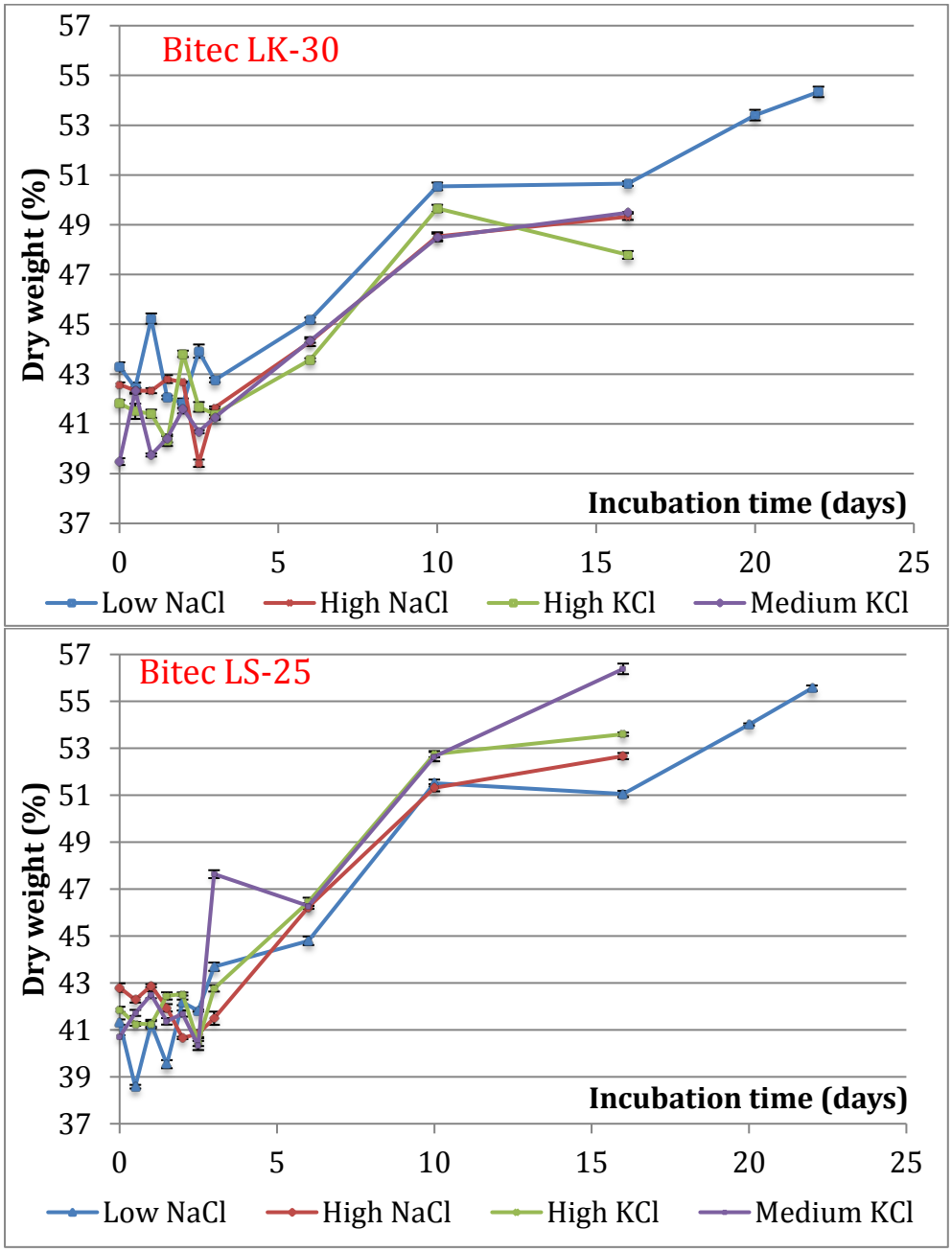

Figure 5.6-2: Dry weight (%) as a function of time for sausages inoculated with the starter cultures LK-30 and LS-25 and added different amounts and types of salts. Low NaCl = 1.5 g NaCl + 1.0 g curing salt, High NaCl = 2.5 g NaCl + 1.0 g curing salt, High KCl = 1.5 g NaCl + 1.28 g KCl + 1.0 g curing salt, and Medium KCl = 2.0 g NaCl + 0.64 g KCl + 0.64 g curing salt /100 g meat mixture. Standard deviation (n=3) is indicated.

5.6.3. Weight losses during ripening and drying

Weight loss is an important aspect from an economic point of view when producing dry fermented sausages. The weight loss is due to water evaporation during ripening and drying. A low degree of evaporation is desirable from an economic point of view, but the key parameter is water activity, which must be reduced below 0.90 before the sausages are considered shelf-stable and microbiologically safe (see below).
Sausages fermented with LS-25 and LK-30 and added low NaCl, were dried for 22 days and lost 38,6 % and 35,9 % of their initial weight, respectively. All the other sausages were dried for 16 days and the weight losses were from 34,7 to 36,7 % (Figure 5.6-3).
All sausages were weighted from the beginning till the end, but the variations between the sausages in the same batch were minimal and not significant.

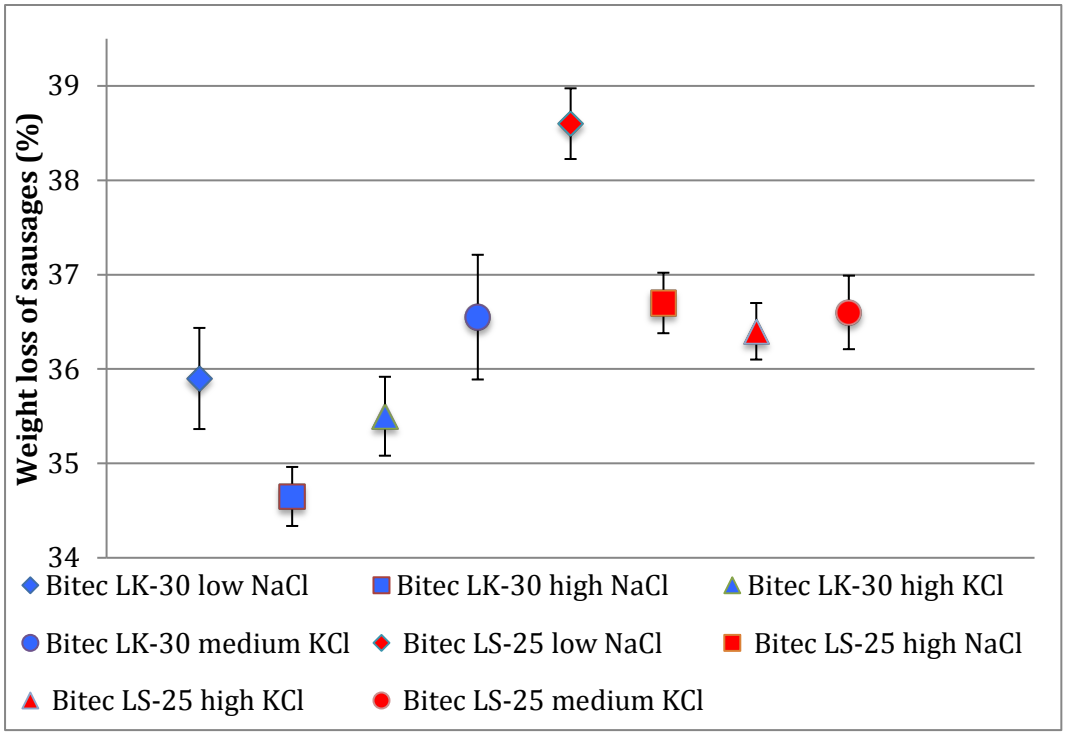

Figure 5.6-3: Weight loss at the end of the drying process for sausages fermented with the starter cultures LK-30 and LS-25 and added different amounts and types of salt. Low NaCl = 1.5 g NaCl + 1.0 g curing salt, High NaCl = 2.5 g NaCl + 1.0 g curing salt, High KCl = 1.5 g NaCl + 1.28 g KCl + 1.0 g curing salt, and Medium KCl = 2.0 g NaCl + 0.64 g KCl + 0.64 g curing salt /100 g meat mixture. The weight loss is based on an average from weighing sausage and remaining sausages with the same composition at the end of the drying process. Standard deviation (n=5-8) is shown.

Weight loss (%) for all sausages inoculated with LK-30 and LS-25 added the different concentrations and types of salt as a function of time can be seen in Appendix D.

5.6.4. Water Activity

The water activity is very important for dry fermented sausages due to microbial safety. A level below 0.90 is considered as safe.
In the batches before addition of salt, the average water activity (n = 12) was 0.967 ± 0.004. Two of the meat mixtures was added salt and mixing started before the meat mixtures were collected, but mixing was not complete before the meat mixtures were taken. These meat mixtures had a brown colour while the others had the pink colour naturally occurred in fresh meat (*See full table with measurements in Appendix C*).

Most of the sausages reached a water activity below 0.90 after 16 days. The sausages added the lowest amount of NaCl was dried for 22 days before they were considered so close to the limit of a_W<0.90 that it was acceptable and the drying terminated (Figure 5.6-4).

The sausages were vacuum-packed, and after two weeks for the sausages added the lowest amount of NaCl, and three weeks for the others, the water activity was analysed again (see Figure 5.6-4). Interestingly, the water activity had decreased considerably during this period of vacuum-packed storage. The statistical analysis showed that there were with 95 % certainty significant difference between the salt concentrations (p=0.000) and the starter cultures (p=0.000) when calculated from day 0 to day 16.

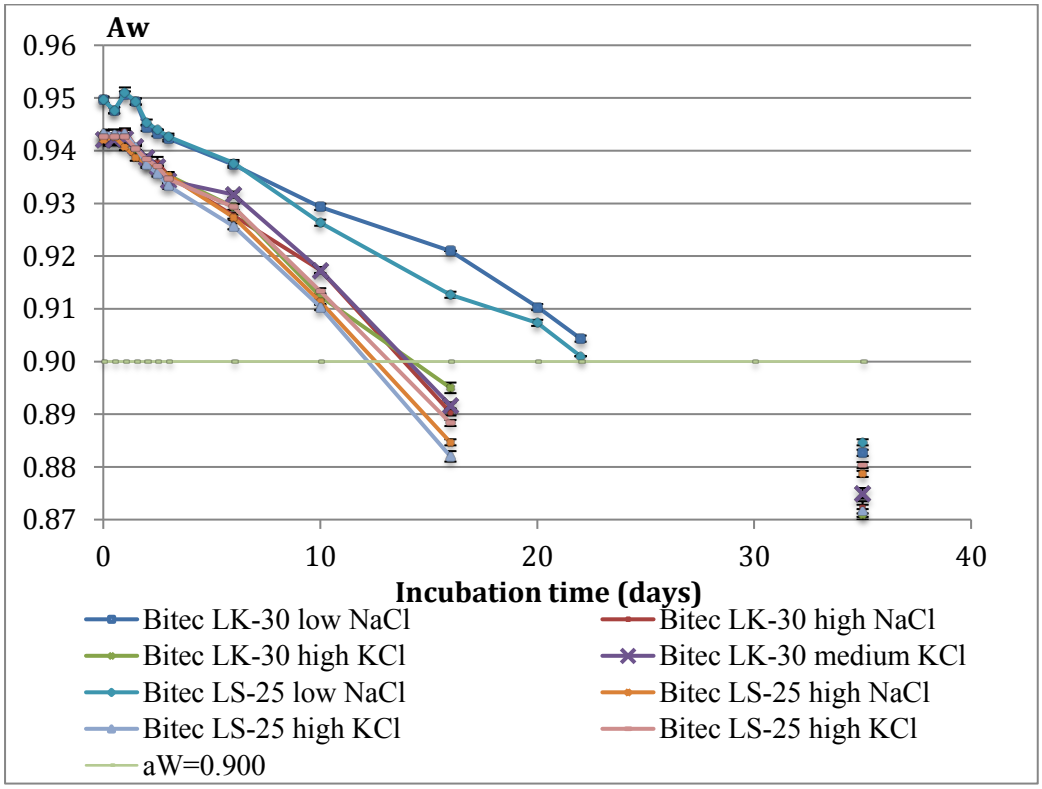

Figure 5.6-4: Water activity as a function of time with meat mixtures inoculated with the starter cultures LK-30 and LS-25 and various concentrations and type of salt. Low NaCl = 1.5 g NaCl + 1.0 g curing salt, High NaCl = 2.5 g NaCl + 1.0 g curing salt, High KCl = 1.5 g NaCl + 1.28 g KCl + 1.0 g curing salt, and Medium KCl = 2.0 g NaCl + 0.64 g KCl + 0.64 g curing salt /100 g meat mixture. At day 35 all sausages were analysed after storage in vacuum packages for two weeks (sausages containing lowest concentration of NaCl) and three weeks. Values are given in A_w ± standard deviation (n=3).

At the end of the drying period, the water activity in two sausages (LK-30 low NaCl and LS-25 medium KCl) were analysed at different locations in the sausages. Considerable differences were found (Table 5.6-1). Based on the test location at the centre of the sausage towards one end, the drying was completed (A_w=0.894 < 0.900), while in the middle it was only 0.921.

Table 5.6-1: Water activity at various locations in the sausages.

	1. Centre of sausage and approx. an equal distance from both ends of the sausage	2. Centre of sausage but towards one end of the sausage	3. Outer edge of the sausage approx. an equal distance from both ends of the sausage
LK-30 low NaCl	0.921	0.894	0.883
LS-25 medium KCl	0.888	0.871	0.851

5.6.5. Organic acids and sugars

Meat mixture without added salt, were analysed to determine the start level of the different compounds.

The meat mixture had a quite high concentration of lactic acid from start (Table 5.6-2). The addition of 0.4 g dextrose/100 g meat mixture (approx. 22.2 µmol/g) gave approx. 29 µmol glucose/g from start. The rest, approx. 7 µmol/g, was presumably present in the meat from start.

Table 5.6-2: Organic acids and glucose in meat mixtures taken directly from the chopper before salt addition. Meat mixture was retrieved from three separate batches added the same amount of dextrose, but the type of starter culture varied. Standard deviation (n=3) is shown.

Compound	Concentration of sugar and acids in meat mixture (µmol/g meat mixture)
Lactic acid	72.2 ± 4.6
Glucose	28.9 ± 1.6
Acetic acid	0.0 ± 0.0
Citric acid	14.0 ± 0.7
Pyruvic acid	3.7 ± 0.7
Succinic acid	3.1 ± 1.7

5.6.5.1. Glucose

Glucose decreased rapidly from start to almost zero in all sausages fermented with LS-25. In sausages fermented with LK-30, however, the concentration glucose never decreased below 10 µmol/g (Figure 5.6-5). The concentration glucose decreased to a lower level in the sausages added KCl. The apparent increase in the concentration of glucose from day 10 is probably due to the drying of the sausages.

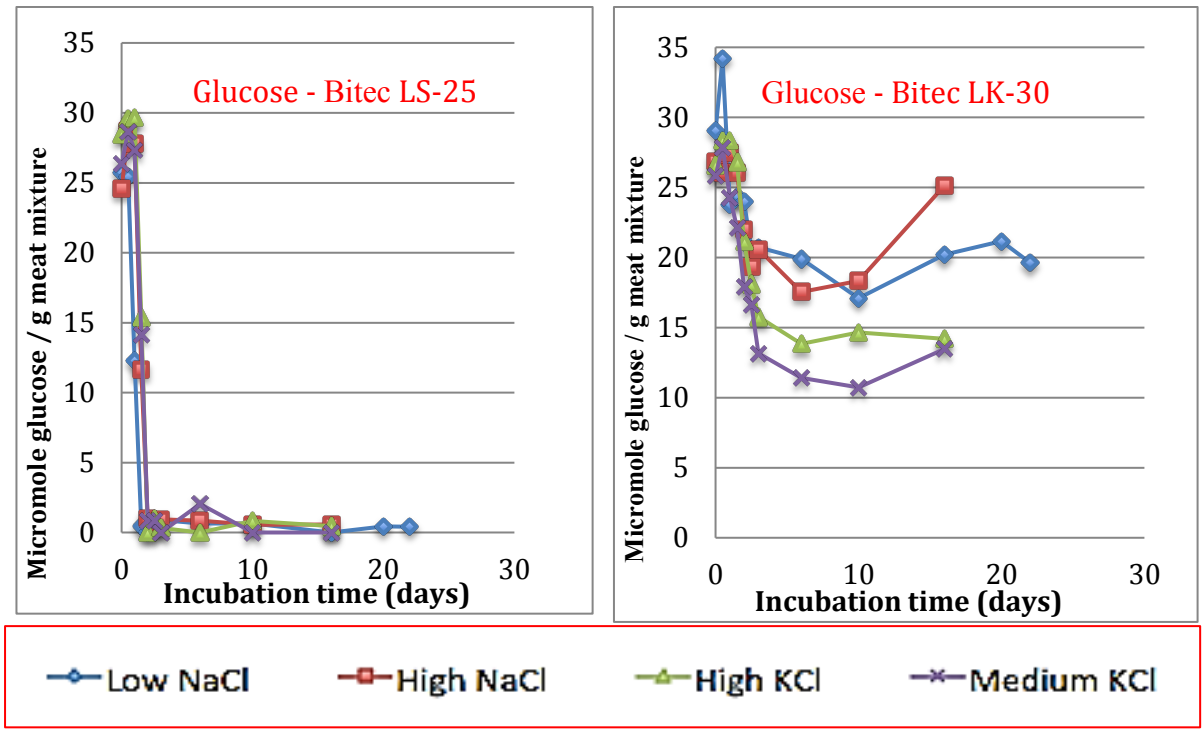

Figure 5.6-5: Glucose as a function of time in the sausages fermented with LK-30 or LS-25 and added different concentrations and types of salt.

5.6.5.2. Lactic acid

The concentration of lactic acid in the initial fermentation phase increased more rapidly in the sausages fermented with starter culture LS-25 than in the sausages fermented with LK-30 (Figure 5.6-6). This is in good agreement with the observed differences in glucose consumption rate (see above). After the initial fermentation period, the concentration of lactic acid continued to increase. Some of the meat mixtures had a peak detected as glycerol that was close to lactic acid. The concentrations of lactic acid that were used were controlled to have a peak on UV to ensure that the peak was lactic acid and not glycerol.
Interestingly, the concentration of lactic acid in sausages fermented with LS-25 was not much higher than in the sausages fermented with LK-30, despite that the glucose consumption by this starter culture was only half of that by LS-25.

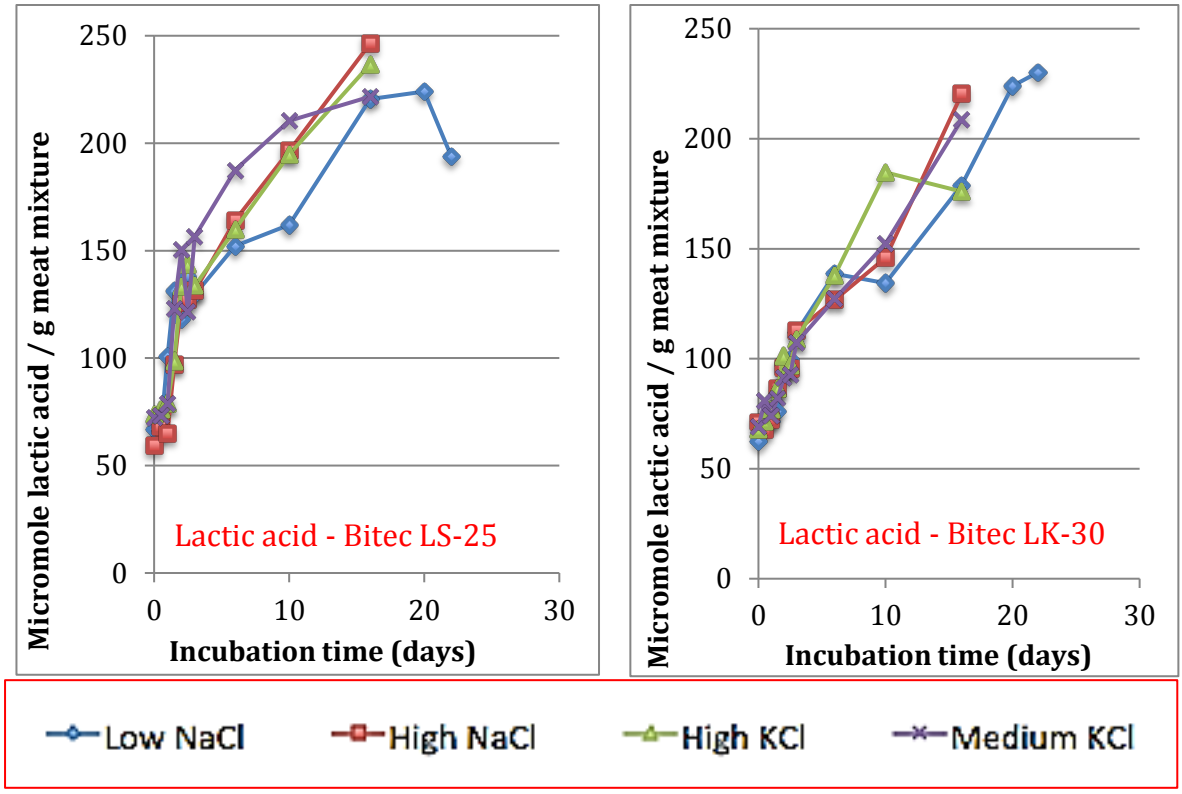

Figure 5.6-6: Lactic acid as a function of time for in sausages fermented with LK-30 or LS-25 and added different concentrations and types of salt.

5.6.5.3. Acetic acid

Acetic acid was produced quite similar for both starter cultures (Figure 5.6-7). Again we have a rapid increase during the initial fermentation period followed by a slower increase during the ripening and drying period.

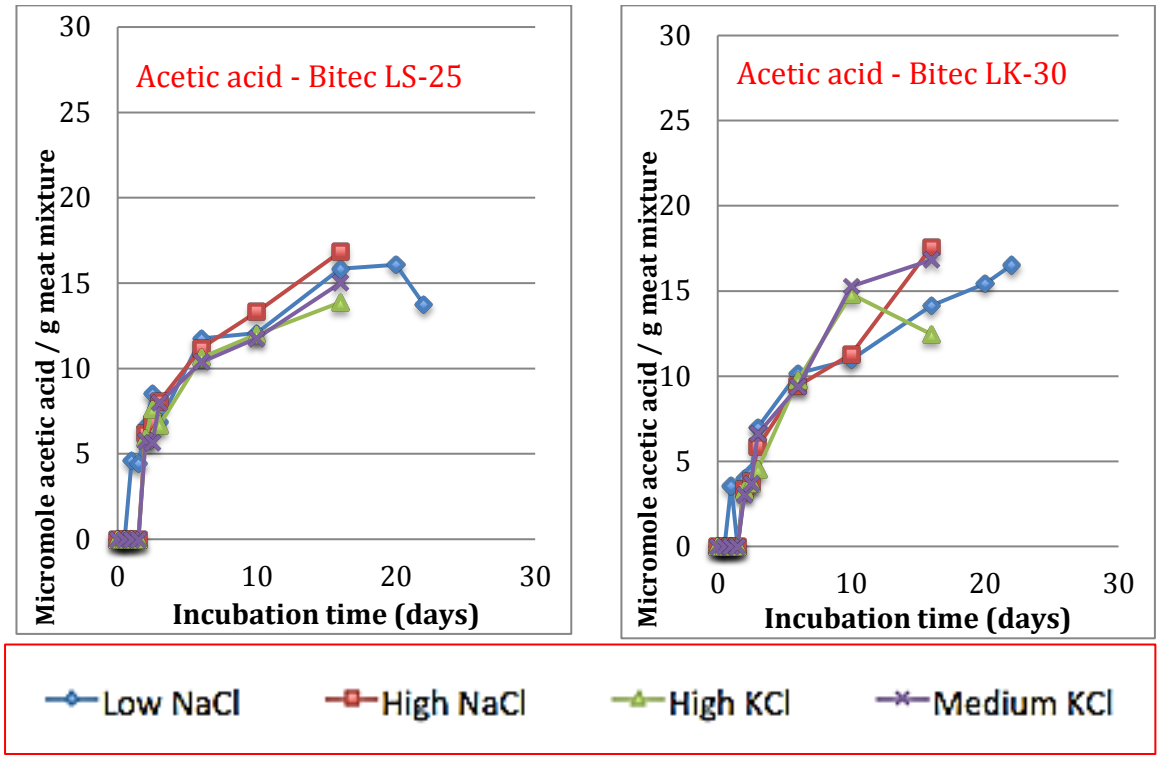

Figure 5.6-7: Concentration of acetic acid as a function of time in sausages fermented with LK-30 or LS-25 and added different concentrations and types of salt.

5.6.5.4. Citric acid

The citric acid content from start was around 14 µmol/g sausage, but increased as water evaporated from the sausage during ripening and drying (Figure 5.6-8). No obvious trend was seen regarding the different salt concentrations.

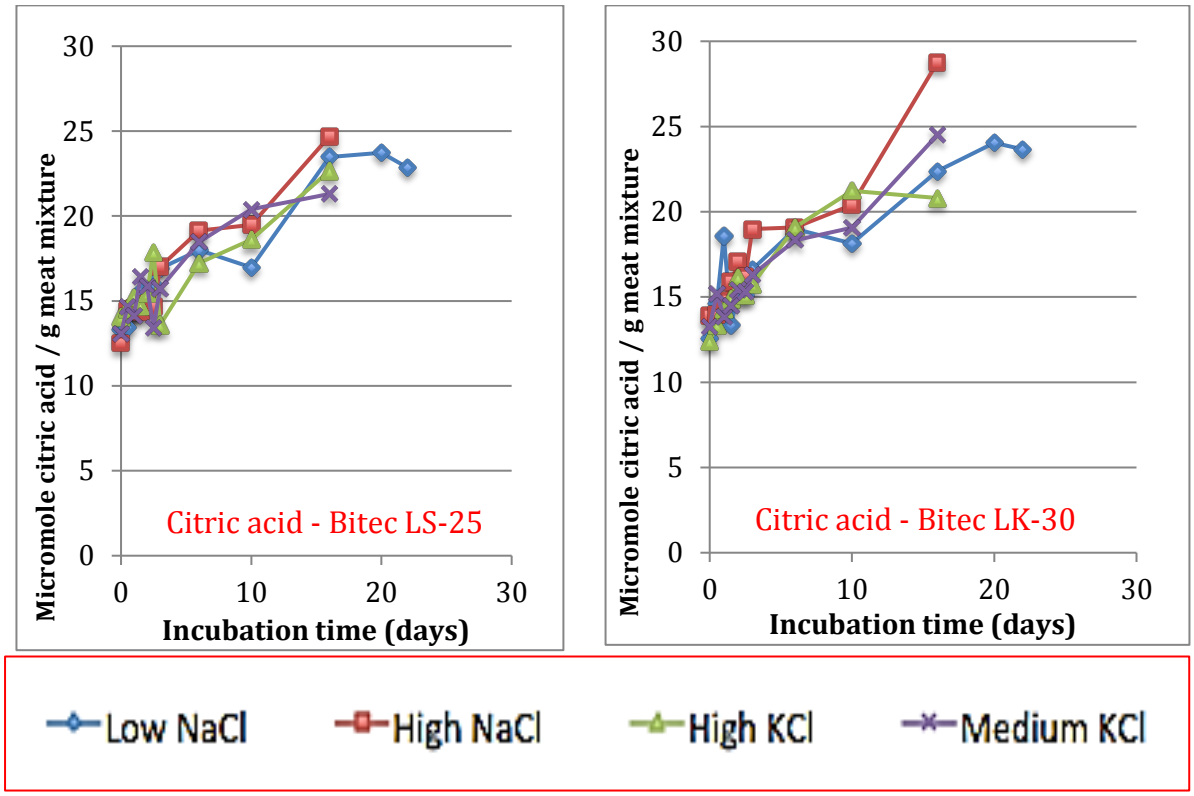

Figure 5.6-8: Concentration of citric acid as a function of time in sausages fermented with LK-30 or LS-25 and added different concentrations and types of salt.

5.6.5.5. Pyruvic acid

The sausages contained 4-5 µmol pyruvic acid/g from start (Figure 5.6-9). In sausages fermented with LS-25 the concentration of pyruvic remained at this level, except for the sausages added high NaCl, where it increased to around 8 µmol/g at the of the ripening and drying period. However, in the sausages fermented with LK-30, pyruvic acid increased during the fermentation phase to around 7 µmol/g, and to 8-10 µmol/g during ripening and drying.

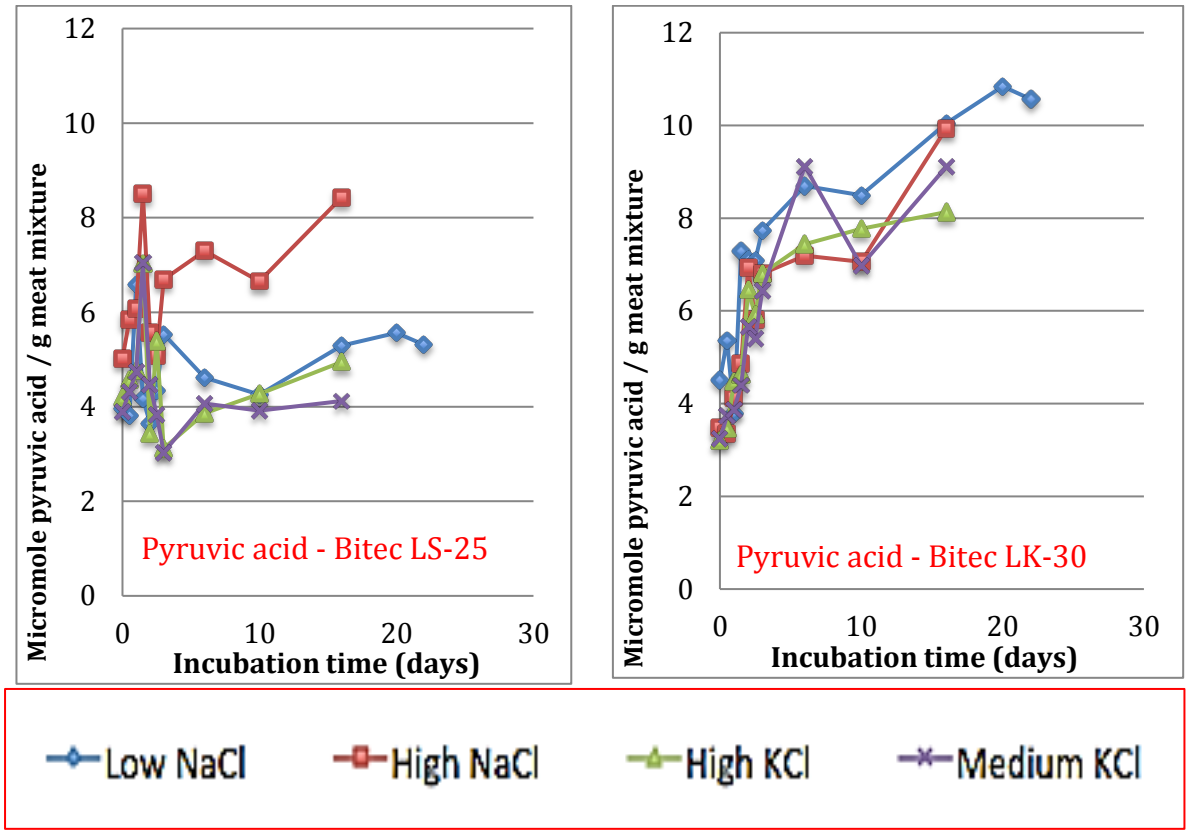

Figure 5.6-9: Concentration of pyruvic acid as a function of time in sausages fermented with LK-30 or LS-25 and added different concentrations and types of salt.

5.6.5.6. Succinic acid

The sausages contained 3-5 µmol succinic acid/g from start, and this increased to 6-8 µmol/g at the end of the ripening and drying period (Figure 5.6-10).

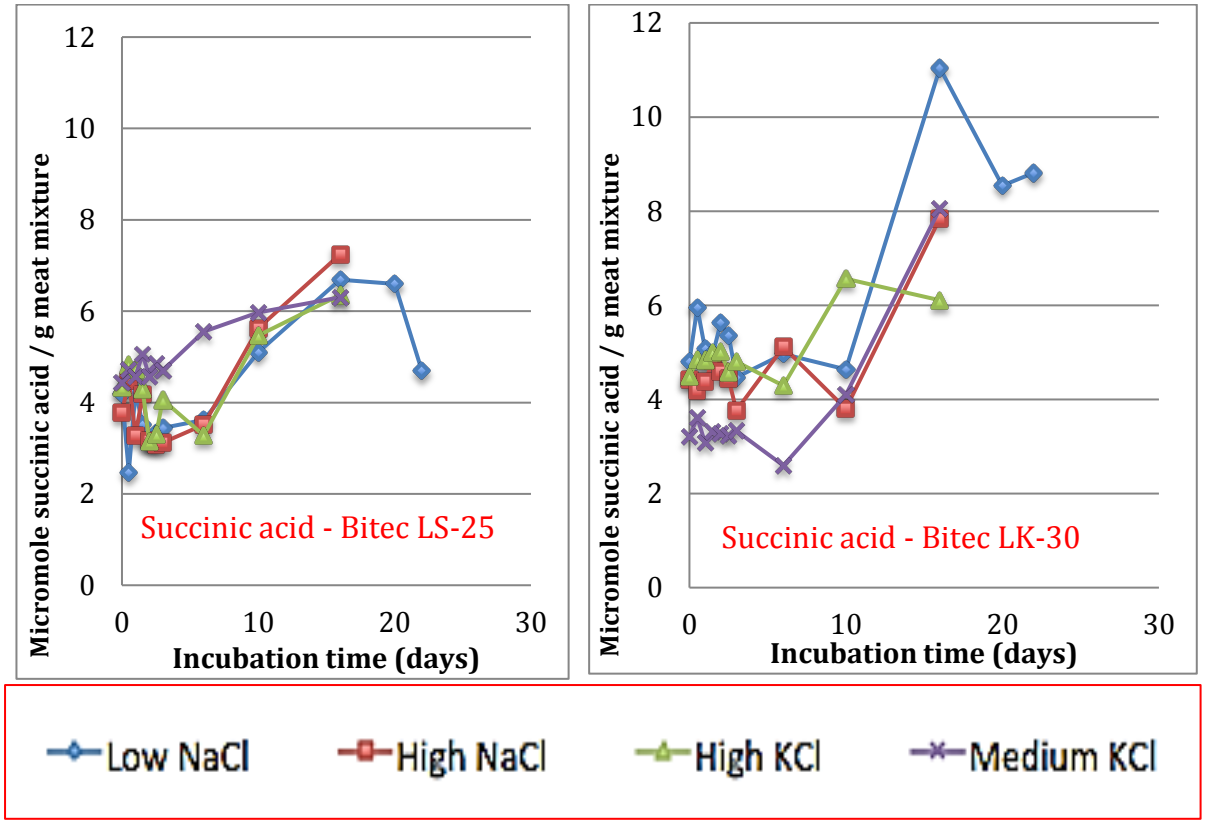

Figure 5.6-10: Concentration of succinic acid as a function of time in sausages fermented with LK-30 or LS-25 and added different concentrations and types of salt.

5.7. Changes in organic acids and glucose during ripening and drying

During ripening and drying, water evaporates from the sausages. This will increase the content of organic acids per g sausage even if no changes in the amount of the compounds occur. The content per g dry weight, however, should remain constant if no changes occur. However, as shown in Table 5.7-1 and Table 5.7-2, the concentration of lactic acid and acetic acid in the sausages increased considerably also per g dry weight during ripening and drying. LK-30 had the highest increase of both lactic acid and acetic acid. This indicates that both lactic acid and acetic acid was produced during this phase, despite that, in the case of LS-25, all glucose was consumed during the initial fermentation phase, and in the case of LK-30, only small amounts of the residual glucose were consumed during the drying and ripening phase.

Table 5.7-1: Content of glucose, lactic and acetic acid in the sausages fermented with Bitec LK-30 after the fermentation phase and at the end of the drying/ripening period.

Sausage	Glucose (µmol/g d.w.)		Lactic acid (µmol/g d.w.)		Acetic acid (µmol/g d.w.)	
	Day 3	Final day[A]	Day 3[B]	Final day[A]	Day 3	Final day[A]
Low NaCl	49	36	262	424	16	30
High NaCl	49	51	276	447	14	36
High KCl	38	30	263	368	11	26
Medium KCl	32	27	260	422	16	34

A 22 days for Low NaCl, 16 days for the others

B The sausages contained 162 ± 13 µmol/g d.w. from start

Table 5.7-2 Content of glucose, lactic and acetic acid in the sausages fermented with Bitec LS-25 after the fermentation phase and at the end of the drying/ripening period.

Sausage	Glucose (µmol/g d.w.)		Lactic acid (µmol/g d.w.)		Acetic acid (µmol/g d.w.)	
	Day 3	Final day[A]	Day 3[B]	Final day[A]	Day 3	Final day[A]
Low NaCl	1	<1	297	349	16	25
High NaCl	1	1	316	468	20	32
High KCl	<1	1	312	442	16	26
Medium KCl	<1	<1	329	393	17	27

A 22 days for Low NaCl, 16 days for the others

B The sausages contained 163 ± 18 µmol/g d.w. from start

5.7.1. Observations of colour and texture

Sausages fermented with LK-30 were found to be softer than sausages fermented with LS-25. Sausages inoculated with LK-30 added low NaCl were remarkable less hard, especially during the fermentation process when protein formed a gel, then the other meat mixtures containing the same salt concentration.
At the end of the drying period, all sausages added the lowest amount of NaCl had more crust at the edges and appearance defects (Figure 5.7-1).

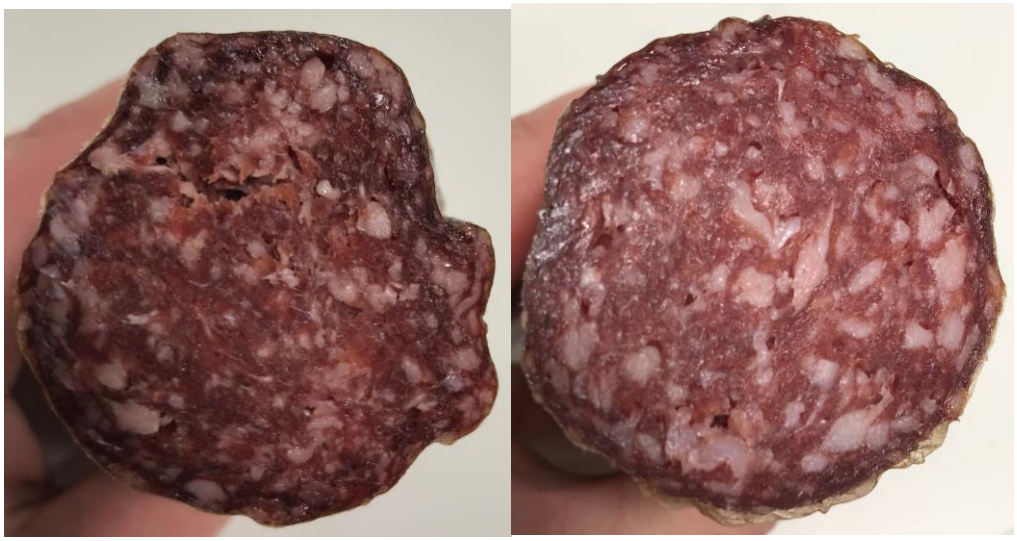

Figure 5.7-1: Left: Sausage with defects in appearances and some crust at the edges. Right: Sample with a little crust at the edges and no defects in appearances. Both pictures are taken from two different sausages with both water activities below 0.90.

Sausages fermented with LK-30 and added low NaCl or high NaCl, seemed to have a darker red colour than the other sausages fermented with LK-30. In general, sausages fermented with LK-30 had a darker red colour than sausages fermented with LS-25.

5.7.2. Sensorial tests

An in-house untrained panel with respect to consistency, colour, taste and aroma assessed the sausages.

Average values for all 15 attributes given by nine assessors (judges) showed that various significant differences between some of the meat mixtures; this is given in Table 5.7-3. How much the assessors (judges) agreed on the meat mixtures for each attribute are given in a Tucker-1 correlation plot in appendix E. Comments given by some of the judges are given in Table 5.7-4 .

Significant differences were found only for some of the attributes and meat mixtures. The differences it could might be explained by both the physiological differences between the assessors and the fact that the panel had no training before the test and might not have a common understanding of the scale. Another cause could be that it actually didn´t was differences regarding the attributes.

Table 5.7-3: Average values for sensorial testing with panel. Nine judges tested eight types of sausages. Meat mixtures with the same letter is not significant different for the attribute. Level of significance is given in the same column as attribute, ns indicates no level of significance found. Meat mixtures with similar attributes regarding taste and odour/aroma, are marked with taste in front of the ones for taste.

Attribute (significant level) sample	LK-30 low NaCl	LK-30 high NaCl	LK-30 high KCl	LK-30 medium KCl	LS-25 low NaCl	LS-25 high NaCl	LS-25 high KCl	LS-25 medium KCl
Colour (p<0.001)	2,89(ef)	5,22 (ab)	3,67(def)	4,11(cd)	5,78 (a)	3,33(ef)	5,56 (ab)	4,67 (bc)
Oily (p<0.01)	6,00(a)	4,33(bc)	3,89(c)	4,76(bc)	5,56(ab)	3,67(c)	3,89(c)	4,00(c)
Lactic acid/salami (p<0.05)	2,78(b)	3,22(b)	2,67(b)	3,78(ab)	4,33(a)	4,56(a)	4,56(a)	3,89(ab)
Chewy (p<0.01)	4,78(ab)	5,56(a)	4,11(bc)	4,78(bc)	3,89(bc)	3,56(cd)	2,67(de)	3,00(de)
Taste lactic acid/salami (p<0.05)	3,78(a)	3,56(a)	4,11(ab)	4,22(ab)	3,89(a)	5,22(b)	5,00(b)	5,22(b)
Sour (ns)	3,00(ab)	2,22(b)	2,33(b)	2,67(ab)	4,33(a)	2,89(ab)	3,11(ab)	2,44(b)
Smoke (ns)	3,67(a)	4,33(a)	4,33(a)	4,89(a)	5,11(a)	4,78(a)	4,22(a)	5,11(a)
Aromatic (ns)	4,56(ab)	4,89(ab)	4,44(ab)	4,00(b)	5,44(a)	4,56(ab)	4,67(ab)	5,00(ab)
Smell intensity (ns)	4,11(a)	4,33(ab)	4,67(ab)	3,78(a)	3,78(a)	4,56(ab)	4,00(a)	5,44(b)
Taste aromatic (ns)	3,89(a)	5,11(ab)	5,00(ab)	4,67(ab)	5,11(ab)	5,00(ab)	4,78(ab)	5,33(b)
Salt (ns)	3,00(a)	4,33(ab)	4,44(ab)	3,89(ab)	4,22(ab)	4,33(ab)	4,89(b)	3,89(ab)
Aftertaste (ns)	3,67(a)	3,89(a)	4,67(a)	4,11(a)	3,89(a)	4,56(a)	4,56(a)	4,78(a)
Taste sour (ns)	3,00(a)	3,11(ab)	2,44(a)	2,78(a)	2,67(a)	4,00(b)	3,11(ab)	3,44(ab)
Taste intensity (ns)	4,44(a)	4,78(a)	5,22(a)	4,44(a)	4,56(a)	5,56(a)	5,11(a)	5,22(a)
Grainy (ns)	4,22(ab)	3,44(ab)	4,11(ab)	3,22(b)	2,78(b)	2,89(b)	3,33(b)	4,89(a)

Table 5.7-4: Comments for some sausages given on the sensorial testing

Sample	Comments
LS-25 low NaCl	Fresh and good cured flavour that tastes like salami, but tastes a bit to salt and lactic acid/sour
LS-25 medium KCl	Good flavour but a hint of too much lactic acid/sour flavour
LK-30 low NaCl	Fat/oily in the edge especially
LK-30 high KCl	An odd bi-taste in the sample, but hard to place what kind of flavour

The results based on the average values and the sample means and LSD-plot can further be analysed using a Bi-plot that allows both attributes and meat mixtures in the same plot. Bi-plot is shown in Figure 5.7-2.

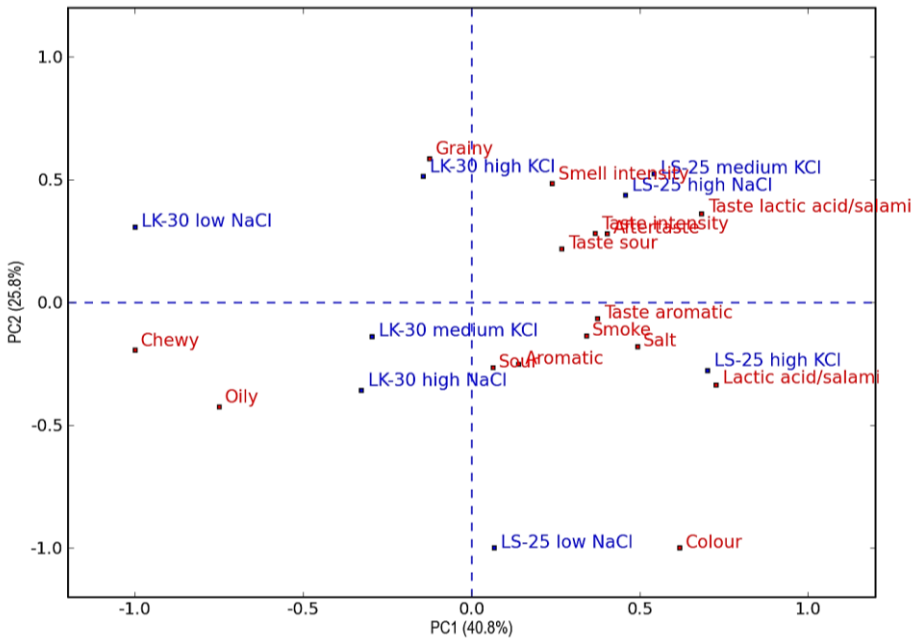

Figure 5.7-2: Bi-plot shows the sensorial evaluation of eight types of sausages containing different concentration of salt, assessed by nine judges. Attributes were evaluated on a scale from one (low intensity) to nine (high intensity). The first axis (PC1) explains 40.8 % of the variance, while the second axis (PC2) explains 25.8 % of the variance. In total explains the plot 66.6 % of the total variance from the original results. Non-trained judges and factors affecting like light, temperature of the sausage *e.g.* may be explanation of the rest of the variance.

Sausages inoculated with LK-30 were perceived as significant more deep red in colour than the sausages inoculated with LS-25. The sausage inoculated with LK-30 added the highest level of NaCl was not significant different from the sausages added LS-25. The sausages added the lowest concentration of NaCl was perceived as oilier than the other sausages. LK-30 was overall found as chewier than LS-25. Sausages inoculated with LS-25 were more or less perceived as significant more tastefull and more odour on the attribute lactic acid/salami. Overall was the sausages inoculated with LK-30 pervcied as more chewy, oily and a deeper red colour, while LS-25 was perceived as a lighter fresher red colour, more taste and odour of lactic acid/salami. Both sausages added low NaCl was found different on the bi-plot, while

the other sausages inoculated with LK-30 seemed to cluster together, the same was seen for LS-25.
Sausages fermented with LS-25 and added low NaCl were perceived as more intense in colour than the other meat mixtures, but the meat mixtures containing LK-30 high NaCl and LS-25 high KCl was found to be like LS-25 low NaCl.

5.7.3. Salt content

To find the amount of salt/sodium in the final product, the sodium content in the sausages was measured. It was also intended to measure the potassium content, but because of problems with the electrode, this was not conducted.
The sausages mostly contained from 0 to 6 % more sodium than predicted from the recipes (Table 5.7-5). For sausages added a low level of NaCl, either alone or in combination with a high level of KCl, the sodium content (calculated as NaCl) was reduced by 13.5-29.8 % relative to the reference of a NaCl content of 5.54 % content of NaCl (high NaCl). The sausages added low NaCl dried to a satisfactory water activity in approx. 22 days, but showed some texture defects.
Sausages added a low addition of NaCl and compensated by a high addition of KCl were reduced compared to the reference sausage with a content of 5.54 % NaCl. These sausages were dried to a satisfactory water activity in 16 days and showed no texture defects.

Table 5.7-5: Sample id, description of the sample, estimated sodium potassium and measured NaCl content in the final meat mixtures after drying were finished.

	Estimated NaCl content (%) [A]	Estimated KCl content (%)[B]	NaCl (%) [C]	Reduction in NaCl compared to reference of 5.54 % NaCl [D]
LK-30 low NaCl	4.00	0.80	4.10	**26.1**
LK-30 high NaCl	5.40	0.80	5.54	**0.0**
LK-30 high KCl	3.90	2.80	4.02	**27.4**
LK-30 medium KCl	4.70	1.80	4.79	**13.5**
LS-25 low NaCl	4.10	0.80	4.35	**21.5**
LS-25 high NaCl	5.50	0.80	5.53	**0.0**
LS-25 high KCl	3.90	2.80	3.89	**29.8**
LS-25 medium KCl	4.70	1.80	4.68	**15.5**

A: The salt content (%) is calculated using the program Prosim (ver. 2016.3). Estimated values are based on the actual weight loss in sausages.

B: The KCl content (%) is calculated based on the added amount of KCl, and an estimate of 0.269 g KCl in the raw meat.

C: Measured sodium content by using a ROSS electrode (4.5.10).

D: Reduction (%) is only based on measurements of NaCl, for meat mixtures containing KCl (high KCl and medium KCl) this would have an affect on the reduction (%) when taken into calculations.

6. Discussion

The aim of the thesis was to study the effects of different starter cultures in combination with sodium reduction, by reducing the amount of added salt (NaCl) or by partial replacement with potassium chloride (KCl), in dry fermented sausages.

Four preliminary studies were conducted in a model system where the meat mixture after mixing with the starter culture, was transferred to a 50-ml plastic tube with a screw cap and incubated at the desired temperature for a few days. The idea was to simulate the first fermentation phase, but the following ripening and drying phase could not be studied in this almost closed system. The four studies investigated the physiochemical properties of the four selected commercial starter cultures when salt and sugar was varied.

In the main study, a total of eight batches of dry fermented sausages were produced, using two levels of NaCl, two combinations of NaCl and KCl, and two commercial starter cultures. The sausages were produced in a production facility, and changes in pH, aW, dry weight, water loss, and consumption of glucose and production of organic acids during fermentation and ripening/drying measured. A sensorial test of the finished sausages was also conducted.

6.1. Dry fermented sausages with reduced sodium content

A primary aim of the study was to produce sausages with a reduced sodium content. The sausages made by adding 2.5 g NaCl + 1.0 g curing salt per 100 g meat mixture (High NaCl) ended up, after ripening and drying, with a sodium content corresponding to approx. 5.5 % (w/w) NaCl. This is slightly above the "normal" salt level in dry fermented sausages (5.2 %). The sausages where the addition of NaCl was reduced to 1.5 g NaCl + 1.0 g curing salt per 100 g meat mixture (Low NaCl), ended up with a sodium content corresponding to 4.1-4.4 % (w/w) NaCl. This is 21-25 % less NaCl than in the "High NaCl" sausages. However, these sausages developed texture defects towards the end of the drying period. The sausages where 1.0 g NaCl was replaced by an equimolar amount of KCl (1.28 g, High KCl) or 0.5 g NaCl was replaced with an equimolar amount of KCl (0.64 g, Medium KCl), ended up with a sodium content corresponding to 3.8-4.0 % NaCl and 4.7-4.8 % NaCl, respectively. This corresponds to a reduction in the sodium content of 27-29 % and 13-15 % relative to the "High NaCl" sausages, respectively. These sausages did not develop any texture defects during drying and reach the required water activity ($a_W < 0.90$) in 16 days, the same as the "High NaCl" reference. The Low NaCl sausages, on the other hand, required 22 days to approach $a_W = 0.90$.

6.1.1. Water activity versus dry weight and weight loss

Water activity is a key parameter in the production of dry fermented sausages. For legal and food safety reasons the water activity in the finished sausages must be below 0.90, and the sausages are dried until $a_W < 0.90$.

The water activity immediately after addition of salt to the meat mixture is a function of the water activity in the meat mixture and the amount of added salt, and the replacement of NaCl with KCl on a molar basis does not affect the initial water activity in the meat mixture (Figure

6.1-1). This is in agreement with Horita et al. (2014b), Corral et al. (2013), and Zanardi et al. (2010), whom all found that when NaCl was partially replaced by KCl on a molar basis, water activity was a function of the added concentration salt molecules and not the type of salt.

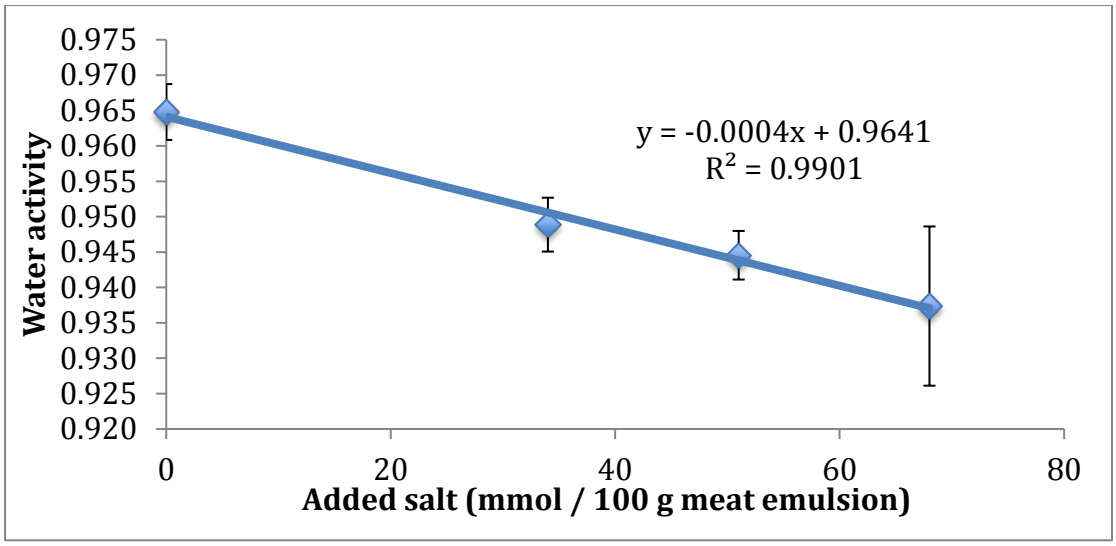

Figure 6.1-1: Water activity as a function of added concentration of salt in the preliminary study one. The more salt molecules added the water activity lowers. Concentrations are given as an average of all meat mixtures containing the same concentration of salt molecules ± standard deviation (n=4 for zero added salt molecules, n=8 for the other concentrations).

During drying the water activity in the sausages decreases. A plot of water activity as a function of weight loss shows that initially water activity decreased only slowly as water evaporated from the sausages (Figure 6.1-2). However, at a water activity of approx. 0.935, water activity started to decrease much more rapidly as water evaporated from the sausages. Apparently, this critical water activity was relatively independent of the initial addition of salt. Initially, the sausages added the lowest concentrations of NaCl (Low NaCl) had a higher water activity than the other sausages, but at the critical point the water activity as a function of the weight loss was almost the same.
The results indicate that the water evaporation from the sausages after they reach the critical water activity was very low. This could be a function of the conditions in the drying chamber (temperature, humidity, air velocity). The measured decrease in water activity after the critical point could then reflect the establishment of a water activity equilibrium in the sausages. The water activity is measured in the centre of the sausages, and if water evaporates rapidly from the sausages the water activity is lower towards the outer edge of the sausage (see Table 5.6-1). When the water evaporation rate decreases, the water activity gradient is likely to decrease, and this may be measured as a water activity decrease in the centre of the sausage despite low evaporation of water. However, the observed decrease in the measured water activity after vacuum packing (see below) indicates that the water activity had not reached an equilibrium when the sausages were vacuum-packed.

After drying, the sausages were vacuum packed in plastic and stored for two or three weeks. During this storage the measured water activity of the sausages decreased, presumably because an equilibrium water activity in the sausages was established when water no longer evaporated from the sausages.

Early vacuum packing could in theory be used to produce salt reduced dry fermented sausages. The texture defects in the sausages added a low level of NaCl were formed during the last week of drying. It is possible that if the drying had been stopped earlier and the sausages vacuum-packed to allow for an equilibration of the water activity, the texture defects could have been avoided. However, the final water activity in the sausages still must be less than 0.90 for legal and food safety reasons, requiring good timing of the termination of the drying process. Furthermore, if the average water activity in the sausages is only slightly lower than 0.90, there is a risk that some sausages in the batch end up with $a_W > 0.90$ due to small variations in water content between different sausages. Since little is known about the microbiological effects when fat/oil or water appears on the outside of the sausage further research should be done.

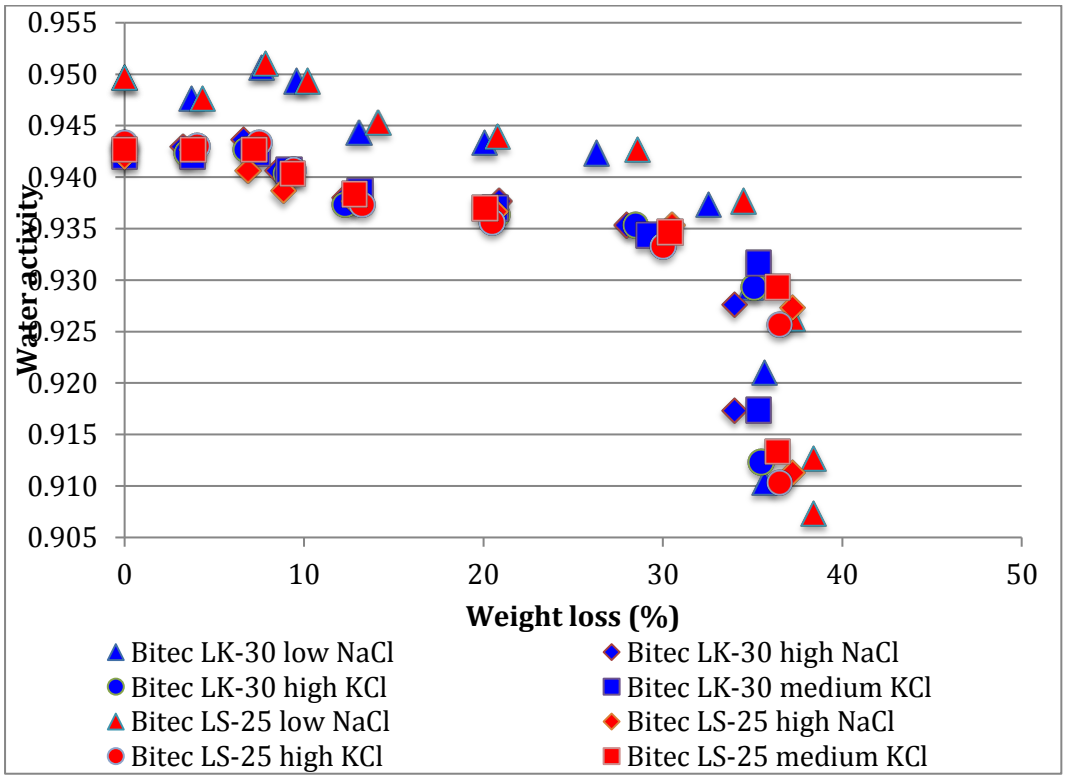

Figure 6.1-2: Weight loss (%) as a function of the water activity for the sausages fermented with LK-30 or LS-25 and added different concentrations and types of salt. (low NaCl=1.5 g NaCl + 1.0 g curing salt, high NaCl=2.5 g NaCl + 1.0 g curing salt, high KCl= 1.5 g NaCl + 1.28 g KCl + 1.0 g curing salt, medium KCl = 2.0 g NaCl + 0.64 g KCl + 1.0 g curing salt / 100 g meat mixture).

The dry weight of the meat mixture immediately after addition of salt, mostly showed good agreement with the hypothesis that it was a function of the dry weight of the meat mixture without added salt plus the amount of salt added on a weight basis (Figure 6.1-3).

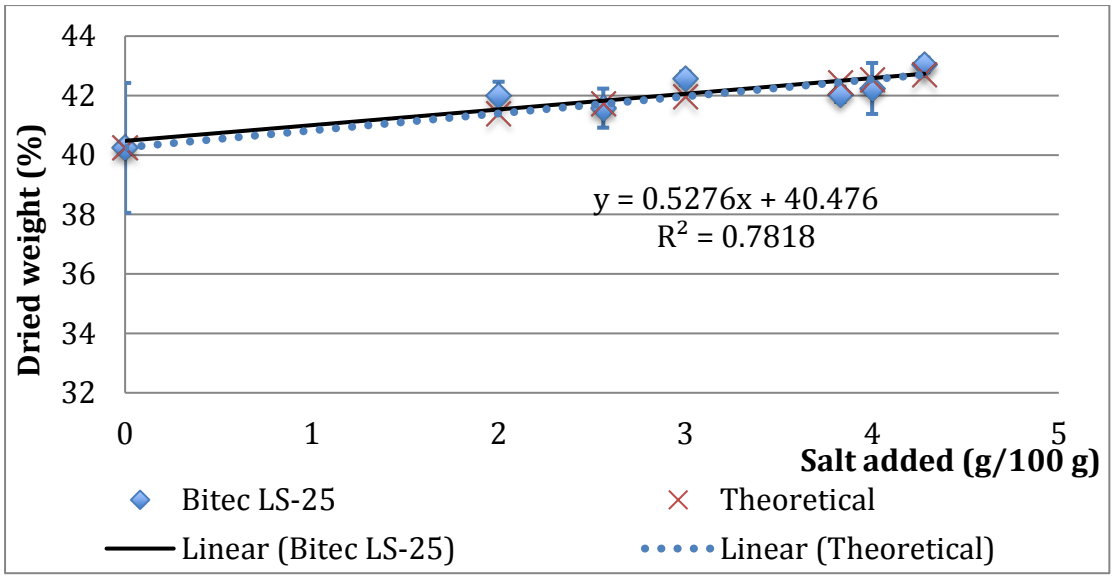

Figure 6.1-3: Measured dry weight (%) as a function of the added concentration of salt (g/ 100 g meat mixture) in the meat mixture inoculated with Bitec LS-25. The theoretical line is based on the measured dry weight in the meat mixture with no added salt plus the calculated amount of added salt. More results are shown in Appendix F.

The dry weight increased as the sausages lost water, and sausages with a high addition of salt had a lower water activity at the same dry weight than sausages added less salt (Figure 6.1-4).

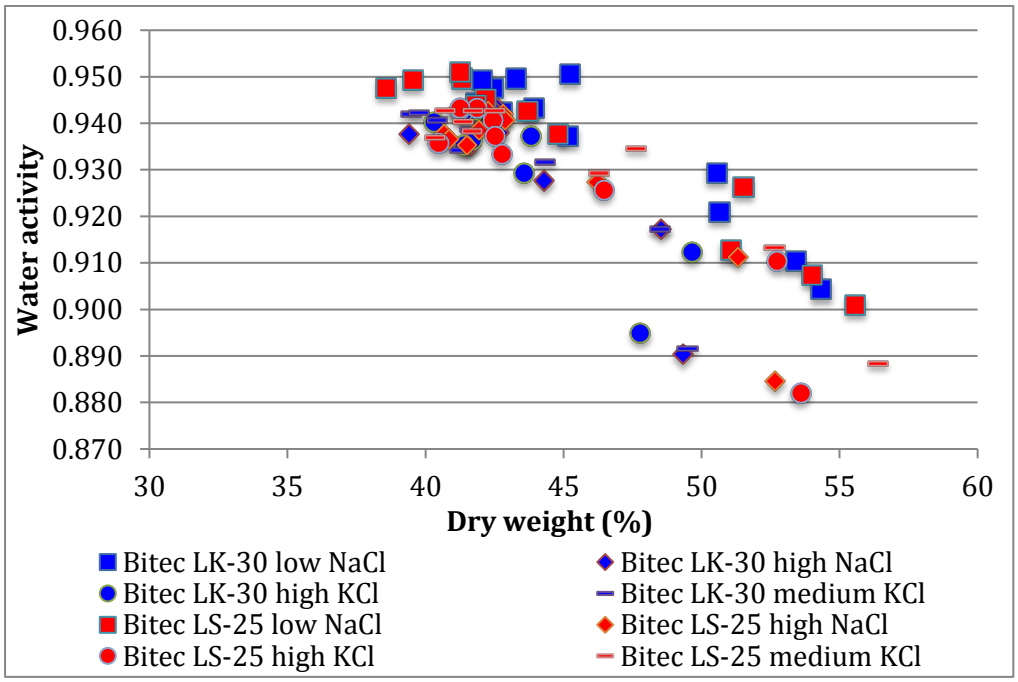

Figure 6.1-4: Water activity as a function of dry weight (%) for sausages fermented with LK-30 or LS-25 and added various concentrations and types of salt. (low NaCl=1.5 g NaCl + 1.0 g curing salt, high NaCl=2.5 g NaCl + 1.0 g curing salt, high KCl= 1.5 g NaCl + 1.28 g KCl + 1.0 g curing salt, medium KCl = 2.0 g NaCl + 0.64 g KCl + 1.0 g curing salt / 100 g meat mixture).

An interesting observation found was when the water activity seen as a function of the dry weight (%) the sausages inoculated with LK-30 added salt on molar weight basis seem to loose most of the water until the water activity reaches approx. 0.92, and then the weight loss

almost stops (Figure 6.1-4). Sausages inoculated with LS-25 shows the same trend, but at a water activity of approx. 0.91.

6.1.2. Effect of addition of NaCl and KCl on pH

Addition of salt to a meat mixture lowers pH, and addition of NaCl and KCl has slightly different effects (Figure 6.1-5). Thus, replacing NaCl with KCl will increase the start pH and may also increase the final pH slightly (Figure 6.1-6).

Keowmaneechai and McClements (2002) state that salts are known to shift the equilibrium constant of water and that positively charged salt ions may displace H+ ions from acid groups on the proteins, which could indicate a different decrease in pH. Stanley et al. (2017) tested partial replacement of NaCl (1.07 % (w/w) with KCl (0.75 and 0.94 % KCl) and found, in good agreement with the results above, that pH in the meat mixtures where NaCl was partially replaced with KCl was a bit higher than the in the sample containing only NaCl. However, the differences were not significant (p=0.698).

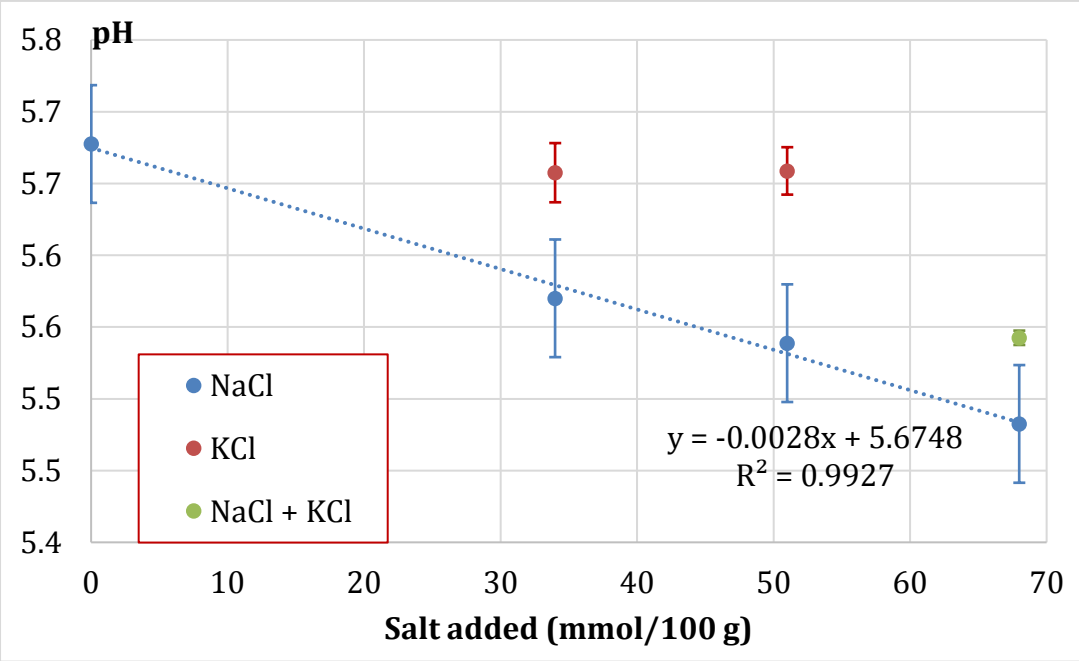

Figure 6.1-5: pH as a function of the molar concentration of added salt in the preliminary study one. Values are given as the average of all starter cultures that has the same concentration of salt ± standard deviation (n=4).

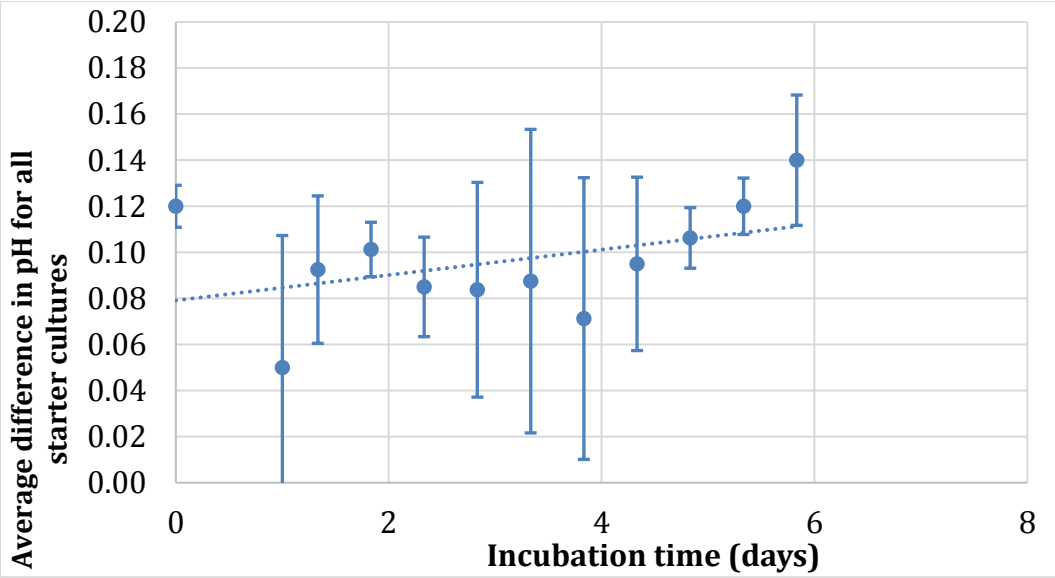

Figure 6.1-6: Average difference in pH added 3.00 g NaCl and 3.83 g KCl / 100 g meat mixture for the meat mixture inoculated with LS-25, Mild & Fast, T-SPX and LK-30 in the preliminary study one as a function of time. Values as given as the average of all starter cultures ± standard deviation (n=4).

6.2. Influence of starter culture

6.2.1. Bitec LK-30

The starter culture LK-30 seemed to produce higher concentrations of acetic acid when added lower concentrations of NaCl added to the meat mixture in the model system, indicating that the low concentration of NaCl induces the fermentation to produce acetic acid. The adding of KCl seemed to be affected by the concentrations, the higher concentrations, the lower concentrations of acetic acid were detected. The trend was not seen in the main study five, but the differences in the concentrations of salt added were not as big.

(Corral et al., 2013) found that when reducing salt (reduced NaCl, and some partial replaced with KCl) in slow fermented sausages, a reduction of 16 % NaCl, did not affect volatiles components like acetic acid, but did effect the textural and the overall quality. However, the substitution of KCl removed the differences regarding the overall quality, except for aroma. The sausages where NaCl was partially substituted with KCl, had the same acceptance as the sausage with high salt.

The starter culture LK-30 seemed to decrease higher concentrations of succinic acid than the other starter cultures. Succinic acid is as known an intermediate of the tricarboxylic acid cycle (TCA) and one of the fermentation end products of anaerobic metabolism in the citric acid metabolic pathway. Succinic acid is known to provide taste in food products like sauerkraut, rhubarb and various cheeses (Song and Lee, 2006).

6.2.2. Bitec LS-25

The starter culture seemed to have a more rapid colour formation in the meat mixtures added KCl, indicating that the bacteria *Micrococcaea* might have been in favour of the environment given when KCl added in the preliminary study one. The detection of lactic acid showed higher concentrations than the slow fermenting starter cultures LK-30 as excepted since the pH was lower, and the concentrations of lactic acid seemed to be higher in the meat mixtures added only NaCl than KCl, this was confirmed in the main study five (see section 6.2.6). Acetic acid seemed to be produced in higher concentrations in the sausages added only NaCl, while in the preliminary study one the concentrations of acetic acid seemed to be higher in the meat mixtures added NaCl, but the sample added the lowest concentration of KCl had also a quite high concentrations of acetic acid so no obvious trend could be said at that stage. This two studies compared could indicate that that the sample added KCl in the preliminary study one might be a fault and that the starter culture LS-25 produces more acetic acid when added NaCl. No articles were found on the bacteria added to the starter culture and production of volatile compounds, so further research has to be conducted to see if the results are correct or not.

Some authors have discussed the changes in flavour compounds like acetic acid when changing different ingredients. Guichard (2002) stated that changing that fat content modified the overall perception of a mixture of flavour compounds from different classes since the fat influences the solubility of aromas and thus the flavour release. Different flavour compounds were released as different types of fat and amounts was changed. Rabe et al. (2003) showed that in higher amount of NaCl were higher amounts of flavour compounds released. The amounts NaCl used was much higher than normal for food, but it showed how the salt affected the effect of salting out.
Lorenzo et al. (2016) found that the fast fermenting starter culture from Chr.Hansen (F-SC-111 Bactoferm) showed a higher concentration of lipid oxidation, microbial beta-oxidation, and carbohydrate fermentation than a slow fermenting starter culture (SM-194) from the same manufacturer. Acetic acid was one of the components that were found in significantly ($p<0.05$) higher concentrations in the sausages produced with the fast fermenting starter culture containing *L. sakei* and *S.carnosus*. The article did not research salt reduction or differences regarding different concentration of salt.

6.2.3. Bitec B Mild & Fast

The starter culture Bitec B Mild & Fast was a fast fermenting starter culture that used 24-48 hours to ferment in the preliminary study one. The formation of colour happened more or less over the same period.
The meat mixture inoculated with the starter culture was quite lower on the dry weight and water activity than the other meat mixtures as mentioned in section 5.2.4,5.2.5. The difference may be due to differences in the composition of the meat mixture, like larger and more fat particles or that the concentration of added salt could have been wrong. The analysis for organic acids and sugar revealed that the starter culture produced the most lactic acid of the tested starter cultures in preliminary study one. The concentration of glucose decreased fastest in the meat mixtures added KCl. The observations for the starter culture Mild & Fast indicates that the starter culture produced quite a lot of lactic acid and other compounds like acetic acid and citric acid, and could give a dry fermented sausage that is quite sour.

6.2.4. Bactoferm T-SPX

No information was found about Bactoferm T-SPX prior to the studies, but it was found to be a "medium rate" fermenting starter culture in the preliminary studies. The starter culture seemed to thrive in meat mixture added NaCl, but in mixtures added KCl or a mix of NaCl and KCl, it took much longer to lower pH. Since NaCl was replaced by KCl on a molar basis, the water activity in the meat mixtures should have been the same. Thus, it seems that KCl somehow interfered with the growth and metabolism of the starter culture.

6.2.5. Changes in organic acids and glucose during fermentation and drying/ripening of dry fermented sausages

The meat mixture contains some glucose, and more is added before the meat mixture is stuffed into the casings. This glucose is assumed to be the main C-source for the lactic acid bacteria during the initial fermentation phase when organic acids are produced, and pH lowered to 4.7-5.1. Normally, all glucose should be consumed during this phase. It was also expected that most of the organic acids would be produced during the fermentation phase. During the ripening and drying phase, the sausages losses water and this will increase the content of organic acids per g sausage. However, unless glucose is consumed, or organic acids produced, the content per g dry weight will be relatively constant.

In the sausages fermented with LK-30, all glucose was not consumed during the initial fermentation phase, and glucose was therefore available during the ripening and drying phase. Somewhat more glucose was consumed in the sausages where some of the NaCl was replaced with KCl, but also in these sausages a substantial fraction of glucose still remained after the initial fermentation phase. Some of this glucose was consumed during the ripening and drying phase and may partly explain the substantial increase in lactic and acetic acid during this phase. However, also in the sausages fermented with LS-25 where the added glucose was consumed during the fermentation phase, the content of lactic and acetic acid increased considerably during the ripening/drying phase (see section 5.7). Here the C-source for the production cannot have been added glucose, but if there was still glycogen present in the meat it may have been hydrolysed to glucose and subsequently metabolised to organic acids. The considerable production of lactic and acetic acid also during the ripening/drying phase was a surprise and need further studies.

As expected, glucose was consumed, and organic acids produced during the fermentation phase. As expected, the content of organic acids also increased per g sausage during the drying phase. However, the content of organic acids also increased when measured per g dry weight, and the increase in lactic and acetic acid was substantial *(*Table 5.7-1,Table 5.7-2). In the sausages fermented with LK-30 there was a substantial amount of glucose left after the initial fermentation phase, and this may partly explain the production of acetic- and lactic acid. However, in the sausages fermented with LS-25 virtually all glucose was consumed during the fermentation phase and still the amount of lactic- and acetic acid per g dry weight increased considerably during the drying and ripening phase.

The results indicate that a fermentation that produced acetic- and lactic acid occurred after the initial fermentation phase. The main C-sources in the sausages during the ripening phase are amino acids/peptides/proteins and lipids, but lactic and acetic acids are not usually

expected to be important fermentation products from these sources. No similar results were found in the literature, and further studies are required.

6.2.6. Comparison of the properties found in the preliminary study one and main study five

Despite the increased concentration of added starter culture of LS-25 did the fermentation go a bit slower than in the initial studies, this could have been the different conditions used during fermentation and drying, and LK-30 was added by using a reactivation medium. The reactivation medium had shown in the preliminary study three and four to improve colour and to make the starter culture stronger against the growth of other microorganisms like mould. The starter culture LK-30 did, on the other hand, ferment a bit faster than it did in the initial studies. The faster development in pH could indicate that a larger inoculum made the starter culture develop faster and that the different temperature caused by the sausages was stored in the manufacturing room while the other sausages were produced, gave the starter culture a faster development.

The determination of acids and sugars was in the preliminary study one and study five quite similar for the almost identical salt contents. In the preliminary study one, a sample was prepared with 3.00 g NaCl / 100 g meat mixture, while in study five a sausage was prepared with 2.5 g NaCl / 100 g meat mixture + 1.0 g curing salt (high NaCl), this two samples was compared based on their salt content.
Both meat mixtures were fermented with the starter cultures LK-30 and LS-25 and showed that the produced concentration acetic acid was quite similar for both starter cultures during fermentation.The meat mixtures inoculated with LS-25 and added 2.00 g NaCl / 100 g meat mixture in the preliminary study one showed some higher concentrations of citric acid in the start sample, but as mentioned earlier the start meat mixtures were taken two hours after inoculation as well as the composition of the meat mixture was different so it might have had an affect. Despite, was the increase and decrease of acetic acid, glucose and citric acid approx. the same concentration during fermentation.
LK-30 did in the preliminary study one decrease the concentration of citric acid, while in study five the concentration of citric acid increased. The concentration of acetic acid was also higher in the preliminary study one, but the concentration at the end of the fermentation seemed to be approx. The same.
The decrease and increase that was seen of citric acid and pyruvic acid for several of the meat mixtures during fermentation in the preliminary study one could indicate that some of the produced concentration converted to the citrate-pathway or the pyruvate-pathway as mentioned in theory in section 3.10. This could lead to higher concentrations of other end products that were not tested for in this thesis.

6.3. Evaluation of model used for the fermentation process

In this thesis a model system consisting of 50 ml plastic tubes were used initially to screen different combinations of starter cultures, salt concentrations and types of salt. The great advantage of this system is that a number of conditions can be tested with a limited amount of work, especially compared to the alternative of full sausage production. A comparison between the preliminary study one and the main study, also shows that there are similarities between the two studies as mentioned earlier, indicating that the system may be used to screen the fermentation process using different starter cultures and salts.

Three challenges were experienced; air/gas pockets in the meat mixture, limited escape possibilities for gas formed during the fermentation, and contamination with other microorganisms. In several cases a grey/brown and soft top layer was formed, while the rest of the sample could be hard, possibly due to protein gel formation, and had the right red/pink colour.
In any future studies, the tubes should be filled entirely and the meat mixture packed by centrifugation to avoid air pockets in the meat. In this way the mould contamination experienced in preliminary study four might have been avoided. The two hours in water bath, could may be left out in future studies as well since the tubes are small and the meat mixture reaches its core temperature faster than a regular sausage will do in industrial manufacturing.

6.4. Sensorial properties

The sausages were assessed by an in-house untrained panel with respect to consistency, colour, taste and aroma

None of the sausages were assessed as significantly different from the others on the attribute aftertaste, which was described to the judges as metallic or bitter. Only one of the judges noted an odd bi-taste in the sausage with a the highest concentration of KCl. Gelabert et al. (2003) found that a substitution of NaCl with KCl did not affect the consumers overall liking before substitution reached 40 %. In this study the substitution level was 14 % (Medium KCl) and 29 % (High KCl).

Sausages fermented with LK-30, and especially the sausages added low NaCl, were found to have a deeper reed colour than the sausages fermented with LS-25. LK-30 contain *Kocuria salcisia,* which is a species in the family *Micrococcaea,* and the darker red colour could come from the bacteria producing more enzymes to reduce nitrite, and especially since the sausages added LK-30 had a higher pH and the starter culture was reactivated before use. Ammor and Mayo (2007) stated that the formation of nitrosomyoglobin is much less pronounced for LAB than for CNC. *Micrococcaea* are in general less sensitive for acid (Martín et al., 2007), so in the sausages inoculated with LK-30 that produced lower concentrations of acid, the bacteria could have been more active and therefore generated more colour

Concerning flavour, the only two attributes with a significance level was taste and odour lactic acid/salami. The attribute is important due to the taste aspect of balancing sour and salami/lactic acid flavour to achieve the desired taste. The composition depends on the sausage and the manufacturer. The sausages fermented with LS-25 were overall highest on both attributes, but the sausages added the lowest concentrations of NaCl were ranked lowest. NaCl is a taste enhancer and a low concentration of NaCl might not have enhanced the lactic acid/salami taste as much as in the other sausages fermented with LS-25.

Sausages fermented with Bitec LK-30 were found to be less chewy/hard than the sausages fermented with Bitec LS-25, indicating a more raw/soft feeling of a slice of the sausage. The results from the sensorial test do not indicate that the sausages inoculated with Bitec LK-30 had a poor consistency, only that it was less hard than those fermented with Bitec LS-25. Horita et al. (2014a) found that 25 % replacement of NaCl with KCl gave sausages with quite similar technological properties and a taste comparable to those with a corresponding molar

amount of NaCl. Zanardi et al. (2010) found that the replacement of NaCl with 13.5 g NaCl, 4.2 g KCl, 2.4 g $CaCl_2$ and 2.4 g $MgCl_2$ / kg meat in fermented Italian salami did not affect the textural parameters during drying. Spaziani et al. (2009) found that low-acid sausages (pH=5-5.1) had a less hard and cohesive texture than control sausages from industrial and artisan sausages. It was suggested that pH values below the isoelectric point increased texture, while pH-values above the isoelectric point gave a decreased texture in hardness. González-Fernández et al. (2006) found differences regarding the use of two different starter cultures on *chorizo,* the starter culture containing *L.sakei K29,* did especially affect the textural parameters by giving an increased hardness and chewiness in the sausages.

6.5. pH measurements in meat mixtures and sausages

Measuring pH in meat mixtures and sausages containing a lot of fat and salt is difficult. Formation of a thin layer of invisible fat on the outside of the pH-probe caused the pH measurements to drift. Frequent washing and cleansing with water and/or soap water was only partly successful in counteracting the lipid film formation, and many of the meat mixtures throughout the whole study had to be measured several times to ensure correct pH measurements.
Several different pH-probes and pH-meters were tested, but the measurements were observed to drift in all, indicating that the problems were due to the meat mixtures. In the end, one pH-meter with a probe designed for measuring in half-solid to solid material was selected.

7. Conclusion

The aim of the thesis was to study the effects of different starter cultures in combination with sodium reduction, by reducing the amount of added salt (NaCl) or by partial replacement with potassium chloride (KCl), in dry fermented sausages.
Replacing 0.5 g (14 %) or 1.0 g (29 %) of the added NaCl (reference 2.5 g NaCl + 1.0 g curing salt per 100 g meat mixture) with an equimolar amount of KCl reduced the final sodium level calculated as NaCl from 5.5 % to approx. 4.8 and 4.0 %, respectively. The sausages achieved $a_W < 0.90$ in the same ripening and drying time (16 days) as the high salt reference, and the sausages were found acceptable in a sensorial test. If the addition of NaCl was reduced by 29 % without replacement with KCl, the final salt level was approx. 4.2 %, but the sausages required a longer drying period (22 days) to obtain $a_W < 0.90$ and developed texture defects towards the end of the drying period. Thus, replacing 29 % of the added NaCl with an equimolar amount of KCl appears feasible to produce dry fermented sausages with a reduced sodium content.
Sausages fermented with Bitec LS-25 and added KCl seemed to have lower concentrations lactic acid and acetic acid than sausages added only NaCl. Bitec LS-25 reduced pH to 4.6-4.8 in 24-48 h. A significantly chewier texture and stronger taste of lactic acid/salami were found in the sensorial evaluation compared to Bitec LK-30.
Sausages fermented with Bitec LK-30 showed no obvious trend regarding the added concentrations of salt, and lowered pH to 4.9-5.0, but the starter culture required less dextrose than Bitec LS-25. The starter culture yielded sausages with a lower concentration of lactic acid, and important, a lower weight loss than the sausages fermented with Bitec LS-25.

An observation found in the thesis was that the water activity decreased after storage in vacuum packing. The sausages added the lowest concentrations of NaCl (1.5 g NaCl + 1.0 g curing salt/100 g meat mixture) had a higher water activity at start since the added salt was lower, but at the water activity approx. 0.93, all sausages actually more or less had the same weight loss and small amount of water had to evaporate from this point. The observations are important for the industry since it could give the opportunity to produce salt reduced sausages without drying a water activity below 0.90 and therefore reduce the risk of texture defects by vacuum packing the sausages at a earlier stage.

An unexpected observation was that the concentration of lactic- and acetic acid increased substantially during the ripening and drying period. The apparent production of lactic acid during this period was comparable to the amount produced during the fermentation phase. In the fermentation phase, glucose was used as C-source for acid production and pH decreased, but the C-source for acid production during ripening and drying is not known. Furthermore, no large pH decrease was observed during ripening and drying. Further studies are required to understand the observation.

A model system to study the initial fermentation phase (2-5 days) based on fermenting the meat mixture in 50 ml plastic tubes was tested. The system can be used to screen starter cultures in combination with different levels of salts (NaCl, KCl, others) and/or glucose. The results in the successful experiments were in reasonable agreement with the results of the sausage production studies, indicating that this may be a useful screening system. However, further refinement of the method is still required.

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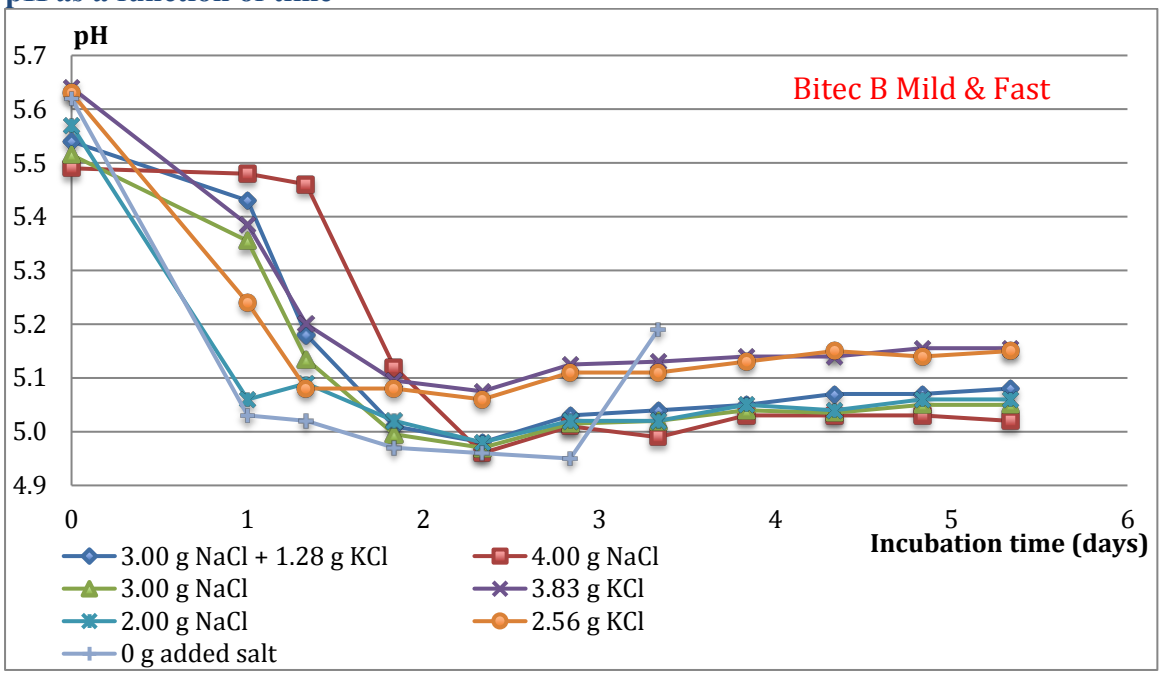
Appendix A: Explanation of the attributes.

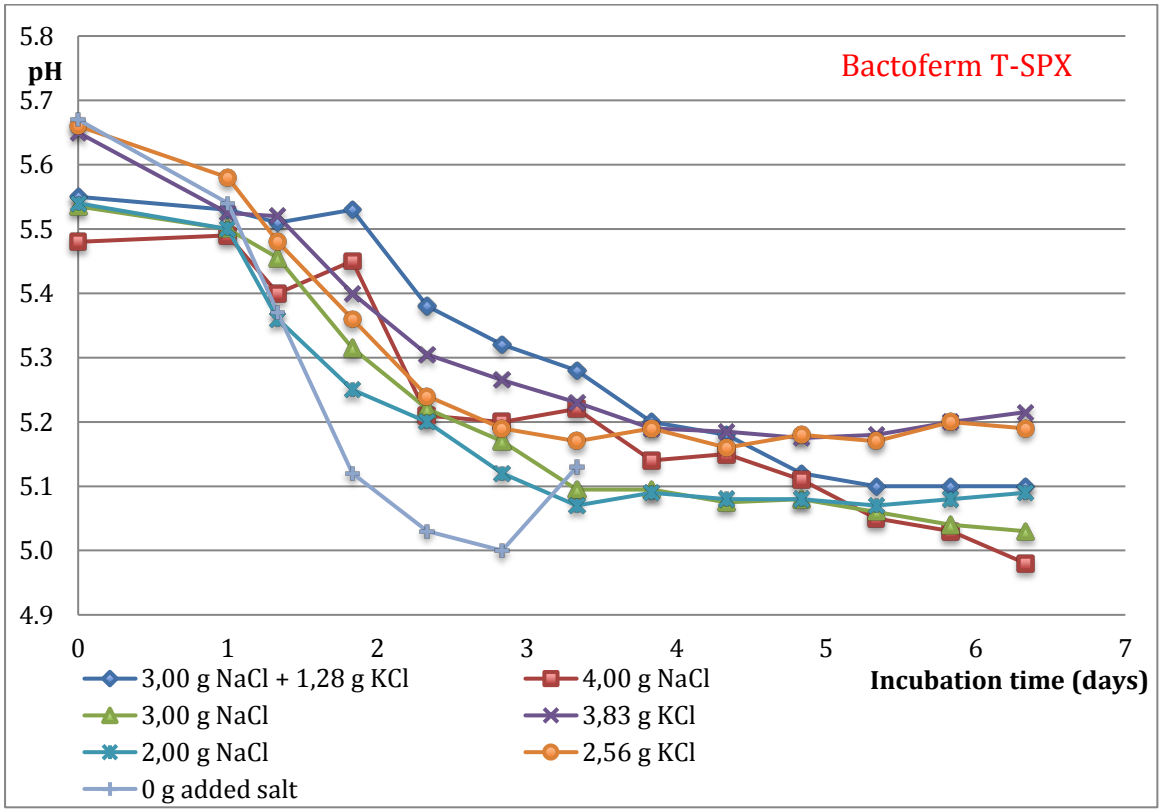
Appendix table A: Explanation of the attributes used in sensorial testing and their definition.

Attribute (ranking)	Definition
Appearance	
Colour (dark red – fresh/bright red)	Colour alterations of the characteristic cured red colour of dry-cured sausages due to chemical, physical and biological processes.
Oily (dry – oily)	It is the exude fat on the lean surface of a slice of sausage.
Odour/aroma	
Lactic acid/salami (Fresh low odour – intense odour)	Lactic acid or other odour components produced by fermentation that could have odour like yoghurt or fresh apples
Sour (low intensity to high intensity)	Basic taste, describes an olfactory complex sensation due presence of organic acids. Odour like mature lemons or apples *e.g.*
Smoke (low intensity to high intensity)	An aromatic that present characteristics of sweet, brown, pungent, acrid, sour, burnt *e.g.*
Aromatic (low intensity to high intensity)	The total aromatic odour when smelled on a slice of sausage, many distinct flavours at once, caused by aromatic compounds.
Intensity (low intensity to high intensity)	The intensity of all odour compounds and how intense they are perceived.
Taste	
Aromatic (low intensity to high intensity)	The total aromatic flavour in a bite, many distinct flavours at once, caused by aromatic compounds.
Salt (low intensity to high intensity)	Describes the basic taste produced by dilute aqueous solutions of various substances such as sodium chloride.
Aftertaste (low intensity to high intensity)	Described the taste received in the mouth when finished chewing a bite of sausage. Taste can be various, but often describes as metallic, acetic acid, butyric acid.
Lactic acid / salami (low fresh/lactic acid taste – high intensity)	Characteristic taste associated with lactic acid. Typical taste is sour characteristics in yoghurt and salami.
Sour (low intensity to high intensity)	Describes an olfactory complex sensation, due to presence of organic acids.
Intensity (low intensity to high intensity)	The intensity of all taste compounds and how intense they are perceived.
Consistency	
Chewy (normal – rubber/chewy)	Describes the feeling when biting through a slice of sausage in this test. The feeling should be easy to bite and chew and not feeling like rubber or like a raw sausage.
Grainy (normal – grainy)	Feeling of the slice of sausage when having it in the mouth of having particles. Feeling should be smooth when chewing. Particles are related to the perception of the quantity of fat exuded during chewing.

Appendix B: Physiochemical properties preliminary study one

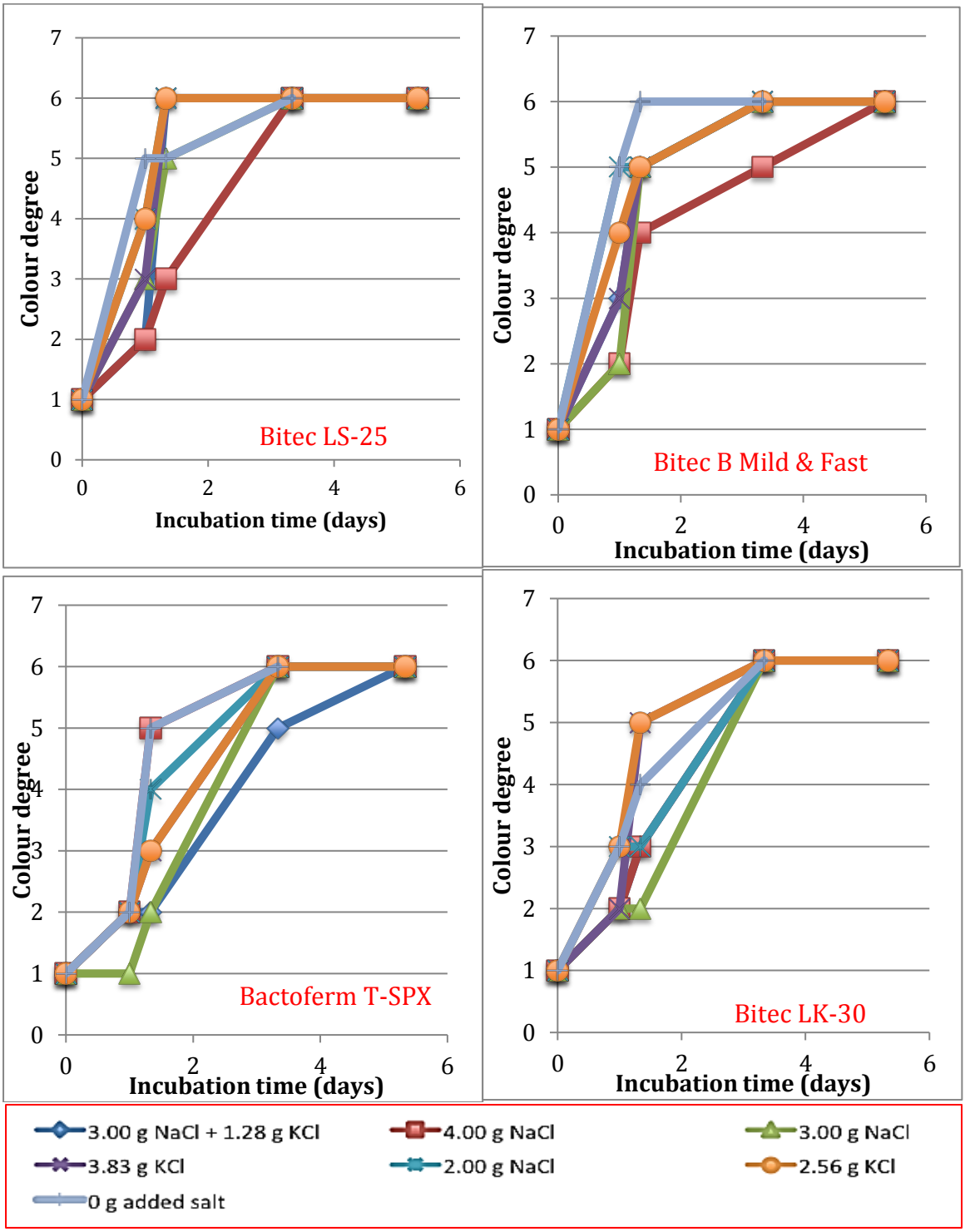
pH as a function of time





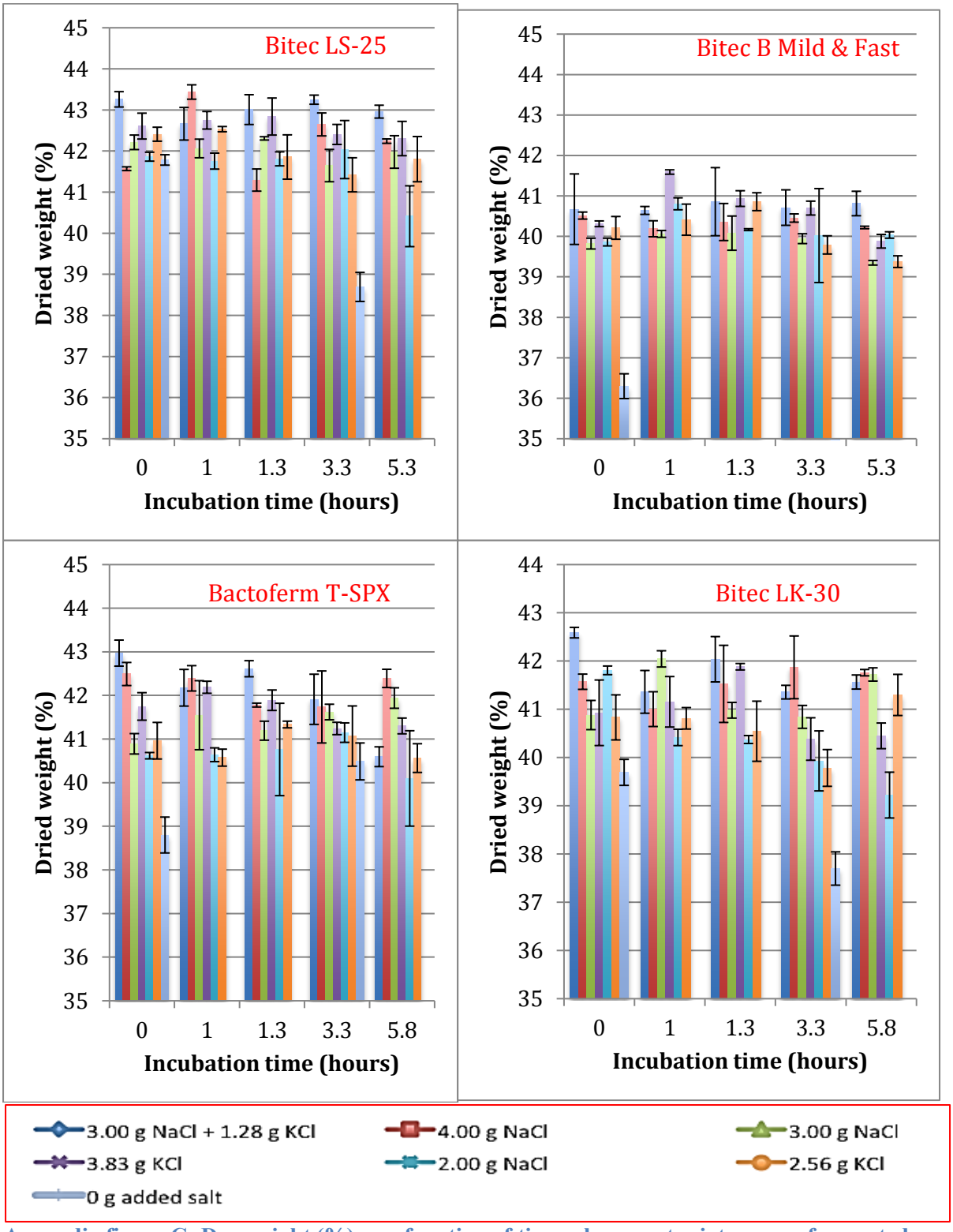
Appendix figure A: pH as a function of time when meat mixtures were fermented in 50 ml tubes, with the starter cultures Mild & Fast and T-SPX and different concentrations and types of salts added.

Colour as a function of time



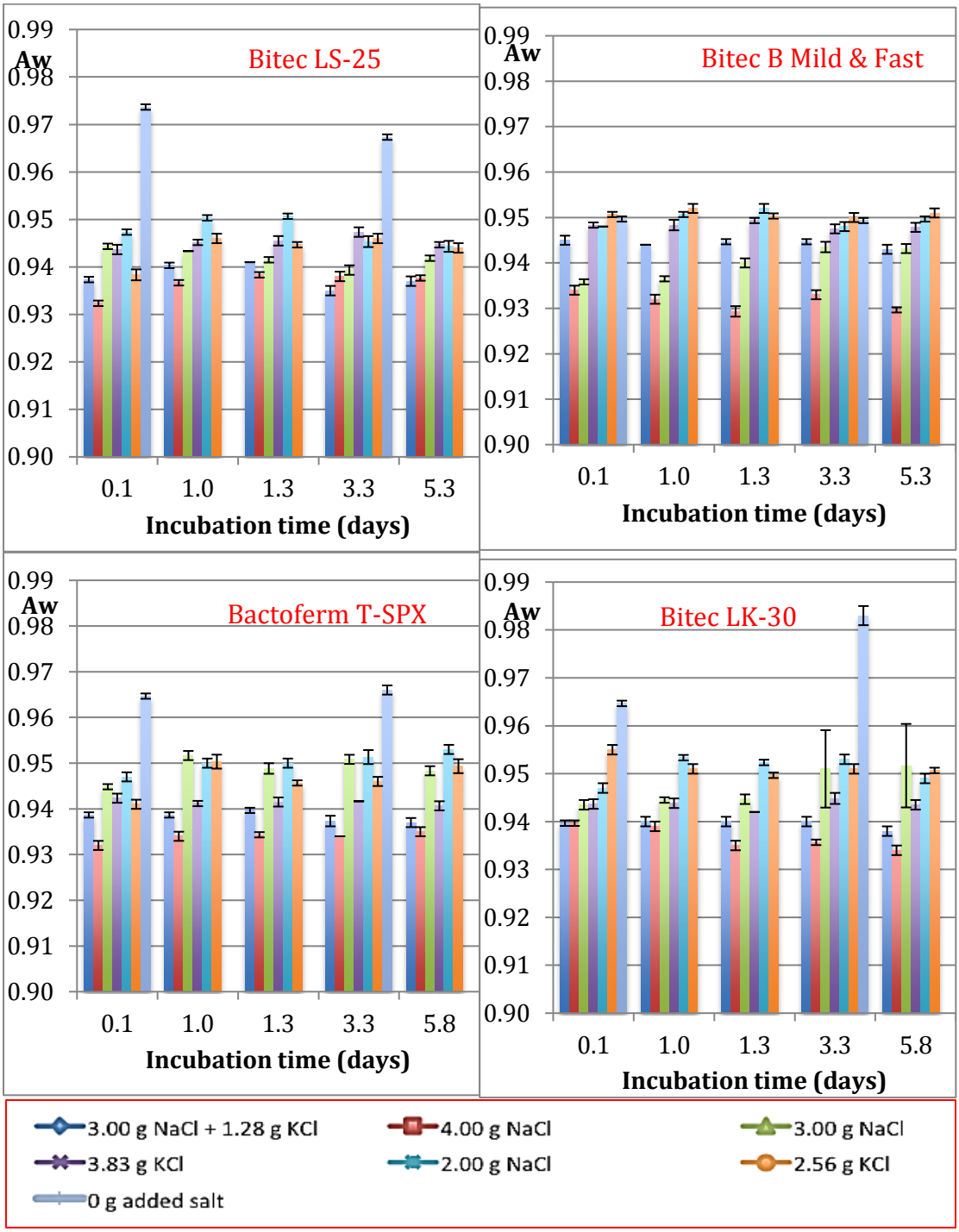
Appendix figure B: Colour degree as a function of time when meat mixture was fermented in 50 ml tubes with different starter cultures and added different concentrations and types of salt.

Dry weight as a function of time



Appendix figure C: Dry weight (%) as a function of time when meat mixture was fermented with starter cultures and added different concentrations and types of salts. Standard deviation (n=3) is indicated.

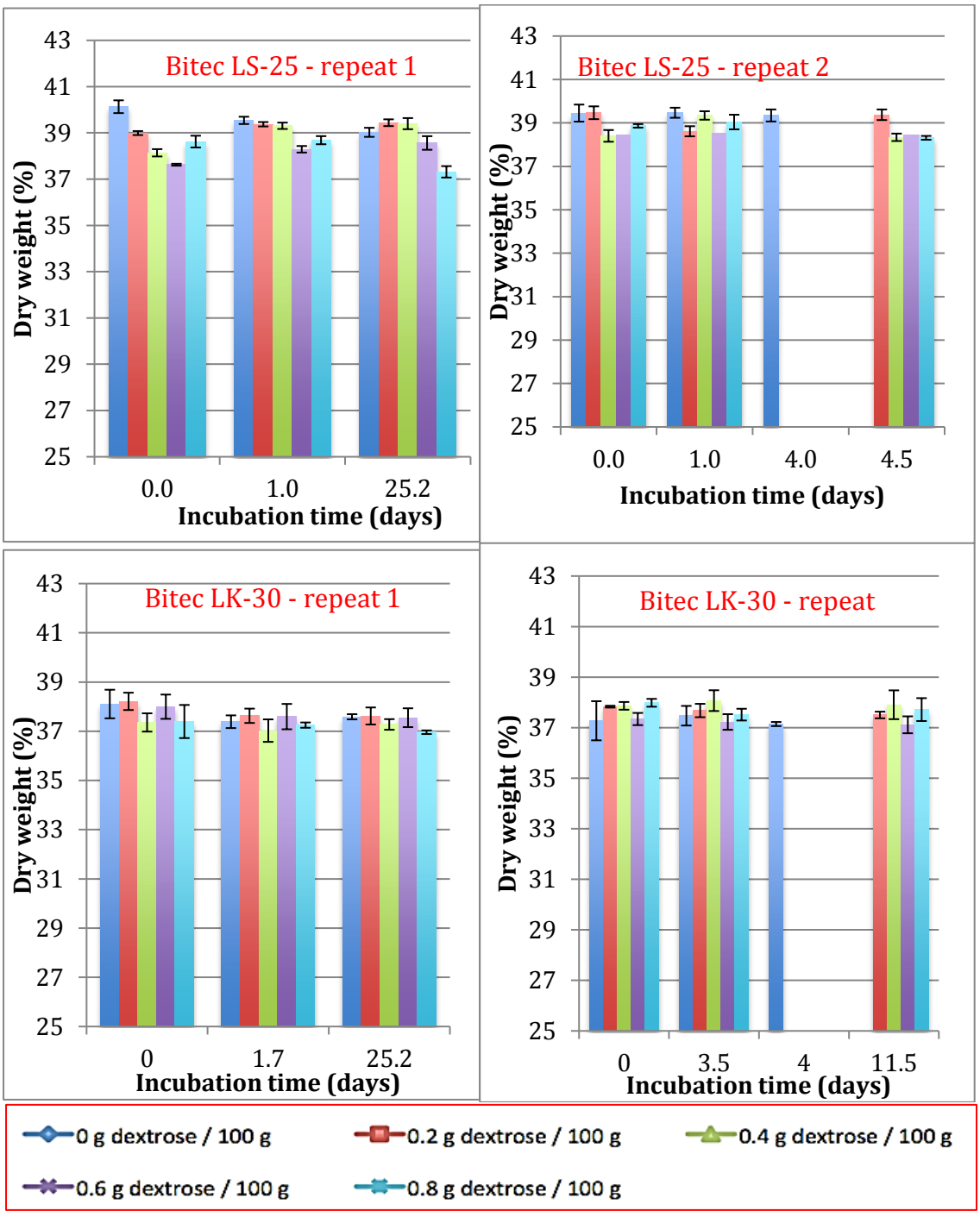
Water activity as a function of time



Appendix figure D: Water activity as a function of time when meat mixture was fermented with starter cultures and added different concentrations and types of salts. Standard deviation (n=3) is indicated.

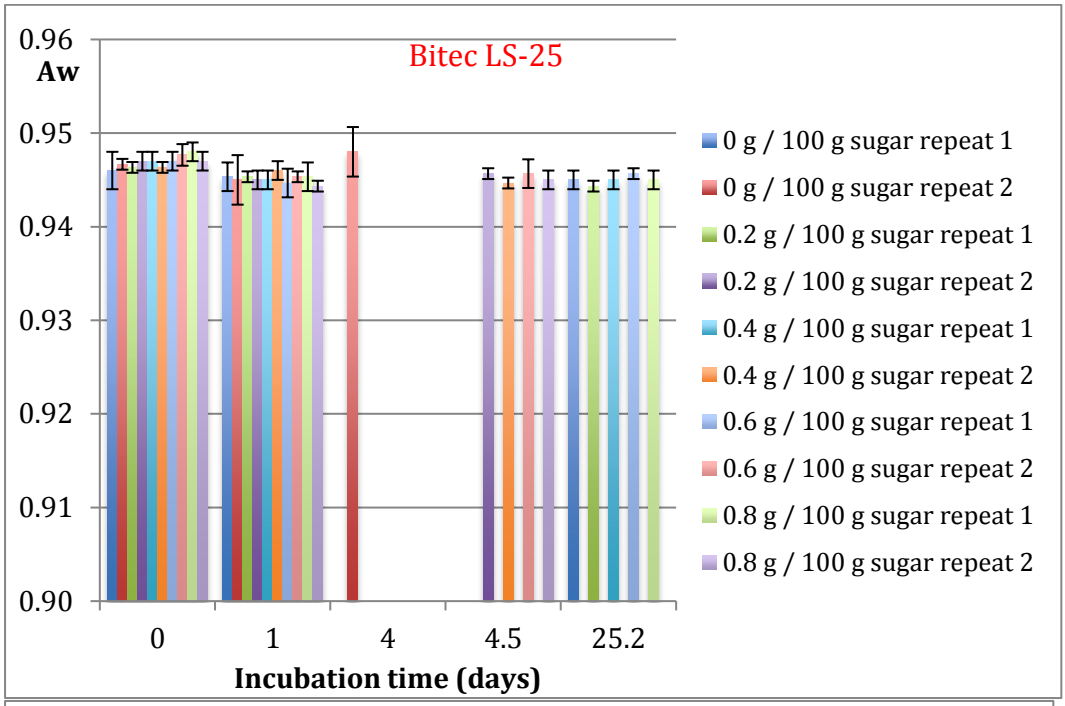
Appendix C: Water activity and dry weight preliminary study three

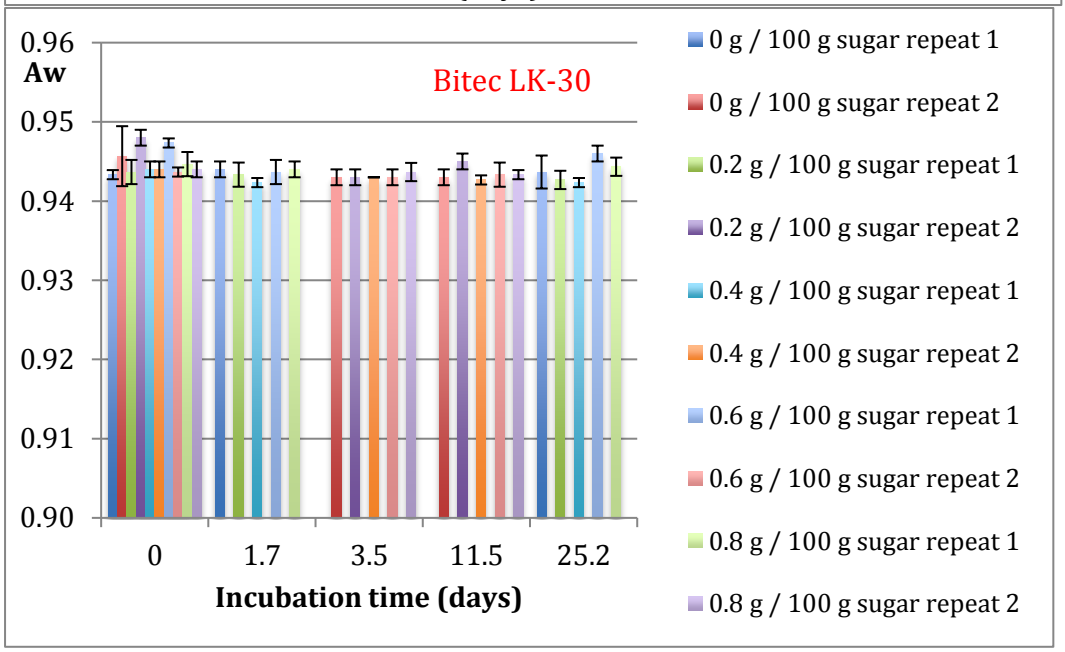
Dry weight (%) as a function of time



Appendix figure E: Dry weight (%) as a function of time in meat mixtures fermented with starter cultures and added 0-0.8 g dextrose / 100 g meat mixture, 3.00 g NaCl / 100 g meat mixture. The study was repeated twice the same way expect for the time.

Water activity as a function of time

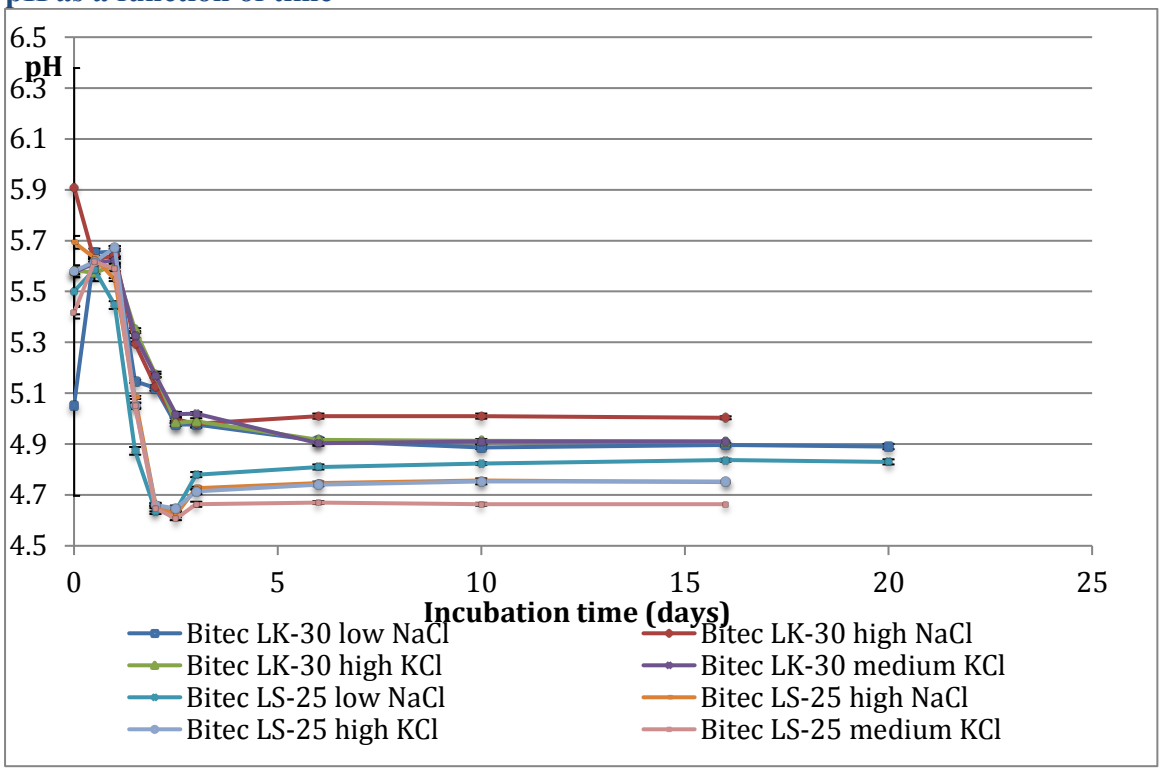




Appendix figure F: Water activity as a function of time with meat mixture inoculated with the starter cultures LS-25 and LK-30 and added various concentrations of dextrose from 0-0.8 g /100 g meat mixture and 3.00 g NaCl / 100 g meat mixture. Standard deviation (n=3) is indicated.

Appendix D: Figures and tables from main study five

pH as a function of time



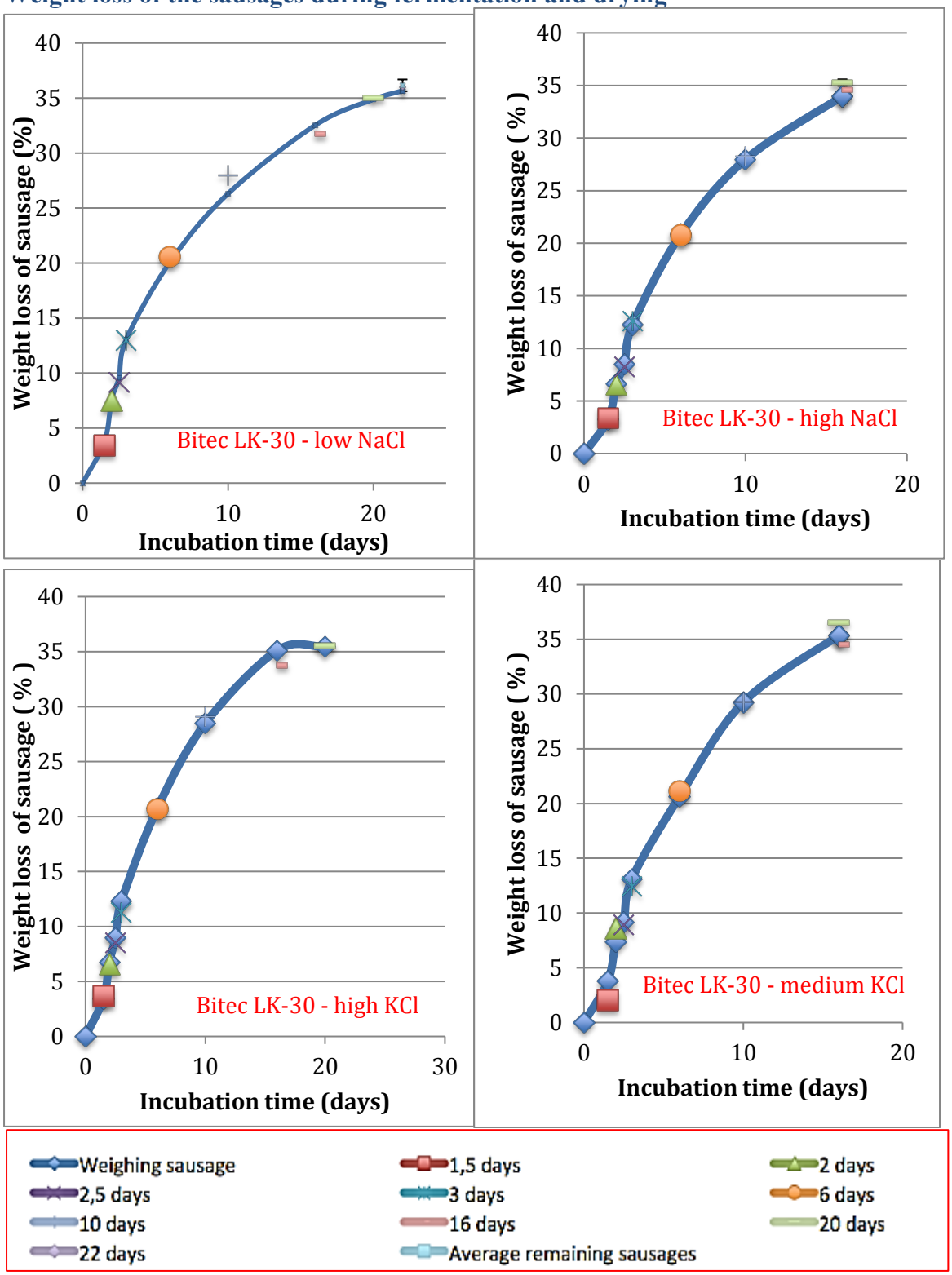
Appendix figure G: pH as a function of time from 0.5 to 6 days after start, for sausages inoculated with the starter cultures LK-30, or LS-25, and added different amounts and types of salt. The standard deviation (n=3) is indicated.

Dry weight and water activity in meat mixture added no salt

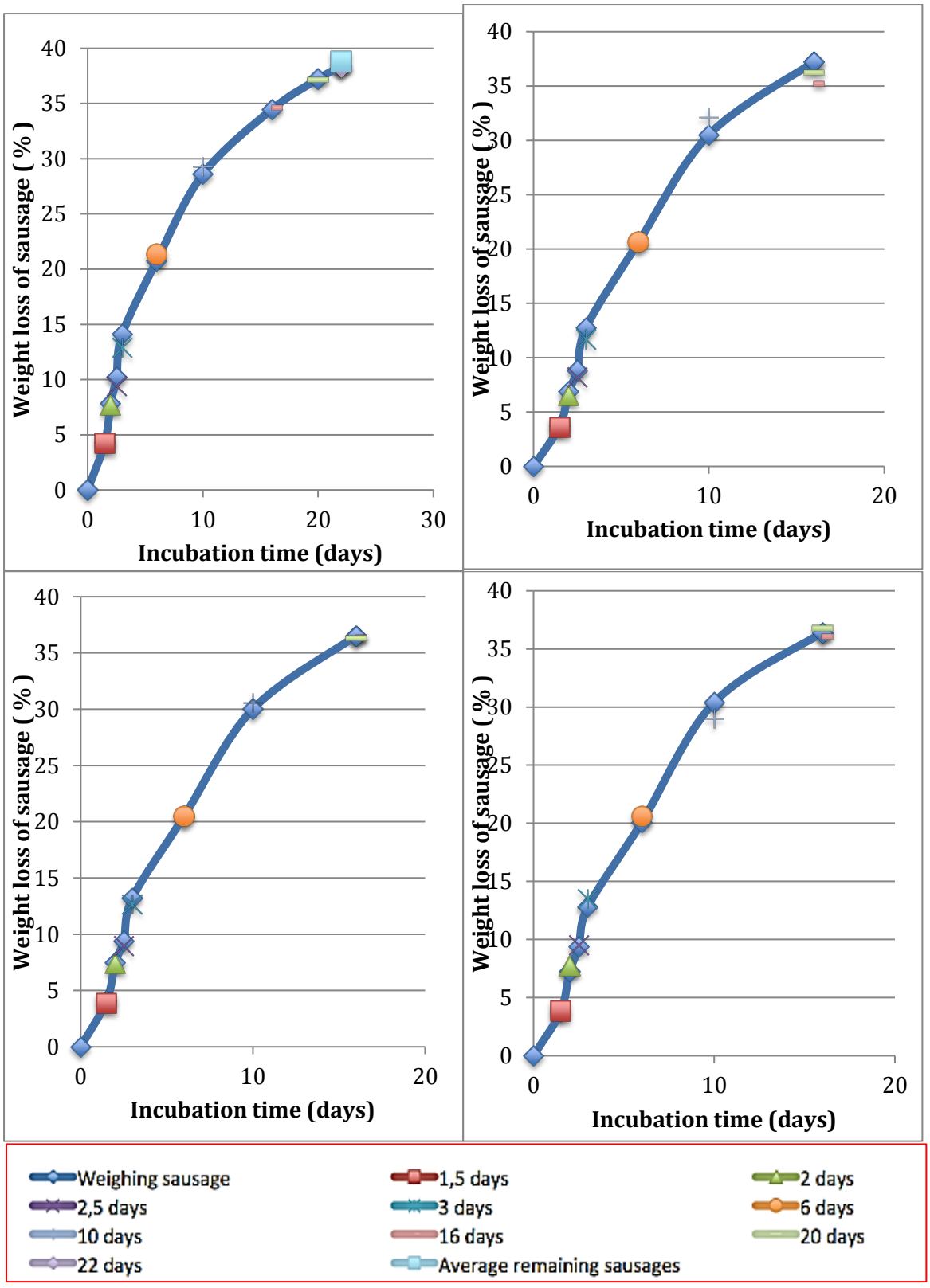
Appendix table B: Dry weight (%) and water activity in meat mixtures used for producing sausages in the main study five. Batches was added starter cultures and sugar (0.4 g dextrose / 100 g).

Sample	Dried weight (%)	Water activity	Comments
1	35.17	0.969	
2	35.53	0.970	
3	35.69	0.968	
4	35.96	0.969	
5	35.14	0.969	
6	35.48	0.967	
7	35.79	0.967	
8	34.88	0.972	
9	35.88	0.971	
10	35.83	0.958	Brown colour
11	35.94	0.959	Brown colour
12	35.23	0.969	
Average	**35.57**	**0.967**	
Stdev	**0.367**	**0.004**	

Weight loss of the sausages during fermentation and drying

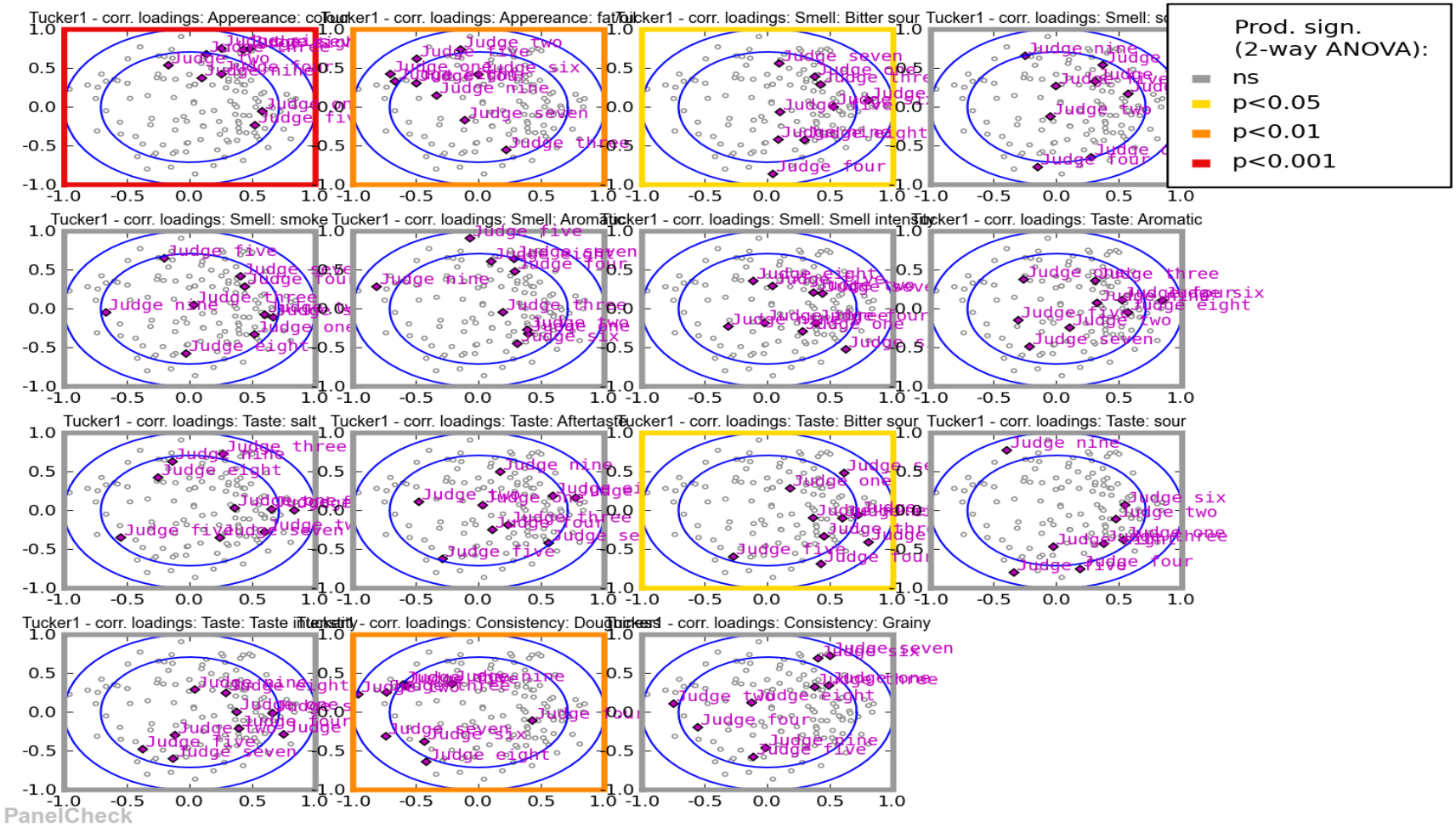


Appendix figure H: Weight loss during fermentation and drying as a function of time for the sausages inoculated with LK-30 and added different concentrations and types of salt. Low NaCl = 1.5 g NaCl + 1.0 g curing salt, High NaCl = 2.5 g NaCl + 1.0 g curing salt, High KCl = 1.5 g NaCl + 1.28 g KCl + 1.0 g curing salt, and Medium KCl = 2.0 g NaCl + 0.64 g KCl + 0.64 g curing salt /100 g / meat mixture.



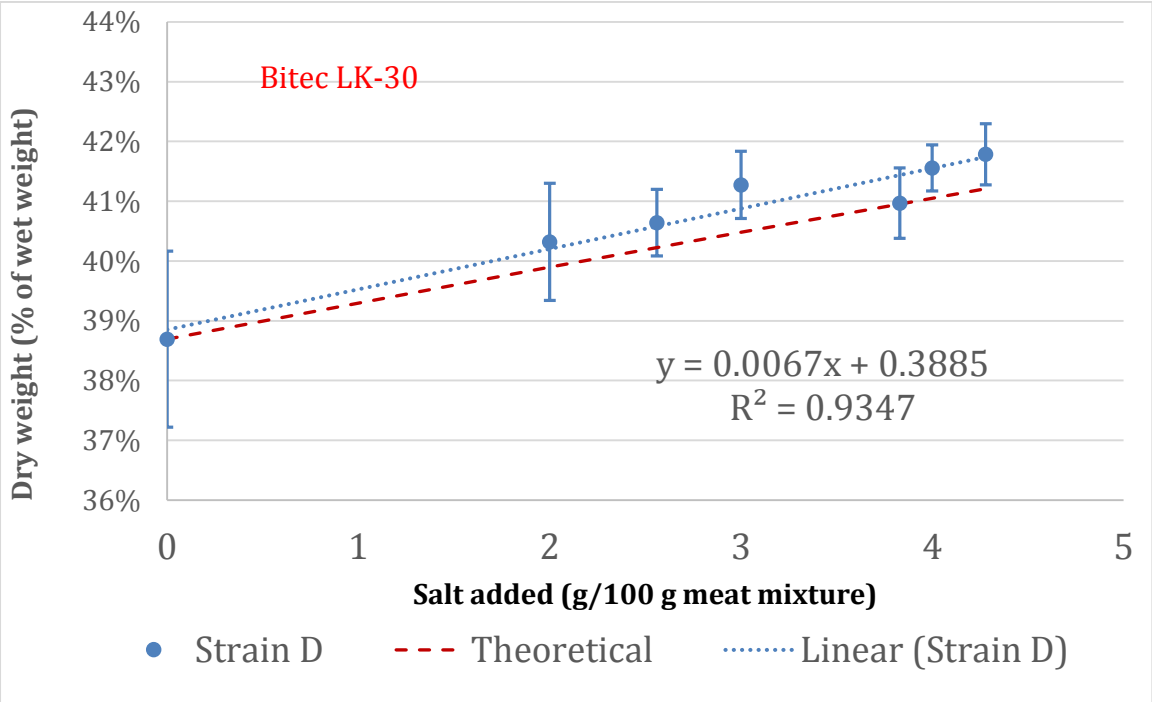
Appendix figure I: Weight loss during fermentation and drying as a function of time for the sausges inoculated with LS-25 and added different concentrations and types of salt. Low NaCl = 1.5 g NaCl + 1.0 g curing salt, High NaCl = 2.5 g NaCl + 1.0 g curing salt, High KCl = 1.5 g NaCl + 1.28 g KCl + 1.0 g curing salt, and Medium KCl = 2.0 g NaCl + 0.64 g KCl + 0.64 g curing salt /100 g / meat mixture.

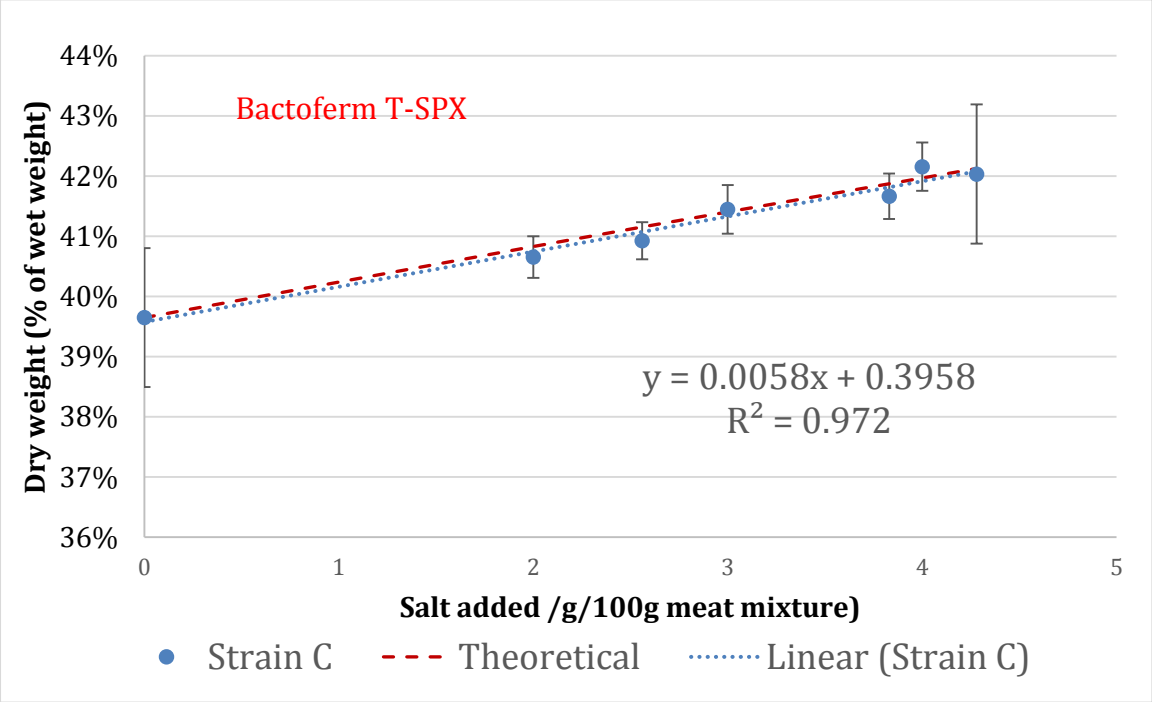
Appendix E: Tucker-1 correlation plot for the sensory evaluation



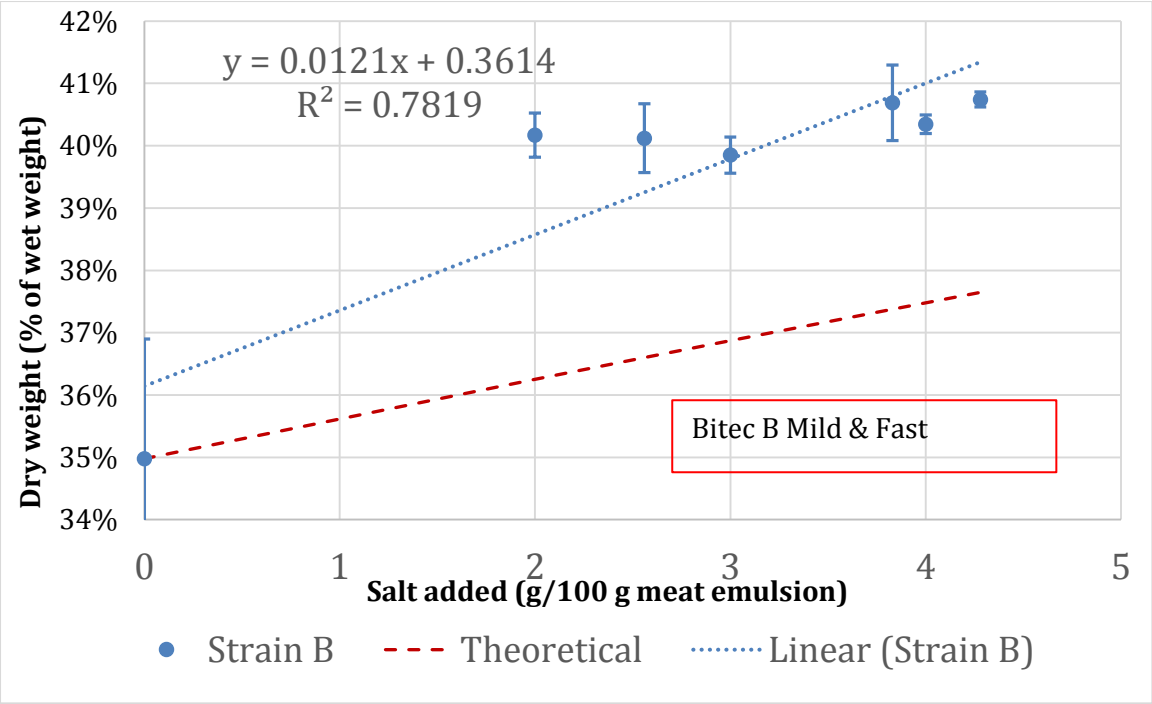
Appendix figure J: Tucker-1 correlation plot for the attributes colour, oily, Odour: lactic acid/salami (bitter sour), sour, smoke, aromatic, intensity; Taste: aromatic, salt, aftertaste, lactic acid/salami (bitter sour), sour, intensity; Consitency: Grainy (doughiness) and grainy.

Appendix F: Dry weight as a function of added salt





Appendix figure K: Dry weight (%) as a function of added concentration of salt (g/100 g meat mixture) in the meat mixture inoculated with LK-30 and T-SPX.



Appendix figure L: Dry weight (%) as a function of added concentration of salt (g/100 g meat mixture) in the meat mixture inoculated with Bitec B Mild & Fast