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Department of Forest Mycology and Plant Pathology
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Abstract

Lyckeby Starch AB is a farmer-owned company that produces products based on potato starch. In order to increase their production efficiency and their international competitiveness the company is investigating the possibilities of keeping their factories open longer. To achieve this, potato has to be stored for a longer period of time than what is practiced today. Starch potato is usually stored in large piles covered by straw and plastic, either in the field or on the farmyard.

The objective was to examine in what way starch yield (starch content*tuber weight) in three different potato varieties was affected by storage in two different types of storage piles. The potato was stored for three months in total and starch content and tuber weight were controlled throughout the period. Stored tubers were also visually examined and prescense of mechanical damage as well as wet rots and dry rots was recorded. Bacteria and fungi were isolated and identified.

In general, varietal differences were greater than differences between the storage piles. Loss of starch yield was greatest during the first weeks of storage, and there was a strong correlation between presence of mechanical damage on tubers and incidence of wet rots and dry rots. Several bacteria and fungi were identified and there was a tendency of difference in bacterial diversity between the different potato varieties, which indicates that the different varieties select for different bacterial species. In order to minimize the loss of starch yield during storage it is important to choose good potato varieties and to harvest them at the right time and under appropriate circumstances in order to avoid mechanical damage.

Sammanfattning

Lyckeby Starch AB är ett medlemsägt företag som tillverkar produkter baserade på potatisstärkelse. För att öka företagets produktionseffektivitet och konkurrenskraft på den internationella marknaden undersöker man möjligheterna att hålla fabrikerna öppna under en större del av året i framtiden. Eftersom att lagringsmöjligheterna i anslutning till fabrikerna är begränsade behöver potatis då kunna lagras under en längre tid hos lantbrukarna. Stärkelsepotatis lagras vanligen i stukor som täcks med halm och plast, antingen i fält eller hemma på gården.

Syftet med uppsatsen var att undersöka hur stärkelskörden (stärkelsehalt*knölvikt) hos tre olika sorters stärkelsepotatis påverkas av lagring i två olika typer av stukor. I ett fältförsök jämfördes en traditionell stuka med en bättre isolerad variant. Lagringen sträckte sig över tre månader och provsäckar togs upp kontinuerligt för kontroll av stärkelsehalt och vikt. Kvaliteten på de lagrade knölarna undersöktes dessutom okulärt med avseende på mekaniska skador och andel knölar med torra respektive blöta rötor. Bakterier och svampar renodlades och identifierades.

Reultaten visade att skillnaderna generellt var större mellan de olika potatissorterna än mellan de båda stukorna. Stärkelseskörden minskade som mest under de första lagringsveckorna, och det fanns ett starkt samband mellan mekaniska skador och såväl torra rötor som blöta rötor. Sortdiversiteten för bakterier skiljde sig åt mellan de olika potatissorterna, vilket indikerar att de är olika mottagliga för olika bakterier. För att lyckas med lagringen av stärkelsepotatis är det viktigt att välja lämpliga potatissorter, att skörda dem varsamt, i rätt tid samt under torra och förhållandevis varma väderförhållanden för att minimera risken för mekaniska skador på knölarna.

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1. Background

The farmer-owned company Lyckeby Starch AB (Kristianstad, Sweden) produce and sells starch products such as potato fiber, potato protein, glue products and construction materials (Lyckeby, 2011). The company has four production industries located in southern Sweden (three in Skåne and one in Blekinge) that are open for production during the autumn, usually from September to the end of December, depending on the amount of starch potato produced. In order to increase the efficiency of the production and to become more competitive on the international market, Lyckeby investigates the possibilities of keeping the factories open for a longer period of time. Since storage capacity within the factories is limited most of the potato tubers are stored in field. An extended production period leads to longer storage periods which introduces greater risks of loss of starch yield and put higher demands on the storage management (Ekelöf, personal communication, 2012).

As stated in the literature review in this thesis, appropriate temperature and humidity are crucial to maintain tubers quality during storage. Presence and growth of pathogenic microorganisms is also very important, and depends on the environmental conditions within the storage pile. There might also be differences between different potato varieties. During the summer of 2011, meetings were held with farmers participating in the companies advisory service where starch potato storage techniques were discussed (ERFA, 2011). Some of the participating farmers shared their experiences, and a number of questions concerning improvements in storage were raised. As a result a storage field trial was set up in Listerlandet, Blekinge, during the autumn/winter 2011-12 where storage quality parameters of three different potato varieties in two different types of storage piles were compared. Studies were complemented by laboratory experiments performed at Swedish University of Agricultural Science (SLU), Uppsala.

2. Litterature review

2.1 Storage of potato

Storage of starch potatoes is generally not prioritized by farmers (ERFA, 2011). Many growers of starch potato also grow ware potato for consumption, and since the quality demands on ware tubers are higher than on starch tubers, farmers usually put more efforts into storage of ware potato. However, storage conditions are highly important for the quality of all potato tubers (Bodin & Svensson, 1996). Starch potato is usually stored in piles, placed directly on the ground either in the field, or the farmyard. The potato pile is covered with straw and plastic to protect the tubers from frost and rain. The straw also absorbs condensation produced by the tubers respiration, which might otherwise enhance the presence of microorganisms (Pringle et al., 2010).

In order to be able to store the tubers for a long period of time it is important to keep the question of storage in mind throughout the cultivation season. Crop rotation, choice of variety, fertilization and crop protection are highly important factors for producing storable tuber yield. Excessive nitrogen fertilization and neglected protection measures against potato late blight can have devastating effects on the storability (Johansson, 1983). A shortage of potassium might decrease the storage capacity of the tubers, although, according to Mulder (1955), this is most likely due to the fact that a deficiency of potassium increases the tubers' sensitivity to mechanical damage, rather than conferring a direct effect on the storage capacity in itself. The maturity rate of the tubers at harvest might affect their storage capacity, but to what extent there are variety dependent differences is not known. However, this effect might be very important for the result of the storage (Driskill et al., 2007).

Tubers should be undamaged, clean, healthy and dry when placed in a storage pile. To minimize the loss of starch, weight and quality during storage it is important to harvest the potato when the weather is dry and warm. If so, the tubers do not need to be dried before storage, and at temperatures above 10˚C the risk for mechanical damage on the tubers is reduced (Dansk Kartoffelstivelse, 2007). According to Johansson (1983) wet tubers should be handled separately and are not suitable for storage. However, drying of wet potatoes for two weeks after harvest has been found to decrease the severity of several commonly occurring post-harvest diseases. The effect was more evident after early harvest than after late harvest (Hide and Boorer, 1991). If the tubers are to be stored for more than 2 to 3 weeks it is recommended to harvest them in September (Dansk Kartoffelstivelse, 2011).

2.2 Temperature and humidity

Respiration of harvested tubers is a process that consumes carbohydrates. At 5˚C the respiration is at its lowest, the intensity increases at higher and lower temperatures (Bodin & Svensson, 1996). To enable respiration, a gas exchange with the surrounding air must be possible. The exchange of gases takes place through small openings in the potato skin, called lenticels. If the potato is covered with e.g. water the respiratory process is impaired. As early as in the 1920's it was shown that the respiratory activity in potato tubers is high directly after harvest, especially if the tubers are harvested before complete maturity has been reached. After 3 to 6 weeks, respiration decreases and stabilizes, even if tubers are stored at high temperatures. Sudden changes in storage temperature have shown to result in temporary increase in respiration intensity. However, the respiration intensity is then reduced again after a short period of time (Kimborough, 1925; Appleman and Miller, 1926).

Table 1. Starch and sugar content (fructose, glucose and sucrose) measured in tuber tissue before and after storage at different temperatures. All tubers were held at 9 ˚C during 28 days, thereafter they were held at different temperatures for 160 days. Means within a row with the same letter are not significantly different from each other at P=0,05 (after Olsen et al., 2003)

	Pre storage	3˚C	7˚C	9˚C
Starch *(µg/mg dry weight)*	550,8 a	455,9 b	545,5 a	518,4 a
Total sugars *(mg/g dry weight)*	25,3 b	69,7 a	34,2 b	15,1 c

At low temperatures sugars are accumulated in the stored tubers. The overall conversion of starch into sugar is a reversible process, and Isherwood (1973) showed that when cold stored potato with accumulated sugar levels were held at temperatures above 10˚C, the sugar was recondensated to starch. Olsen et al. (2003) examined the impact of different storage temperatures on the content of starch and sugars in tuber tissue before and during storage (Table 1). They found that tubers stored at low temperatures contained significantly lower concentrations of starch than tubers stored at higher temperatures. The authors explained this by the impact of high temperatures on starch transformation, increased respiration losses and the fact that sugar had been used for germination by tubers stored at high temperatures. Increased storage temperature results in decreased accumulation of sugar, but it also shortens the life of the potato tubers since high storage temperatures activate the physiological processes within the tubers and increase their ability to start germinating (Bodin & Svensson, 1996, Fogelfors, 2001).

The relative air humidity is largely regulated by the potato tubers themselves, at or around 94-97%, and is generally difficult to control (Pringle et al., 2009). According to Fogelfors (2001) a temperature of 4˚C and a relative air humidity of 92-95% are considered optimal once the wound healing period is over. At high temperatures the air humidity generally decreases, which causes the tubers to dry out. As water is lost from the tubers they lose weight and become soft. If the air humidity exceeds 95% there is an increased risk for water condensation from the tubers (Fogelfors, 2001). The risk for attacks by post-harvest pathogens increases both at too high and too low air humidity, since many pathogenic microorganisms thrive under warm and moist conditions (Bodin & Svensson, 1996; Pringle et al., 2009).

2.3 Mechanical damage and wound healing

Mechanical damage on the potato tubers usually consists of cuts, crush damage and bruises caused by wounding during harvest and handling of the tubers. Mechanical wounds cause a direct loss of quality, and also constitute entry sites for disease-causing microorganisms (Johansson, 1983). The fungi *Phoma* spp. and *Fusarium* spp. exemplify pathogens that take benefit of mechanical damage (Agrios, 2005). Mechanical damage can also activate latent infections in the tubers. According to Pringle et al. (2009), large and long tubers tend to be more sensitive to damage.

Uninfected wounds generally heal during the wound healing period under suitable storage conditions. Wound healing is a rapid process where a layer of cork tissue, suberin, is formed in the undamaged tissue at the wound site. The suberin layer provides a durable barrier which protects the tuber from bacterial and fungal infections (Taiz and Zeiger, 2006). The protection seems to be greater against bacteria than against fungi, and the level of protection has been found to vary between different potato varieties (Escande and Echandi, 1988).

During the process of wound healing it is important to maintain favorable environmental conditions. Good air circulation, relative humidity higher than 90% and a temperature above 10˚C are important factors for successful wound healing (Loria, 1993). An optimum temperature between 10-15˚C can compensate for deficient humidity (Johansson, 1983; Bodin

& Svensson, 1996). The wound healing process is more rapid in younger than in older tubers, and it is therefore important to minimize handling and damage of the tubers during storage. The development of suberin and wound periderm is at its slowest 5 to 6 months after harvest (Pringle et al., 2009). Cuts heal more rapidly than damage caused by crushing and bruising (Bodin & Svensson, 1996). Tubers that have not undergone the wound healing process are very sensitive to condensation and temperatures above 15˚C. Unhealed potatoes also face a high risk of suffocation (Johansson, 1983).

Wound healing is an energy demanding process that might cause a decrease of yield (Dansk Kartoffelstivelse, 2007). Lutman (1926) investigated the effect of mechanical damage on the respiration intensity of potato tubers. He found that the respiration intensity increased 3 to 4 times during the first three days after the tuber got damaged. The respiration intensity decreased after another couple of days, but remained 1.5 to 2 times as high as the respiration intensity of undamaged tubers. Cracks and outs in the tubers also caused increased respiration intensity, while damage caused by bruising did not increase the respiration intensity, as long as the skin was intact.

Immature tubers are especially sensitive to mechanical wounding, and it is therefore desirable not to harvest until the tubers are fully mature (Appleman and Miller, 1926; Dansk Kartoffelstivelse, 2007). During the first weeks after harvest even gentle handling causes a loss of water from the tubers three times greater per time unit than during the subsequent storage period. Wounded, unhealed tubers can lose more than 300 times as much water as intact tubers (Bodin & Svensson, 1996).

2.4 Microorganisms associated with potato tubers

Endophytic bacteria, i.e. bacteria that live within the plant tissue, are ubiquitous in the nature and their colonization does not necessarily cause harmful effects to plants. De Boer and Copeman (1974) investigated the endophytic bacterial flora in potato and found that the bacterial populations varied significantly between different plants, which was thought to be due to the onset of senescence predisposing plants to bacterial colonization of differing degrees. All bacteria found were non-pathogenic, and infection of the tuber tissue was thought to be due to contamination from the soil micro-flora. Weinert et al. (2010) investigated the impact of variety on the bacterial colonization of the tuber surface and of the potato rhizosphere. They found that the the colonization of tuber-associated bacteria in general was only weakly affected by the plant genotype. However, some varieties used in their trial significantly affected the size of bacterial population and the size of their antagonistic bacterial population. Several bacteria associated with soft rot on potatoes were also listed by Hooker (1981). In general, *Bacillus* and *Pseudomonas* seems to be common bacterial associates of potato tubers (Table 2).

Table 2. Different types of bacteria found associated with potato tubers in three different studies

Genus	de Boer & Copeman (1974)	Hooker (1981)	Weinert et al. (2010)
Agrobacterium	x	-	-
Bacillus	x	x	x
Clostridium	-	x	-
Erwinia	-	x	-
Flavobacterium	-	x	x
Micrococcus	x	-	-
Pseudomonas	x	x	x
Streptomyces	-	-	x
Xanthomonas	x	-	-

An endophytic lifestyle is beneficial for the bacteria as it confers stable temperature and protection from the competition from the surrounding microflora and from the grazing soil microfauna. In order to live inside the plants tissue, however, endophytic bacteria have to overcome the defense system of the plant, and this might confer beneficial effects of the bacteria on the host plant. The beneficial effects of endophytic bacteria have been measured as plant growth promotion (Kloepper et al., 1991; Höflich et al., 1994). This growth promoting effect can be direct through their production of growth stimulatory compounds and/or through their ability to facilitate the uptake of certain nutrients from the environment. The effects can also be indirect through decrease or prevention of infections of one or several pathogenic organisms (Agrios, 2005).

2.4.1 Starch-degrading microorganisms

Amylases are starch-degrading enzymes produced by all kinds of micro-organisms and commonly occurring in nature (Sarian et al., 2011). Potato starch granules are quite resistant to degrading enzymes (Taniguchi et al., 1982). As the tubers sprout, however, starch granules are degraded, which indicate that there are infact degrading enzymes produced in the tubers. Taniguchi et al. (1982) identified *Bacillus circulans* that was able to degrade potato starch granules. *B. subtilis*, and *B. amyloliquefaciens* along with *Microbacterium aurum* and *Streptomyces* sp. are other examples of amylase producing bacteria (Hayashida et al., 1988; Primarini & Ohta, 2000; Sarikaya et al., 2000; Sarian et al., 2011). There are also fungal species, e.g. *Aspergillus*, known to produce amylolytic enzymes (Ueda et al., 1974; Sarian et al., 2011).

Storage at 3˚C increases the activity of β-amylase in potato tubers (Nielsen et al., 1997). However, this increase was explained to occur due to a general stress response in the tubers that coincides with cold sweetening, but the enzyme was not directly involved in the degradation of starch. The biological process of starch degradation in potato tubers is generally poorly understood (Taniguchi et al., 1982; Nielsen et al., 1997).

2.4.2 Post-harvest diseases

During short periods of storage bacteria are considered to cause more harm than fungi, since their growth is faster (Pringle et al., 2009). Dry rots are usually caused by fungi and wet rots are generally caused by bacterial infections (Andersson, personal communication, 2012). Post-harvest disease development is generally promoted by high moisture in combination with warm temperature (Pringle et al., 2009). Global losses due to postharvest bacterial and fungal soft rots are estimated to vary between 15 to 30% of harvested crop (Agrios, 2005). There is a difference, however, in sensibility towards diseases and pathogens between different potato varieties. Synthesis of pathogen-suppressive compounds and production of thick protective surface layers around damaged areas are some of several mechanisms to acquire disease resistance. Since potato tubers are usually stored at low temperatures it may take weeks or even months before disease symptoms are visible on the tubers (Pringle et al., 2009).

2.4.2.1 By bacteria

Some bacteria cause post-harvest soft rot in potato. Soft rot is due to secondary infections which infect tubers that are already infected with other primary pathogens such as fungi (Pringle et al., 2009). The bacteria are able to survive in infected tuber tissue, in soil, on contaminated equipment and containers. Artificial wounding of subsoil plant tissue contributes to an increased colonization of endophytic bacteria within plants (Gagné et al., 1987). Wounds do not only serve as entry sites for bacteria, they also enable leakage of plant exudates, thus creating a favorable and nutrition-rich environment. Once infection has occurred, the bacteria multiply in the intercellular spaces of the tuber tissue and produce

degrading enzymes that enables their further spread. Symptoms of bacterial soft rot often begin as small watery lesions. The lesions grow rapidly and enlarge in size and depth, the infected tissue becomes soft and decayed. The process is rapid and a whole tuber may be decayed within 3 to 5 days (Agrios, 2005). There are no direct control measures against bacterial soft rot in potatoes other than to keep the crop healthy throughout the growing season and to to minimize mechanical damage during harvest. The tubers should be dry, and humidity and temperature should be low during storage (Pringle et al., 2009).

Black leg on potato is caused by *Pectobacterium atrosepticum*, (syn. *Erwinia carotovora subsp*. *atroseptica*), *P. carotovorum* subsp. *carotovorum*, (syn. *E. carotovora* subsp. *carotovora*) and *Dickeya* spp. (syn. *E. chrysanthemi*) (Persson, 2010). These bacteria produce pectolytic enzymes to degrade the plant tissue. Infection can occur on the tuber surface in the soil as bacteria spread with water. Black leg can cause patches of dry, dark rot spreading from the stem end of the tuber. A lighter zone often surrounds the darker part of the rot, and is seen when the tuber is cut in half. There is a characteristic smell associated to the rot. Sometimes the rot appears as a brown discoloration and the tuber is then firm and without smell. The severity depends on the time of infection and the conditions during growth and storage. Under appropriate storage conditions a corky barrier can be formed that restricts attacks (Persson, 1990; SMAK a), 2006).

2.4.2.2 By fungi

Various *Fusarium* spp. cause dry rot on potato (Schöber and Turkensteen, 1992; Olvång, 2000). *Fusarium* infection usually starts in wounds or cuts in the field before or during harvest and develops during storage. Dry rot may cause heavy losses in potato that is stored for a long period of time. Lesions appear moist and light brown at first, but become darker and somewhat dry with time. As the area of affected tissue enlarges, it often becomes sunken and the skin shows concentric wrinkles (Olvång, 2000). White, pink or yellow mold may appear and eventually parts of the tuber or the entire tuber is destroyed. There seems to be some resistance to dry rot in young tubers, and the disease usually progresses remarkably faster towards the end of the storage season (Loria, 1993). Chemical fungicides can be used to control dry rot, although integrated crop management is preferred to control the disease, and successful protection is achieved by careful crop management. Choosing potato cultivars with high levels of resistance to dry rot and to minimise the mechanical damage are some control means (Schöber and Turkensteen, 1992).

Gangrene is caused by *Phoma exigua* var. *foveata*, which is a secondary pathogen that requires damage on the tuber to infect (Agrios, 2005). The disease is problematic in areas where potato tubers are harvested and stored at low temperatures. The pathogen can be soil- or seed-borne. Healthy looking seed tubers may still carry a latent infection, which might spread in the tuber if wounded, and/or transmit the disease to developing tubers. Soil-borne infections increase when potato crop is grown in monoculture (Bång, 1989). The disease usually spreads during harvest (Olvång, 2000) but it can also be transmitted to healthy tubers during storage (Schöber and Turkensteen, 1992). Gangrene used to be problematic in Sweden during 1970-80, but is now less frequent since the production system for seed potato changed in 1984 to laboratory meristem-based cultivation. The mother plant originating from the meristem is then free from viruses, bacteria and fungi and thus forms healthy tubers that can be used as seed (Jönsson, 1987). However, the disease still occurs occasionally and may cause major losses during storage (Bång, 1989). Its symptoms differ depending on variety and age of the rot, but in advanced stages the affected tissue is generally hollow and sharply defined from surrounding healthy tissue, while the infected peel often is wrinkled (Olvång, 2000).

The oomycete *Phytophthora infestans* is devastating to potato cultivation as it infects both plants and tubers. Tubers generally get infected in the field when sporangia are transported

with water from late blight infected leaves through the soil or when the tubers come in contact with contaminated leaves (Andersson and Sandström, 2000). The earliest symptoms of tuber blight are small reddish or brownish patches under the skin of the potato. The affected tissue grows darker and somewhat depressed as the rot progresses. A cut through the diseased tissue shows an irregular brown dry rot, which can spread in the entire tuber. The blight develops strongly at high temperatures and often serves as entry for soft rot bacteria (SMAK b), 2006).

Pythium spp. is another oomycete that infects potato tubers and causes watery wound rot. Tubers are usually resistant to the pathogen as long as they are healthy and intact, but get easily infected when wounded. In warm and moist environments symptoms are visible a few days after infection. The risk of infection is high when tubers are harvested under moist conditions and can lead to heavy losses of the yield. Watery wound rot develops inside the tuber and is visible when the tuber is cut in half. Infected tissue is light grey and darkens when exposed to air. The rotted, central portion of the tuber is dark-brown to black. A sharp line distinguishes healthy tissue from the infected tissue. The infection sometimes develops towards the surface of the tuber and can then be seen through the potato skin. Tubers infected by *Pythium* spp. are often infected by secondary pathogens, such as pathogenic bacteria (Twengström, 2003).

2.5 Storage in field piles

It is important to place the storage pile in a good location where it can remain until the tubers are delivered to the factory. In order to prevent water from gathering underneath the pile it is important to choose a location with permeable soil. To minimize the risk of stagnant water under the pile during storage, the surface of the ground should be higher in the middle of the pile (Dansk Kartoffelstivelse, 2007). The surfaces of the pile should be as even as possible in order to minimize the risk for accumulation of condensed water vapours and frost. The storage pile should be placed in a north-south direction in order to maximize the air flow (Dansk Kartoffelstivelse, 2011). During night time and when there is a risk of rain the pile should be covered with plastic or other suitable material. However, during day time and when the weather is good the storage pile should preferably be uncovered. When the temperature decreases it is important to protect the tubers from cold and frost. Hence, the storage pile should be covered with a thick layer of straw. One of the purposes of the straw layer is to absorb moisture moving upwards as the tubers respire and during the period of wound healing. Hence, the depth of the straw should be largest at the top of the pile. If the straw gets wet it should be replaced. In order to facilitate uncovering a net could be placed between the tuber pile and the covering straw (Persson, 1997).

Farmers should be aware of the condition of the tubers throughout the period of storage to ensure that the quality of the tubers is good enough when they are taken out of storage. There is a higher risk for the tubers to get destroyed due to too high temperatures than to be destroyed by damage caused by frost. An ideal temperature inside the storage pile is 3-4˚C (Dansk Kartoffelstivelse, 2007).

Field trials with storage piles in Denmark during 2003 and 2004 showed that the highest risk factors for storage in piles were that the tubers were too cold at time of harvest, limited ventilation in the piles and tuber infection by *Phytophthora infestans*. Hence, it is important to harvest the tubers at appropriate time, which in the Danish trials was before October 10th, and that the tubers are dry when placed in the piles. The trials in Denmark also showed that the piles should be covered during night time in order to minimize changes in temperature, and that it is important to choose suitable potato varieties (AKV, 2004).

3. Objective

The objective with this thesis was to investigate to what extent starch yield (starch content*tuber weight) in potato tubers is affected by storage. A comparison was made between three different potato varieties stored in two different types of field storage piles.

The hypothesis was that changes in starch yield depend on storage temperature, relative humidity within the storage pile and prescence and growth of starch degrading bacteria and fungi. Since the period of storage was relatively short, bacteria were considered to be mainly responsible for reduction in tuber quality.

4. Materials and methods

4.1 Field trial

Two different types of storage piles, A and B, were constructed on a farmer's field in Blekinge, Sweden. Figure 1 illustrates pile A in the dome shaped manner it is usually constructed for field storage of starch potato. Harvested tubers were placed directly on the ground, covered with a net and an approximately 20 cm thick straw cover and thereafter covered with perforated plastic. Pile B was somewhat more advanced and formed in a way recommended by crisp processing companies and is illustrated by Figure 2. Tubers were placed on the ground in a rectangle shape formed by square bales of straw. The tubers were then covered with a net and thereafter an approximately 60 cm thick cover of straw. Finally the pile was covered with perforated plastic.

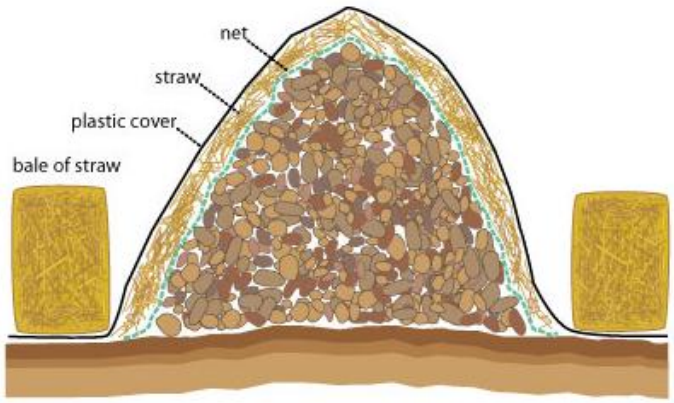

Figure 1. Illustration of a typical dome shaped pile for storage of starch potato in field. Pile A.

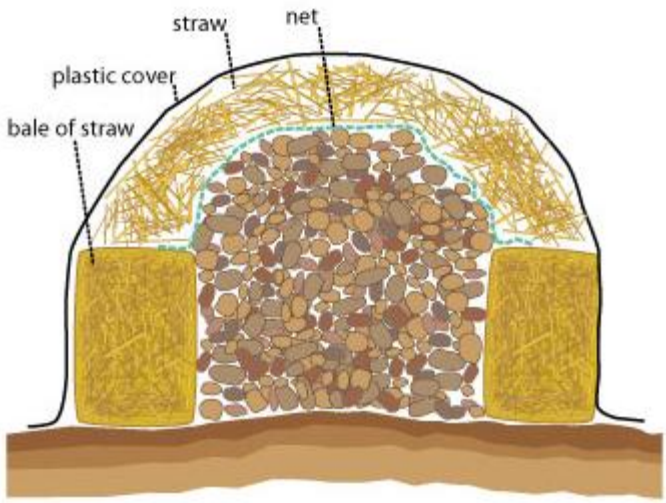

Figure 2. Illustration of a rectangular pile usually recommended by commercial companies for field storage of tubers meant for crisp production. Pile B.

Figure 3. Perforated bags with potato tubers placed in storage pile B (left). The pile was then filled up with potato tubers (approximately 30 cm) and covered with a net, straw and plastic cover (right).

The three starch potato varieties Kuras, Merano and Novano were included in the study. Kuras is a late maturing variety with potential for a high starch yield. Merano is a very late maturing variety, which is still not well known under Swedish conditions, but it is said to have potential for high starch yields as well. Novano is a fairly early maturing variety, also with potential for high starch yield (Nordström, personal communication, 2012). All three varieties were grown in a variety trial in a field nearby the site where the storage field trial was set up under identical growing conditions. All tubers were harvested on October 17th 2011 under identical conditions. After harvest potato tubers of the cultivar Kuras was stored in both piles. Perforated bags with Kuras, Merano and Novano respectively, containing approximately 10 kilos each, were then placed in both piles (Figure 3) and covered with approximately 30 cm of potatoes of the variety Kuras. Each storage pile contained four blocks corresponding to the different occasions on which the sample bags were going to be collected from the pile (Figure 4). The sample bags were randomly placed within each block.

	Storage pile A				**Storage pile B**		
Block I	Novano	Merano	Kuras	Block I	Kuras	Merano	Novano
	Merano	Kuras	Novano		Novano	Kuras	Merano
	Kuras	Novano	Merano		Merano	Novano	Kuras
	Novano	Kuras	Merano		Merano	Novano	Kuras
Block II	Kuras	Novano	Merano	Block II	Kuras	Novano	Merano
	Merano	Novano	Kuras		Merano	Novano	Kuras
	Merano	Novano	Kuras		Novano	Kuras	Merano
	Novano	Merano	Kuras		Kuras	Merano	Novano
Block III	Merano	Kuras	Novano	Block III	Merano	Kuras	Novano
	Kuras	Merano	Novano		Novano	Kuras	Merano
	Kuras	Novano	Merano		Novano	Merano	Kuras
	Novano	Merano	Kuras		Merano	Kuras	Novano
Block IV	Novano	Merano	Kuras	Block IV	Merano	Kuras	Novano
	Novano	Kuras	Merano		Kuras	Merano	Novano
	Kuras	Merano	Novano		Merano	Novano	Kuras
	Kuras	Merano	Novano		Novano	Merano	Kuras

Figure 4. Experimental set up of the storage trial in field. Net bags containing 10 kg potato tubers of each of the varieties Kuras, Merano and Novano respectively were randomly placed within four different blocks in the storage piles A and B.

4.2 Tuber weight and starch content

Starch content was measured for each of the varieties at time of harvest on October 17th 2011. The sample bags were collected from the storage piles on four occasions: October 25th, November 9th, November 24th 2011 and January 17th 2012. At the time of collection the bags were weighed and starch content was measured in order to compare changes in weight and starch content over time. Starch yield, i.e. the amount of starch (kg) present in the tubers, was calculated by multiplying the actual starch content with the weight at the time of measurement.

4.3 Temperature and relative humidity during storage

Sensors that registred temperature and relative humidity sensors (USB Temperature and Humidity Data Logger, Claes Ohlsson) were placed within the two storage piles. Six sensors were placed in each storage pile. Weather data for the area during the period of storage was gathered from LantMet at SLU/Fältforsk.

4.4 Mechanical damage on tubers

At the time of harvest four bags of each potato variety were sent to SLU, Uppsala, for visual inspection of tuber quality. Incidence of mechanical damages, i.e. number of damaged tubers, was recorded. Additional examinations of number of tubers with mechanical damage were made on November 24th 2011 and January 25th 2012.

4.5 Wet rots and dry rots on tubers

Incidence of wet rots and dry rots, i.e. number of infected tubers, and severity, i.e. area of infected tuber tissue, were recorded visually at two occasions: of November 24th 2011 and January 25th 2012. At the final quality assessment after three months of storage, ten tubers from each of the 24 trial bags (three varieties*four repetitions) were randomly sampled and stored at 6˚C.

Figure 5. Potato peels were plugged out in a uniform manner from ten randomely chosen tubers of each treatment and replicate.

4.5.1 Characterisation of culturable bacteria associated with tubers

Three plugs of peel tissue (5 mm diameter) were taken from each tuber (Figure 5). In total, thirty plugs resulted from each sample bag, they were pooled together and thoroughly blended. The suspensions thus obtained were diluted serially in phosphate buffered saline solution (PBS/l destilled water; 8.0 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4). Appropriate dilutions were spread aseptically on sterile diluted trypticase soy broth agar (TSA/l destilled water; 20 g TSA broth (Oxoid Microbiology Products), 7.5 g agar (Oxoid

Microbiology Products)) for assessing culturable bacterial populations and on potato dextrose agar (PDA; 39 g PDA (Oxoid Microbiology Products)/l distilled water) for fungal populations. Each dilution was spread in two replicates and all agar plates were incubated at 12˚C.

Bacterial colony forming units (CFU) were counted 48 hours after spreading (Figure 6) and the data was transformed to calculate CFU per gram dry weight of potato tissue. Morphologically different colonies were selected and transferred to new agar plates for purification and further analyses (Figure 6). This procedure resulted in 72 pure isolates of bacteria. The plates were kept at 4˚C for storage.

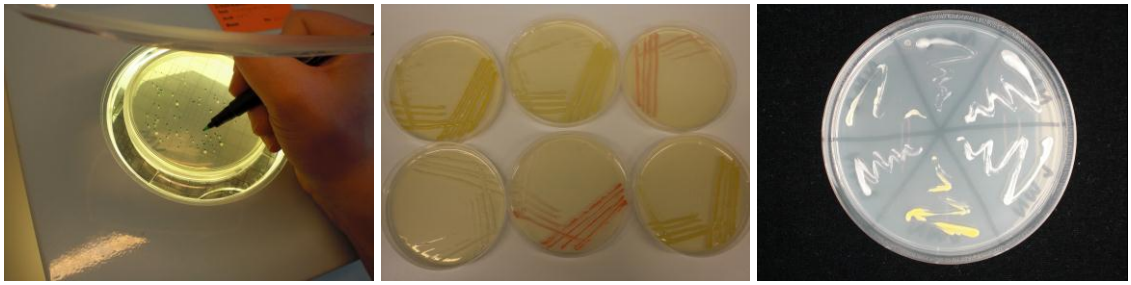

Figure 6. Counting of bacterial colonies (left), purification of morphologically differing bacterial colonies (middle and right).

4.5.1.1 DNA extraction and amplification

The bacterial DNA was amplified by polymerase chain reaction (PCR), which enables particular segments of DNA to replicate by nucleic acid synthesis (Saiki, 1990). Double-stranded DNA is denatured by heating and converted into single strands. During an annealing period two primers; one corresponding to the 5' end and one corresponding to the 3' end of the DNA, bind to the nucleotides flanking the targeted DNA sequence. The primers serve as starting points for synthesis of new DNA strands complementary to the target DNA sequence. Finally, a heat-stable DNA polymerase binds nucleotides to the primers and produces a double-stranded copy of the target DNA sequence (Klug et al., 2007).

Fragments of 16S rRNA gene sequences were PCR amplified by using the universal bacteria-specific primers 27F (5´-AGA GTT TGA TCM TGG CTC AG-3´) and 907R (5´-CCG TCA ATT CMT TTR AGT TT-3´). The PCR solution consisted of: 11.25 µl sterile H_2O, 5 µl PCR buffer (RB), 5 µl deoxynucleoside triphosphate (dNTP) [0.2 mM], 1.5 µl $MgCl_2$ [20mM], 0.25 µl of DreamTaq™ DNA Polymerase (Fermentas Molecular Biology Products) [5u/µl], 1 µl of each primer and 25 µl bacteria sample at a concentration of 1^{-10}. The 2720 Thermal Cycler (Applied Biosystems®) was used as follows: initial DNA denaturation at 94˚C for three minutes; 28 cycles of 94˚C for 45 seconds; 52˚C for 30 seconds; 72˚C for 30 seconds; and a final extension step at 72˚C for seven minutes. Thereafter the samples were cooled down to 4˚C, all according to the method described by Saiki (1990). Following this, 4 µl of each of the PCR samples were used to screen for amplification efficiency and amplimer size by electrophoresis of 250 voltage for 30 minutes on a 1.2% agarose (Agaros Standard, Saveen Werner AB)-sodium boric acid buffer (Brody and Kern, 2004 [4.6 mM]) gel. The gels were stained with ethidium bromide and visually analysed under UV light (GelDoc 2000, BioRad laboratories).

4.5.1.2 Purification of PCR products

In order to get rid of salts, unincorporated dNTPs and unused primers the PCR-products were purified with Agencourt® AMPure Xp® (Beckman Coulter Inc.). In this method DNA is

bound to small magnetic beads, and by using a magnet it is possible to wash and dry the DNA. Thereafter the DNA can be extracted from the beads. The PCR-products (50 µl) were mixed with 85 µl AMPure® magnetic bead solution and incubated at room temperature for 5 minutes. The samples were then placed on a magnetic plate for 10 minutes, after incubation the plate was turned upside down to get rid of the liquid. Following this 200 µl 70% ethanol was added to each sample and incubated for 30 seconds at room temperature. When liquid had been removed the plate was removed from the magnet and the samples were dried at 37˚C for 60 minutes. When dry, 50 µl of elution buffer was added according to recommendation to each sample and carefully mixed. Finally 45 µl of each sample was transferred to a new plate and dried over night at 37˚C.

The dry samples were then sent to Macrogen Inc., South Korea, for sequencing.

4.5.1.3 Species diversity analyses

In order to compare the composition of the bacterial communities in the three varieties in two piles Shannon's diversity index was calculated. Shannon's diversity index measures the species diversity in a community; higher values of H represent greater diversity (Ricklefs, 2007). The species richness, i.e. number of species present, as well as the relative abundance of the different species is taken into account.

$$H = \sum_{i=1}^{s} -(P_i * lnP_i)$$

H = Shannon´s diversity index
P_i = fraction of the entire population made up of species i
S = number of species encountered
Σ = sum from species 1 to species S

4.5.1.4 Estimation of starch degrading bacterial population

With an aim to estimate proportion of tuber associated bacteria with starch hydrolyzing ability, all the 72 isolates were examined *in vitro* by using Gram´s iodine solution. Starch amended agar was prepared by adding 0.44% soluble wheat starch (Sigma-Aldrich®) to TSA (Oxoid Microbiology Products). Bacteria were inoculated on the starch amended agar and incubated for 72 hours. The agar surface was flooded with Gram´s iodine (1 g iodine, 2 g potassium iodine, 300 ml sterile water) and incubated in dark for ten minutes. The iodine reacts with starch and forms a dark blue-colored complex. A clear yellowish halo formed around the bacterial colony after flooding with Gram´s iodine solution was considered as a positive reaction indicating that starch had been hydrolyzed by the bacteria (Figure 7).

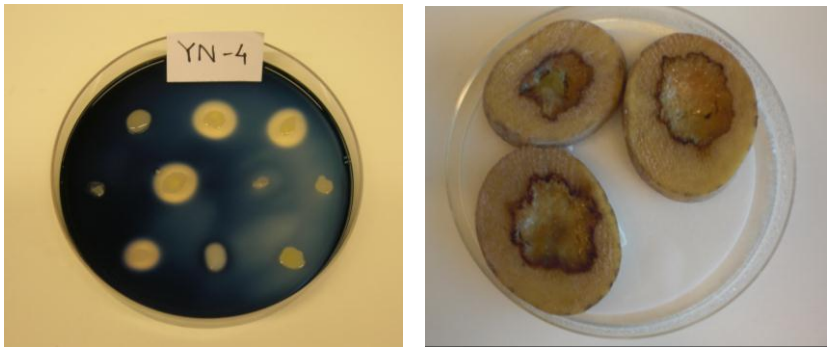

Figure 7. Starch hydrolysation by four tuber associated bacteria *in vitro* (yellow halo) (left). *In vivo* examination of starch hydrolysing ability of bacterial isolates by inoculation of potato slices (right).

In vitro tests were complemented with an *in vivo* assay to ascertain the starch hydrolysing ability of the bacteria. Half a cm thick slices of a commercial ware potato variety Melody were co-inoculated with each bacterial isolate. Tubers were surface-sterilised with ethanol before cutting slices. Care was taken to retain moisture and minimise oxidation of sliced tissue during inoculation. Each isolate was suspended in 1 ml PBS, and 20 µl of each suspension was applied in a shallow well (1.5 cm in diameter*5 mm depth) made in the centre of each potato slice. Each combination was prepared in three replicates and in a uniform manner. The inoculated slices were incubated in moist environment and dark at 20˚C for one week after which the affected tuber tissue was estimated qualitatively as a measure of the starch-hydrolyzing ability (Figure 7).

4.5.2 Characterisation of culturable fungi associated with tubers

Apparently morphologically different fungi appearing on TSA or PDA (Oxoid Microbiology Products) were selected and purified for the purpose of identification by aseptical inoculation on sterile diluted PDA (20 g PDA/l destilled water, 7.5 g agar/l destilled water) amended with antibiotics (Rifampicin [5 ppm] and Streptomycin [50 ppm]) followed by incubation at 12˚C. Reinoculation was carried out to ensure the purity of the fungi. Fungal mycelium was later harvested directly from the agar surface and placed in small plastic vials together with four glass pearls before lyophilizing the fungal tissue at -60 ˚C for 18h until further analyses.

4.5.2.1 DNA extraction and amplification

The mycelia was homogenized by mixing for 20 seconds. To each of the homogenized samples 1 ml CTAB-buffer [3%] was added and the samples were incubated at 65˚C for one hour. The samples were shaken every 20-30 minutes during incubation. The samples were then centrifuged at 13 000 rpm for five minutes and the supernatants (approximately 800 µl) were transferred to new 1.5-ml Eppendorf tubes. One volume of chloroform was added and the solution was mixed by shaking the tubes carefully. The samples were then centrifuged at 8000 rpm for 10 minutes and the upper phase (approximately 500 µl) was transferred to new Eppendorf tubes. DNA was precipitated by adding 1.5 volumes of cold isopropanol-mix and the samples were kept at -22˚C for one hour. After freezing the samples were centrifuged at 13 000 rpm for 20 minutes, and the supernatants were carefully poured out. The DNA had then formed a pellet which was washed by adding 200 µl 70% cold ethanol and centrifuged at 6500 rpm for five minutes. The ethanol was then poured out and the tubes were left up-side down on absorbent paper for one hour in order for the pellets to dry. When dry, the pellets were resuspended in 50 µl sterile water.

The DNA solutions were run through a Spectophotometer (NanoDrop, ND-1000) in order to evaluate the concentration of DNA in the samples. The samples were then diluted with sterile water according to their concentration in order to reach final concentrations as even as possible, approximately 0.5 ng/µl.

The PCR solution consisted of: 11.25 µl sterile H_2O, 5 µl PCR buffer (RB), 5 µl deoxynucleoside triphosphate (dNTP) [0,2 mM], 1.5 µl $MgCl_2$ [20 mM] 0.25 µl of DreamTaq^{TM} DNA Polymerase (Fermentas Molecular Biology Products) [5u/µl], 1 µl of each of the primers ITS IF (5´-CTT GGT CAT TTA GAG GAA GTA A-3´) and ITS 4 (5´-TCC TCC GCT TAT TGA TAT GC-3´) and 25 µl fungal DNA sample at a concentration of 0.5 ng/µl. The samples were run through PCR in a 2720 Thermal Cycler (Applied Biosystems®) at the program ITS 55; initial DNA denaturation at 94˚C for five minutes; 35 cycles of 94˚C for 30 seconds; 55˚C for 30 seconds; 72˚C for 30 seconds; and a final extension step at 72˚C for seven minutes. Thereafter the samples were cooled down to 10˚C, according to the method described by Saiki (1990). Following this, 4 µl of the PCR samples were used to screen for amplification efficiency and amplimer size by electrophoresis of 250 voltage for 30 minutes on a 1.2% agarose (Agaros Standard, Saveen Werner AB)-sodium boric acid buffer (Brody and Kern, 2004 [4.6 mM]) gel. The gels were stained with ethidium bromide and visually analysed under UV light (GelDoc 2000, BioRad laboratories).

4.5.2.2 Purification of PCR products

The fungal PCR products were purified with Agencourt® AMPure Xp® (Beckman Coulter Inc.), in the same way as the bacterial PCR products (see above; 4.5.1.2).

The dry fungal samples were then sent to Macrogen Inc., South Korea, for sequencing.

4.6 Statistical analyses

All statistical analyses were performed with JMP, version 10.0.0.

5. Results

5.1 Starch yield

Starch yield measured after three months of storage, on January 17th 2012, did not differ significantly between storage pile A and B, but there were significant differences between the three varieties within the different piles (Figure 8). The reduction in starch yield was significant in all three varieties during the first three weeks of storage. Starch yield tended to reduce continuously in all three varieties, but these reductions were no longer significant. The initial starch yield was reduced with 7% in the potato variety Kuras, 8% in the variety Merano and 5% in Novano after three months of storage (Table 3, Figure 9).

The starch content in the tubers at harvest, on October 17th 2011, differed significantly between the three potato varieties, the variety Novano had the highest initial content of starch and Kuras the lowest (Table 3). Starch reduction was not affected by type of storage pile and there was no statistical correlation between change in starch content and time of storage (Figure 9).

Tuber weight decreased rapidly during the first weeks of storage, probably due to loss of water. After this initial loss there were no significant differences in losses between the subsequent measurements. There were no significant differences in weight loss between the two piles, except for Kuras on November 24th, where there was a significant difference between pile A and B. Significant differences between the two piles were found only during the process of storage. After three months of storage no significant differences in starch content and weight loss were found between storage pile A and B (Table 3).

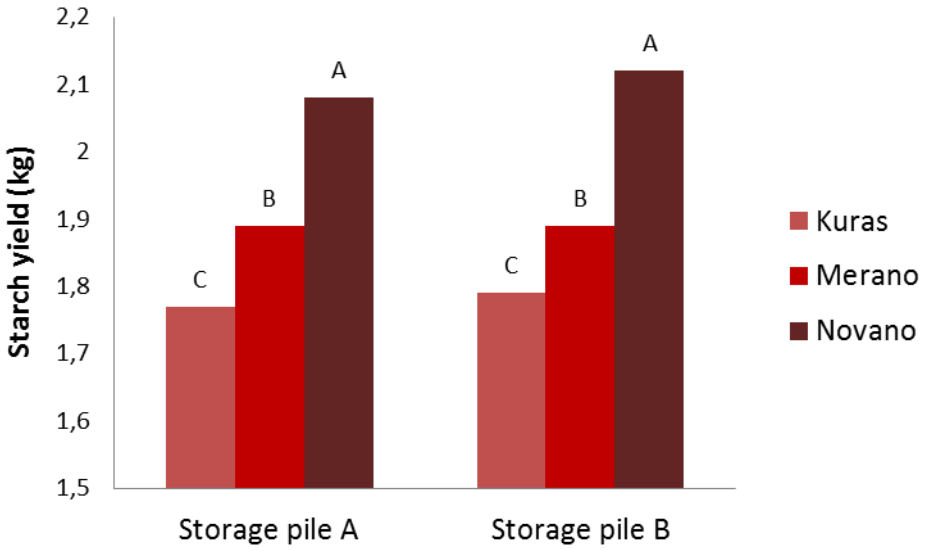

Figure 8. Starch yield (kg) from three different potato varieties, Kuras, Merano and Novano stored for three months in storage pile A and B. Calculations were based on percentage of weight loss and starch content (weight loss calculated from an initial amount of 10 kg tubers of each variety). Within each storage pile, bars marked with different letters are significantly different at P > 0.001.

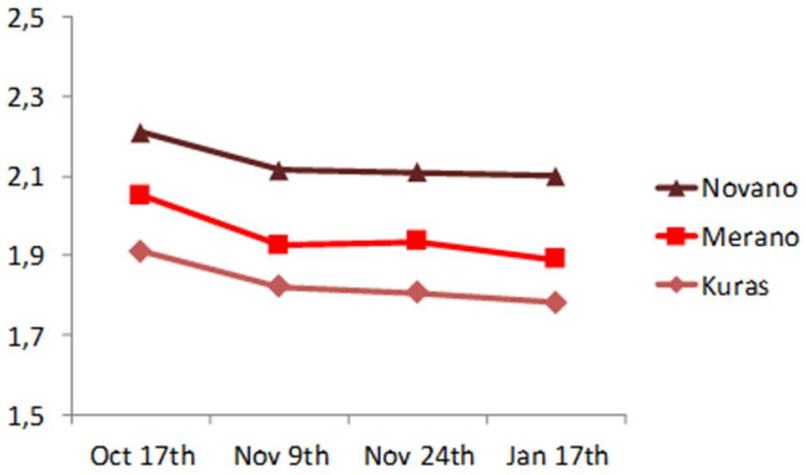

Figure 9. Change in starch yield (kg) over three months of storage in three different potato varieties.

Table 3. Starch yield, starch content and tuber weight loss in three potato varieties stored in two types of storage piles during a period of three months. Starch content was not measured on October 25th. No statistical analyses were made on the initial values (October 17th)

		Starch yield (kg)		p-value	Starch content (%)		p-value	Weight loss (%)		p-value
	Sampling	Pile A	Pile B		Pile A	Pile B		Pile A	Pile B	
Kuras	2011-10-17	1.91	1.91	-	19.12	19.12	-	0	0	-
	2011-10-25	-	-	-	-	-	-	4.91	4.07	0.1427
	2011-11-09	1.81	1.83	0.1724	17.80	18.00	0.4198	5.53	4.40	0.1453
	2011-11-24	1.82	1.79	0.0149*	18.72	17.88	0.0047*	6.09	5.14	0.0323*
	2012-01-17	1.77	1.79	0.3995	18.40	18.02	0.1239	7.91	7.41	0.5017
Merano	2011-10-17	2.05	2.05	-	20.45	20.45	-	0	0	-
	2011-10-25	-	-	-	-	-	-	4.57	5.09	0.0903
	2011-11-09	1.93	1.92	0.0344*	19.22	19.65	0.4445	6.00	6.20	0.4873
	2011-11-24	1.93	1.94	0.7311	19.02	18.92	0.8532	6.25	5.44	0.7061
	2012-01-17	1.89	1.89	0.9721	19.50	19.40	0.8471	7.87	7.95	0.9419
Novano	2011-10-17	2.21	2.21	-	22.08	22.08	-	0	0	-
	2011-10-25	-	-	-	-	-	-	3.34	4.26	0.2799
	2011-11-09	2.10	2.13	0.1148	21.22	20.88	0.2946	5.05	3.61	0.1431
	2011-11-24	2.10	2.12	0.4249	20.92	21.35	0.2743	4.89	3.87	0.3920
	2012-01-17	2.08	2.12	0.2035	21.82	21.42	0.4310	5.87	4.09	0.2156

- Not measured
* Statistically significant difference between piles within a variety

5.2 Temperature and relative humidity during storage

There was a correlation between the temperatures in storage pile A and B throughout the period of storage (Figure 12). The temperature decreased with time in both storage piles. The temperature in pile A fluctuated more than in pile B during the entire period. Figure 12 and 13 also show that the decrease in temperature within the storage piles was closely correlated with the decrease in air temperature.

The relative humidity was constantly high in both piles, although it reached 100% earlier in pile A than in pile B (Figure 12). There seems to be no correlations between the relative humidity within the storage piles and the relative air humidity (Figure 12 and 13).

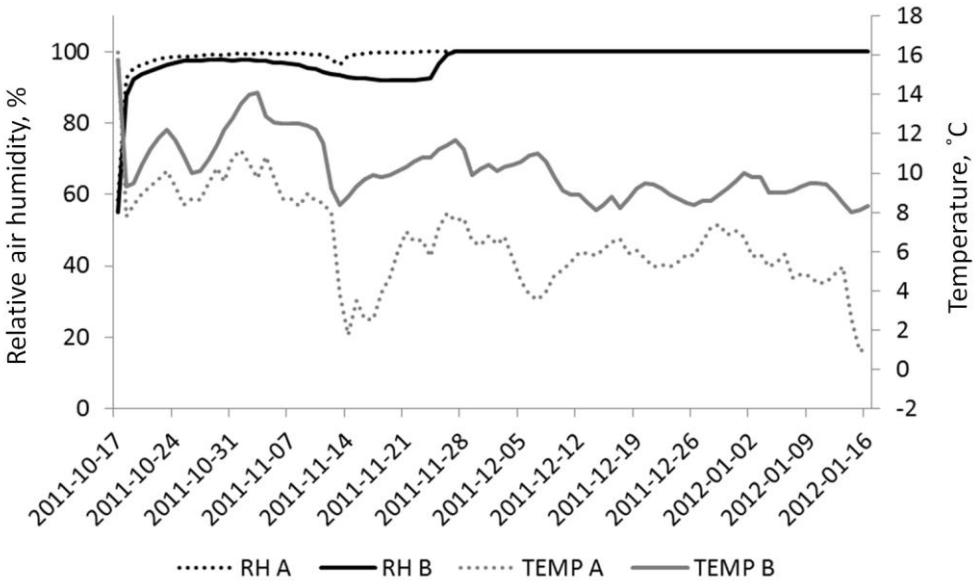

Figure 12. Temperature and relative humidity during three months in potato piles A and B stored at field site, Listerlandet. Data from LantMet vid SLU/FältForsk.

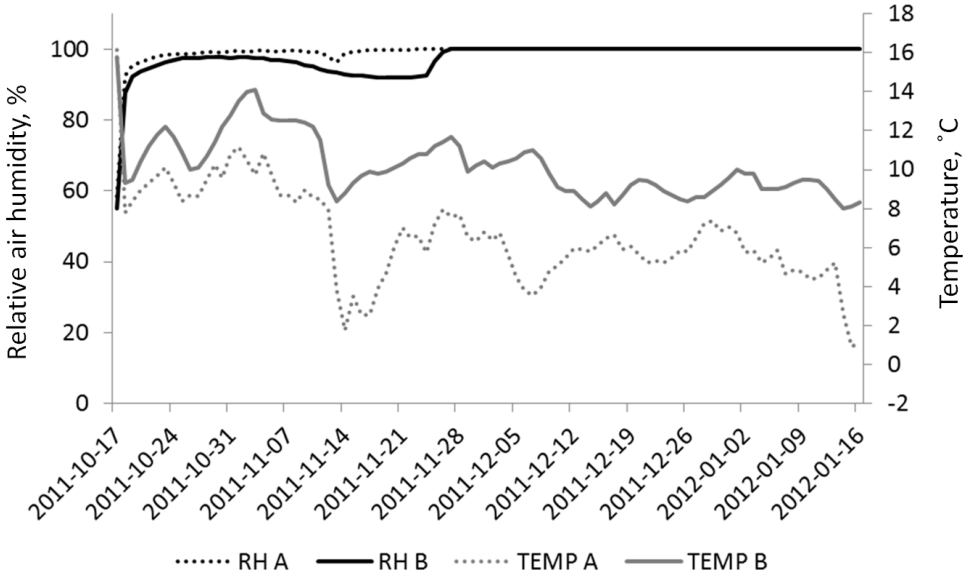

Figure 13. Air temperature and relative air humidity during three months in potato piles A and B stored at field site, Listerlandet. Data from LantMet vid SLU/FältForsk.

5.3 Estimation of mechanical damage on tubers

In average for all three varieties 67% of the tubers showed mechanical damage. The levels of mechanical damage was assessed as number of tubers with mechanical damage, and did not include the amount of tuber tissue damaged (Figure 10). As shown in Figure 11, the three different varieties of potato differed significantly from each other, where Merano carried the highest percentage of damaged tubers (82%), followed by Kuras (69%) whereas Novano carried the lowest percentage of damaged tubers (59%).

Figure 10. Mechanical damage on potato tubers.

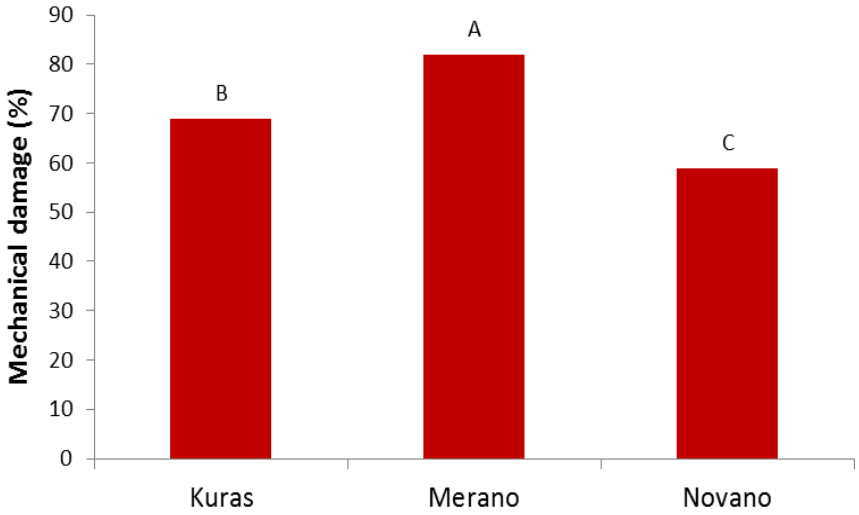

Figure 11. Percentage tubers with mechanical damage at harvest. Bars marked with different letters are significantly different from each other ($p<0.0001$).

5.4 Wet rot and dry rots on tubers

5.4.1 Incidence and severity

There was no significant difference in the number of tubers affected by wet rots or dry rots between storage pile A and B. Significant differences were evident, however, between the different varieties (Figure 14). The variety Novano showed significantly lower incidence of wet rots than the other two varieties irrespective of the type of pile. The severity of wet rot infections was also significantly lower in the variety Novano in pile A, but not in pile B. There were significant differences in incidence of dry rots in storage pile A between the varieties Merano and Novano, where Novano had the lowest amount of infected tubers. The varieties Novano and Kuras also showed lower severity of dry rots in storage pile B than the variety Merano.

Incidence of wet rots or dry rots rot did not increase significantly in the two storage piles during the period of storage. The severity of dry rots increased significantly from November 24th to January 17th in storage pile B. The severity of dry rot in storage pile A, as well as wet rots in both storage piles, tended to increase during the period of storage, but the increases were not statistically significant.

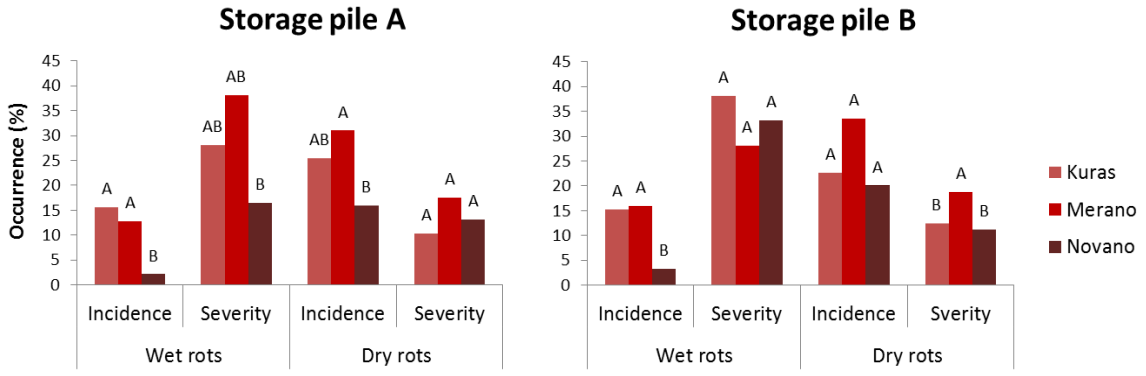

Figure 14. Incidence (number of infected tubers) and severity (area of infected tuber tissue) of wet rots and dry rots respectively in three different potato varieties and two different types of storage piles, measured after three months of storage. Bars within each group marked with different letters are significantly different at p=0.05.

5.4.2 Wet rots and dry rots in relation to mechanical damage

A significant positive correlation was observed between incidence of mechanical damage on tubers and incidence of wet rots in the variety Merano. Similar tendency was observed in the variety Kuras but the correlation was not significant. No significant correlation between incidence of mechanical damage and wet rots was found in the variety Novano (Figure 15).

Incidence of mechanical damage on tubers in relation to incidence of dry rots did not differ significantly between the two piles. Again the correlation between the two parameters differed between the varieties. The correlation was significant in the varieties Kuras and Merano, but not significant in Novano (Figure 16).

No correlations were established between incidence of dry rots and incidence of wet rots in either of the potato varieties.

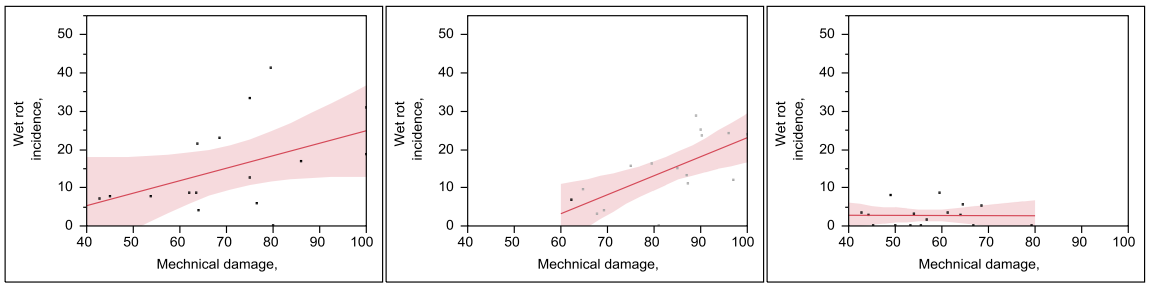

Figure 15. Incidence of wet rots (number of infected tubers) compared to incidence of mechanical damage (number of damaged tubers); from left to right potato variety Kuras (p=0.0722, R^2=0.21), Merano (p=0.0042, R^2=0.45) and Novano (p=0.9679, R^2=0.00) respectively. Shadowed areas indicate the 95% confidence interval.

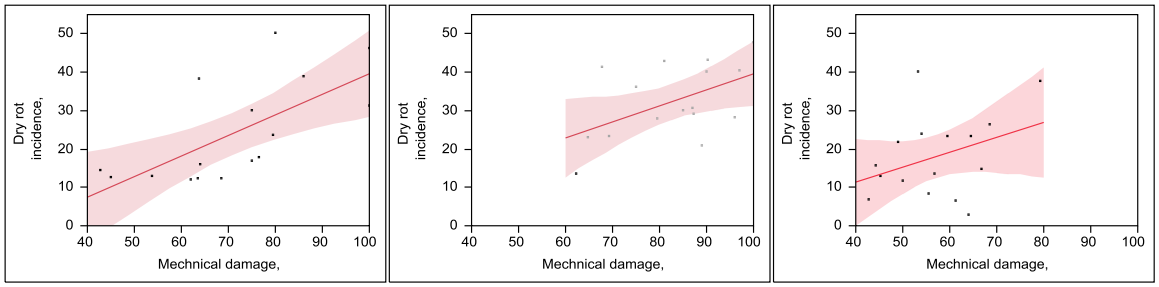

Figure 16. Incidence of dry rots (number of infected tubers) compared to incidence of mechanical damage (number of damaged tubers); from left to right potato variety Kuras (p=0.0042, R^2=0.45), Merano (p=0.0438, R^2=0.26) and Novano (p=0.1691, R^2=0.13) respectively. Shadowed areas indicate the 95% confidence interval.

5.4.3 Culturable bacterial and fungal communities in stored tubers

5.4.3.1 Bacterial population size

Bacterial population size as estimated on TSA by the culture-dependent approach differed statistically between the three varieties in pile B, with the variety Merano harbouring the highest population (5 x Kuras and 24 x Novano, Figure 17). The population size in the variety Merano also differed between the two storage piles (Table 4). There was a general tendency of higher bacterial counts in the varieties Kuras and Novano in pile B but the differences were not significant.

Estimation of bacterial population on PDA also showed the same pattern as on TSA. In general the bacterial counts were lower on PDA than on TSA.

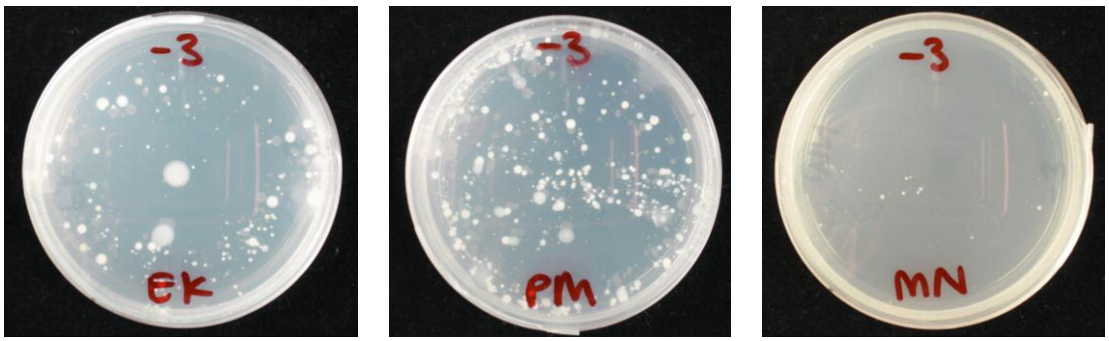

Figure 17. Bacterial colonies appearing after 48 hours on TSA in from left to right potato variety Kuras, Merano and Novano.

Table 4. Number of viable bacterial cell counts (colony forming units, cfu x10^6) per gram dried potato peel tissue on TSA. Tubers were stored for three months in field in two pile types, pile A and B. Values followed by different letters indicate significant differences between varieties in each storage pile. P-value shown in table indicate statistical significance between the piles

	Storage pile A		Storage pile B		p-value
Kuras	2.73	A	8.73	B	0.1234
Merano	3.67	A	47.32	A	0.0173
Novano	1.76	A	1.95	B	0.8883

5.4.3.2 Bacterial species diversity

In total, 27 different genera and 19 different species from ten different bacterial families were identified from the stored potato tubers. All bacterial species isolated and identified are listed in Table 5. Bacteria known to be pathogenic to potato, such as *Pectobacterium atrosepticum* that cause Blackleg on potato (Persson, 2010) were among the identified species. Among the identified bacteria *Pseudomonas flourescens* and *P. putida* were also found present.

The bacterial diversity (those identified) seemed to be higher in the varieties Merano and Novano than in Kuras (Table 6). A tendency of higher diversity was also seen in pile B. It is possible that the higher diversity was due to a higher number of culturable bacterial populations obtained from tubers stored in pile B than from A.

Table 5. Bacterial families identified from tubers of the potato varieties Kuras, Merano and Novano three months after storage in two different piles in field. The quantity of identified species within each potato variety is indicated by number

			Kuras		Merano		Novano	
Family	**Genus**	**Species**	**Pile A**	**B**	**A**	**B**	**A**	**B**
Bacillaceae	*Bacillus*	*simplex*					2	
Enterobacteriaceae	*Erwinia*	*spp.*		1				
Enterobacteriaceae	*Erwinia*	*billingiae*					1	1
Enterobacteriaceae	*Enterobacter*	*amnigenus*			2	2		
Enterobacteriaceae	*Pectobacterium*	*atrosepticum*			1			
Enterobacteriaceae	*Rahnella*	*aquatilis*	1				2	
Enterobacteriaceae	*Serratia*	*proteamaculans*		1				
Enterobacteriaceae					4			
Flavobacteriaceae	*Chryseobacterium*	*balustinum*	1			1		1
Flavobacteriaceae	*Chryseobacterium*	*spp.*			1	1		
Flavobacteriaceae	*Chryseobacterium*	*piscicola*						2
Flavobacteriaceae	*Flavobacterium*	*spp.*						1
Flavobacteriaceae						1		
Leuconostocaceae	*Leuconostoc*	*gasicomitatum*				1		
Leuconostocaceae	*Leuconostoc*	*mesenteroides*						1
Micrococcineae	*Arthrobacter*	*spp.*	2		1			1
Micrococcineae	*Cellulomonas*	*spp.*			1			
Moraxellaceae	*Acinetobacter*	*iwolffii*						1
Moraxellaceae	*Acinetobacter*	*spp.*						1
Oxalobacteraceae	*Janthinobacterium*	*spp.*						1
Propionibacterineae	*Propionibacterium*	*acnes*						1
Pseudomonadaceae	*Pseudomonas*	*spp.*	2	3	1		1	4
Pseudomonadaceae	*Pseudomonas*	*brenneri*			1			
Pseudomonadaceae	*Pseudomonas*	*fluorescens*				2		2
Pseudomonadaceae	*Pseudomonas*	*fragi*	1					
Pseudomonadaceae	*Pseudomonas*	*grimontii*	1					
Pseudomonadaceae	*Pseudomonas*	*lundensis*				1		
Pseudomonadaceae	*Pseudomonas*	*putida*	1	1		1		
Xanthomonadaceae	*Stenotrophomonas*	*maltophilia*	1		1	1	1	1
Unidentified bacterial isolates				1		2	1	4

Table 6. Bacterial diversity (based on 72 cultured isolates) in potato tissue of three varieties three months after of field storage in two pile types in field. Only bacterial isolates identified at genera level were included in the Shannon diversity index, bacterial isolates identified only at family level were counted as unidentified bacterial isolates

	Kuras	**Merano**	**Novano**	**Storage pile A**	**Storage pile B**
Number of isolates counted	10	13	15	15	20
Shannon diversity index	3,07	3,65	4,32	4,23	5,03

5.4.4.3 Proportion of starch hydrolyzing bacterial population

The variety Kuras seemed to harbour a smaller starch degrading bacterial population than Merano and Novano, when tested *in vitro* and *in vivo* (Table 7). *In vitro* the populations of starch degraders constituted about 1/3rd of the total bacterial population in Kuras while the ratio was 2/3rd in Merano and Novano. However, these differences were not statistically significant. No significant difference was noticed between the population sizes in the two piles independent of the method of analysis.

Table 7. Percentage starch hydrolyzing bacteria of total bacteria isolated from three different potato varieties after three months of storage in two pile types in field

	Storage pile A		**Storage pile B**	
	In vitro	***In vivo***	***In vitro***	***In vivo***
Kuras	31	11	40	11
Merano	67	29	70	36
Novano	71	33	66	27

5.4.4.4 Fungi

Results from fungi cultured from the three varieties on PDA and TSA are summarised in Table 8. No attempt was made to calculate diversity index for fungi as a total 11 species were identified and this was considered to be too small material to give conclusive results as regards varietal and pile differences. Most fungi identified are common soil inhabitants but some are also reported to be common plant inhabitants. Fungi with known pathogenic potential were identified, such as *Cladosporium cladosporioides*, *Colletotrichum coccodes*, *Colletotrichum gloeosporioides*, *Mucor hiemalis*, *Mucor racemosus*, *Phoma eupyrena* and *Phoma herbarum*.

Table 8. Fungi isolated on PDA and TSA from three potato varieties stored for three months in piles in field

Species	**Occurrence**	**Source of information**
Cladosporium cladosporioides	Soil, plants	Domsch et al. (1980)
Colletotrichum coccodes	Soil, potato, tomato	Ingram et al. (2011)
Colletotrichum gloeosporioides	Fruits, post-harvest diseases	Phoulivang et al. (2010)
Galactomyces geotrichum	Plants, soil, insects, mammals	Fungal Biodiv. Centre (2012)
Mucor hiemalis	Soil	Domsch et al. (1980)
Mucor racemosus	Wood, soil, dung, litter	Agrios (2005)
Penicillium freii	Cereals	Intern. Mycol. Assoc. a) (2000)
Phoma eupyrena	Soil, plants	Q-bank a) (2011)
Phoma herbarum	Soil, air, water	Q-bank b) (2011)
Scytalidium lignicola	Plants, decaying wood	Intern. Mycol. Assoc. b) (2000)
Umbelopsis sp.	??	??

6. Discussion

The major objective with this study was to compare the storage efficiency between two different types of field storage piles and three different varieties of starch potato. In general, the differences between the two piles were small, but the differences between the three potato varieties were significant. The variety Novano was found to be the best suited, and Kuras the least suited variety for long-term storage.

That the differences between the two piles were not as great as expected in terms of storage capacity indicates that the form of the pile has no impact on the final storage result. Another more likely explanation behind the results is that the weather during the experimental period was unusually mild. With such weather it becomes unnecessary to insulate the piles in the way pile B was done.

6.1 Starch yield

The final starch yield after three months of storage differed significantly between the three potato varieties (Figure 8), which was also found in Danish starch potato storage trials (AKV, 2004). This indicates that the choice of variety is highly important in order to ensure a high starch yield in the end. The loss of starch yield increased throughout the period of storage. The largest losses, however, occurred during the first weeks of storage (Table 3) which might be due to wound healing (Dansk Kartoffelstivelse, 2007). The starch content also decreased during the first few weeks, which was probably due to increased respiration directly after wounding of the tuber (Lutman 1926). The second recording of starch content in the tubers was done after 23 days of storage. The decrease in starch content probably occurred earlier. The level of starch content got stabilized after a few weeks, and in some cases it even increased again (Table 3). The increase in starch content towards the end of the storage period was probably caused by the loss of tuber weight due to loss of water through respiration and thus leading to a concentration of the starch content in the tubers.

6.2 Temperature and relative humidity during storage

There was a co-variation in temperatures between pile A and B throughout the storage period (Figure 12). However, the temperature in pile A was constantly lower than in storage pile B, and reached at its coldest almost 0˚C. The internal fluctuations were also less pronounced in pile B, which was very likely due to its extended insulation. Initially the temperature in pile B was on average 10˚C which seems to have been high enough to promote the wound healing process. The relative humidity at >90% was also appropriate for wound healing. The average temperature in pile A was slightly lower than in pile B but also appropriate for wound healing.

Temperature decreased strongly in both piles around November 14th (Figure 12) which coincided with an intense decrease in air temperature (Figure 13). Following this decrease the air temperature tended to decrease but fluctuate strongly for the rest of the storage period. This fluctuation had no practical impact on the pile temperatures, although the average temperature declined continuously in both piles. After the period of wound healing a temperature of 4˚C is considered to be optimal (Fogelfors, 2001; Dansk Kartoffekstivelse, 2007), which indicates that the temperature in pile A probably was more appropriate for potato storage. It should be noticed, however, that the autumn of 2011 was unusually warm. If the weather would have been colder pile B would probably have had a temperature more suitable for storage of potato. At lower temperatures, i.e. about 3˚C, there would have been an increased risk for starch degradation due to the transformation of starch into sugar (Marquez

and Añon, 1986; Olsen et al. 2003). Such low temperatures were reached only occasionally in this field trial.

The relative humidity within the piles was high throughout the period of storage (Figure 12) and there was no effect of the fluctuation in air moisture (Figure 13), both piles seems to have been satisfactoryily insulated. It reached to 100% sooner in pile A than in pile B. Storage pile B was better insulated, which was probably the cause of the slower increase of relative humidity. The greatest humidity affecting factor seems to have been the tubers own respiration (Pringle et al. 2009).

6.3 Mechanical damage on tubers

Tubers of all three potato varieties carried high incidence of mechanical damage, on average 67%. According to Pringle et al. (2009), large and long tubers are extra sensitive to mechanical damage. Starch potato tubers are usually quite large which partly explains the high incidence of damages in tubers. At the time of harvest there had also been a few cold nights and the tubers were probably affected by frost, which most likely made them vulnerable to handling. The same problem occurred in storage trials in Denmark (AKV, 2004). Despite the high damage frequency in all varieties there was a significant varietal difference (Figure 11). Since all potato tubers were harvested under identical conditions this indicates that the variety Merano is more sensitive to mechanical damage than the other varieties which may be because it sets the tubers shallower than others and thus makes the tubers more vulnerable to cold temperatures. Merano might also have larger tubers, thus more sensitive to mechanical damage (Pringle et al., 2009). Another possible explanation might be that Merano matures slower than the other two varieties. Immature tubers are more sensitive to mechanical damage than fully mature ones (Appleman and Miller, 1926; Dansk Kartoffelstivelse, 2007).

Mechanical damage often constitutes entry gates for pathogens, and increased damage hence likely leads to increased incidence of rots (Johansson, 1983). Latent infections might also get activated by mechanical damage on the tubers. The variety Merano had high incidence of both wet rots and dry rots in both piles (Figure 14), and there was a significant positive correlation between percentage of damaged tubers and incidence of rots (Figure 15 and 16). The total culturable bacterial population size was significantly higher in Merano in pile B than in the other varieties (Table 4), which was probably due to the favorable microclimate for bacterial colonization and survival that was created in the damaged potato tubers. The tubers of Novano showed a lower percentage of tubers with mechanical damage and low incidence of rots, indicating that these factors are important for the final starch quality in the tubers.

6.4 Wet rots and dry rots on tubers

Presence of primary pathogens, such as fungi, is likely to increase the incidence of bacteria and wet rots (Andersson, personal communication, 2012). In this study, however, there were no correlations between incidence of dry rots and incidence of wet rots in any of the varieties, or in the two storage piles. It is possible that the experimental period of three months in this study was too short. Ware potatoes are usually stored up to 12 months, and fungi and dry rots then become more problematic.

Incidence (number of infected tubers) of wet and dry rots, did not increase significantly during storage in either of the storage piles in the field trial, but rot sverity (the area of infected tuber tissue) did increase. These results indicate that pathogenic infection occurred prior to storage and that the post-harvest diseases at harvest did not infect new tubers during storage. The disease intensity increased with time, however, and since mechanical damage on

tubers generally increased the presence of both types of rots it therefore becomes important to harvest the tubers intact and to avoid damage during harvest and handling (Figure 15 and 16).

In general, the estimation of bacterial population size was lower on PDA than on TSA, indicating that TSA is better for measuring bacterial diversity than PDA. Furthermore, the diversity as calculated on the basis of identified species was lower in the variety Kuras than in the other two varieties, which indicates that certain bacteria are selected by certain potato varieties. A tendency of higher species diversity was observed in pile B than in pile A which was possibly due to a warmer microclimate and hence a higher bacterial population size in tubers in pile B.

The results of estimation of starch hydrolyzing population differed when performed *in vitro* which is an environment that lacks competition compared with the *in vivo* slice assay (Table 5) in which the test organism has to face competition from the surrounding endophytic microflora. *In vitro* assay was based on hydrolysis of wheat starch while in the *in vivo* assay the bacteria were tested for degradation of potato starch, which is considered to be more difficult (Taniguchi et al., 1982) and hence one reason for low correlation between the two assays. The potato variety Melody was chosen to enable comparison of the isolates which originated from three different potato varieties mentioned above.

Several of the tuber associated fungal species identified in this study are ubiquitous in soil (Table 7). No attempt was made to quantify their population size and it is not known if the different potato varieties selected for different fungi and to what extent the isolation procedure favoured only certain genera. Choice of nutrient media other than TSA and PDA might have facilitated detection of oomycetes, such as *Phytphthora infestens* and *Phytium* spp., if present.

Fungi such as *Phoma* spp. and *Fusarium* spp. benefit easily from mechanical damage (Agrios, 2005), and were therefore expected to occur in abundance in the tubers. *Phoma* spp. were detected in the tuber samples, but no attempt was made to quantify its population in relation to other microorganisms in this experiment. Some *Fusarium* spp. are also common potato pathogens. However, no *Fusarium* species were found in the tubers samples in this study. A likely explanation is that young tubers have some resistance to *Fusarium*, and hence the dry rot develops after a longer period of storage than was used in this study (Loria, 1993). If *Fusarium* was present it would have appeared during isolation on agar plates, even if it was latent. Schöber and Turkensteen (1992) mention control of *Fusarium* spp. by antagonistic organisms. *Tricoderma* fungi are mentioned as potential biocontrol agents, as well as *Pseudomonas flourescens* and *P. chlororaphis* (Leben et al., 1987; Chatterton et al., 2003; Pringle et al. 2009; Jaya Prakash et al., 2009). Bacetria such as *Pseudomonas flourescens* and *P. putida* were found to occur in the tubers of Merano and Novano. The role of such bacteria in tubers in terms of restricting populations of e.g. *Fusarium* remains to be investigated. Non-pathogenic microorganisms will probably have an increased importance as biocontrol agents in the future, either in the soil during potato cultivation, or as tuber surface treatments before storage (Pringle et al., 2009).

7. Conclusions

- Starch yield was significantly reduced after three months of storage, due to loss of weight in combination with loss of starch content in the tubers. The largest losses occurred during the first weeks of storage

- There were generally greater differences between potato varieties than between the two storage piles. It should be noticed however that the weather during the autumn of 2011 was unusually mild

- There were great differences between the three potato varieties in initial starch content, sensibility to mechanical damage, prescense and growth of microorganisms and final starch yield. This implies the importance of choosing good varieties

- There was a positive correlation between mechanical damage on tubers and incidence of wet rots and dry rots. Tubers seemed to get infected by rots before they were placed in the storage piles and it is therefore important to keep tubers undamaged before storage

- Both pathogenic and beneficial micro-organisms were isolated and identified in the stored potato tubers. The importance of varietal effect on endophytic populations at harvest and during storage needs further attention in order to obtail high starch yields in the future

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