Muhammad Ikhsan Sulaiman

EFFECT OF CALCIUM FERTILIZATION ON THE QUALITY OF POTATO TUBERS (*Solanum tuberosum* L.) CV. SATURNA

The brown centre inside potato tuber

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EFFECT OF CALCIUM FERTILIZATION ON THE QUALITY OF POTATO TUBERS (*Solanum tuberosum* L.) CV. SATURNA

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Muhammad Ikhsan Sulaiman

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Abbreviations

AA	Amino acid
Abs	Absorbance
ATP	Adenosine triphosphate
df	Degree of freedom
DIP	2,6-dichlorophenol indophenol
DM	Dry matter
FW	Fresh weight
GO	Gross Oesingen
HKB	Hankensbuettel
PEP	Phospho-enol-Pyruvate
KAS	Ammonium nitrate with lime
KS	Calcium nitrate
LGW	Langwedel
Max	Maximum
Min	Minimum
MS	Mean square
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
N_{min}	Nitrogen in mineral form
OPA	*O*-phtaldialdehyde
R	Pearson's coefficient of correlation
R^2	Coefficient determination
RH	Relative humidity
RP-HPLC	Reverse phase – high performance liquid chromatography
Stdv	Standard deviation
s.e.	Standard error
uv	Ultra violet

Amino acids:

Ala	Alanine
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine
His	Histidine
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
Met	Methionine
Phe	Phenylalanine

Ser	Serine
Thr	Threonine
Tyr	Tyrosine
Val	Valine

Units:

°C	degree Celcius
cm	centimeter
g	gram
ha	hectare
kg	kilogram
km	kilometer
L	Liter
L*	Lightness unit based on CIE 1976
m	meter
M	molar
mg	milligram
Mg	megagram
mL	milliliter
min	minute
mm	millimeter
µ	Micro
N	Normality
RVU	rapid visco unit
Tg	Terragram
v/v	volume per volume

Elements:

B	Boron
Ca	Calcium
Cu	Copper
Fe	Iron
H	Hydrogen
I	Iodine
K	potassium
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
Na	Sodium
O	Oxygen
P	Phosphorus
S	Sulphur
Zn	Zinc

1 Introduction

1.1 Relevance of potato production in Germany

The potato (*Solanum tuberosum*) has been cultivated for several thousand years. The potato originated in highlands along the Andes Mountains from Peru to Bolivia, in South America (Burton, 1966) was introduced to Europe by Spanish explorers in the middle of the 16th century. Suitable climatic and soil conditions allowed the spread of the potato progressively on all continents, mostly in temperate regions and highland areas (Lisinska and Lezczynski, 1989).

The potato has become an important food crop in the world. The tuber as edible part of the potato plant is a staple food for many people, particularly in Europe and North and South America. World production in 2004 was more than 328 million tons, with China, Russia, India, USA and Ukraine being the five biggest potato producers. They account for more than 50% of world potato production (FAO-stat, 2004). Now the potato has become important as more than just a staple food. Food and non-food industries based on the potato are growing fast all over the world. The expansion of the utilizations of the potato has made the quality of the potato tuber of key importance.

Germany is the second biggest potato producer in Europe after Poland. Approximately 20% of the agricultural companies in Germany are working in potato production. In Germany, potatoes are the third most important crop after wheat and sugar beet, with a harvest area of 295,000 ha and a production rate of almost 13 Tg in 2004 (FAO-stat, 2004). From the total production, almost 25% is consumed directly as fresh or table potatoes and more than 42% goes to industry. More than 50% of the industrial potatoes are used in starch production. The second most important products are dried and frozen potatoes, which account for about 28% of the total for industrial potatoes (Schmitz and Wronka, 2002).

The Bundesverband der Deutschen Suesswarenindustrie (2004) in its annual report described potato chip production as important industries in the snack foods production. The potato chip production needs more than 5% of the German industrial potato output, or about 341,600 tons in 2001–2002. From that, about 100,500 tons potato chips were produced (Weber and Putz, 2003), which amounts to 234 million Euro a year. The Bundesverband der Deutschen Suesswarenindustrie (2004) calculated that the average per capita chip consumption in Germany accounts for about 880 g per person, or about 28% of total snack consumption. Compared to peanuts, cakes, and extruder products, potato chips become the most popular snack food for German people.

1.2 Factors affecting the quality of potato chips

For food products, appearance, taste, aroma, flavor, and texture, as well as nutrient value, are important quality parameters and become decision criteria for the food acceptance of consumers. The processing quality determines the quality of food products. On the processing line, potato slices are deep fried in oil at a temperature of 180–185°C. Water is removed from the slice and is replaced by oil amounting to as much as 35–40% of the finished chips (Bennett, 2001). A micro structural study of frying potatoes (Buchan and Aguilera, 2001) showed that water removal and volume alteration (shrinkage) of potato slices start at a temperature of 70–80°C, when the starch granules are completely gelatinized. Moreover, the oil is located mainly in the crust, while the core is virtually free from oil. The oil content of chips shows a negative relationship to the specific gravity of the potato tuber and to the thickness of the chip slice (Smith, 1987). When the temperature of the slice is near 150°C, the vacuole of the tuber cell begins to breakdown, sugars are released and the chip starts to develop color (Bennett, 2001). Costa et al. (2001) reported that crust formation starts when the potato surface reaches approximately 143°C. At a temperature of 180°C, potato chips show a crust-type structure only.

Bennett (2001) described the quality of potato chips as having a saddle-shape curl, being light gold in color, having no blemishes, being crisp and tender in the mouth, leaving a pleasant aftertaste, and having a slight potato flavor. From 30 volatile compounds of potato chips, pyrazine and aldehyde groups are reported to be the main compounds in the flavor formation of chips and their concentration in the potato chips depends on the fry oil used (Melton et al., 1993). Frying oil, as reported by Weber and Putz (2003), has an influence on the sensory acceptance of chips, which may be due to the fatty acid composition of the oil. However, the most difficult problem in the potato chip industry, according to Smith (1987), is the maintenance of a desirable color of chips throughout the year. Dark chips caused by the Maillard reaction are unattractive to the consumer and often have an undesirable flavor (Bennett, 2001).

The Maillard reaction is a non-enzymatic reaction between reducing sugars and amino acids which causes browning or darkening. The chemistry of the reaction is known to be a complex series of reactions leading to the formation of a variety of products, including flavors, aromas and colors. Color occurs due to the formation of high molecular weight (> 12,000 Daltons) polymeric compounds also known as melanoidins. These are generally formed by the reaction of the Amadori product or other dicarbonyls with amino acids (Fayle and Gerrard, 2002).

Chip darkening is described as a function of fry temperature and time, where lightness is decreased with increases in frying time and temperature (Sahin, 2000). The study of Roe et al. (1990) on chips from potatoes grown under different nitrogen regimes showed that the chip's color correlated to the total content of reducing sugars and free amino acids. The correlation is stronger than that from either reducing sugar or free amino acid alone. A study by Rodriguez-Saona et al. (1997) using artificial chips reported that the level of reducing sugars (D-glucose and D-fructose) and amino acids explained 75–88% of the color formation of chips, where an increase of 0.1 mg g^{-1} fresh

weight of reducing sugars causes a 1 unit decrease in mean L*–value and hue angle, while the amino acids asparagine and glutamine have only little effect on the chips' browning. Roe and Faulks (1991) reported that lysine, γ-amino-butyric acid and glycine were the main contributors to the browning of chips. Sucrose has also been reported to cause color formation, although the intensity is less than that caused by reducing sugars (Leszkowiat et al., 1990). During frying, sucrose may be hydrolyzed in the presence of glycine to yield glucose and fructose at temperatures as low as 150°C. Moreover, Rodriguez-Saona et al. (1997) suggested that ascorbic acid may also cause darkening, particularly when reducing sugars are at low concentration, but ascorbic acid concentration is usually not high enough to produce unacceptable darkening.

Asparagine and glutamine are known to be the major amino acids in potato tuber (Davies, 1977). The content of free asparagine and glutamine as well as glucose and fructose has been a more substantial quality parameter of chips since Swedish researcher in 2002 found acrylamide formation in a range of foods heated during production or preparation (Amrein, et al., 2003). Acrylamide is a toxic and carcinogenic compound for humans. It is established now that acrylamide can be formed during the frying of potato slices or pieces at temperatures of 120 to 150°C, accompanied by the Maillard reaction chain (Gertz and Klostermann, 2002).

1.3 Factors affecting the internal quality of potato tubers

Most research on chip's potato is focused on producing potatoes which have light chip color or a low content of reducing sugars. A carefully selection of varieties for chip production is important for processors. Some varieties for chips mainly grown in the USA are Kennebec, Chippewa, Russet Burbank, Katadhin, and Cherokee (Smith, 1987). In 2004, there were 41 varieties suitable for the chips processing in Germany and they were classified as very early, early, middle early and late varieties. The selection of varieties is based

on the content of dry matter and reducing sugars during storage, and sprouting problems (Putz, 2004).

Moreover, it was reported that several cultural factors such as drought and flood stress and nitrogen and potassium fertilization may have an effect on the color of potato chips. Drought stress during plant growth may have an influence on the maturity of the tuber, while immature tubers have high sugars content (Iritani, 1981; Schock et al., 1993). A pot experiment of Sulaiman (2000) with three potato varieties grown in water flood (100% water holding capacity = WHC) and water deficit (3% WHC) showed that growing potatoes in water flood led to an increase of D-glucose of 48–99% and D-fructose of 77–136%. On the contrary, potato growth in water deficit led to a decrease of D-glucose about 21–52% and D-fructose about 32–54%. After six months of storage at 4°C, the total reducing sugars content was kept lower in tubers grown under water deficiency than in tubers grown at sufficient water supply and water flood. Roe et al. (1990) reported about the effect of nitrogen fertilization on the increase of free amino acids and on the decrease of reducing sugars concentration in tubers, whilst the effect of storage on free amino acids in two varieties is described to be minor (Talley, et al. 1984). A study by Stanley and Jewell (1989) shows a trend of decreasing dry matter and total reducing sugars with an increasing rate of potassium fertilizer.

However, a wide range of investigations have focused on the behavior of the potato tuber in storage. It is already established that cold storage at 4–5°C causes sweetening of the tubers and reduce the color quality of chips (Coffin et al., 1987; Brown et al., 1990; Edwards et al., 2002). Cottrell et al. (1993) reported that the increasing of reducing sugars during cold storage is related to a remarkable increase in the activity of starch hydrolyzing enzymes such as α-amylase, β-amylase, and debranching enzyme during the first week of storage. The activity of these enzymes is reported to increase only slightly if the tuber is stored at a high temperature (10–11°C). Ross and Davies (1987) suggested

that α-amylase is responsible for the initiation of starch granule degradation, while β-amylase activity is high in unsprouted tubers, prior to the onset of rapid starch depletion. On the other hand, Sowokinos (2001) described the role of phosphorolytic enzymes in starch degradation. He suggested that starch conversion at a low temperature is following more the hexogenesis pathway (formation of glucose and fructose from sucrose) than the respiration pathway. The hexogenesis pathway is controlled by the enzymes UDP-glucose pyrophosphorylase, sucrose-6-phosphate synthase, and acid invertase in the cytoplasm and vacuole. In the respiration pathway, where the rate declines at a low temperature and *vice versa*, sugars are converted completely to CO_2, water, and energy. Moreover, varieties that resist sweetening in the cold have shown a higher rate of respiration in storage. However, there are some advantages to cold storage because it can prolong storage time, reduce the respiration rate and water loss, and inhibit sprouting (Van Es and Hartmans, 1981).

Copp et al. (2000) reported that in most cases the point at which increasing respiration rates during storage are observed to correspond to the point at which chip color quality starts to decline, although they did not get any correlation with the tubers' sugars content. They concluded also that an increase in the respiration rate is not always associated with the end of dormancy and sprouting, as had been concluded from previous studies.

Mechanical stress during harvest, handling, transportation and storage may cause an increase in reducing sugars accumulation during storage due to increasing invertase activity as reported by Hironaka et al. (2001). However, research related to mechanical stress is more focused on blackspot discoloration inside the tuber. Blackspot is a type of enzymatic discoloration as the result of oxidation of phenolic amino acids such as tyrosine by polyphenol oxidase. Deficiency of potassium increases the potential for blackspot formation in the tuber due to increasing of the free tyrosine in the tubers (McNabnay, et al., 1999). In contrast, blackspot potential is decreased for the water-stressed tuber from a potato grown in a pot (Pawelzik and Delgado, 1999). Further-

more, the study of Delgado et al. (2001) concluded that chlorogenic acid may contribute to blackspot formation in the tuber grown under water stress. For the chip industry, blackspot causes obvious quality deterioration and great losses. However, mechanical stress may be reduced through strengthening the outside of potato tubers; the increasing of the tuber Ca may be promising.

1.4 The importance of calcium for the plant and plant cell

Calcium in potato tuber tissue, according to the study of Davies and Millard (1985), can be divided into physiologically active and inactive forms. Physiologically active Ca occurs as free ion and as the water soluble Ca salts of organic acids, chlorides and nitrates and may be reversible bound to proteins and pectin. Calcium bound to phosphate, oxalate, carbonate and silicate salts exist in an inactive form. Davies and Millard (1985) found that more than 90% of tuber Ca is in a physiologically active form and very little is in the insoluble inactive form, such as Ca oxalate. Calcium oxalate and phosphate and water soluble Ca salts are mostly present in the vacuole (Marschner, 1995). Most of the Ca is a cell wall substance and is located in the appoplast, in the middle lamella and at the outside surface of the plasma membrane. In the middle lamella, Ca is bound to polygalacturonic acids in the pectin as Ca pectate. Calcium in small concentrations is present in the matrices of mitochondria and on plastid envelopes, in the vicinity of amyloplast, in plastids and in the endoplasmic reticulum, but not found within amyloplast (Oparka and Davies, 1988; White and Broadley, 2003). In the potato tuber, the concentration of Ca in the outer tissues (periderm and in the vascular ring) is approximately 400% higher than in the inner tissues (pith) (Oparka and Davies, 1988).

Calcium in a physiologically active form has been known to take part in the activities of many enzymes in the plant cell. Lopez-Nicolas et al. (2000) reported an increase of lipoxygenase activity in potato tubers in the presence of Ca^{2+}. Moreover, Marschner (1995) listed a number of enzymes that are activated by Ca^{2+}, i.e. α-amylase, phospholipase, ATPases, hexodiphosphatase

and PEP carboxylase. High Ca content in plant cells is also known to activate the pentose phosphate pathway and the biosynthesis of amino acids (Allan and Trewavas, 1987). A study of Wei and Sung (1993) on rice cultivated in solution with Ca supplement showed that carbohydrate metabolism enzymes such as sucrose synthase and invertase are induced by long-term Ca application. Furthermore, recent research showed that Ca stress (deficiency and excess of Ca) in *Mentha pulegium* L. leaves induced antioxidant enzymes and lipoxygenase activity, and furthermore Ca might take part in the senescence processes (Candan and Tarhan, 2005).

Calcium as a cell wall substance has a function in cell wall and membrane stabilization, and therefore it has an influence on tissue firmness and resistance against environmental stresses, microbial and nematode diseases. Many investigations reported the improvement of tissue integrity and firmness for pears, melons, apples, pineapples, as well as potatoes by the spraying of Ca directly on the skin surface or immersion of the fruits in the Ca solution (e.g. Gerasopoulos, 1999; Lester, 1999; Roy, 1999; Ahrne et al., 2003). It has been reported that Ca application may increase the pectin content in the cell wall and therefore increase the resistance of the potato tuber to nematodes and *Erwinia carotovora* (Fatemy and Evans, 1986; McGuire and Kelman, 1986). Calcium has been also reported to improve the resistance of the potato tuber to heat stress, while Ca deficiency led to internal disorders in potatoes, bitter pit in apples, and some physiological disorder in many fruits and vegetables (Kleinhenz and Palta, 2002; Sterrett and Henninger, 1991; Davies, 1998; Yuri et al., 2002).

Moreover, it was reported that Ca may delay the ripening of pears and as well as membrane lipid catabolism in apples during storage (Gerasopoulos, 1999; Picchioni et al., 1998) because Ca decreases the respiration rate and ethylene production (Recasens et al., 2004).

Recent research on the function of plant Ca is focused on the mechanism and the transport of Ca^{2+} in the cell as a second messenger if a plant is

under stress. The Ca concentration in the cytosol in plant cells is an indicator for environmental challenges, such as cold shock, salt, or mechanical stress. The concentration of Ca in the cytosol is very low, ranging between 0.1–1 µM (White and Broadley, 2003). Efflux or influx of Ca^{2+} from or to the cytosol occurs through different Ca^{2+}-channels from or to the appoplast, endoplasmic reticulum, vacuole or mitochondria and is regulated by Ca^{2+}-ATPases and H^{+}/Ca^{2+} antiporters. Some Ca-binding proteins such as calmodulin, calcineurin B-like proteins, Ca-dependent protein kinase, and some other Ca-binding proteins have also been implicated in cellular responses to diverse environmental, developmental and pathological challenges.

However, investigations on the effect of Ca on the quality of potato tubers were more focused on improving resistance against diseases and less to the chemical and biochemical attributes of tubers. Concerning the role of Ca^{2+} in respiration, carbohydrate metabolism enzymes, biosynthesis of amino acids, and firmness of plant tissue, Ca application on the potato plant may influence tuber quality, long-term storage stability of tubers, and certainly chips and starch quality.

1.5 Calcium fertilizer and calcium uptake of potato tuber

Calcium is classified as a macronutrient. Its availability in common soil is high and therefore Ca deficiency is rare in nature but may occur in soils with low base saturation or high level of acidic deposition (McLaughlin and Wimmer, 1999). The Ca concentration in a soil solution may reach 25 mmol L^{-1} but in average 1.25–2.5 mmol L^{-1}, or about ten times higher than potassium (Barber, 1985). However, Ca concentration in the tuber is very low compared to other macronutrients and sometimes Ca deficiency may occur. The content of Ca in the tuber falls in a wide range, between 75 and 3,060 mg kg^{-1} DM (Bamberg et al., 1998). The immobility of Ca in the phloem is the reason for the low Ca content of tubers. Calcium taken up from the soil is transported to leaves via xylem but cannot be re-translocated to the tuber (Krauss and Marschner,

1973). Moreover, the capacity of the tuber for Ca uptake is controlled genetically (Bamberg et al., 1998). The mechanism of tuber Ca uptake is not yet established and is still being discussed. Nevertheless, it is accepted that Ca storage in tubers involves either a xylem transport system due to Ca mobility in the xylem during tuber development or direct uptake trough the tuber surface area from the surrounding soil (Busse and Palta, 2004; Habib and Donnelly, 2002; Kratzke and Palta, 1985; Krauss and Marschner, 1973).

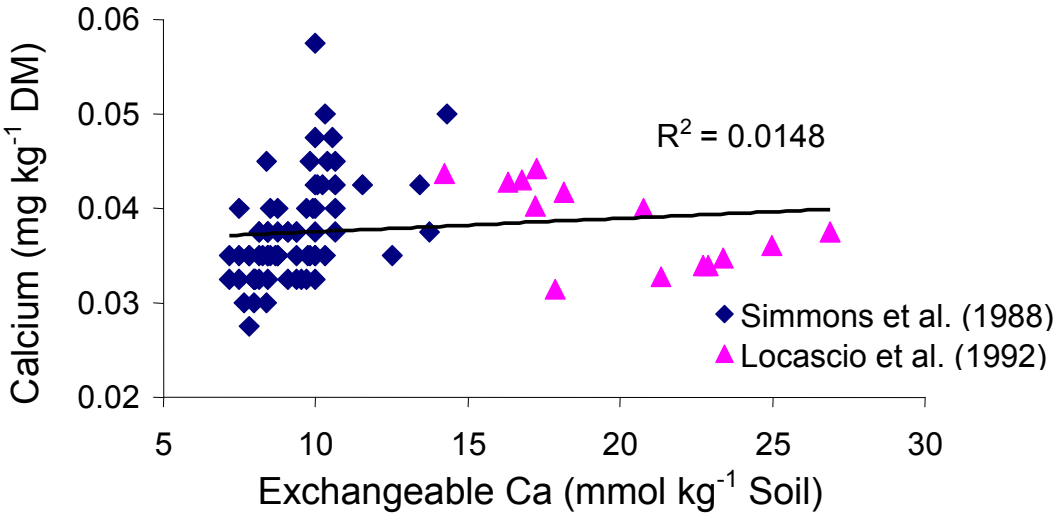

Figure 1 Relationship between exchangeable calcium and calcium concentration of potato tuber periderm from previous studies

Some Ca fertilizers such as gypsum, lime, and nitrogen fertilizer containing Ca with different application times and methods have been already studied by many investigators (e.g. Clough, 1994; Kleinheinz et al., 1999; Locascio et al., 1992; Simmons and Kelling, 1987; Simmons et al., 1988). It is reported that Ca application did not always increase the Ca content of tubers, especially from potatoes that are grown in sandy soil with a low cation exchange capacity. Most of the studies investigated the exchangeable Ca in the soil and found a low relationship to tuber Ca concentration (Figure 1). Claassen (1990) reported that Ca in the soil is transported to the root mainly through water flow; therefore, investigations should also consider the Ca in the soil solution and not only the exchangeable cation.

1.6 Aims of the research and outline of the thesis

Concerning the numerous functions of Ca in the plant cell wall, carbohydrate metabolism, and protein synthesis, this research aims to study the direct and/or indirect effects of Ca application on the quality of potato tubers in relation to the Ca concentration in the tuber. The relationship between Ca in the soil and the concentration of nutrients in the tuber is studied in Chapter 3. A wide range of nutrient concentration in the tuber as an impact of Ca fertilizer may show a deficiency of some nutrients, as with brown centre; this is also studied in this chapter.

In Chapter 4, the effect of Ca fertilizer on tubers quality related to some quality parameters of the tuber before and after storage is described. The relationship between the quality parameters of the potato tuber as well as the relationship between mineral levels in the tuber and quality parameters of potato tuber are discussed in Chapter 5.

The effect of Ca fertilizer on the color quality of potato chips as well as the factors influencing the browning of potato chips is discussed in Chapter 6. Chapter 7 characterizes the influence of Ca fertilizer on the pasting properties of potato starch and flour.

The thesis concludes in Chapter 8 with a general discussion of the results and some recommendations for future studies.

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2 Material and methods

2.1 Material

Potato tuber (*Solanum tuberosum*) variety Saturna was investigated in this study. Variety Saturna is a commercial Dutch variety and has the following parentage: [Maritta x (Record x CPC 1673-1 (adg)]. It is an old variety which came to the market in 1970 (Bundessortenamt, 2004). The tuber has a middle deep sprout eye and has a round oval form. The skin is yellow and smooth. This variety has a middle yield potential with middle starch content and is frequently used in chip production due to its resistance to sweetening during long-term storage. Variety Saturna is a table potato, has a light yellow color of tuber parenchyma and is classified in the middle-late to very late maturity group. The cooked potato is soft and floury and has low potential to the after cooking blackening. Variety Saturna has good storage properties at high temperatures (8–11°C).

2.2 Field experiment

The field experiment was located in three locations in Lower Saxonia: Gross Oesingen, Langwedel, and Hankensbuettel. The soil was classified as loamy sandy soil. The properties of the soil are presented in Table 1. As basic fertilizers, all plots received 44, 100, and 24 kg ha^{-1} P, K and Mg respectively. Nitrogen fertilizer was applied at a rate of 160 kg N ha^{-1} minus N_{min} as ammonium nitrate with lime (KAS). Potatoes were planted in April in plots consisting of six rows 0.75 m apart and 8 m long. During growing, the plants were irrigated with a sprinkler to maintain the soil humidity. The potatoes were harvested in September. Soil analysis was done before fertilizer application, 56 days after planting, and after harvest. The soil was sampled by boring the soil to depths of 30 and 60 cm.

2.2.1 Field experiment 2001

The experiment was located in Langwedel and Gross Oesingen. In this year, two experiments were done. The first experiment was aimed to observe the effect of application method of nitrogen fertilizers on the Ca uptake of tubers. Two nitrogen fertilizers containing Ca, calcium nitrate (KS) and ammonium nitrate with lime (KAS) were applied as band application after hilling, broadcast before hilling, and 2/3 applied broadcast before hilling and 1/3 inducted in the tubers area at the onset of tuberization. The second experiment was aimed to compare two Ca fertilizers: gypsum ($CaSO_4.2H_2O$) in powder form and granulate $CaCO_3$. They were applied at rates of 450 and 900 kg Ca ha^{-1}. Both Ca fertilizers were applied by hand before planting. The treatments consisted of two types of Ca fertilizers, two application rates, and six replications, and were conducted according to randomized block design of experiment.

2.2.2 Field experiment 2002 and 2003

In 2002, the field experiment was located in Langwedel and Hankensbuettel. Gypsum and $CaCO_3$ were applied at rates of 560, 1120, and 2240 kg Ca ha^{-1}. Plants without gypsum and $CaCO_3$ application were used as controls. To see any effect of KAS on the Ca concentration in the tuber, application of KAS was compared with urea and Ca nitrate (KS) with the same rate of application.

In 2003, the field experiment was located in Hankensbuettel and Gross Oesingen. Three Ca fertilizers were used in this year: gypsum, $CaCO_3$ and Basic slag. Gypsum was applied with the same rate as in 2002. $CaCO_3$ and Basic slag were applied at rates of 1120 and 2240 kg Ca ha^{-1}. In both years, the experiment was conducted according to randomized block design of experiment with four replications.

2.3 Storage conditions and potato sample preparation

After harvest, all tubers were graded based on diameters of < 35 mm, 35 – 50 mm, and > 50 mm for small, medium, and big tuber size, respectively. The medium tubers were stored in a conditioning room with a temperature of 8 ± 1°C and relative humidity of 92% for seven months. Quality parameters of tubers were analysed before storage and after three, five, and seven months of storage.

Analysis of dry matter, ascorbic acid, blackspot potential, and starch extraction was carried out with fresh tubers. For the other analyses, freeze-dried material was used. For freeze-drying, five or six tubers were washed and peeled with a household peeler. Afterwards, the tubers were cut into small cubes, and frozen by immersing immediately in liquid nitrogen. The frozen potatoes were freeze-dried by an Epsilon 2-40 Christ freeze-dryer (Germany), until the water content reached 2 to 4%. The dried material was ground using a household grinder and stored in plastic flasks at room temperature.

2.4 Soil analysis

2.4.1 Soil solution displacement

Soil solution was obtained by miscible displacement similar to the procedure described by Adams (1974) as shown in Figure 2. The fresh soil samples were sieved with a 2 mm sieve and utilized within 24 hours for soil solution investigation. After sieving, the soil was filled evenly in a 250 mL volumetric cylinder with a hole in the bottom. The hole was covered with ashless filter paper 593[3] (Schleicher & Schuell, Germany). Using a peristaltic pump, water was allowed to drop slowly on the soil. The water displaced the soil solution downwards and the soil solution was collected in a glass beaker. To exclude soil solution dilution by the added water, 4% potassium thiocyanate was added as a marker to the water. The obtained soil solution was then tested with 5% iron (III) chloride. A red coloration in the solution indicated the presence of the

marker. With repeated measurements, the marker was not detected in a solution volume below 20 mL. The soil solution was filtered and the pH was determined. Calcium and potassium concentration of the solution were measured using an Eppendorf Elex 6361 flame photometer (Germany), and magnesium was measured using a Unicam m series atomic absorption spectrophotometer (Cambridge, UK).

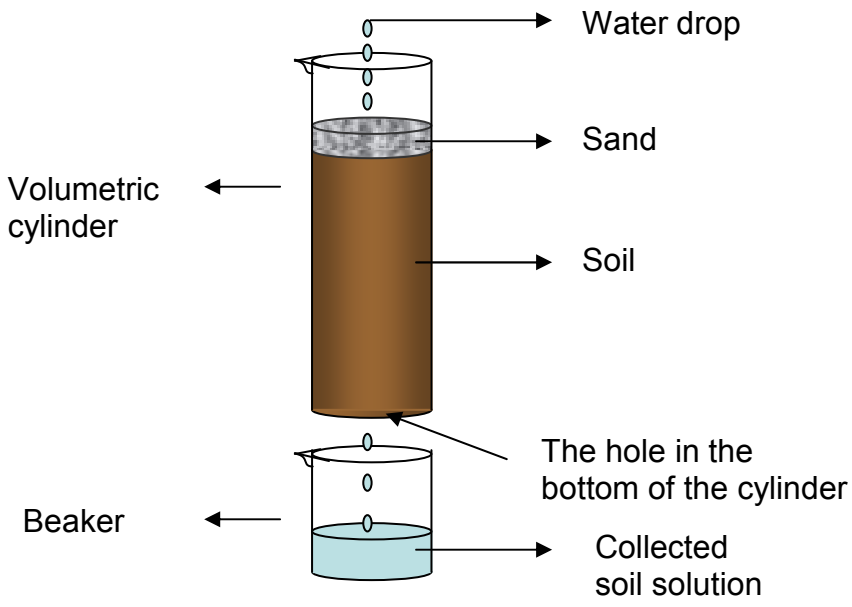

Figure 2 Displacement of soil solution according to the method described by Adams (1974)

2.4.2 Determination of exchangeable soil nutrients

The exchangeable soil nutrients were extracted with 1 N ammonium acetate according to Claassen (1990). The fresh soil was air dried and one gram was weighed on the ashless filter paper 593[3] (Schleicher & Schuell, Germany). Ten mL 1 N ammonium acetate (Merck, Germany) was poured slowly on the soil every 15 min for five times. The filtrate was collected in a 50 mL volumetric flask and ammonium acetate was added to the flask up to the mark. The exchangeable Ca and K were determined by using an Eppendorf Elex 6361flame photometer (Germany) and Mg by using a Unicam m series atomic absorbance spectrophotometer (Cambridge, UK).

2.5 Tuber Analysis

2.5.1 Determination of dry matter content

Dry matter content of the potatoes was determined according to EAPR (1974). Five tubers were washed, dried, and cut into cubes. The cubes were homogenized in a laboratory mixer until a homogenous pulp was obtained. About ten grams of the pulp was poured into a petri dish and then heated at 60°C for 15 hours. Afterwards, the oven temperature was raised to 105°C. After three hours at 105°C, the petri dish with the dry potato was cooled to room temperature in the desiccators and weighed. The total dry matter content of potatoes was calculated according to formula (1):

$$DM = \frac{D}{W} x100 \qquad (1)$$

DM = dry matter [%]
D = weight of dry sample [g]
W = weight of wet sample [g]

2.5.2 Determination of L-ascorbic acid

L-ascorbic acid was determined by titration with solution 2,6-dichlorophenol-indophenol (DIP) according to Diemair (1963). Five tubers were peeled and minced. Five grams of the mince were sampled and immersed in 20 mL 5% metaphosphoric acid (Merck, Germany). The mixture was homogenized by ultra turrax (Janke & Kunkel TP 18/10, Germany) in a 100 mL cylinder for two minutes. After homogenizing, distilled water was added to the homogenate to a volume of 50 mL, and the homogenate was filtered with filter paper no. 595 ½ (Schleicher & Schuell, Germany). Ten mL of the clear solution was titrated with 0.21% 2,6-dichlorophenol-indophenol (Merck, Germany). Ascorbic acid content was calculated using formula (2):

$$AA = \frac{V_{DIP}}{F \cdot W_s} \times D_l \qquad (2)$$

AA = Ascorbic acid (mg kg^{-1} FM)
V_{DIP} = Titration volume of DIP (mL)
F = Titration volume for one mg ascorbic acid (mL mg^{-1} AA)
W_s = Weight of sample (kg)
D_l = Dilution factor

The factor (F) was the titration volume of 1 mL 0.1% standard solution ascorbic acid (Merck, Germany) in a mixed solution of 1 mL 5% metaphosphoric acid and 9 mL distilled water.

2.5.3 Determination of citric acid

Citric acid was determined enzymatically by the spectrophotometric method according Roche catalogue no. 0139076 (Germany). The method is based on the enzymatic conversion of citric acid to oxaloacetate and acetate by citrate lyase. Freeze-dried potato flour (500 mg) was extracted with 18 mL distilled water by horizontal shaking for 30 minutes. After shaking, the mixture was cleared by adding 2 mL 60% perchloric acid (Merck, Germany). The suspension was filtered with filter paper no. 593 ½ (Schleicher & Schuell, Germany) and the filtrate was used for the determination using the enzyme kit consisting of glycylglycine buffer at pH 7.8 and citrate lyase. The concentration of NADH oxidized in the enzymatic reaction is stochiometric equal to the concentration of citrate. NADH was determined by means of its light absorbance at 340 nm with a Hewlett Packard 8453 uv-spectrophotometer (Germany).

2.5.4 Determination of chlorogenic acid

Chlorogenic acid was determined spectrophotometrically according to Griffith et al. (1992). Freeze-dried potato flour (100 mg) was suspended in 2 mL solution consisting of 0.17 M urea (Merck, Germany) and 0.1 M acetic acid (Merck, Germany). After adding 1 mL distilled water, the suspension was shaken for 15 s. After shaking, 1 mL 0.014 M sodium nitrite (Merck) was added and mixed well. After two minutes of reaction, 1 mL 0.5 M sodium hydroxid (Roth, Germany) was added to the suspension. The suspension was then cen-

trifuged at 2250 gravities in a centrifuge (Eppendorf 5416, Germany) for ten minutes. The absorbance of the clear solution was measured at 510 nm with a Hewlett Packard 8453 uv-spectrophotometer (Germany). The concentration was calculated from the standard curve produced by measuring 50 to 400 ppm of 5-caffeic acid (Sigma).

2.5.5 Potato chip producing and color measurement

Potato chips were produced according to EAPR (1974). Representative samples of five to six tubers were peeled with a hand peeler and sliced with a home slicer into regular slices of 1.2–1.3 mm thickness. The slices were washed for one to two minutes under running tap water and dried superficially. Afterwards, they were fried with 2.5 kg vegetable fat (Lidl, Germany) in an electrical household fryer at 175 ± 5°C. The weight ratio of potato slices and frying fat was one to fifty. The chips were sufficiently fried when the water content of 2% was achieved, shown by no more air bubbles appearing in the frying oil. The frying oil was replaced after about 40 tests.

The lightness (L*) of the chips was measured with a Minolta CR-300 chroma meter (Japan). The measurement was repeated ten times.

2.5.6 Determination of starch

Starch determination was conducted according to ICC-Standard no. 123 (Arbeitsgemeinschaft für Getreideforschung, 1994). Freeze-dried potato flour (2.5 g) was weighed in a 100 mL volumetric flask. Fifty mL of 1.124% HCl (Merck, Germany) was added to the flask. The starch was hydrolyzed by cooking in boiling water for 15 minutes. During the first eight minutes, the flask was shaken horizontally. After cooling to room temperature, the suspension was cleared by adding 2 mL 10% Wolframatophosphoric acid (Roth, Germany). Distilled water was added to fill the flask to the mark and the suspension was then filtered with filter paper no. 593 ½ (Schleicher & Schuell, Germany). The optical rotation of the solution containing monosaccharide was measured with

a polarimeter (Carl Zeiss type V Dr Na, Germany). The starch content was calculated using formulas (3) and (4):

$$C = \frac{\alpha}{[\alpha]_D^{20}.l} \quad (3)$$

C = concentration [g mL^{-1}]
α = optical rotation of the solution
$[\alpha]_C^{20}$ = specific rotation of hydrolyzed potato flour at 20°C, which equals 181.8°
l = the polarimeter tube length [dm]

$$\text{Starch content} = \frac{C.V_{ext}.1000}{W} \text{ [g } kg^{-1}\text{]} \quad (4)$$

V_{ext} = extractions volume [mL]
W = weight of sample [kg]

2.5.7 Determination of amylose and amylopectin ratio

Determination of the amylose and amylopectin ratio was done by staining the starch with I_2–KI and measuring the absorbance at 550 and 618 nm according to Hovenkamp-Hermelink et al. (1988). Twenty-five milligrams of freeze-dried potato flour or potato starch was immersed in 0.5 mL 45% perchloric acid (Merck, Germany). After four minutes of reaction, 8 mL distilled water was added. I_2–KI staining was achieved by mixing 4 mL of the starch solution and 5 mL of a diluted (1:2 v/v) Lugol's solution (Merck, Germany), which is a mix of 2 g KI and 1 g I_2 diluted in 300 mL distilled water. The absorbance was measured immediately after mixing in a spectrophotometer (Hewlett Packard 8453, Germany).

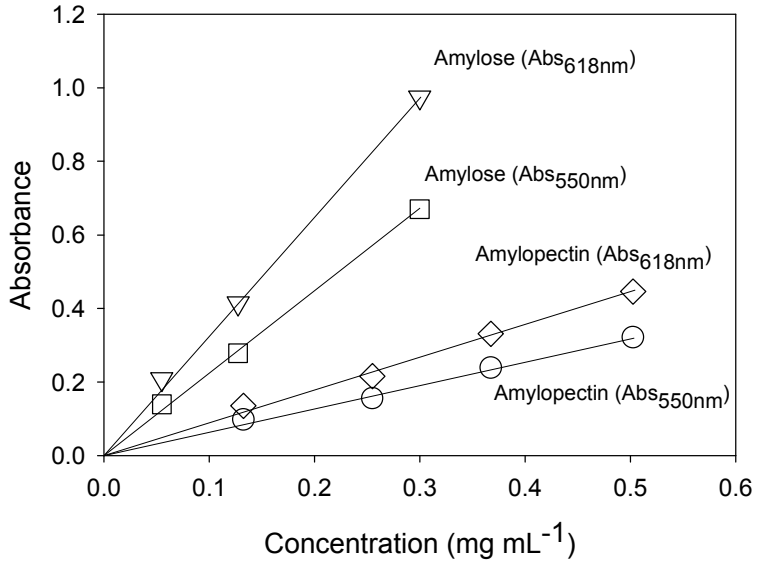

Figure 3 Standard curve for the determination of amylose and amylopectin ratio

A standard curve was produced by measuring the pure amylose and amylopectin of the potato (Merck, Germany) with concentration gradients of 0.06, 0.13, and 0.30 mg mL^{-1} for amylose and 0.13, 0.26, 0.37, and 0.50 mg mL^{-1} for amylopectin. The standard curve is presented in Figure 3. The ratio (R) of amylose and amylopectin was calculated according to formula (5):

$$R = \frac{PxGxa_{am618} + (1-P)xGxa_{ap618}}{PxGxa_{am550} + (1-P)xGxa_{ap550}} \quad (5)$$

P = fraction of amylose
G = starch concentration
a = absorbance coefficient [abs.mL.mg^{-1}]
am = amylose
ap = amylopectin

2.5.8 Enzymatic determination of sugars

Two and a half grams of freeze-dried potato flour was stirred with 70 mL distilled water in a 100 mL glass beaker for one hour. The suspension was transferred to a 100 mL volumetric flask and water was added to the mark. Afterwards, the suspension was filtered with filter paper no. 595 ½ (Schleicher &

Schuell, Germany). Ten mL of the filtrate was transferred into a 25 mL volumetric flask and cleared with 1.25 mL Carrez I solution (85 mM potassium hexacyanoferrate (II) ferrocyanide [Roth, Germany]) and 1.25 mL Carrez II solution (250 mM zinc sulfate [Roth, Germany]). The clear solution was filtered with filter paper no. 593 ½ (Schleicher & Schuell, Germany) and the filtrate was used for the determination using the enzyme kit for sugars analysis no. 716260 (Roche, Germany).

D-glucose was determined before and after enzymatic hydrolysis of sucrose. Sucrose was hydrolyzed by β-fructosidase to D-glucose and D-fructose. First, D-fructose was converted to D-glucose-6-phosphate by phosphoglucose-isomerase. The hexokinase catalyzes the phosphorylation of D-glucose with ATP at pH 7.6 to D-glucose-6-phosphate. The D-glucose-6-phosphate is oxidized to D-gluconate-6-phosphate by glucose-6-phosphate-dehydrogenase. In this reaction, $NADP^+$ is reduced to NADPH. The NADPH occurring in the reaction is equivalent to the content of D-glucose and is determined spectrometrically at the absorbance of 340 nm. The sucrose concentration is calculated from the difference of the D-glucose concentration before and after enzymatic inversion.

2.5.9 Determination of micro- and macronutrients

The micro- and macronutrients were determined according to Abu-Samra, et al. (1975) by digestion of freeze-dried potato flour using a microwave. About 750 mg of freeze-dried potato flour was weighed in a special container (MPV 100, Germany) for microwave use containing 5 mL distilled water. After adding of 8 mL 65% nitric acid (Merck, Germany), the sample in the closed container was digested in the Mega 1200 microwave for 18 hours (Germany). The digested solution was diluted in a 50 mL volumetric flask. The micro- and macroelements were measured at Hydro Agri Hydro- Agri GmbH & Co. KG (now Yara GmbH &

Co. KG) with a Perkin Elmer Optima 3000 inductively coupled plasma-atomic emission spectroscopy (USA).

2.5.10 Determination of nitrogen

The nitrogen content was determined according to the Dumas combustion method described by Sweeny and Rexroad (1987) with using a LECO® CN-2000 nitrogen analysator (USA). For this determination, three hundred milligrams of freeze-dried potato flour were combusted in the combustor. The Dumas combustion method is based on the principle of oxidative digestion of the sample under a controlled oxygen supply at high temperatures (approx. 900°C). The resulting combustion gases are directed with the CO_2 carrier gas over copper oxide, which acts as a catalyst, and thus are extensively oxidized.

2.5.11 Free amino acid determination

Extraction of free amino acid

Free amino acid was extracted from freeze-dried potato flour using hydrochloric acid as described by Anonymous (1993). One gram of freeze-dried potato flour was suspended in a centrifuge tube with 4 mL 1 N hydrochloric acid. The tube was shaken horizontally for one hour and centrifuged at 15,000 gravities for 30 min (Du Pont Sorvall RC-5B, USA). The supernatant was collected in a 10 mL volumetric flask. The extraction was done three times, with the last two extractions being done with 3 mL hydrochloric acid. Finally, the hydrochloric acid was added to the flask to adjust the final volume to 10 mL. To get a clear solution, the supernatant was centrifuged again at 15,000 gravities for 30 min.

Determination of free amino acids by HPLC

The HPLC separation of fluorescent *o*-phtaldialdehyde (OPA) derivatives had been applied to the assay of free amino acids according to the method described by Fisher et al. (2001). The procedure was based on the reaction of

a reducing agent β-mercaptoethanol, to give a complex which can be measured by fluorescence.

The OPA solution was prepared by dissolving 125 mg of OPA in 22 mL of methanol, then mixing it with 500 µL of β-mercaptoethanol and 2.5 mL 0.5 M borate buffer at pH 9.5.

The extract of amino acids was derivate with the fluorescence reagent (*o*-phthalaldehyde) for two minutes. Ten microliters of the mixture was injected into the HPLC. The HPLC system consists of degasser WellChrom K-5004 (Knauer, Germany), multisolvent delivery system 600E (Waters, USA), autosampler 2157 (Pharmacia LKB, Sweden), temperature control module (Waters, USA), 5 µm column LiChroCart 250-3 and pre-column LiChroCart 4-4 (Merck, Germany), and a fluorescence detector 474 (Waters, USA). The gradient eluents, which consisted of methanol (71/29 and 20/80, v/v) in 50 mM sodium acetate buffer (pH 7.0), were used to separate the amino acids at a flow rate of 0.6 mL min^{-1} and a temperature of 45°C. The amino acids were standardized using the Perbio Amino Acid Standard H (USA).

2.5.12 Investigation of pasting properties of potato flour and starch

The pasting properties of potato flour and starch, such as peak viscosity, peak time, pasting temperature, peak temperature, holding strength, breakdown, setback, and final viscosity, were determined by using rapid visco analyzer super 3 (Newport Scientific, Australia) according to ICC Standard no. 162 (ICC Standards, 1999). Before testing, the water content of the flour must be determined. Two grams of flour was weighed in a vessel and then transferred to the test canister. Twenty-five grams of water was added to the canister. The canister with the paddle was set up in the equipment. The equivalent sample mass (S) and water mass (W) were corrected with 14% moisture basis and calculated according to formulas (6) and (7):

$$S = \frac{86 \times A}{100 - M} \quad (6)$$

$$W = 25 + (A - S) \quad (7)$$

S = corrected sample mass [g]
A = sample weight at 14% moisture basis [g]
M = actual moisture content of the sample [%]
W = corrected water mass [g]

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3 Effect of calcium fertilizers on mineral composition and occurrence of brown centre in potato tubers

Abstract

Three years of field experiments were conducted in sandy soil at two locations each year in 2001, 2002 and 2003. In 2001, ammonium nitrate with lime and calcium nitrate were applied in three different application methods: band application after hilling, broadcast application before hilling, and 2/3 broadcast application before hilling and 1/3 inducted to the tuber area. In 2002 and 2003, gypsum, $CaCO_3$, and basic slag were applied at rates between 560 and 2240 kg Ca ha^{-1}. Soil was sampled 56 days after planting and after harvest, and the cation concentration in the soil solution and exchangeable cation in the soil were analyzed. The results show that lime and basic slag failed to increase Ca concentration in the soil solution during tuber growth and failed to increase the Ca concentration of the tubers. In contrast, application of gypsum kept and increased the Ca concentration in the soil solution during tuber development. Consequently, application of gypsum increased up to 65 and 112% the Ca in the tuber parenchyma and periderm tissue, respectively. It was observed that tubers with Ca concentrations lower than 0.1 g kg^{-1} DM and K concentrations lower than 18 g kg^{-1} DM were susceptible to the occurrence of brown centre discoloration in the fresh potato tuber.

3.1 Introduction

It is well known that Ca is an essential nutrient for plant growth. Deficiency of Ca is associated with disorders of tuber form development and a decrease in resistance against diseases and stress such as cyst nematodes (Fatemy and Evans, 1986), tissue maceration by *Erwinia carotovora* (McGuire and Kelman, 1986), heat stress (Kleinhenz and Palta, 2002; Sterrett and Henninger, 1991), internal brown spot (Tzeng at al., 1986), and internal necrotic disorder (Davies, 1998). However, potato tubers have low Ca content, compared to other parts of the potato plant. Immobility of Ca in the phloem may be a reason for low levels of Ca concentration in the fruit as well as in the potato tuber (Marschner, 1995).

To obtain its requirement of Ca, the potato tuber has a mechanism that is different from the mechanism of leaves and stems. Calcium that is absorbed

through the main root system is translocated mainly into leaves and only in small amounts (0.1–0.2%) into the tuber (Krauss and Marschner, 1973). Busse and Palta (2004) reported that tubers obtain Ca only from the soil in the area of the stolon and tubers and it is already agreed that Ca is not obtained through retranslocation from the leaves. Kratzke and Palta (1985) concluded that Ca is absorbed simultaneously with water by the tuber root or stolon through the xylem system. The intensity depends mainly on the transpiration rate of the tuber and the length of the stolon as well as the tubers roots. Calcium uptake by the tuber through the stolon occurs when the transpiration rate of the tuber is higher than the leave (Krauss and Marschner, 1974). Meanwhile, Habib and Donnelly (2002) reported that Ca can be absorbed directly from the soil solution through the tuber periderm but the rate is very low. Their experiment with Ca^{45} showed, in contrast to Krauss and Marschner (1973) and Busse and Palta (2004), that the basal root may provide Ca for the tuber in a larger quantity than does the stolon. These results demonstrated that the uptake mechanism of Ca and Ca accumulation in the tuber are still in discussion and are not well-established.

Under field condition, particularly on sandy soil with low cation exchange capacity, application of Ca fertilizer does not always increase the tuber Ca, even if the extractable Ca levels in the soil are 12–25 mmol Ca kg^{-1} of soil (Simmons and Kelling, 1987). Low and no correlation were found between soil and tuber Ca concentration (calculated from Simmons et al., 1988). The source of Ca fertilizer, form of the fertilizer, application method and timing, soil type and annual climate may influence the Ca uptake of the tuber. Many studies reported that tuber Ca can be increased but the results were not consistence from year to year (e.g. Clough, 1994; Kleinhenz et al., 1999; Locascio et al., 1992). The inconsistency may result from lack of information about the interactions between the fertilizer and soil, and the availability of Ca in the soil solution that can be taken up by the tuber. Because Ca in soil is transported to roots mainly by mass flow (Claassen, 1990) and it can be absorbed directly

from the soil solution by the tuber and tuber root, it seems to be important to investigate the relationship between fertilizer and Ca status in the soil solution during tuber development. In this study, three Ca sources with different solubility and influence on soil acidity were compared.

3.2 Material and methods

3.2.1 Field experiment

Potato cv. Saturna was grown in loamy sandy soil in 2001, 2002 and 2003 in Northern Germany. The experiment was conducted according to randomized block design with six replications in 2001 and four replications in 2002 and 2003. Each plot consisted of six rows 0.75 m apart and 8 m long.

In 2001, ammonium nitrate with lime (KAS) and calcium nitrate (KS) were applied in three different application methods. They were band application after hilling, broadcast application before hilling, and 2/3 broadcast application before hilling and 1/3 inducted to the tuber area at the onset of tuberization.

In 2002, the experiment was located in Hankensbüttel and Langwedel. Calcium was applied by hand broadcast preplant in form of sieved gypsum ($CaSO_4.2H_2O$) and granulates $CaCO_3$ with rates of 560, 1120 and 2240 kg Ca ha^{-1}. All plots received 44, 100, and 24 kg ha^{-1} P, K, and Mg, respectively. Nitrogen was applied at a rate of 160 kg N ha^{-1} as ammonium nitrate with lime (KAS). The plants used as controls did not receive any additional Ca fertilizer instead of KAS. Application of KAS as the control was compared with Ca nitrate (KS) and urea to see the effect of KAS application on the Ca concentration in the soil.

In 2003, the experiment was located in Hankensbüttel and Groß Oesingen. In addition to gypsum and $CaCO_3$, basic slag was also applied. Basic slag is a by-product of the iron industry and contains about 32% SiO_2 and 7% MgO (Havlin et al., 1999). Gypsum was applied at rates of 560, 1120, and 2240 kg Ca ha^{-1}. Meanwhile, $CaCO_3$ and basic slag (Thomasduenger GmbH, Ger-

many) were applied at rates of 1120 and 2240 kg Ca ha^{-1}. All plots received the same rates of N, P, K and Mg as in 2002.

The plants were harvested in mid-September corresponding to the physiological maturity of potato tubers. Tubers were graded based on diameters of < 35, 35 – 50 and > 50 mm for small, middle and large tuber size fractions, respectively. From the middle tuber size fraction, five tubers were subsampled from the bulk sample, washed, and peeled using a hand peeler. The periderm and parenchyma parts were separately freeze-dried and ground. The freeze-dried samples were digested with 65% HNO_3 in microwave (Mega 1200, Germany) according the method of Abu-Samra (1975). The macro- and micronutrients of the digested samples were analyzed using an inductively coupled plasma atomic emission spectrometer (Perkin Elmer Optima 3000, USA).

3.2.2 Soil analysis

Exchangeable cations

Soil was sampled before planting, 56 days after planting (July) and after harvest (September) in the tuber formation area (30 cm depth) by taking four cores per plot. Table 1 and Table 2 show the soil properties and the nutrient concentration before planting. The fresh soil samples were sieved and utilized within 24 hours for soil solution investigation. Soil solution was obtained by miscible displacement similar to the procedure described by Adams (1974). Meanwhile, some samples were air dried and subsampled for a soil test of exchangeable Ca, Mg, and K with 1 M NH_4OAc, pH 7 according to Claassen (1990). The concentrations of Ca and K in the extract were analyzed using a flame photometer (Eppendorf Elex 6361, Germany) and the concentration of Mg was analyzed using an atomic absorption spectrophotometer (AAS Unicam m Series, England).

Soil solution displacement

The fresh soil was filled into a 250 mL cylinder with a hole in the bottom. The hole was covered by filter paper no. 593[3] (Schleicher & Schuell, Germany). By using a peristaltic pump, water was allowed to drop slowly on the soil, displacing the soil solution downwards (Figure 2). It was collected in a glass beaker. To test whether the soil solution was diluted by the added water, 4% potassium thiocyanate was added as a marker to the water and the soil solution obtained was then tested with 5% iron (III) chloride. A red coloration in the solution indicated the presence of the marker. With repeated measurements, the marker was not detected in a solution volume below 20 mL. The solution was filtered and the pH and Ca and K concentrations were measured using a flame photometer. The concentration of Mg was measured using an atomic absorption spectrophotometer.

Table 1 Properties of the soil

Location	Humus (%)	Clay (%)	Silt (%)	Sand (%)	Coarse (%)
Hankensbüttel	1.60	5.62	37.34	57.05	0.50
Langwedel	1.70	8.05	14.24	77.72	1.52
Gross Oesingen	2.50	5.14	10.33	84.53	1.37

Table 2 Initial soil test values of pH and exchangeable cations in the plow layer (0 – 30 cm depth)

Location	2002				2003			
	pH_{CaCl}	Ca	K	Mg	pH_{CaCl}	Ca	K	Mg
		---- mmol kg^{-1} soil ----				--- mmol kg^{-1} soil ---		
Hankensbüttel	5.7	18.89	3.79	1.64	5.6	13.32	1.97	1.19
Langwedel	5.2	14.77	2.30	0.90	-	-	-	-
Gross Oesingen	-	-	-	-	5.4	8.75	2.79	3.61

3.2.3 Brown centre determination

Brown centre is an internal tuber disorder shown by a browning in the center of the tuber parenchyma. This symptom is a serious defect of the tuber

quality because it reduces the appearance and color quality of the chips. Brown centre was determined in this study by halving 35 tubers from the bulk sample after three months of storage at 8°C and relative humidity of 92%. The samples used in the counting of brown centre were taken from the potatoes planted in 2002 and 2003, but were also from the field experiment at the same site in 2001 (data are not presented). The results are presented as percentage of tubers with brown centre from 35 tubers.

3.2.4 Statistical analysis

Analyses of variance were computed for data analysis using a complete block design by general linear model procedure of statistical software SAS release 8.02. Data were presented as mean value and standard error from all treatments.

3.3 Results

3.3.1 Yield and size of tuber

The effect of Ca fertilizer on the yield and tuber size of potatoes is presented in Table 3 and Table 4. In contrast to the application of $CaCO_3$, the number of small tubers was reduced significantly by application of gypsum in 2002 ($P<0.05$) but in 2003 the effect was not significant and showed only a tendency. In 2003, the number of small-sized tubers was affected by location ($P<0.001$), where the number in Hankensbuettel was lower than in Gross Oesingen.

Calcium fertilizer had no significant effect on total yield in both planting seasons. Effect of location was significant in 2002, when the average yield in Langwedel was 43% higher than in Hankensbuettel ($P<0.001$). The significant effect of location probably occurred due to different irrigation treatments by the farmer. However, the effect of Ca fertilizer was observed in the percentage of small-sized tubers.

Table 3 Effect of calcium fertilizer on yield and tuber size of potatoes from planting season 2002

Fertilizers	Rates	Hankensbuettel				Langwedel			
		Yield	Tuber size			Yield	Tuber size		
			Small	Medium	Large		Small	Medium	Large
Control		37	13	39	48	49	16	50	34
CaCO3	560	35	14	50	50	48	16	43	40
	1120	36	13	55	45	45	15	50	35
	2240	30	17	55	45	47	18	49	33
Gypsum	560	31	11	45	55	48	14	41	46
	1120	33	12	45	55	53	10	41	49
	2240	34	10	42	58	48	12	48	39
s.e.		2.14	1.21	3.42	4.00	2.86	2.13	3.21	3.87

Rates, yield, and tuber size are presented in kg Ca ha^{-1}, Mg ha^{-1}, and %, respectively.
s.e. is standard error.

Table 4 Effect of calcium fertilizer on yield and tuber size of potatoes from planting season 2003

Fertilizers	Rates	Hankensbuettel				Langwedel			
		Yield	Tuber size			Yield	Tuber size		
			Small	Medium	Large		Small	Medium	Large
Control		46	13	57	30	44	8	47	45
CaCO3	1120	42	15	62	23	43	10	44	46
	2240	42	14	59	27	43	9	54	36
Gypsum	560	44	13	56	31	43	7	51	42
	1120	45	12	59	29	44	10	51	39
	2240	47	9	52	39	47	9	47	44
Basic slag	1120	41	13	57	30	46	9	47	44
	2240	44	8	47	45	45	8	45	47
s.e.		1.47	4.63	3.36	1.58	2.32	3.86	3.27	1.14

Rates, yield, and tuber size are presented in kg Ca ha^{-1}, Mg ha^{-1}, and %, respectively.
s.e. is standard error.

3.3.2 Soil Analysis

Soil pH

Figure 4 shows the soil pH after Ca fertilization at the onset of tuberization and after harvest. The soil pH was significantly affected by fertilizer ($P<0.001$) and the interaction of fertilizer x rate ($P<0.001$), but the effect of the interaction of location x fertilizer x rate was not significant ($P>0.05$). Soil pH increased corresponding to the increasing rate of $CaCO_3$ application. At the same time, gypsum and basic slag application had little effect on soil pH.

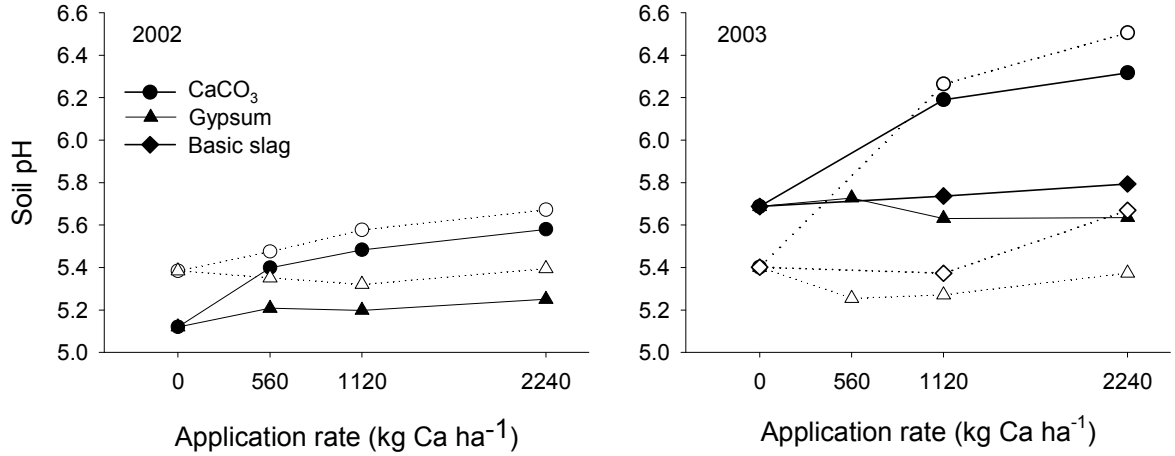

Figure 4 Effect of calcium fertilization on soil pH from planting seasons 2002 and 2003. The continuous line shows the pH at the onset of tuberization and the dotted line shows the level after harvest

Calcium concentration in soil solution and exchangeable calcium

Mean square analysis of Ca concentration in the soil solution and exchangeable Ca is presented in Table 5. Between both planting seasons, the effect of treatments was relatively similar at all locations. The variability of Ca concentration in the soil solution at the onset of tuberization came from fertilizer and the interaction of fertilizer x rate. In contrast to 2002, the interaction of location x fertilizer x rate was high significant ($P<0.01$) in 2003. However, the effect of fertilizer on Ca status in the soil was more obvious after harvest, when the effect of the interaction of fertilizer x rate was high significant ($P<0.001$).

Table 5 Mean square analysis of calcium concentration in soil solution and exchangeable calcium at the onset of tuberization and after harvest

Main effect	df	soil solution Ca concentration		Exchangeable Ca	
		Onset	Harvest	Onset	Harvest
---------- 2002 ----------					
Location (L)	1	2.84	0.22	11.11*	1.58
Fertilizer (F)	1	9.38*	183.94***	0.40	0.99
Rate (R)	3	2.59	16.54***	6.91***	11.92***
L x F	1	0.01	2.09	0.27	1.27
L x R	3	1.81	3.05	0.25	0.69
F x R	3	3.68*	22.28***	0.26	0.35
L x F x R	3	2.89	2.05	0.43	0.60
---------- 2003 ----------					
Location (L)	1	1.08	4.91*	2.11	0.02
Fertilizer (F)	2	24.70***	58.48***	7.19***	2.55
Rate (R)	3	2.52	20.54***	7.48***	11.35***
L x F	2	11.50***	4.03	0.09	1.42
L x R	3	2.40	0.89	0.36	1.87
F x R	4	9.78***	18.24***	3.33*	2.49
L x F x R	4	3.93**	2.50	0.88	0.76

*, **, *** are F tests significant at P<0.05, P<0.01, and P<0.001, respectively

In addition to the mean square analysis in Table 5, the Ca concentration in the soil solution and exchangeable Ca in the soil is presented in Figure 5. While the application of gypsum and basic slag increased the Ca in the soil solution at the beginning of tuberization, application of $CaCO_3$ could not increase the Ca in the soil solution at any application rate. After harvest, Ca concentration in the soil solution had decreased and the intensity depended on the Ca sources. After application of $CaCO_3$ and basic slag, the decrease of Ca in the soil solution ranged between 64 and 70% in 2002 and between 27 and 48% in 2003. In contrast to the effects of $CaCO_3$ and basic slag, the decline of Ca concentration in the soil solution could be reduced to only 10% by increasing rate of gypsum application to the highest application rate. This result showed that the use of gypsum can maintain the Ca concentration in a soil solution during tuberization time.

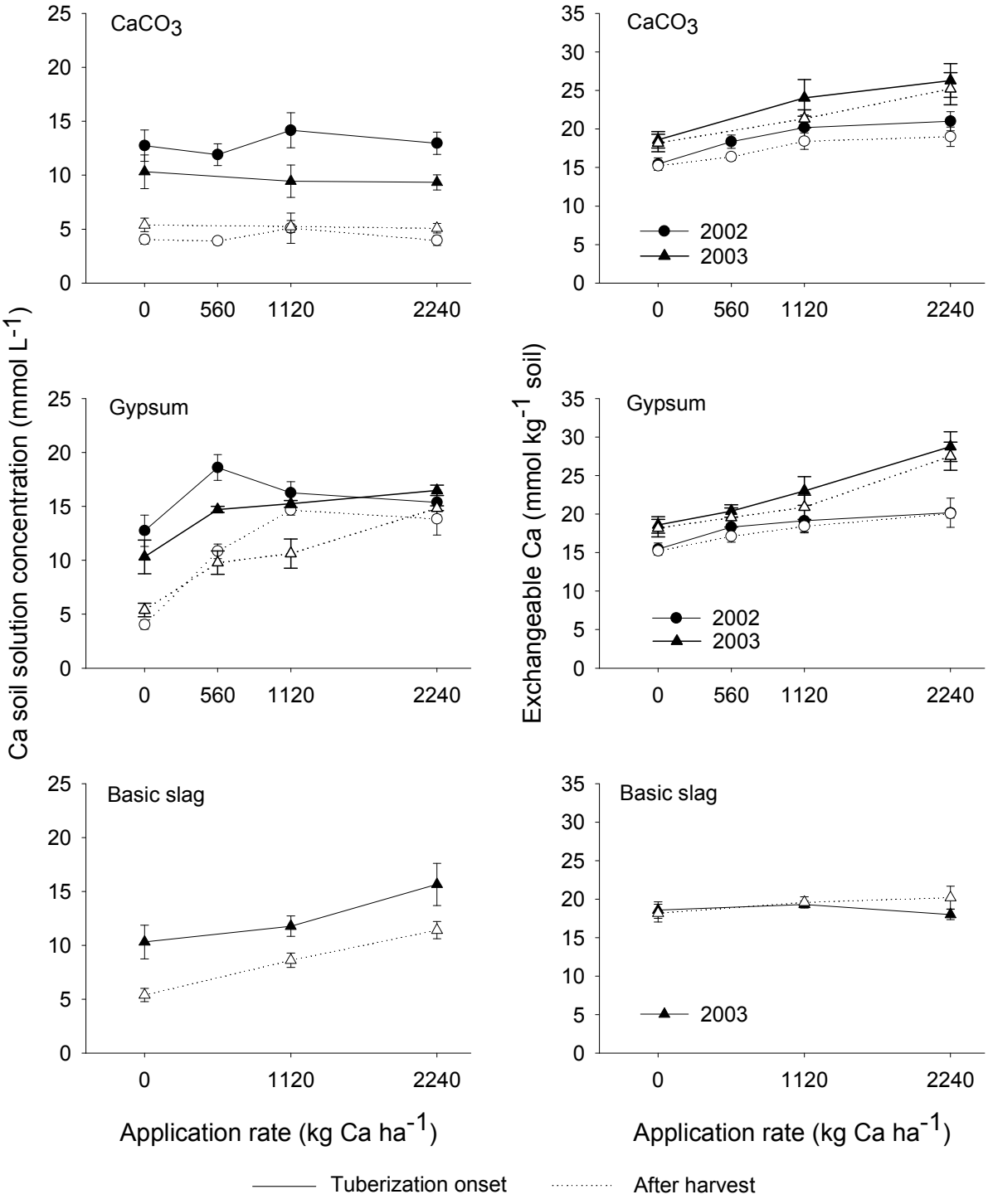

Figure 5 Effect of calcium fertilization on calcium concentration in soil solution and exchangeable calcium

The effects of $CaCO_3$ and gypsum on exchangeable Ca were not different. Exchangeable Ca increased significantly with rising rates of $CaCO_3$ and gypsum ($P<0.001$), while basic slag had little effect on exchangeable Ca. After harvest, exchangeable Ca had decreased only slightly.

Exchangeable K and Mg were generally not affected by Ca applications. Exchangeable K and K concentration in the soil solution were 2.72 ± 1.01 mmol kg^{-1} soils and 1.71 ± 1.12 mmol L^{-1} at the onset of tuberization, and they had decreased by 46 and 67% after harvest, respectively. Significant increases in exchangeable Mg were observed by the addition of Mg from basic slag applications. The exchangeable Mg and Mg concentration in the soil solution were 1.90 ± 0.88 mmol kg^{-1} soil and 2.66 ± 1.30 mmol L^{-1} at the onset of tuberization, respectively. As with K, the exchangeable Mg decreased only slightly after harvest and Mg concentration in the soil solution decreased about 43%.

3.3.3 Tubers mineral composition

Macronutrient concentration of potato tubers

Generally, location had a major effect on the macronutrient composition in this study. The climate between both locations can be assumed to be similar because the distance between the locations was only about 5 km. Soil type and irrigation may be reasons for the variability.

The composition of macronutrients in the parenchyma and periderm tissue of the potato tubers is shown in Table 6 and Table 7. Calcium concentration in the tuber parenchyma and periderm was increased by gypsum application up to 1120 kg Ca ha^{-1} in all locations and years. Application of more than 1120 kg Ca ha^{-1} had no significant effect on the Ca concentration in the tuber parenchyma and periderm. The increase was up to 65% in the parenchyma and 112% in the periderm. Application of basic slag led to a slight increase of tuber Ca only in Gross Oesingen. In contrast to gypsum, $CaCO_3$ application could not increase the tuber Ca; it even led to a decrease in Langwedel in 2002.

Calcium levels in the tuber parenchyma and periderm had high correlation with the Ca concentration in the soil solution during tuber development as shown in Figure 6 and Figure 7. In these figures, Ca availability is described

as relative Ca concentration in the soil solution. It is the percentage of Ca levels in the soil after harvest relative to the control at the onset of tuberization. The relative tuber Ca concentration is the comparison between tuber Ca of treated samples and the untreated sample (control), in which the control had a relative value of 100%.

Both figures show that most of the gypsum application was in quadrant I in the graph. This means that the increase in the Ca level in the tuber was an impact of the increase of the Ca concentration in the soil solution during tuber growth after gypsum application. In contrast, lime application is more in quadrants II and III close to the axis. The quadrant II results mean that although the Ca concentration in the soil solution decreased during tuber growth, it can still improve the Ca in the tuber. However, the level was not as high as with gypsum application. This means that the Ca uptake occurred mainly at the onset of tuberization or by the young tuber, when the Ca concentration in the soil solution from the $CaCO_3$ application was still high. The quadrant III results mean that a decrease in Ca concentration in the soil solution was followed by a decrease in Ca in the tuber. However, an exception was observed in Hankensbuettel in 2003, when all Ca fertilizers had no significant effect on any macronutrients.

Moreover, Ca fertilization was observed to have an effect on S, Na and Mg composition in the potato tuber (Table 6 and Table 7). In particular, gypsum application led to a small increase in S concentration to 21% and 32% in the tuber parenchyma and periderm, respectively. However in 2003, the effect was observed only in the periderm of tubers grown in Gross Oesingen.

Table 6 Macroelements concentration in potato tuber parenchyma and periderm and mean square analysis from planting season 2002

		Parenchyma tissue (g kg^{-1} DM)						Periderm tissue (g kg^{-1} DM)					
Fertilizer	Rate	Ca	K	Mg	Na	P	S	Ca	K	Mg	Na	P	S
	(kg Ca ha^{-1})	Hankensbuettel											
Control		0.129^{b}	18.17	0.92	0.029^{ab}	2.35	1.61^{b}	0.580^{c}	27.54	0.99^{c}	0.027	2.34	1.80^{b}
$CaCO_3$	560	0.136^{b}	18.51	0.93	0.032^{a}	2.40	1.61^{b}	0.780^{c}	31.43	1.13^{ab}	0.026	2.34	2.07^{ab}
	1120	0.138^{b}	18.36	0.94	0.032^{ab}	2.39	1.64^{ab}	0.790^{c}	31.31	1.14^{a}	0.029	2.36	2.01^{ab}
	2240	0.144^{b}	18.11	0.91	0.028^{ab}	2.40	1.60^{b}	0.820^{bc}	31.02	1.12^{ab}	0.027	2.39	2.14^{ab}
Gypsum	560	0.180^{ab}	19.29	0.93	0.028^{ab}	2.48	1.78^{ab}	0.800^{c}	29.27	0.97^{c}	0.024	2.44	2.06^{ab}
	1120	0.206^{a}	19.67	0.95	0.025^{b}	2.55	1.89^{a}	1.150^{ab}	31.27	1.02^{bc}	0.025	2.54	2.38^{a}
	2240	0.213^{a}	19.04	0.95	0.027^{ab}	2.29	1.80^{ab}	1.230^{a}	31.18	1.00^{c}	0.021	2.32	2.29^{a}
		Langwedel											
Control		0.134^{ab}	18.51	0.97	0.024^{a}	2.22	1.44^{bc}	0.680^{bcd}	30.93	1.16^{a}	0.021	2.34	1.85^{ab}
$CaCO_3$	560	0.096^{b}	17.28	0.96	0.021^{ab}	2.04	1.43^{c}	0.510^{d}	28.48	1.12^{ab}	0.022	2.24	1.76^{ab}
	1120	0.120^{ab}	18.66	0.98	0.018^{ab}	1.99	1.43^{c}	0.570^{cd}	28.53	1.03^{bc}	0.018	2.11	1.68^{b}
	2240	0.095^{b}	17.70	0.99	0.020^{ab}	2.05	1.39^{c}	0.470^{d}	27.48	1.10^{abc}	0.020	2.20	1.58^{b}
Gypsum	560	0.158^{ab}	18.02	0.98	0.020^{ab}	2.14	1.69^{ab}	0.870^{ab}	29.63	1.01^{bc}	0.016	2.26	2.04^{a}
	1120	0.177^{a}	18.31	1.02	0.015^{b}	1.96	1.74^{a}	0.990^{a}	28.45	0.99^{c}	0.017	2.05	2.09^{a}
	2240	0.157^{ab}	18.83	1.01	0.016^{b}	2.10	1.63^{ab}	0.830^{abc}	29.11	1.02^{bc}	0.016	2.23	1.85^{ab}
Mean square analysis													
Treatments	df												
Location (L)	1	19.84***	1.88	19.25***	78.23***	35.30***	37.31***	22.54***	2.24	4.50	27.31***	10.96*	39.92***
Fertilizer (F)	1	61.67***	5.44	4.90	32.74***	0.69	38.93***	63.48***	0.02	44.12***	8.09*	0.43	30.74***
Rate (R)	3	4.84*	0.68	1.22	4.36**	0.62	6.76***	13.33***	0.38	0.56	1.25	0.54	6.18***
L x F	1	0.01	0.47	0.25	0.62	0.00	0.42	2.05	1.20	3.63	0.04	0.47	3.03
L x R	3	4.54*	1.62	0.26	2.45**	2.98	0.12	11.03***	10.70***	13.15***	0.51	3.00	6.56***
F x R	3	7.26**	1.85	0.97	4.90	1.27	6.44***	9.47***	0.58	5.59***	1.04	0.35	6.04***
L x F x R	3	0.26	1.75	0.50	2.29	1.96	0.07	2.14	1.06	0.60	0.68	1.07	0.94

ns, *, **, *** are F tests not significant and significant at P>0.05, 0.01 and P<0.001, respectively.
The same letter in the same column shows no significant difference.

Table 7 Macroelements concentration in potato tuber parenchyma and periderm and mean square analysis from planting season 2003

		Parenchyma tissue (g kg^{-1} DM)						Periderm tissue (g kg^{-1} DM)					
Fertilizer	Rate	Ca	K	Mg	Na	P	S	Ca	K	Mg	Na	P	S
	(kg Ca ha^{-1})						Gross Oesingen						
Control		0.106^{b}	20.78	1.02	0.041^{a}	2.28	1.57	0.740^{c}	35.51	1.45	0.045^{ab}	2.14	1.99^{b}
$CaCO_3$	1120	0.113^{b}	20.81	1.07	0.039^{ab}	2.20	1.60	0.860^{c}	35.36	1.46	0.050^{a}	2.20	2.09^{b}
	2240	0.129^{ab}	20.32	1.01	0.038^{ab}	2.14	1.55	0.940^{bc}	35.36	1.41	0.050^{a}	2.18	2.04^{b}
Gypsum	560	0.148^{ab}	20.66	1.03	0.031^{bc}	2.15	1.72	1.000^{bc}	35.40	1.36	0.031^{c}	1.94	2.31^{ab}
	1120	0.169^{a}	20.57	0.98	0.028^{c}	2.01	1.65	1.150^{ab}	35.51	1.28	0.028^{c}	1.91	2.30^{ab}
	2240	0.171^{a}	21.21	1.02	0.026^{c}	2.12	1.72	1.280^{a}	36.96	1.37	0.028^{c}	2.04	2.46^{a}
Basic slag	1120	0.150^{ab}	21.25	1.02	0.032^{abc}	2.14	1.58	0.950^{bc}	34.50	1.39	0.038^{bc}	2.06	2.08^{b}
	2240	0.155^{ab}	21.46	0.96	0.031^{abc}	2.30	1.59	0.930^{bc}	36.38	1.38	0.039^{abc}	2.09	2.16^{ab}
							Hankensbuettel						
$CaCO_3$	0	0.144	18.83	0.94	0.037	2.12	1.58	1.000	33.78	1.31	0.041	2.09	2.14
	1120	0.144	18.62	0.88	0.039	1.96	1.54	1.040	34.66	1.25	0.047	2.03	2.05
	2240	0.151	18.42	0.93	0.042	1.99	1.56	1.070	33.80	1.26	0.042	1.94	2.04
Gypsum	560	0.152	19.19	0.95	0.035	2.10	1.63	0.980	32.45	1.23	0.041	1.99	2.02
	1120	0.147	19.33	0.93	0.037	2.11	1.61	1.000	33.89	1.27	0.042	2.02	2.05
	2240	0.149	19.70	0.99	0.037	2.19	1.71	1.010	34.38	1.29	0.045	2.13	2.10
Basic slag	1120	0.154	19.33	0.89	0.040	2.01	1.52	1.160	34.44	1.29	0.042	1.98	2.07
	2240	0.147	19.47	0.86	0.038	2.05	1.55	1.070	34.98	1.25	0.042	2.01	2.13
Mean square analysis													
Treatments	df												
Location (L)	1	4.95*	82.69***	80.73***	3.79	8.41*	1.49	11.83***	12.98***	37.70***	1.54	2.36	2.16
Fertilizer (F)	2	8.23**	5.62*	4.99*	9.22***	0.39	9.66***	6.02**	0.17	0.91	16.22***	0.90	7.83***
Rate (R)	3	3.60*	0.49	1.02	1.57	2.50	0.90	6.99***	1.27	2.27	0.67	3.11	1.24
L x F	2	7.53**	0.94	3.82	3.32	3.80	0.11	15.90***	0.52	2.26	13.40***	4.51*	7.35***
L x R	3	2.40	0.05	1.77	3.23*	0.24	0.65	3.04	0.48	0.35	1.79	0.08	4.23*
F x R	4	2.45	2.40	4.97***	2.63	1.22	4.06*	2.66	0.66	0.99	8.19***	1.92	3.01*
L x F x R	4	1.97	0.34	2.37	0.99	2.86*	0.13	4.08*	0.14	1.17	6.89***	1.87	2.16

*, **, *** are F tests significant at P>0.05, 0.01 and P<0.001, respectively.
The same letter in the same column shows no significant difference.

Table 8 Microelement concentration in potato tuber parenchyma and periderm and mean square analysis from planting season 2002

		Parenchyma tissue (mg kg^{-1} DM)						Periderm tissue (mg kg^{-1} DM)					
Fertilizer	Rate	B	Cu	Fe	Mn	Mo	Zn	B	Cu	Fe	Mn	Mo	Zn
	(kg Ca ha^{-1})	----------	----------	----------	----------	----------	Hankensbuettel	----------	----------	----------	----------	----------	----------
Control		3.98	3.00	17.63	7.63	0.68	17.04	6.70^{d}	5.65	95.54	8.77	0.12	27.72
Lime	560	3.95	3.11	17.54	7.45	0.67	17.28	8.81^{a}	6.40	102.95	10.05	0.15	29.18
	1120	3.72	3.11	17.94	7.71	0.60	15.72	8.77^{a}	5.93	134.33	10.97	0.26	26.95
	2240	3.66	2.95	17.44	7.49	0.58	14.72	8.29^{ab}	5.98	135.50	10.40	0.26	24.36
Gypsum	560	3.90	3.46	20.57	7.80	0.45	17.07	6.90^{cd}	5.84	102.02	9.00	0.11	24.96
	1120	3.90	3.75	19.12	7.96	0.43	19.08	7.49^{bcd}	6.39	119.22	11.42	0.13	29.92
	2240	4.10	3.07	17.64	7.74	0.39	17.36	7.85^{abc}	5.96	103.10	10.65	0.12	29.25
		----------	----------	----------	----------	----------	Langwedel	----------	----------	----------	----------	----------	----------
Control		4.26	2.83	17.99	7.77	0.45^{ab}	16.80	8.49	5.72	45.14	8.48	0.18	38.38
Lime	560	4.38	2.45	17.52	7.66	0.52^{ab}	14.79	7.86	5.13	38.23	6.96	0.26	26.57
	1120	4.33	2.48	19.34	7.96	0.46^{ab}	15.17	7.38	6.54	33.03	6.53	0.25	24.42
	2240	4.27	2.42	17.88	7.78	0.66^{a}	15.87	7.37	5.74	36.33	6.45	0.29	25.02
Gypsum	560	4.38	2.92	18.60	8.79	0.30^{b}	17.25	7.75	5.63	34.61	9.40	0.18	28.24
	1120	4.37	2.56	18.01	8.48	0.32^{b}	16.66	7.51	5.97	33.68	9.56	0.12	27.11
	2240	4.47	2.71	17.41	7.86	0.43^{b}	17.95	7.62	6.14	28.89	6.78	0.15	23.83
Mean square analysis													
Treatments	df												
Location (L)	1	50.72***	53.93***	0.02	4.94	14.91***	0.57	0.59	0.16	115.49***	27.75***	0.98	0.69
Fertilizer (F)	1	2.65	21.43***	0.76	8.59*	25.77***	6.08*	14.59***	0.05	1.14	2.74	8.98*	1.07
Rate (R)	3	0.21	1.40	1.76	1.41	3.19*	0.11	0.44	0.38	0.52	1.37	0.32	3.35*
L x F	1	0.45	0.71	1.30	0.96	0.00	0.00	19.39***	0.24	0.47	3.47	0.06	0.01
L x R	3	0.84	4.50*	0.51	0.56	6.07***	0.87	13.32***	0.27	2.03	4.02	0.26	3.05
F x R	3	1.67	2.99	3.18	2.87	3.96*	1.63	4.52**	0.23	0.42	0.85	1.51	1.25
L x F x R	3	0.27	2.58	0.91	1.18	0.06	0.96	3.25*	3.54*	0.23	1.15	0.04	2.21

*, **, *** are F tests significant at P>0.05, 0.01 and P<0.001, respectively.
The same letter in the same column shows no significant difference.

Table 9 Microelement concentration in potato tuber parenchyma and periderm and mean square analysis from planting season 2003

		Parenchyma tissue (mg kg^{-1} DM)						Periderm tissue (mg kg^{-1} DM)					
Fertilizer	Rate	B	Cu	Fe	Mn	Mo	Zn	B	Cu	Fe	Mn	Mo	Zn
	(kg Ca ha^{-1})	---------- Gross Oesingen ----------											
Control		4.07	2.92	17.58	7.02	0.15 ^{ab}	14.69 ^{ab}	9.70	6.20	122.18	10.61	0.15 ^{ab}	28.67
Lime	1120	3.93	2.87	18.13	7.46	0.18 ^{ab}	13.90 ^{b}	9.41	6.56	146.48	11.15	0.21 ^{ab}	23.49
	2240	4.13	3.02	18.11	7.13	0.21 ^{a}	14.16 ^{b}	9.71	6.66	142.83	10.63	0.27 ^{a}	23.75
Gypsum	560	4.07	2.95	17.51	7.09	0.10 ^{ab}	14.54 ^{ab}	9.68	6.02	108.79	10.20	0.11 ^{bc}	22.21
	1120	3.79	2.98	17.01	6.87	0.06 ^{ab}	13.37 ^{b}	9.17	6.21	116.20	9.83	0.02 ^{c}	21.44
	2240	3.99	3.01	17.90	6.93	0.04 ^{b}	15.54 ^{ab}	9.79	6.76	132.24	10.81	0.09 ^{bc}	24.56
T. Lime	1120	4.05	2.96	18.13	7.09	0.12 ^{ab}	14.07 ^{bc}	9.32	6.43	132.83	10.35	0.23 ^{ab}	21.82
	2240	4.04	3.47	17.92	6.49	0.16 ^{ab}	17.65 ^{a}	9.43	6.95	125.81	11.25	0.15 ^{abc}	25.55
		---------- Hankensbuettel ----------											
Control		4.23	2.75	16.86	7.16	0.04	19.09 ^{a}	10.01	6.59	136.22	10.74	0.11	38.72
Lime	1120	4.04	2.73	16.18	6.66	0.07	13.14 ^{c}	10.05	6.53	171.54	12.20	0.11	23.89
	2240	4.14	2.59	16.76	7.04	0.12	17.70 ^{ab}	9.72	6.29	182.36	12.35	0.17	45.35
Gypsum	560	4.09	2.91	18.63	7.19	0.04	18.32 ^{ab}	9.18	6.21	147.35	11.01	0.12	34.98
	1120	4.16	2.92	17.14	6.95	0.04	15.08 ^{bc}	9.75	6.51	160.73	11.40	0.07	24.85
	2240	4.10	3.01	17.14	7.51	0.09	17.19 ^{ab}	9.87	7.07	176.85	12.17	0.11	32.40
T. Lime	1120	4.02	2.78	17.95	6.58	0.04	15.52 ^{abc}	9.84	6.53	201.13	13.17	0.11	28.86
	2240	3.91	2.95	20.50	6.69	0.07	14.92 ^{bc}	9.82	6.86	197.15	13.14	0.10	27.88
Mean square analysis													
	df												
Location (L)	1	0.94	5.01 *	0.05	0.05	16.46 ***	30.34 ***	0.86	1.11	52.23 ***	10.53 ***	4.54	15.94 ***
Fertilizer (F)	2	0.32	1.70	2.35	2.51	4.20 *	0.77	0.15	0.38	2.56	2.36	9.28 ***	1.37
Rate (R)	3	0.73	1.23	0.80	0.46	0.57	14.34 ***	0.59	3.62 *	11.57 ***	2.09	0.56	3.40 *
L x F	2	2.37	0.79	2.03	2.32	2.07	1.75	0.06	0.72	2.65	0.79	3.34	1.16
L x R	3	0.55	0.40	0.51	1.87	1.21	7.96 ***	0.90	0.66	4.10 *	1.50	0.03	0.84
F x R	4	0.82	1.78	1.35	2.80 *	2.66	0.59	0.47	1.10	1.96	1.74	9.83 ***	2.04
L x F x R	4	0.74	0.76	2.02	1.16	1.65	6.47 ***	0.18	0.28	1.14	0.79	2.80 *	3.51 *

*, **, *** are F tests significant at P>0.05, 0.01 and P<0.001, respectively.
The same letter in the same column shows no significant difference.

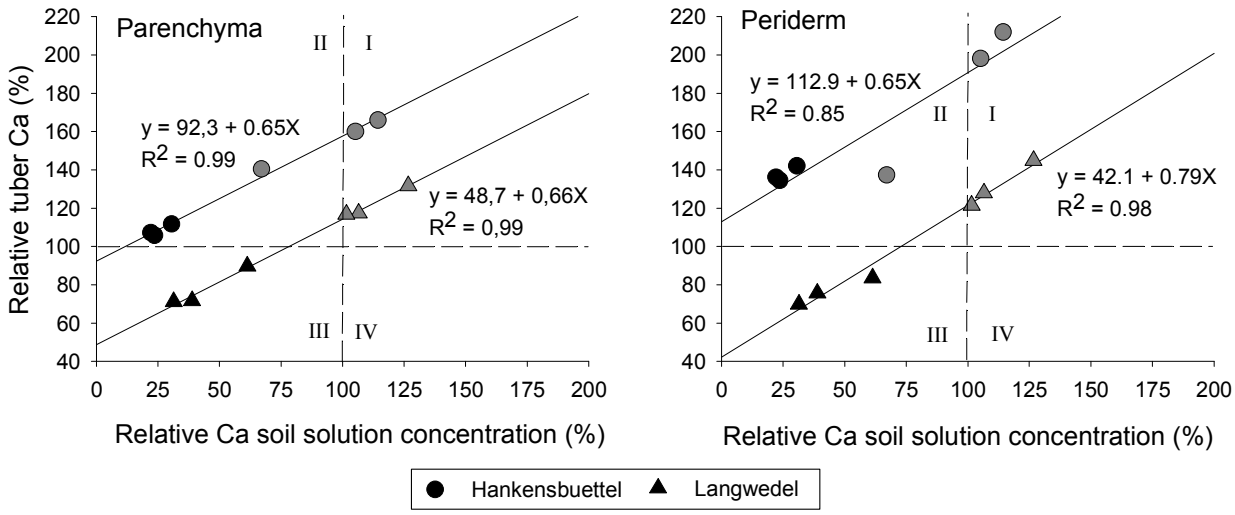

Figure 6. Relative tuber calcium as a function of relative calcium concentration in soil solution during tuber growth fertilized with $CaCO_3$ (black) and gypsum (grey) from planting season 2002. The concentration of Ca in the soil solution of the control treatment in Hankensbuettel and Langwedel was 13.9 and 11.6 mmol L^{-1}. The Ca concentration of the untreated potato parenchyma and periderm was 0.13 and 0.58 g kg^{-1} DM in Hankensbuettel and 0.13 and 0.68 g kg^{-1} DM in Langwedel. They related to relative value of 100% in the graph which was divided in four quadrants: I, II, III, and IV.

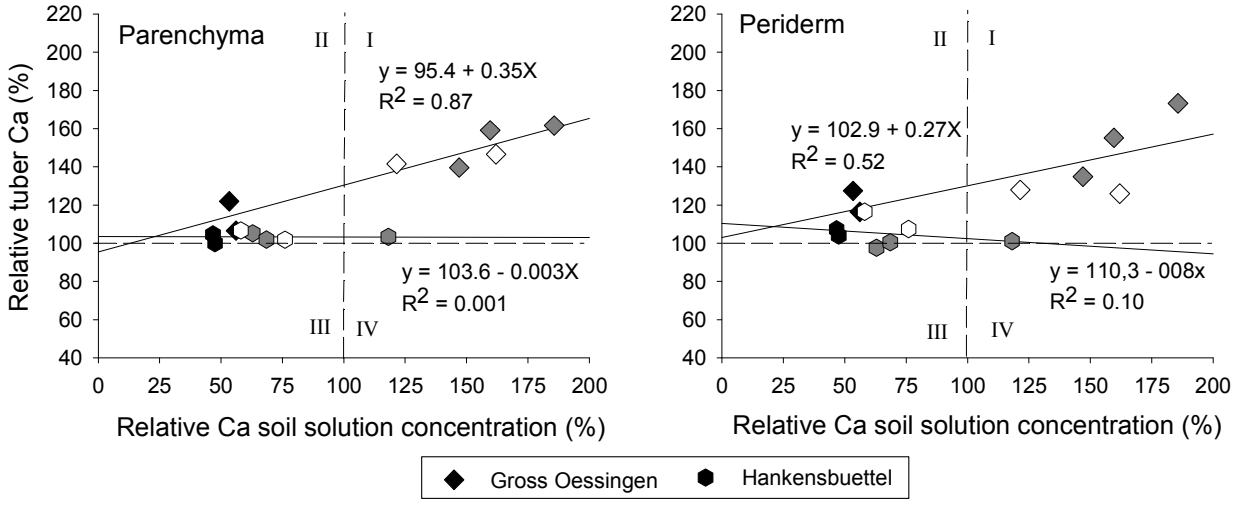

Figure 7. Relative tuber calcium as a function of relative calcium concentration in soil solution during tuber growth fertilized with $CaCO_3$ (black), gypsum (grey), and basic slag (white) from planting season 2003. The concentration of Ca in the soil solution of the control treatment in Hankensbuettel and Langwedel was 7.8 and 12.8 mmol L^{-1}. The Ca concentration of the untreated potato parenchyma and periderm was 0.11 and 0.74 g kg^{-1} DM in Hankensbuettel and 0.14 and 1.00 g kg^{-1} DM in Langwedel. They related to relative value of 100% in the graph which was divided in four quadrants: I, II, III, and IV.

Application of gypsum was observerd to reduce Na content in the tuber parenchyma and periderm, while application of $CaCO_3$ had less effect on Na concentration in tuber.

Unlike with S and Na, the effect of Ca fertilization on Mg was found only in the tuber periderm of tubers from planting season 2002. The effect varied depending on location. The concentration of Mg was increased by $CaCO_3$ application in Hankensbuettel and decreased by $CaCO_3$ and gypsum application in Langwedel.

Micronutrients concentration

Variability of micronutrient concentration in the tubers was mainly caused by the effect of location (Table 8 and Table 9). Every location might have a different concentration of micronutrients in the soil. The effect of Ca fertilization was observed to be significant on the concentration of Zn, Mo, B, Cu, and Mn, but the effect was not consistent and different between the years. Application of gypsum reduced the concentration of Mo in the tuber parenchyma, in both years but not in all locations, while application of $CaCO_3$ had less effect on the concentration of Mo in the tubers. Zinc was reduced in the tuber parenchyma by all Ca fertilizers but only in 2003. Boron was significantly increased in the tuber periderm by the application of $CaCO_3$ but only in Hankensbuettel in 2002. Copper and Mn were affected by the Ca fertilization but with low significance.

3.3.4 Effect of nitrogen fertilizers

In planting season 2001, the interaction between nitrogen fertilizers and the application methods on the Ca concentration in the tuber parenchyma and periderm was statistically not significant ($P > 0.05$). Between fertilizers, application of KS resulted in higher Ca content in the tuber parenchyma and periderm than application of KAS (Table 10).

In planting season 2002, N sources had no significant effect on the Ca concentration in the soil solution either at the onset of tuberization or after harvest (P>0.05). Hence, Ca concentration either in the parenchyma or in the periderm was not significantly affected by the nitrogen sources (P>0.05). However, average Ca content in the tuber was higher when potatoes were applied with KAS and KS than with urea which does not contain Ca (Table 11).

Table 10 Effect of nitrogen fertilizers and application methods on tuber Ca concentration from planting season 2001

Fertilizers	Application methods	Tuber Ca (g kg^{-1} DM)	
		Parenchyma	Periderm
Ammonium nitrate with lime	Band	0.084 [b]	0.46 [b]
	Induction	0.091 [ab]	0.52 [a]
	Broadcast	0.088 [b]	0.50 [ab]
Calcium nitrate	Band	0.099 [a]	0.54 [a]
	Induction	0.097 [a]	0.54 [a]
	Broadcast	0.097 [a]	0.50 [ab]
s.e.		0.003	0.02

The same letter in the same column shows no significant difference
s.e. is standard error

Table 11 Effect of different sources of nitrogen on calcium concentration in soil solution and in potato tubers from planting season 2002

Fertilizers	Ca in soil solution (mmol L^{-1})		Calcium tuber (g kg^{-1} DM)	
	Tuber initiation	After harvest	Parenchyma	Periderm
Ammonium nitrate with lime	12.47 [a]	4.03 [ab]	0.13 [a]	0.63 [a]
Calcium nitrate	16.80 [a]	2.98 [b]	0.12 [ab]	0.65 [a]
Urea	13.28 [a]	4.36 [a]	0.11 [b]	0.53 [a]
s.e.	1.46	0.41	0.01	0.05

The same letter in the same column shows no significant difference
s.e. is standard error

3.3.5 The occurrence of brown centre

The brown centre symptom inside the tubers was observed in the tubers grown in planting seasons 2001, 2002 and 2003 (Figure 8). Tubers with this

symptom were found in large number in 2001, when most of the tubers came from plants that were treated without Ca and also from plants that were treated with low rates of Ca fertilizer of 450 and 900 kg Ca ha^{-1} as gypsum or lime. Respectively, 15–52, 4–14, and 1–13 % of the tubers in 2001, 2002, and 2003 were found with this symptom.

Figure 8 Brown centre inside potato tuber

In this study, the nutrients Ca and K obviously reduced the occurrence of brown centre. The relationship between tuber Ca and K concentration and the incidence of brown centre was strong, with Pearson coefficients of 0.73 and 0.71, respectively. Figure 9 shows that tubers containing less than 0.10 g Ca kg^{-1} DM were more susceptible to brown centre. The incidence of brown centre could be reduced noticeably down to 5%, when the tubers contained more than 0.15 g Ca kg^{-1} DM. Figure 9 indicates also that the incidence of brown centre depended on the K concentration in the tuber. Concentration of K in this study was affected less by Ca fertilization and more by location. Concentration of more than 18 g K kg^{-1} DM sharply reduced the incidence of brown centre below to 10%.

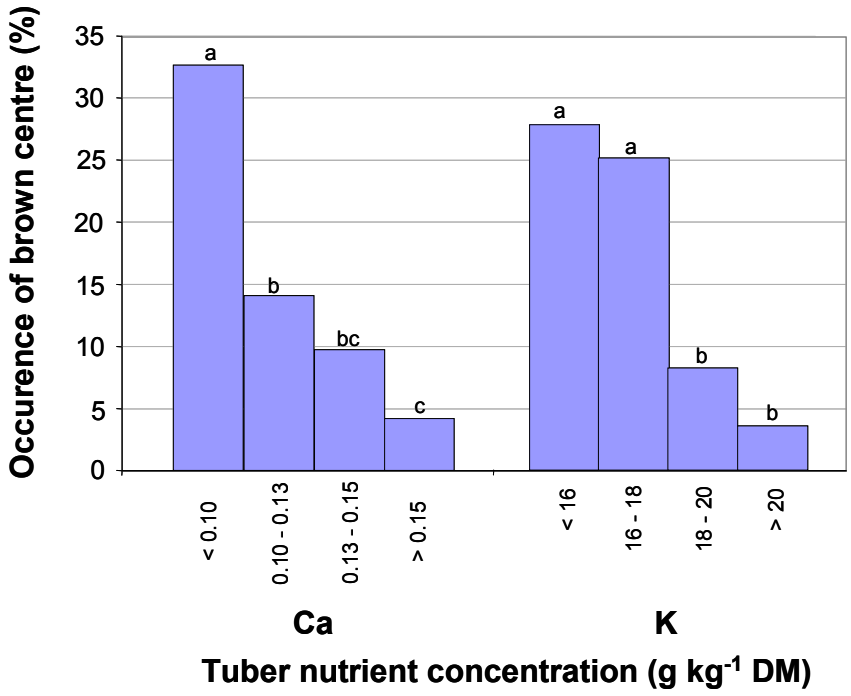

Figure 9 Relationship between calcium and potassium concentration in tuber parenchyma with occurrence of internal brown centre

3.4 Discussion

Calcium is a major nutrient in the soil. Its concentration is about ten times higher than that of K and Mg. However, the Ca concentration in the potato tubers in this investigation was low, about 200 times lower than K and 10–20 times lower than Mg, P, and S. If the Ca accumulates in the tuber through xylem transport or direct absorption via the tuber stolon or tuber surface, this indicates that water influx into tuber was very slow and, according to Addiscott (1974) and Krauss and Marschner (1974), the flow of solution into the tubers through the xylem occurred for only very limited periods, mainly at night when the tops do not transpire. Therefore, Ca concentration in the soil solution as available Ca for the tuber during growth was very important for sustaining a continuous Ca supply into the tuber (Figure 6 and Figure 7). Calcium fertilizer chosen to increase Ca in the tuber should have the capability to maintain high Ca concentration in the soil solution during tuberization.

Gypsum has shown an ability to maintain Ca concentration in the soil solution until harvest. This capability is probably due to moderate solubilization of

gypsum that is ten times more soluble than $CaCO_3$. In steady state condition, the molar solubility of gypsum and $CaCO_3$ in a solution is 5.25 x 10^{-3} and 6.3 x 10^{-4} moles per liter solution at 20°C (Adams, 1974), respectively. Dissolution of Ca-fertilizers occurs when the concentration of their anion and cation is lower than their molar solubility. This condition occurs when water content of soil is increased by the rain and irrigation or when Ca^{2+} concentration is decreased through absorption of the plant root or binding at the negative charge on the soil particle surface. When water content of the soil was low or Ca supply by mass flow was higher than uptake, precipitation of Ca in the root area had been reported by Malzer and Barber (1975). Therefore, in the dry planting season in 2003, the effect of Ca-fertilizer on the Ca level in the tubers was not shown clearly.

Moreover, the results showed that $CaCO_3$ and gypsum reacted with a similar response to the Ca absorption of the negative charge on the soil surface (Figure 5). According to Brady and Weil (2002), exchangeable cations that are available to the plant are replaced by hydrogen ions from root hairs and are released into the soil solution. Because of the application of $CaCO_3$, the Ca ion bound in the negative charge of the soil failed to be released into the soil solution. This happened because of the pH effect of $CaCO_3$, by which H^+ released from root hairs was neutralized by OH^- from $CaCO_3$.

Furthermore, an increase in pH followed by $CaCO_3$ application may affect the plants uptake of macro and micronutrients from the soil (Tyler and Olsson, 2001) but the effect was not shown in the tuber. In contrast to $CaCO_3$, application of gypsum had a little effect on the soil pH. Application of gypsum that increased the Ca level in the soil solution showed an antagonistic effect with Na and therefore it depressed Na uptake of tuber. This result was supported by Cabanero et al. (2004) with their study on pepper plants grown under saline condition.

Many studies reported that increasing the level of Ca in the soil may influence the nutrient uptake by the plant: negatively for K, Mg, P, Na, Zn, Cd,

and Cu and positively for Fe, Mn, Cl, and B (Koenig and Pan, 1996; Escrig and Morell, 1997; Lopez-Lefebre et al., 2001). The influence may be seen clearly in the leaf, but in the tuber the influence did not approved, as also reported by Addiscott (1974) and Clough (1994). The investigation of Locascio et al. (1992) showed also a different response between petiole leaf and tuber tissue in the nutrients concentration. Immobility of Ca in the phloem and different mechanisms of Ca absorption by the tuber has probably caused less correlation between Ca and other nutrients. Moreover, the contribution of direct uptake of the tuber to the concentration of other nutrients is very small due to the low influx via stolons from the soil into the tuber compared to the influx via the phloem.

This study showed that the application methods had no effect on the Ca content in the tubers. Lower Ca content in the tuber when applied with KAS was caused by depression of the ammonium supply from KAS (Havlin et al., 1999). In contrary, Ca content in the tuber was increased by the nitrate supply from KS.

Small response of Ca application in 2003 might be caused by low rainfall in that year. The same result was also found in the study of Locascio et al. (1992) and they argued that with low rainfall soluble salts would be expected to accumulate in the bed near the soil surface and the difference due to Ca rate would be minimized.

The shoots of potato plants in this experiment did not show any symptoms in nutrients deficiency; however the brown centre occurred inside the tubers. This showed a different Ca status between shoots and tubers. However, there exists only little information about the reasons for this symptom, but many studies have reported that Ca fertilizer or tuber Ca reduces the occurrence of brown centre, internal brown spot and internal necrosis disorder (e.g. Clough, 1994; Tzeng et al., 1986; Davies, 1998; Kleinhenz et al., 1999). In this study, the brown centre is caused by tuber deficiency in Ca and K. Brown centre is initiated by cell death and tissue necrosis from tissue that is deficient in

Ca (Bangerth, 1979; Olsen et al., 1996). There is no data that indicates at which Ca level brown centre occurs. As comparison, Seling et al. (2000) reported from their study that necrosis in potato leaves can occur when the plants are growing in soil with Ca-supply levels of 30 and 60 µM Ca or when leaves contain less than 5 g Ca kg^{-1} DM. Furthermore, the Ca-deficient leaves suffer a reduction of Ca in the apoplast and cell wall, followed by increasing diffusible pectin fragments due to increasing polygalacturonase activity.

Kleinhenz et al. (1999) who investigated the Ca concentration in each tuber were found that Ca concentration was not similar between the tubers from one plant. In this study, Ca concentration of each treatment was measured from only 5–6 tubers while brown centre was investigated in each tuber therefore brown centre was also found although the tubers is high in Ca. However, this experiment showed the occurrence of brown centre can be reduced when Ca in the tuber could be increased.

This study also indicated an contribution of K in the occurrence of brown centre. The involvement of K was not caused by Ca-fertilization, because K accumulation in the tubers was affected less significant by Ca-fertilization, only in one location in 2003, but affected more significant by location. The occurrence of brown centre was higher at the locations and in the years when K content of tuber was lower. The reason was not clear and need more investigation but it may be related to the function of K in the tuber tissue in osmoregulation and cation-anion balance.

3.5 Conclusion

Calcium concentration in the potato tubers depended on the Ca concentration in the soil solution during tuber growth. The exchangeable Ca could not be used as a parameter to the Ca concentration in the soil solution. Gypsum could maintain the Ca concentration in the soil solution during tuber growth; therefore it was an effective source for increasing Ca concentration in the potato tubers. These results are supported by Simmons et al. (1988). Moreover,

the concentration of Ca and K had relationship with the occurrence of internal tuber disorder brown centre.

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4 Effect of calcium fertilization, year, and location on internal quality of fresh and stored potato tubers

Abstract

Potatoes cv. Saturna were grown on sandy soil in Northern Germany in three years of field experiments from 2001 to 2003. Three Ca fertilizers were compared in this study; they are gypsum, $CaCO_3$, and basic slag with application rates of 450 and 900 kg Ca ha^{-1} in 2001 and 560, 1120, and 2240 kg Ca ha^{-1} in 2002 and 2003. Dry matter, starch, crude protein, sugars, organic acid, and free amino acid composition were analyzed in fresh and stored potato tubers.

Application of $CaCO_3$ can be a disadvantage for the processing of potato tuber because it may increase the concentration of reducing sugars as well as chlorogenic acid in the fresh and long-term stored tubers. In contrast, application of gypsum had no influence on the reducing sugars during storage. In the dry planting season, application of $CaCO_3$, gypsum and basic slag may reduce the starch synthesis.

4.1 Introduction

Soil acidity is one of the main constraints to crop production in the humid areas of the world. Calcium in the form of calcium oxide, hydroxide, or carbonate are often applied by the farmer not only to adjust the soil pH, but also to accomplish the requirement of plants to Ca. Brady and Weil (2002) suggested that a pH range of about 5.5 to 7.0 seems to be best to promote the availability of plant nutrients. The plants need hydrogen ions to replace the nonacid cations (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) on the cation exchange sites of humus and clay, in that the cations can be available for the plants.

Calcium is essential for plants due to its function in the structure of cell walls and cell membranes, its involvement in many enzyme reactions in the cell, and its function as a secondary messenger in stress conditions (Marschner, 1995). The effect of Ca in potato tubers on the improvement of resistance against disease and environmental stress has been supported by many studies (e.g. Kleinhenz and Palta, 2002; Sterret and Henninger, 1991; Davies, 1998). Therefore, it seems to be important to increase the Ca concentration in

the potato tuber. Several studies have investigated the effectiveness of sources and application methods of Ca fertilizers and its effect on the yield and tuber size (Simmons and Kelling, 1987; Simmons et al., 1988; Kleinhenz et al., 1999; Clough, 1994).

The effect of Ca application on the cations uptake and the function of Ca as activator in many enzyme reactions may influence the chemical composition of the potato tubers. Changes in the chemical composition are related to the quality of the tubers. There are no reports about the effect of Ca on the quality of potato tubers during storage. This chapter discusses the effect of Ca fertilization on the quality of fresh potato tubers after harvest and quality changes during storage.

4.2 Material and methods

Potatoes cv. Saturna were grown in sandy soil in Northern Germany in three years field of experiments from 2001 to 2003. The experiment was performed in plots consisting of six rows 0.75 m apart and 8 m long according to randomized block design with six replications in 2001 and four replications in 2002 and 2003. Three Ca fertilizers were compared in this study: gypsum, $CaCO_3$, and basic slag with application rates of 450 and 900 kg Ca ha^{-1} in 2001 and 560, 1120, and 2240 kg Ca ha^{-1} in 2002 and 2003. Basic slag was applied only in 2003. All plots received 160, 44, 100 and 24 kg ha^{-1} of N, P, K, and Mg, respectively.

The tubers were planted in April and harvested in mid-September, when they achieved their physiological maturity. The climate data are presented in Table 12. The harvested tubers were graded based on sizes of <35, 35 – 50, and > 50 mm. Tubers of middle size were used for the chemical analysis. Investigation was performed on the freshly harvested potatoes and after three, five, and seven months of storage at 8°C and at relative humidity (RH) of 98%. While dry matter and ascorbic acid determination was performed on fresh tu-

bers, the other analyses were performed on the freeze-dried tubers without periderm.

Table 12 Precipitation rate, sunshine length, and temperature during planting seasons 2001 to 2003[a]

	Precipitation (mm)			Sunshine length (hours)			Average Temperature (°C)		
Month	2001	2002	2003	2001	2002	2003	2001	2002	2003
April	51	69	19	147	154	179	8	9	9
May	35	68	26	287	181	212	15	15	15
June	81	72	28	163	198	285	15	18	20
July	35	201	51	277	128	218	20	19	21
August	70	88	25	221	206	267	20	21	21
Total	271	497	148	1095	867	1161			

[a] Data were obtained from http://www.wetteronline.de wheater station Celle

The dry matter was determined according to EAPR (1974). Starch was analyzed polarimetrically according to ICC-Standard no. 123 (Arbeitsgemeinschaft für Getreideforschung, 1994). The crude protein was determined by multiplication of total nitrogen by the factor 6.25. Total nitrogen was analyzed according to the Dumas combustion method by LECO® CN-2000 (Sweeny and Rexroad, 1987). L-ascorbic acid was determined by titration with solution 2,6-dichlorophenol-indophenol (DIP) according to Diemair (1963). Citric acid and sugars (D-glucose, D-fructose, and sucrose) were determined enzymatically by the spectrophotometric method of Roche catalogue no. 0139076 and 716260, respectively. Chlorogenic acid was determined spectrophotometrically according to Griffith et al. (1992). Free amino acid was extracted from freeze-dried potato flour with 6 M hydrochloric acid according to Anonymous (1993) and analyzed with RP-HPLC with the method described by Fisher et al. (2001).

The data are presented as mean ± standard error. The analysis of variance was performed for data analysis and differentiated with Tukey posthoc test at $p<0.05$ using the software SAS release 8.02.

4.3 Results and discussion

4.3.1 Tuber dry matter

Figure 10 shows the change in dry matter of potato tubers fertilized with Ca during storage. The dry matter of potato tubers before storage was not affected by Ca fertilization at any rate of application in 2001, 2002 and 2003 ($p>0.05$). Maier et al. (2002) also found that lime application up to 2000 kg ha^{-1} had no effect on the dry matter of potato tubers.

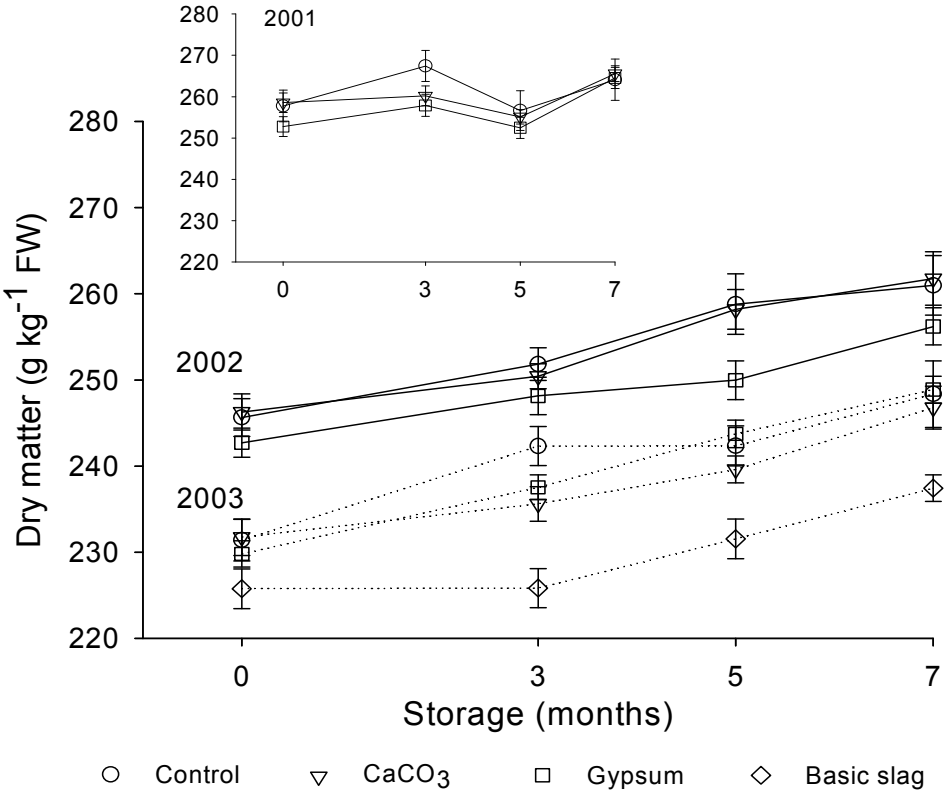

Figure 10 Effect of calcium fertilization on dry matter content of potato tubers during storage. The data are presented as mean value from all Ca levels of application.

In 2003, tuber dry matter was 230 g kg^{-1} FW, the lowest from all seasons. The reason may have been the low precipitation rate in 2003 (Table 12); although sprinkler irrigation was applied, it was insufficient. Water stress during plant growth might have occurred. This is supported by Lynch et al. (1995), who argued that mid-season water stress might decrease the specific gravity of tubers that was correlated to dry matter and starch content. The tu-

bers dry matter in 2002 was lower than in 2001, although the precipitation rate was higher in 2002. There was no indication of water insufficiency in 2001. Higher tuber dry matter in 2001 may have been caused by longer sunshine length which led to a higher photosynthesis rate. The effect of temperature and sunshine length on the increase in dry matter of potato tubers was also described by Kooman et al. (1996).

Generally, dry matter content increased significantly ($P<0.001$) and linearly during storage at 8°C and 95% RH. This result disagrees with the study of Kolbe et al. (1995) on potatoes stored at 8°C and 95% RH, which showed a decrease in the dry matter of tubers during storage time. However, the dry matter content depends on the balance between weight loss due to water evaporation from tubers and weight loss due to starch catabolism or respiration, since starch is the major component of tuber dry matter. Ratovski (1981) suggested that weight loss due to respiration does not exceed 0.5 to 0.6% of the fresh weight of tubers stored at temperatures of 5 to 10°C, while evaporation of water causes 90% of weight loss; therefore, the increase in dry matter was occurred.

The effect of Ca fertilizer on the dry matter content during storage was not significant ($P>0.05$). In 2002 and 2003, application of gypsum and basic slag, which had a lower dry matter before storage, had also a lower dry matter content during storage time than control and application of $CaCO_3$. The dry matter content increased linearly with almost the same slope. This means that the change in dry matter occurred in the same rate and there were no indication that Ca-fertilizers had an influence on the change in dry matter during storage.

4.3.2 Starch

Starch is the main component of dry matter in potato tubers. The effect of Ca fertilization on the starch concentration in potato tubers differed in 2001, 2002, and 2003 (Figure 11). The highest starch concentration was achieved in

2001 but the effect of Ca fertilization at rates up to 900 kg Ca ha^{-1} was not significant. Higher starch concentration in 2001 might have been caused by adequate water availability and longer sunshine length during plant growth. There was no difference between the starch concentrations of the untreated tubers in 2002 and 2003. A positive effect of Ca fertilization was observed when it was applied during the wet planting season in 2002, when application of $CaCO_3$ at a rate of 560 kg Ca ha^{-1} and gypsum at 2240 kg Ca ha^{-1} increased the starch concentration of tubers up to 5%. In contrast, the starch concentration was reduced up to 5.7% when Ca fertilization was performed during the dry planting season 2003.

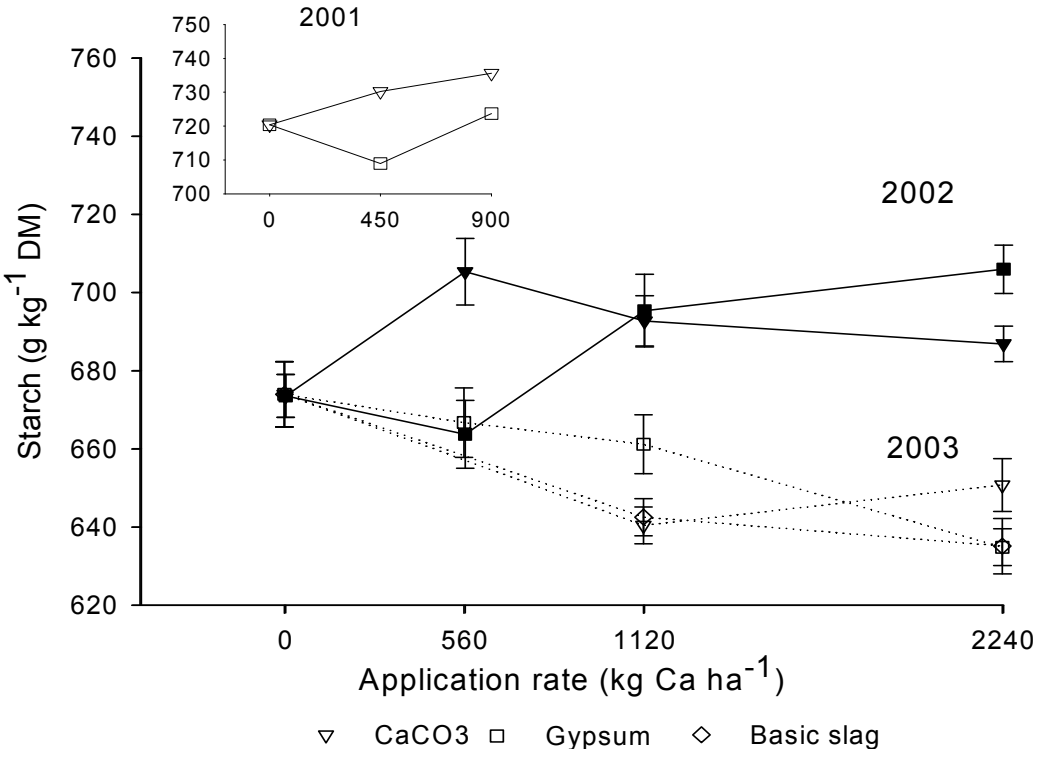

Figure 11 Effect of calcium fertilization on starch concentration of potato tubers before storage.

During storage, tuber starch concentration decreased especially during the first three months accompanied by dormancy breakage that occurred during the third month's storage. After three months of storage, the tuber starch concentration was relatively constant, with a slight increase in the fifth month of storage. This result is supported by Biemelt et al. (2000), who found that

starch breakdown occurred rapidly during the first three months of storage and afterwards it could increase depending on the cultivar. Moreover, the starch breakdown is controlled by hydrolytic (Cottrell et al., 1993) and phosphorolytic enzymes (Sowokinos, 2001), and results in the formation of sucrose and reducing sugars as well as energy that is required for the developing sprouts.

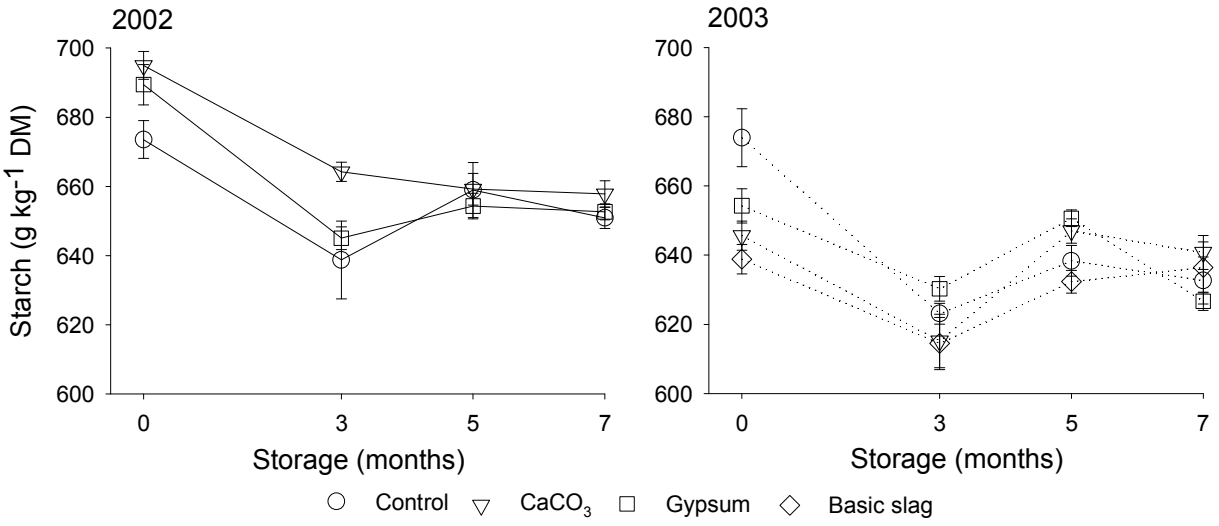

Figure 12 Effect of calcium fertilization on starch concentrations in potato tubers during storage. The data are presented as mean value from all Ca levels of application.

In this study, the alteration of tuber starch concentration during storage was similar between 2002 and 2003 (Figure 12). In 2002, $CaCO_3$ application led to higher tuber starch concentration than in the untreated control and after gypsum application during the first three months' storage, but the effect of Ca fertilization was not significant after five months of storage. In 2003, the effect of Ca fertilization on the tuber starch concentration was not significant compared to the control throughout the storage time.

4.3.3 Sucrose and reducing sugars

Sucrose

Sucrose concentration in the tubers was more affected by the year than by the Ca fertilization (Figure 13). The highest sucrose concentration was ob-

served in the tubers from the dry planting season in 2003, and the lowest was from the season 2001. This result reinforced the presumption that water stress occurred in 2003. According to the study of Geigenberger et al. (1997), water stress led to the partitioning of carbon favor to sucrose synthesis via activation of sucrose 6-phosphate synthase. At the same time, water stress reduced starch synthesis (Figure 11) by inhibiting reactions subsequent to ADP-glucose pyrophosphorylase.

The effect of Ca fertilization on the sucrose concentration was not significant during storage (Figure 14). Sucrose concentration decreased linearly up to five months of storage and afterward the change was not significant. The decrease in sucrose concentration during storage was due to translocation of sucrose from the parenchyma to the vascular tissue to provide energy for the developing sprout (Biemelt et al., 2000).

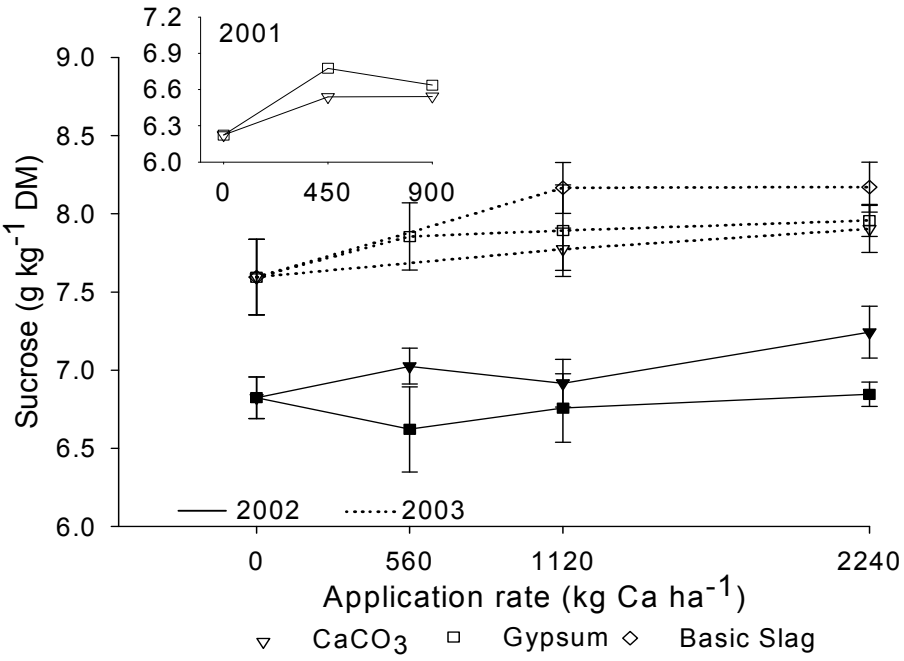

Figure 13 Effect of calcium fertilization on sucrose concentrations in potato tubers before storage.

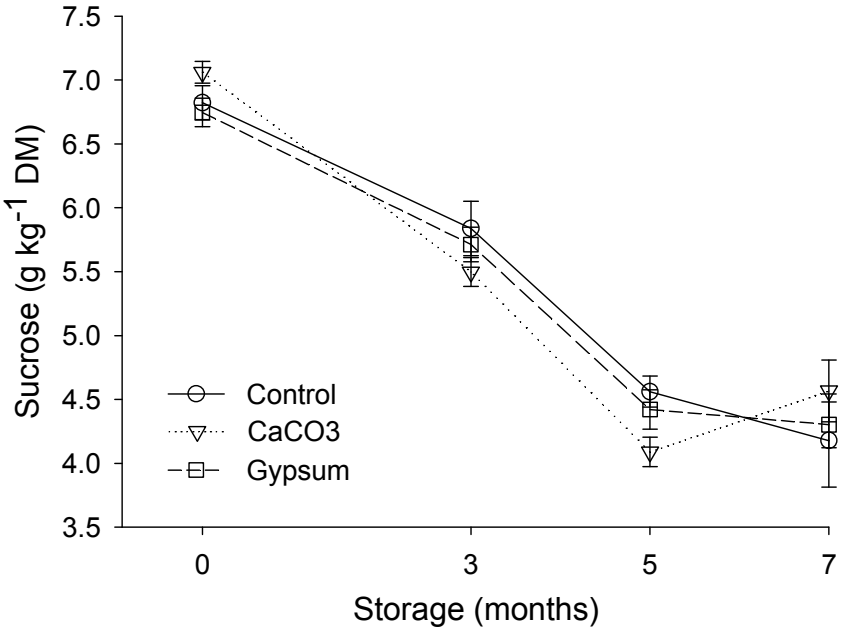

Figure 14 Effect of calcium fertilization on sucrose concentrations in potato tubers during storage from planting season 2002. The data are presented as mean value from all Ca levels of application.

Reducing sugars

Effect of Ca fertilization on reducing sugars accumulation in the tubers before storage is presented in Figure 15. Reducing sugar concentrations in tubers before storage were significantly affected by the application of $CaCO_3$ ($p<0.001$) in 2002, when an increasing rate of $CaCO_3$ increased the concentration of reducing sugars in the tuber. Application of $CaCO_3$ up to 2240 kg Ca ha^{-1} increased the reducing sugars concentration in the tubers almost 200%. Although the effect of $CaCO_3$ application was not significant in 2001 and 2003, the application of $CaCO_3$ led on an average to higher reducing sugars than the use of gypsum.

A comparison between the planting seasons shows that reducing sugars were much higher in 2001, although according to many studies, the dry season in 2003 might have been expected to increase the reducing sugars as found in former studies (Iritani, 1981; Lynch et al., 1995; Sowokinos et al., 2000). In our investigations, in 2003 the effect of water stress on the accumulation of reducing sugar did not occur. This result is supported by Shock et al. (1993), who found that water stress at early, mid-season and late bulking had no effect on

the accumulation of reducing sugars after harvest. The effect was shown after storage.

During storage the concentration of reducing sugars in the tubers increased more than 300% during the first three months of storage and afterwards remained relatively constant, with a small decrease during the following fifth month's storage (Figure 16). In the third and fifth month's storage, there was no significant difference between Ca fertilizers with regard to the reducing sugars concentration. The effect of $CaCO_3$ was observed after seven months of storage, when the reducing sugars concentration was 30% higher than in the control tubers. The reason for the effect of $CaCO_3$ on the reducing sugar accumulation before and after seven months of storage was not clear and more investigations are required for the explanation.

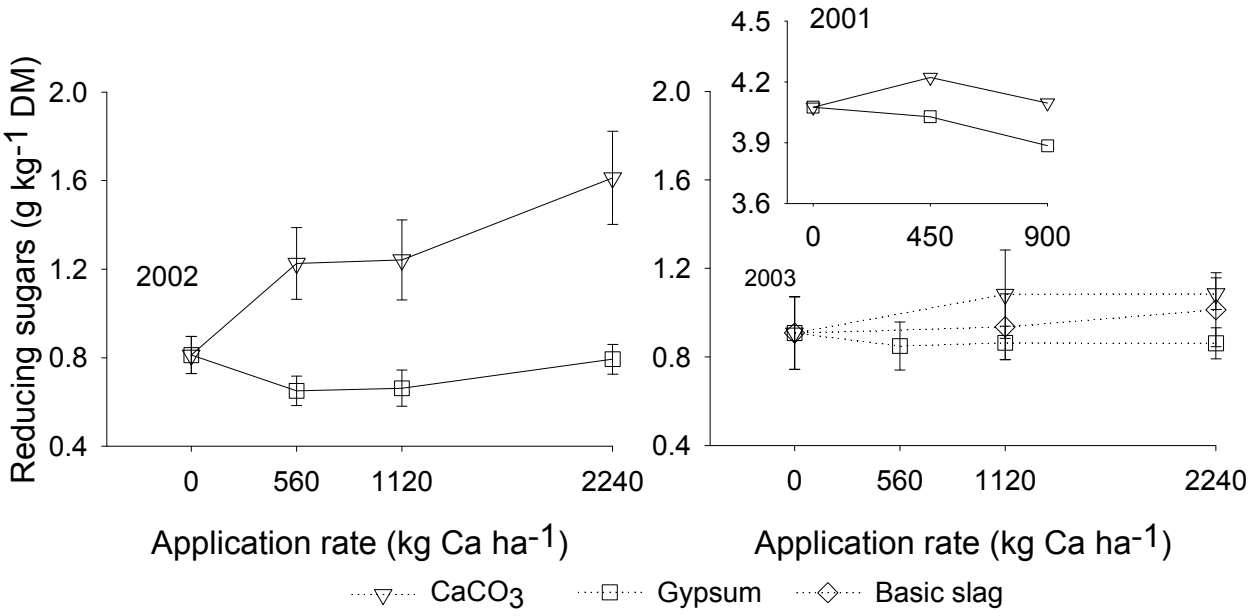

Figure 15 Effect of calcium fertilization on the reducing sugars concentrations in potato tubers before storage.

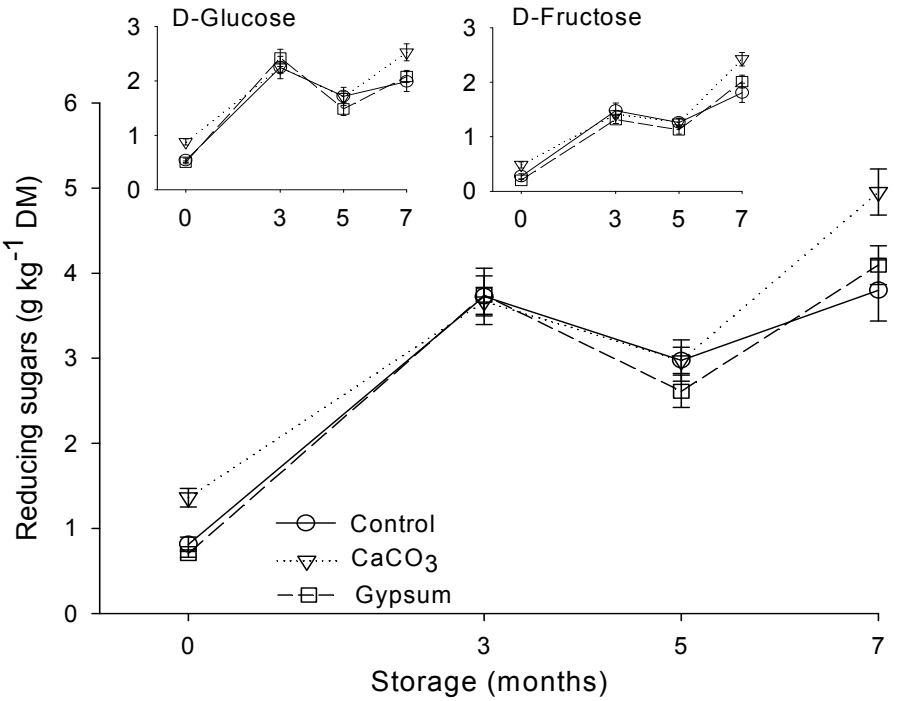

Figure 16 Effect of calcium fertilization on reducing sugars concentrations in tubers during storage. The data are presented as mean value from all Ca levels of application.

4.3.4 Crude protein and free amino acids composition

Crude protein

The effect of fertilizer on the crude protein concentration in the tubers before and after seven months of storage is illustrated in Figure 17. Crude protein was determined by measuring the total nitrogen and multiplying by factor of 6.25. The crude protein in this study ranged between 70 and 90 g kg^{-1} DM before storage and 86 and 112 g kg^{-1} DM after seven months of storage. The crude protein concentration increased significantly ($P<0.01$) in 2002 by gypsum application at the rate of 560 kg Ca ha^{-1}, but there was no effect of Ca fertilization in 2003. The effect of storage was significant ($p<0.001$) in both years: after seven months of storage, crude protein concentration in the tubers increased up to 17% in both years, with the exception of gypsum application at the rate of 560 kg Ca ha^{-1} in 2002. However, there was no indication that Ca fertilization had an affect on the crude protein concentration after storage.

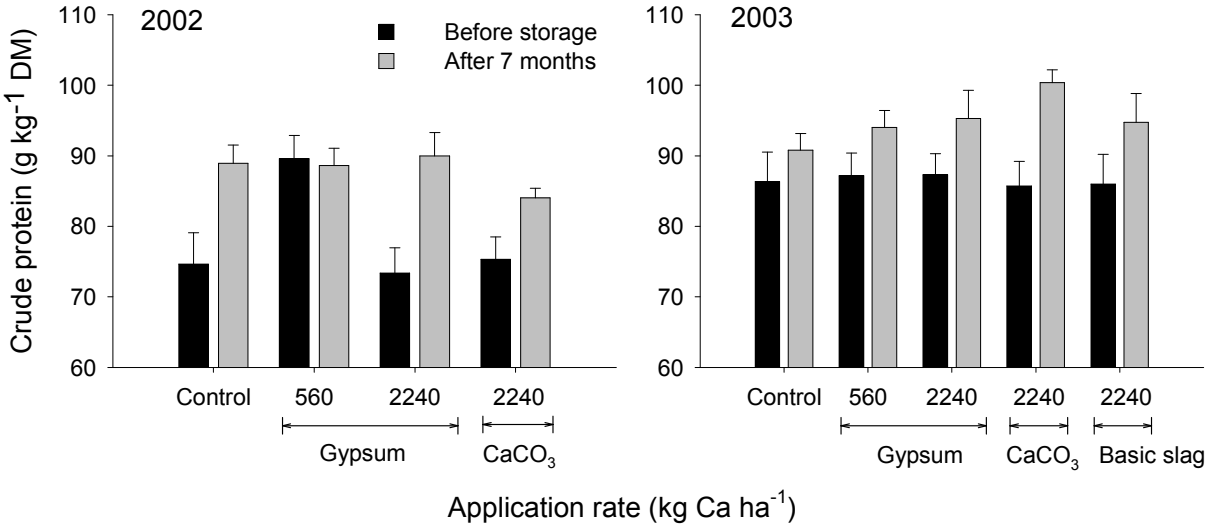

Figure 17 Effect of calcium fertilization on crude protein concentrations in potato tubers before and after seven months storage

According to Meineke (1995) protein concentration in the potato tuber ranged between 70 and 110 g kg^{-1} DM and is particularly affected by nitrogen fertilization. However, the nitrogen fertilization did not affect the pure protein concentration in the tubers, which ranged between 35 and 50% of crude protein. Seibles (1979) divided the tuber protein into soluble and insoluble forms where the soluble form consisted of globulins and albumins in a ratio of 3:1. The study of Ortiz-Medine and Donnelly (2003) with 20 potato cultivars showed that the soluble proteins were higher in the tuber periderm than in the cortex and pith with ranges of 38–73 and 30–49 g kg^{-1} DM, respectively and the content of soluble proteins increased to 16–18% depending on cultivars during long-term storage. The half of crude protein is the nonprotein-N fraction that consists of free amino acids, nitrate, purine derivates (adenine, guanine, xanthaine, hypoxanthine), and glycoalkaloids such as α-solanine and α-chaconine (van Es and Hartmans, 1981). Nevertheless, the free amino acids of tubers are particularly important in potato processing due to their contribution to the Maillard reaction that may lead to dark browning of processed products.

Free amino acids composition

The contents of 17 free amino acids, excluding Pro and Cys, of tubers from planting season 2002 and 2003 are presented in Table 13 and Table 14, respectively. The total free amino acids ranged between 13.0 and 37.6 g kg^{-1} DM. They accounted for 15.2 – 46.3% of crude protein.

The effect of Ca fertilizers on the free amino acids composition was not significant in 2002. In 2003, Ca fertilizers reduced Glu, Val, and Ser content significantly. Whilst, the effect of application rate was not significant in both years.

Table 13 Effect of calcium fertilization and storage on free amino acids and amides compositions of potato tubers from planting season 2002

Amino acids	Before storage			Between fertilizers	After 7 months storage			Between fertilizers	Between storages
	Control	Gypsum	CaCO3		Control	Gypsum	CaCO3		
	---------- % ----------				---------- % ----------				
Asn	47.2	44.3	38.2	ns	33.3	42.7	44.8	ns	ns
Tyr	12.5	12.2	10.8	ns	11.9	10.9	12.4	ns	ns
Gln	8.5	9.5	12.3	ns	12.6	8.7	8.7	ns	ns
Glu	7.1	7.9	8.0	ns	9.8	9.5	8.6	ns	*** (+)
Asp	6.6	7.5	8.0	ns	8.4	7.7	7.7	ns	ns
Arg	5.3	5.8	6.3	ns	8.1	7.5	6.3	*	*** (+)
Val	2.4	2.3	3.1	ns	2.7	2.3	2.2	ns	* (-)
Lys	2.4	2.3	3.0	ns	4.4	3.3	3.0	*	*** (+)
Phe	2.3	2.4	2.9	ns	2.0	2.0	1.5	ns	*** (-)
Ser	0.9	1.1	1.2	ns	1.0	8.4	8.3	ns	** (-)
Ile	1.6	1.3	1.3	ns	1.3	1.2	1.0	ns	*** (-)
Met	1.3	1.4	1.5	ns	1.0	0.7	0.6	**	*** (-)
Thr	0.9	0.9	1.6	ns	1.7	0.8	0.8	ns	ns
Leu	0.7	0.7	0.9	ns	1.0	0.9	0.8	*	*** (+)
Gly	0.3	0.3	0.4	ns	0.6	0.4	0.4	*	** (+)
Ala	0.3	0.3	0.3	ns	0.3	0.3	0.3	ns	* (+)
His	nd	nd	nd		nd	nd	nd		
Total AA	23.3	21.2	21.6	ns	21.5	25.5	24.8	ns	ns

ns, *, **, *** are F tests not significant, and significant at P<0.05, P<0.01 and P<0.001, respectively.
Total AA is total amount of amino acids presented in g kg^{-1} DM.
nd is under detection limit.
(+) and (-) show an increase and a decrease, repectively.

Table 14 Effect of calcium fertilization and storage on free amino acids and amides compositions of potato tubers from planting season 2003

Amino acid	Before storage				Between fertilizers	After 7 months storage				Between fertilizers	Between storages
	Control	Gypsum	CaCO3	Basic slag		Control	Gypsum	CaCO3	Basic slag		
Asn	38.9	43.0	41.5	44.0	ns	42.9	36.9	46.3	45.6	***	ns
Tyr	12.6	13.0	13.9	12.5	ns	9.8	10.5	8.9	8.5	ns	*** (-)
Gln	9.3	9.0	9.6	9.3	ns	10.9	13.1	11.4	11.9	ns	*** (+)
Glu	10.4	8.5	7.9	8.0	***	9.6	10.2	8.7	7.9	*	ns
Asp	7.1	5.6	5.6	5.4	ns	6.4	6.8	5.7	6.0	ns	ns
Arg	6.5	6.9	7.2	7.5	ns	6.8	8.5	7.4	7.1	*	ns
Val	2.7	2.4	2.4	2.2	*	2.2	2.5	2.1	2.2	ns	ns
Lys	2.7	2.8	3.1	2.7	ns	3.1	3.5	3.0	3.0	*	** (+)
Phe	2.5	2.4	2.3	2.2	ns	1.5	1.9	1.4	1.6	**	*** (-)
Ser	1.7	1.2	1.3	1.2	*	1.7	1.2	1.4	1.1	ns	ns
Ile	1.3	1.2	1.2	1.1	ns	1.1	1.2	1.0	1.1	ns	ns
Met	1.3	1.3	1.3	1.3	ns	0.7	0.8	0.7	0.7	**	*** (-)
Thr	1.1	1.0	1.0	1.0	ns	1.0	1.0	1.0	0.9	ns	ns
Leu	0.8	0.8	0.8	0.7	ns	0.9	0.9	0.8	0.9	ns	** (+)
Gly	0.6	0.5	0.5	0.5	ns	0.6	0.3	0.4	0.3	ns	* (-)
Ala	0.6	0.5	0.5	0.5	ns	0.9	0.7	0.4	0.4	***	ns
His	nd	nd	nd	nd		nd	nd	nd	nd		
Total AA	25.3	28.2	28.7	28.7	ns	27.3	26.8	33.0	30.7	ns	ns

ns, *, **, *** are F tests not significant, and significant at P<0.05, P<0.01 and P<0.001, respectively.
Total AA is total amount of amino acids presented in g kg^{-1} DM.
nd is under detection limit.
(+) and (-) show an increase and a decrease, repectively.

Free amino acids increased for about 20% in the tubers which were grown under insufficient water availability in 2003. Increasing of free amino acids, especially Pro, by plants grown under stress is already known, for example in soybean (Jia et al., 2001), in cotton (Showler, 2002), and in spinach (Di Martino, 2003). In this study, in addition to the total content of free amino acids, the free amino acids' composition was altered due to the stress. The major contributors for the increase in free amino acids were Gly and Ala. They increased more than 100%. Phe, Met, Val, Thr, Leu, Tyr, Lys, Arg, and Gln increased 17 – 66% in the dry planting season 2003.

However, the total free amino acids were not affected either by the Ca fertilization or by the storage ($p>0.05$). Brierley et al. (1996) found that the total free amino acid content increased during the first 11 weeks or 25 weeks of storage, afterwards the accumulation of free amino acids was back to the level

before storage. The authors interpreted the increase as a result of the increase in proteinase activity at the onset of dormancy breakage. Therefore in this study, the total free amino acids after seven months of storage were not different from the total free amino acids before storage.

The main free amino acids in the tubers were Asn, Tyr, Gln, Glu, Asp and Arg. They accounted for more than 85% of total free amino acids in the tubers. The composition of free amino acids was altered significantly during storage. Lys and Leu increased, in contrast to Phe and Met, which decreased during storage in both years. Brierley et al. (1997) found the accumulation of the free amide amino acids Asn and Gln during storage of Pentland Dell potato tubers, while the study of Davies (1977) recorded an increase in Arg during storage. However, the study of Davies (1977) on 91 analyses of free amino acids of potato tubers showed the evidence of variation of Gln, Pro, Ala, Val, Tyr, 4-aminobutyric acid and His in relation to location and cultivar as well as weather and soil conditions.

4.3.5 Organic acids

L-ascorbic acid

L-ascorbic acid (vitamin C) in potato tubers can be a major and cheap source of vitamin C in the human diet. Figure 18a shows that Ca fertilization had no influence on the L-ascorbic acid concentration in the potato tubers. The L-ascorbic acid concentration in 2002 was 55% higher than in 2001. This shows that the L-ascorbic acid concentration in potato tubers was strongly affected by the year. The effect of storage on its concentration was high significant ($p<0.001$); the concentration decreased rapidly about 40–50% in the first five months in 2001 and in the first three months in 2002, and thereafter decreased more gradually over the whole storage period. This result is in agreement with Keijbets and Ebbenhorst-Seller (1990). Loss of L-ascorbic acid during storage is related to its function in the cell as an antioxidant and

enzyme cofactor, in electron transport for mitochondria, and in the biosynthesis of oxalate and tartrate (Smirnoff, 1996).

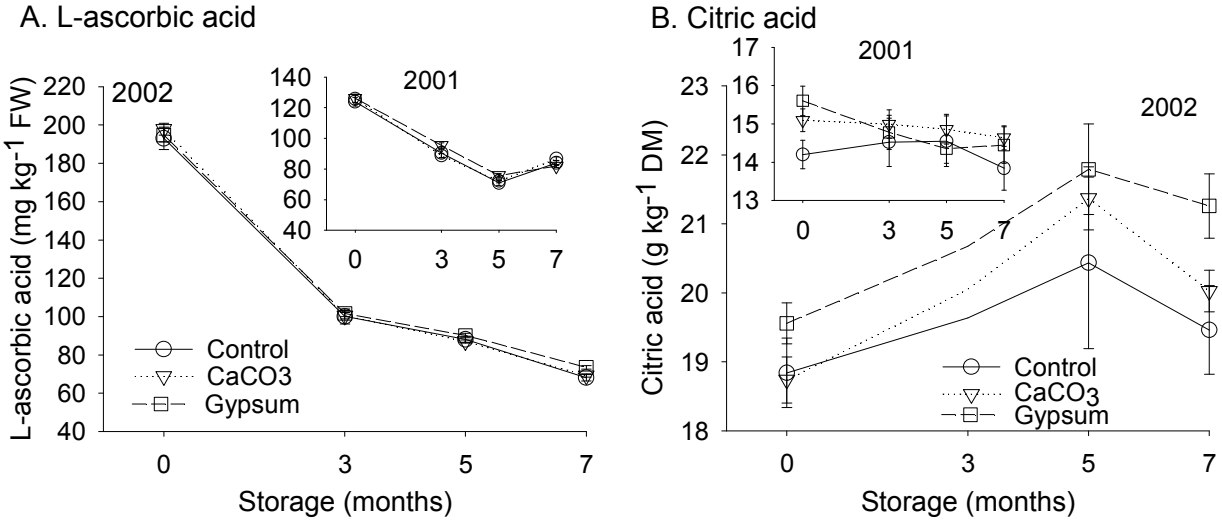

Figure 18 Effect of calcium fertilization on L-ascorbic acid (a) and citric acid (b) concentrations in potato tubers during storage. The data are presented as mean value from all Ca levels of application.

Citric acid

Citric acid is a major organic acid in potato tubers. Citric acid can compete with chlorogenic acid in binding iron to make a colorless complex. Potato tubers high in citric acid have low susceptibility to darkening after cooking (Lisinska and Leszczynski, 1989). Although in the study of Zhang (1989) the concentration of citric acid was affected by N, P, and K fertilization, in this experiment there was no evidence that Ca fertilization had a significant effect ($p>0.05$) on the citric acid concentration in tubers (Figure 18b). During storage, the concentration of citric acid in the tubers was altered slightly in 2001, but in 2002 the citric acid concentration increased by up to 12% during the fifth month of storage. This result is confirmed by van Es and Hartmans (1981).

Chlorogenic acid

Chlorogenic acid is a phenolic compound and well-known for causing darkening after cooking and it seems to be involved in the blackspot formation (Delgado et al., 2001). Chlorogenic acid is concentrated more in the cortex

than in the parenchyma (Lewis et al., 1998) and related to the defense mechanism of the tuber against disease (Jonasson and Olsson, 1994). In this study, the highest chlorogenic acid was found in dry planting season 2003 (Figure 19). This result supported Delgado et al. (2001) who found that tubers from drought-stressed plant had higher concentrations of chlorogenic acid.

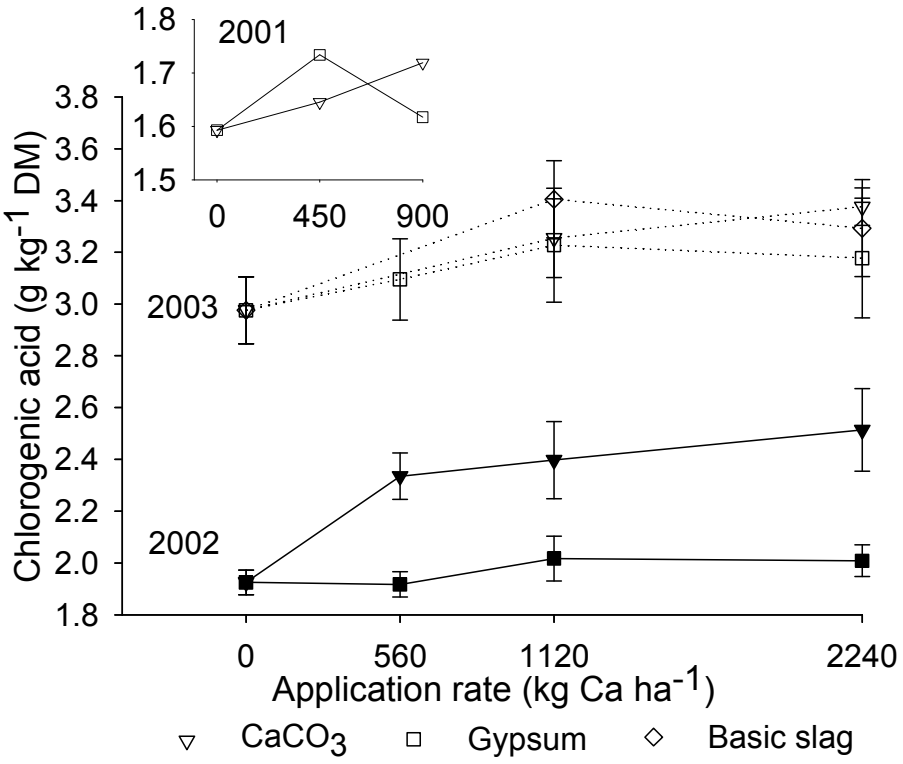

Figure 19 Effect of calcium fertilization on chlorogenic acid concentrations in potato tubers before storage

The effect of Ca fertilizer on the tuber concentration of chlorogenic acid was observed only in 2002, when application of $CaCO_3$ led to a 25% increase in the chlorogenic acid concentration in the tubers. The rate of $CaCO_3$ application had only a slightly effect and was not significant. In contrast, application of gypsum had no effect on the chlorogenic acid concentration in the tuber.

The changes of chlorogenic acid content during storage were different for tubers from planting seasons 2002 and 2003 (Figure 20). In 2002, chlorogenic acid increased during the first three months and decreased thereafter. In 2003, the concentration decreased and achieved the lowest level in the fifth month of storage. Other investigations have reported an increase (Delgado et

al., 2001) and a decrease (Dale et al., 1998) in the tuber concentration of chlorogenic acid during storage. The concentration of chlorogenic acid during storage may depend upon the potato cultivar. Clifford (1999) reported that the concentration of chlorogenic acid rose during storage, especially in the light and during wound healing.

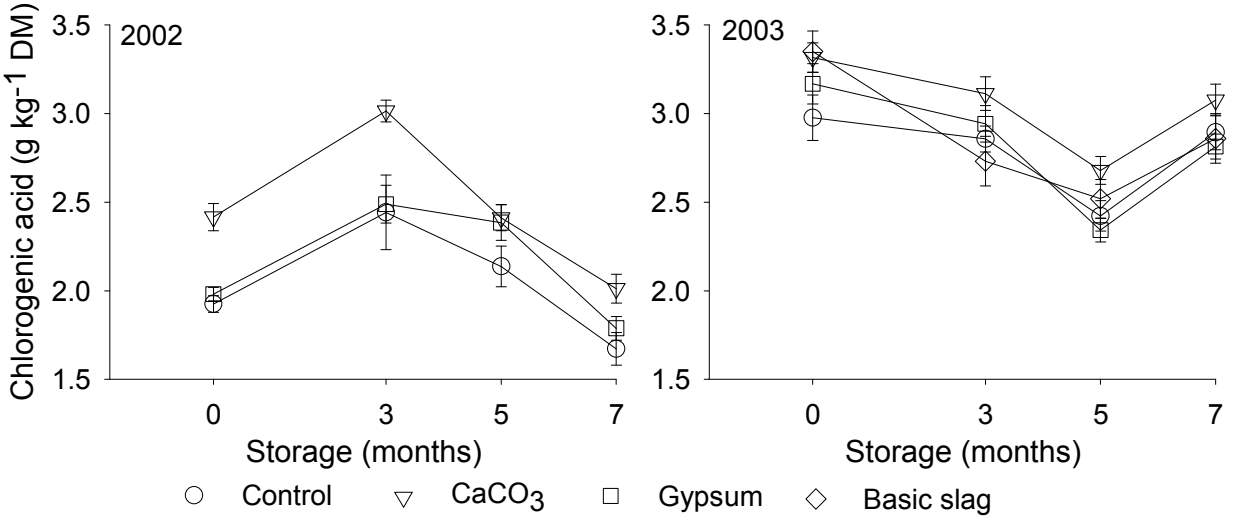

Figure 20 Effect of calcium fertilization on chlorogenic acid concentrations in potato tubers during storage. The data are presented as mean value from all Ca levels of application.

4.3.6 Effect of year and location

Year factor may be defined in older literatures (e.g. Burton, 1966) as annual climate including temperature, relative humidity, day length, light intensity, precipitation rate, and the complex interaction between them and with the plant. The effect of year and location was investigated from the untreated samples to separate it from the effect of Ca fertilization and results are shown the Table 15. The results show that year was the main factor that affected the quality of potato tubers. The dry matter, starch, and reducing sugars were the highest in 2001 and the lowest in 2003. On the other hand, crude protein, organic acid, and sucrose were the highest in 2003 and the lowest in 2001. The year 2002 was intermediate in all these factors. The effect of annual climate was also observed by Kolbe (1990) on dry matter, reducing sugars, and nitrogen protein.

Kooman et al. (1996) concluded that dry matter is a product of photosynthesis, the production depended on day length at emergence, light intensity, temperature and vegetation time. In 2003, although longer sunshine might lead to longer photosynthesis, the precipitation was the lowest. Low in precipitation might cause water stress and reduce the photosynthesis. In 2001, although sunshine length was almost similar to in 2003, the precipitation rate was two times higher than in 2003. In 2002, precipitation was the highest especially in July. High rate of precipitation reduced the sunshine length in this year.

Table 15 Quality parameters of potato tubers from different locations and planting seasons

Parameter	2001		2002		2003		Between years	Between locations
	GO	LGW	LGW	HKB	GO	HKB		
Dry matter	269^{a}	247^{b}	250^{b}	242^{bc}	232^{c}	230^{c}	***	***
Starch	740^{a}	701^{ab}	672^{b}	675^{b}	691^{ab}	657^{b}	***	*
Crude protein	53^{c}	64^{bc}	77^{ab}	72^{abc}	80^{ab}	93^{a}	**	*
D-fructose	1.7^{a}	1.72^{a}	0.24^{b}	0.3^{b}	0.32^{b}	0.09^{b}	***	ns
D-glucose	2.6^{a}	2.13^{a}	0.56^{b}	0.53^{b}	0.86^{b}	0.54^{b}	***	ns
Sucrose	6.1^{c}	6.32^{bc}	7.06^{b}	6.58^{bc}	8.12^{a}	7.06^{b}	***	***
Ascorbic acid	118^{b}	129^{b}	188^{a}	198^{a}			***	ns
Citric acid	14^{b}	14^{b}	20^{a}	18^{a}			***	ns
Chlorogenic acid	1.8^{cd}	1.4^{d}	2^{c}	1.9^{c}	2.7^{b}	3.2^{a}	***	***

***, **, *, and ns are significant at $p<0.001$, $p<0.01$, $p<0.05$, and not significantly different, respectively.
Different letters in the same row showed significant difference at $p<0.05$.
GO, LGW, HKB are the abbreviations for Gross Oesingen, Langwedel, Hankensbuettel, respectively.

Effect of temperature on the dry matter was found by Kolbe (1990) that an increase of temperature for 1°C (from temperature range between 15 and 17°C) led to shorten a vegetation time for one week. Shorter vegetation time means shorter photosynthesis time. During the experiment time, average temperature was almost similar from 2001 to 2003, but in June, the average temperature was the lowest in 2001 and the highest in 2003. At this time, vegetation phase of potato plant entered to the phase of tuber development

and had been showed an effect on the biochemical properties of the tubers. High temperature followed by high transpiration could lead to water stress and decrease the dry matter production.

The effect of location might be defined as all soil factors, including the type of soil and soil acidity, structure, fertility, composition, and also water availability, that influence the nutrient acquisition of plants. The effect of location on the content of dry matter, sucrose, and chlorogenic acid was high significant, but on the content of starch and crude protein it was less significant. The soil type where the potatoes were grown was almost similar, sandy soil. The climate in the locations could be assumed as similar because the distances between them were only few Kilometers. Nutrient composition of the soil and irrigation applied by the farmers and variation of the annual climate were the reason for the variation in the quality parameter of potato tubers.

4.4 Conclusion

Application of Ca for improvement of the Ca concentration in the potato tuber might reduce the quality of the potato tuber, especially after the application of $CaCO_3$. Application of $CaCO_3$ could be a disadvantage for processing potatoes because it might increase the concentration of reducing sugar as well as chlorogenic acid in the fresh harvested and long-term-stored tubers. In dry planting seasons, application of $CaCO_3$, gypsum and basic slag might reduce the starch production.

In contrast to the use of $CaCO_3$, application of gypsum had a no effect on the biochemical properties of potato tubers. However, gypsum was applied due to its effectivity to improve Ca concentration in the potato tubers, while application of $CaCO_3$ led to increase in the soil pH but it failed to increase Ca in the tubers (see Chapter 3). The chemical and biochemical properties of potato tubers might be affected more by the environmental factors such as the soil pH, location, and year, than by the Ca concentration of potato tubers.

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5 Correlation analysis on nutrient composition of potato tubers fertilized with calcium in three years of field experiments and three locations

Abstract

Application of gypsum, $CaCO_3$ and basic slag up to 2240 kg Ca ha^{-1} in three years of field experiment in three locations varied the mineral and chemical composition of potato tubers. The treatments led to a broad range of Mo, Fe, Na, Zn, N, Cu, and Mn concentrations in the potato tubers as well as a wide range of reducing sugars, chlorogenic acids, ascorbic acid, citric acid concentration and free amino acid compositions. The relationship of Mo and Na with chemical compounds in the potato tubers confirmed the role of these nutrients in the metabolism of potato tubers.

5.1 Introduction

The tuber is the storage organ of a potato plant. Chemical compounds accumulated in the tuber are affected by many factors. Particular attention has been paid to the effects of nutrients on the yield and quality of the tubers. Improving yield, quality, and storability of potato tubers as an effect of macronutrients such as N, P, and K has been established and modeled by Kolbe et al. (1995) and Kolbe (1995). Recently, investigations on the effect of Ca became more intensive due to the function of Ca in plant cell metabolism, in the building of cell walls and membranes, and as an activator in many enzyme reactions (White and Broadley, 2003; Marschner, 1995). However, compared to other macronutrients, Ca concentration in the potato tuber is the lowest due to immobility of Ca in the phloem.

Attempts to increase Ca in the potato tuber by application of Ca fertilizers have been carried out by many investigators (e.g. Simmons et al., 1988; Kleinhenz et al., 1999; Locascio et al., 1992). Mineral composition of potato tubers after Ca fertilization and its effect on the yield and the occurrence of internal disorders has been investigated by Clough (1994), but information about the effect of a broad range of mineral nutrient concentration as an impact of Ca

treatment on the quality parameters of potato tubers, as well as on the relationships between mineral nutrients and other quality parameters are not available.

Correlation analysis is a useful method to describe the relationship between two or more factors, and it has been used in many agricultural experiments (Mead et al., 2003). This chapter focused on the relationship between mineral nutrients and quality parameters after Ca fertilization across years and locations.

5.2 Material and methods

Potato cv. Saturna was grown in sandy soil in Northern Germany in three years of field experiment from 2001 to 2003. The experiment was performed in plots consisting of six rows 0.75 m apart and 8 m long according to randomized block design with six replications in 2001 and four replications in 2002 and 2003. Three Ca fertilizers were compared in this study: gypsum, $CaCO_3$, and basic slag (which was applied only in 2003) with application rates of 450 and 900 kg Ca ha^{-1} in 2001 and 560, 1120, and 2240 kg Ca ha^{-1} in 2002 and 2003. All plots received 160, 44, 100, and 24 kg ha^{-1} of N, P, K, and Mg, respectively. The tubers were planted in April and harvested in mid-September when the tubers achieved their physiological maturity. Investigations were performed on the freshly harvested tubers.

The dry matter was determined according to EAPR (1974). Starch was analyzed polarimetrically according to ICC-Standard no. 123 (Arbeitsgemeinschaft für Getreideforschung, 1994). The crude protein was determined by multiplication of total nitrogen by the factor 6.25. Total nitrogen was analyzed according to the Dumas combustion method by LECO® CN-2000 (Sweeny and Rexroad, 1987). L-ascorbic acid was determined by titration with solution 2,6-dichlorophenol-indophenol (DIP) according to Diemair (1963). Citric acid and sugars (D-glucose, D-fructose, and sucrose) were determined enzymatically by the spectrophotometric method of Roche catalogue no. 0139076 and

716260, respectively. Chlorogenic acid was determined spectrophotometrically according to Griffith et al. (1992). Free amino acid was extracted from freeze-dried potato flour with 6 M hydrochloric acid according to Anonymous (1993) and analyzed with RP-HPLC with method described by Fisher et al. (2001). Mineral nutrients of the tubers' parenchyma and periderm were determined by digestion of freeze-dried samples in 65% HNO_3 by using a microwave (Mega 1200) according to Abu-Samra, et al. (1975) and the macro and micronutrients of the solution were analyzed on an inductively coupled plasma atomic emission spectrometer (Perkin Elmer Optima 3000).

The relationship between quality parameters and mineral nutrients from all Ca treatments was correlated to each other across years and locations. The Pearson correlation coefficient as well as the F-distribution ratio and the significance were calculated by using the procedure correlation of release SAS 8.02. The relationship was explained by the linear relationship Pearson's coefficient correlation more than 0.70. The variation of the quality parameters was described as percentage of standard deviation from the mean value.

5.3 Results and discussion

5.3.1 Mineral nutrients in the potato tuber

The effect of Ca fertilization, location, year, and the interaction between them led to broad variation in mineral nutrient concentrations in the potato tubers as shown in Table 16. The most affected mineral nutrient was Mo with a variation of 70 and 63% in the tuber parenchyma and periderm, respectively. Moderately-impacted mineral nutrients were Fe, Na, Zn, and Ca, as well as N, Cu, and Mn. For these nutrients, the variation ranged between 15 and 56%.

Potassium and N have important roles as main macronutrients in the parenchyma and periderm of potato tubers. They accounted for almost 90% of the macronutrient composition of the parenchyma and periderm. The main micronutrients were Fe and Zn. They accounted for 70 and 82% of the micronutrient composition of the parenchyma and periderm, respectively. Generally,

micronutrients were concentrated more in the periderm than in the parenchyma. Their contents in the periderm and parenchyma were 2.39 and 0.13% of the total mineral nutrients, respectively. While Ca, Fe, B, and Cu were mineral nutrients which concentrated in the tuber periderm, Mo was concentrated in the tuber parenchyma. All mineral nutrients had a highly significant correlation ($P < 0.001$) between their concentrations in the periderm and in the parenchyma, but only Ca, K, S, N, and Na had a high Pearson's correlation coefficient. However, all mineral nutrients have specific functions and in certain contents they are essential for the potato tuber or plant (Marschner, 1995). Inadequacy or toxicity (excess) of the minerals is always associated with nutritional disorders.

Table 16 Mean, standard deviation, minimum, and maximum concentrations of macro- and microelements as well as ratio of mineral concentration in tuber parenchyma and periderm and the correlation between them

Element	Tuber parenchyma				Tuber periderm				Pearson corr.†	Ratio p/m††
	Mean	Stdv.	Min	Max	Mean	Stdv.	Min	Max		
Ca	0.12	0.04	0.06	0.23	0.71	0.25	0.39	1.42	0.83	5.87
K	17.71	2.07	12.90	22.51	29.24	4.23	20.40	38.06	0.88	1.65
Mg	0.91	0.08	0.70	1.10	1.14	0.15	0.84	1.56	0.51	1.26
P	2.04	0.27	1.49	2.77	2.18	0.25	1.57	3.06	0.61	1.07
S	1.44	0.23	0.90	1.97	1.84	0.32	1.09	2.69	0.87	1.27
N	11.26	2.50	5.93	17.42	20.59	4.23	10.95	30.40	0.88	1.82
Na	0.03	0.01	0.01	0.05	0.03	0.01	0.01	0.06	0.86	1.04
B	3.94	0.45	2.82	5.38	8.36	1.07	6.10	11.27	0.17	2.12
Cu	2.47	0.60	1.31	4.42	5.36	1.21	2.85	8.13	0.75	2.17
Fe	16.13	2.90	10.03	36.39	84.58	47.22	24.67	223.17	0.34	5.24
Mn	6.67	1.02	4.87	9.22	8.92	2.03	5.12	15.23	0.35	1.34
Mo	0.33	0.23	0.01	0.88	0.16	0.10	0.01	0.59	0.31	0.50
Zn	14.93	2.95	8.00	31.80	25.88	8.69	10.98	77.37	0.36	1.73

Ca, K, Mg, P, S, N, and Na are in g kg^{-1} DM

B, Cu, Fe, Mn, Mo, and Zn are in mg kg^{-1} DM

† Pearson correlation between mineral nutrients in the parenchyma and periderm

†† Ratio p/m is the ratio between the concentration of a mineral in the periderm and the parenchyma

Stdv, Min and Max are standard deviation, minimum and maximum concentration

Table 17 Correlation matrix between mineral nutrients in potato tuber parenchyma

	Ca	K	Mg	P	S	N	Na	B	Cu	Fe	Mn	Mo
K	0.34**											
Mg	0.04	0.57***										
P	-0.02	0.17	0.17									
S	0.58***	0.40***	0.32**	0.38**								
N	0.15	0.30*	0.04	-0.01	0.41***							
Na	0.48***	0.26*	-0.13	-0.03	0.13	0.44***						
B	0.04	0.13	0.39**	-0.01	-0.08	0.06	-0.25*					
Cu	-0.10	0.51***	0.16	0.55***	0.50***	0.10	0.14	-0.03				
Fe	0.21	0.19	-0.05	0.16	0.25*	0.51***	0.15	0.00	0.21			
Mn	0.28*	-0.22	0.36**	0.35**	0.31*	0.17	-0.37**	0.37**	0.06	0.16		
Mo	0.03	-0.53***	-0.12	0.37**	-0.15	-0.51***	-0.53***	-0.06	-0.03	-0.13	0.44***	
Zn	-0.28*	0.17	0.26*	0.12	0.35**	0.31*	0.02	0.31*	0.11	0.08	0.35**	-0.08

*, **, *** show a significant correlation at P<0.05, P<0.01, and P<0.001

The concentration of mineral nutrients in the plant tissue depends on many factors, for instance, nutrient availability in the soil, soil type and condition, the complexity of the uptake mechanism of the plant root, and some environmental factors such as water availability, temperature, evaporation rate, etc. Moreover, interactions between minerals during their uptake and transport are reported to determine the nutrient composition in the plant tissue. Regression analysis in Table 17 and Table 18 was carried out to determine the interactions between minerals in the potato tubers.

In the tuber parenchyma, highly significant correlation of some minerals was found but the Pearson's correlation coefficient was low. The low correlation coefficient may be due to the fact that most of the minerals concentrated more in the periderm than in the parenchyma. For example Mo, a mineral which was concentrated more in the parenchyma, had a highly significant correlation with K, P, N, Na, and Mn, but the correlation coefficient was lower and not high enough to explain the correlation. To explain the interaction between mineral nutrients in the potato tuber, the analysis must be focused on the periderm (Table 18).

Table 18 Correlation matrix between mineral nutrients in potato tuber periderm

	Ca	K	Mg	P	S	N	Na	B	Cu	Fe	Mn	Mo
K	0.71***											
Mg	0.45***	0.86***										
P	-0.49***	-0.44***	-0.49***									
S	0.89***	0.73***	0.46***	-0.33**								
N	0.59***	0.59***	0.45***	-0.43***	0.52***							
Na	0.37**	0.57***	0.69***	-0.46***	0.29*	0.55***						
B	0.59***	0.85***	0.89***	-0.60***	0.53***	0.56***	0.65***					
Cu	0.40***	0.60***	0.48***	-0.13	0.36**	0.32*	0.35**	0.47***				
Fe	0.60***	0.64***	0.54***	-0.42***	0.59***	0.66***	0.74***	0.56***	0.42***			
Mn	0.66***	0.71***	0.53***	-0.34**	0.68***	0.68***	0.60***	0.60***	0.48***	0.83***		
Mo	-0.12	-0.09	-0.03	0.09	-0.12	0.07	0.01	-0.07	-0.04	-0.06	0.02	
Zn	0.21	0.34**	0.25*	-0.13	0.14	0.39**	0.31*	0.37**	0.35**	0.25*	0.38**	0.02

*, **, *** show a significant correlation at P<0.05, P<0.01, and P<0.001

In the potato periderm, correlation between Ca and S resulted from the application of gypsum. Correlation of Ca and S by the gypsum application was also reported by Clough (1994). Although Ca had a higher coefficient correlation with K than with S, due to the different transport mechanism of Ca to the tubers (Kratzke and Palta, 1985; Busse and Palta, 2004), the correlation must be carefully discussed. An increase in K was followed by an increase in Mg. Moreover, increases in K and Mg were associated with increases in B, Mn, and Fe. Furthermore, no indications of antagonism between elements were found in the potato tubers with exception of P in this study. Significant negative correlations of P with S, N, Na, B, Fe and Mn were observed but the Pearson's coefficient correlations were low.

Antagonism between K with Ca and Mg has been reported especially by high levels of Ca and Mg in the soil solution that may reduce K uptake by the plant because cations tend to compete against one another for uptake by roots (Brady and Weil, 2002). In the potato leaves, antagonism of K with Mg and Ca had been reported by Nogueira et al. (1996) but nutrients concentration in the leaves did not show the concentration in the tubers. The study of Simmons

and Kelling (1987) showed a not significant relationship between Ca in the leaf and in the tubers. In potato tubers, reduction in K and Mg concentration after addition of Ca had been reported by Simmons et al. (1988) and Simmons and Kelling (1987), but it was not consistently occurred as reported by Locascio et al. (1992) and Clough (1994).

5.3.2 Relationship between quality parameters of potato tubers

The variation of contents of some quality parameters of potato tubers is presented in Table 19. The variation as effects of Ca fertilization, location, year, and the interaction between them were found for reducing sugars. For D-fructose, the variation was 83% and for D-glucose, it was 65%. Moderately affected compounds were chlorogenic acid, crude protein, ascorbic acid, citric acid, and total free amino acids with considerably lower variation.

Table 19 Mean, standard deviation, minimum and maximum concentrations of potato tubers' quality parameters

Parameters	Mean	Stdv	Min	Max
Dry matter (g DM kg^{-1} FW)	247.1	15.2	214.4	281.6
Starch (g kg^{-1} DM)	694.7	42.4	613.5	812.9
Sucrose (g kg^{-1} DM)	6.76	0.61	5.34	8.43
D-glucose (g kg^{-1} DM)	1.59	1.03	0.30	4.07
D-fructose (g kg^{-1} DM)	1.00	0.83	0.01	3.25
Ascorbic acid (in mg kg^{-1} FW)	151.7	37.3	100.5	236.3
Citric acid (g kg^{-1} DM)	16.32	2.56	11.16	22.40
Chlorogenic acid (g kg^{-1} DM)	21.83	6.90	12.21	40.37
Crude Protein (g kg^{-1} DM)	70.4	15.6	37.0	108.9
Total free amino acids (g kg^{-1} DM)	25.59	5.37	12.99	37.39

Stdv, Min and Max are abbreviation of standard deviation, minimum and maximum concentration

The relationship between the quality parameters of potato tubers is presented in Table 20. A high relationship could be seen between D-fructose and D-glucose, which are components of reducing sugars. Sucrose formed as a product of starch degradation through the hexogenesis pathway was converted to reducing sugars in the vacuole (Sowokinos, 2001). A positive correla-

tion was observed between reducing sugars and dry matter as well as starch as the main component in the dry matter of potato tubers. This result was supported by Sowokinos et al. (2000) and Pritchard and Scanlon (1997), who found that dry matter in the tubers correlated with the reducing sugar concentration. Moreover, D-glucose was observed to have a negative correlation with L-ascorbic acid. This may be related to the biosynthesis of L-ascorbic acid from D-glucose in the cytosol, chloroplast, vacuole, mitochondria, and cell wall (Smirnoff, 1996).

In dry matter composition, starch and crude protein correlated negatively to each other. This result is supported by the study of Zahedi et al. (2004) on wheat and Meineke (1995) on potato tubers, who found that starch deposition and protein accumulation were different in responses to environmental factors and confirmed that these two components accumulate independently of each other. Meanwhile, protein had a relatively high correlation with free amino acid content in the potato tuber.

Table 20 Correlation matrix between quality parameters of potato tubers

No	Parameters	1	2	3	4	5	6	7	8	9
1	Dry matter									
2	Starch	0.68***								
3	Sucrose	-0.48***	-0.48***							
4	D-Glucose	0.60***	0.56***	-0.34***						
5	D-Fructose	0.54***	0.53***	-0.31***	0.96***					
6	Ascorbic acid	-0.51***	-0.48***	0.26***	-0.79***	-0.74***				
7	Citric acid	-0.61***	-0.55***	0.43***	-0.76***	-0.72***	0.74***			
8	Chlorogenic acid	-0.60***	-0.53***	0.52***	-0.53***	-0.58***	0.48***	0.45***		
9	Protein	-0.73***	-0.70***	0.50***	-0.77***	-0.75***	0.63***	0.71***	0.69***	
10	Total AA	-0.36**	-0.63***	0.23	-0.20	-0.33*	-0.27	0.67***	0.57***	0.77***

*, **, *** show a significant correlation at P<0.05, P<0.01, and P<0.001

AA is an abbreviation of free amino acids

Although the total content of free amino acids was affected only moderately by the treatments, more effects could be seen in the composition of free amino acids (Table 21). The most-affected free amino acids were Ser, Gly and Ala with a variation between 50 and 55%, respectively. Moderately af-

fected free amino acids were Asn, Glu, Arg, Thr, and Leu, which had a variation between 30 and 40%.

The relationships between free amino acids in the potato tubers are presented in Table 22. The total free amino acids had a high relationship with most of free amino acids. Between the free amino acids, positive relationships were observed. However, the explanation for the relationships was difficult because the free amino acid composition of the potato tubers varied over a wide range depending on cultivar and environmental factors, as reported by Davies (1977) and Synge (1977). Free amino acid composition may be related to the amino acid composition of the potato protein due to the activity of proteinase as reported by Brierley et al. (1996 and 1997). Moreover, some amino acids will be accumulated more under abiotic stress conditions, for example Gly and Ser at salt stress (Di Martino et al., 2003), Tyr at K deficiency (McNabnay, 1999) and Pro at water deficit stress (Showler, 2002).

Table 21 Mean, standard deviation, minimum, and maximum concentration of free amino acids in the potato tuber

Amino acids	Mean	Std	Min	Max
Asn	10.92	3.55	1.24	18.83
Tyr	3.08	0.86	0.29	4.89
Gln	2.43	0.64	0.70	4.54
Glu	2.09	0.63	0.40	3.47
Asp	1.60	0.37	0.31	2.41
Arg	1.70	0.57	0.44	3.13
Val	0.62	0.16	0.11	0.96
Lys	0.68	0.19	0.15	1.23
Phe	0.60	0.15	0.14	0.91
Ser	0.32	0.16	0.16	1.20
Ile	0.31	0.08	0.06	0.48
Met	0.33	0.08	0.09	0.51
Thr	0.26	0.08	0.06	0.51
Leu	0.19	0.06	0.04	0.39
Gly	0.11	0.06	0.01	0.41
Ala	0.11	0.06	0.03	0.44

Stdv, min and max are abbreviation of standard deviation, minimum and maximum concentration in g kg^{-1} DM

Table 22 Correlation matrix between free amino acids of potato tubers

	Total AA	ASN	TYR	GLN	GLU	ASP	ARG	VAL	LYS	PHE	SER	ILE	MET	THR	LEU	GLY
ASN	0.93***															
TYR	0.76***	0.67***														
GLN	0.83***	0.68***	0.59***													
GLU	0.75***	0.52***	0.62***	0.68***												
ASP	0.73***	0.60***	0.42**	0.69***	0.80***											
ARG	0.88***	0.72***	0.64***	0.88***	0.74***	0.60***										
VAL	0.83***	0.65***	0.60***	0.82***	0.76***	0.78***	0.82***									
LYS	0.73***	0.55***	0.69***	0.80***	0.60***	0.40**	0.86***	0.75***								
PHE	0.87***	0.75***	0.62***	0.78***	0.67***	0.75***	0.81***	0.93***	0.72***							
SER	0.62***	0.50***	0.55***	0.69***	0.74***	0.63***	0.68***	0.68***	0.65***	0.64***						
ILE	0.80***	0.70***	0.48***	0.75***	0.52***	0.67***	0.77***	0.90***	0.69***	0.93***	0.55***					
MET	0.84***	0.72***	0.66***	0.79***	0.63***	0.67***	0.83***	0.88***	0.73***	0.92***	0.63***	0.86***				
THR	0.64***	0.45***	0.46***	0.81***	0.73***	0.72***	0.68***	0.82***	0.69***	0.73***	0.71***	0.63***	0.70***			
LEU	0.73***	0.64***	0.48***	0.71***	0.50***	0.51***	0.79***	0.76***	0.78***	0.79***	0.66***	0.88***	0.73***	0.59***		
GLY	0.52***	0.40**	0.59***	0.55***	0.67***	0.47***	0.57***	0.57***	0.63***	0.52***	0.90***	0.41**	0.54***	0.66***	0.56***	
ALA	0.59***	0.46***	0.56***	0.62***	0.71***	0.50***	0.68***	0.60***	0.69***	0.56***	0.95***	0.49***	0.55***	0.63***	0.69***	0.89***

*, **, *** show a significant correlation at P<0.05, P<0.01, and P<0.001
AA is abbreviation of free amino acids

5.3.3 Relationship between mineral nutrients and quality parameters of potato tubers

The relationship between the mineral nutrients and quality parameters of potato tubers is presented in Table 23. Ca was observed to have no correlation with any of the quality parameters in the potato tuber with the exception of citric acid, total free amino acid as well as Asn, but the coefficient of correlation was low. High positive correlation was recorded between K and citric acid. This result is supported by the study of Zhang (1989) on potatoes fertilized with N, P, and K. A relatively high correlation was found between Mo and chlorogenic acid.

Table 23 Correlation between mineral nutrients and quality parameters of fresh potato tubers

	Dry matter	Starch	Sucrose	Glucose	Fructose	Ascorbic acid	Citric acid	Chlorogenic acid
Ca	-0.17	-0.13	0.10	-0.17	-0.21	-0.14	0.49**	0.10
K	-0.48***	-0.29*	0.54***	0.05	-0.20	0.09	0.87***	0.39**
Mg	-0.05	0.04	0.26*	-0.02	-0.10	0.18	0.56***	-0.07
P	0.22	0.06	-0.30*	0.00	0.09	-0.15	0.24	-0.24
S	-0.13	0.01	0.03	-0.11	-0.15	-0.18	0.33*	0.06
Na	-0.47***	-0.21	0.41***	0.26*	0.00	-0.26	-0.16	0.67***
B	-0.05	-0.12	-0.07	-0.38	-0.38**	0.10	0.24	-0.05
Cu	-0.17	-0.14	0.10	-0.02	-0.10	-0.02	0.39*	0.00
Fe	-0.18	-0.41	0.12	0.03	-0.09	-0.09	0.41*	0.13
Mn	0.36**	0.09	-0.34**	-0.27*	-0.06	-0.12	0.43**	-0.47***
Mo	0.65***	0.48***	-0.62***	-0.01	0.33**	-0.06	-0.30	-0.73***
Zn	0.01	-0.12	-0.16	-0.33**	-0.27	0.29	0.48**	0.01
N	0.00	-0.55***	-0.06	0.11	0.10	-0.45***	0.50***	0.48***

*, **, *** show a significant correlation at P<0.05, P<0.01, and P<0.001

Table 24 Correlation between mineral nutrients and free amino acids of potato tubers

	Ca	K	Mg	P	S	Na	B	Cu	Fe	Mn	Mo	Zn
Asn	0.53***	0.13	-0.25	0.08	0.37*	0.28*	-0.15	0.12	0.41**	0.13	-0.34**	0.28*
Tyr	0.03	0.28*	-0.01	-0.04	0.04	0.45***	0.03	0.06	0.11	-0.22	-0.61***	0.16
Gln	0.16	0.08	-0.09	-0.07	0.15	0.21	0.06	-0.08	0.43**	0.18	-0.35**	0.18
Glu	0.05	0.17	-0.24	-0.24	0.00	0.61***	-0.02	-0.17	0.11	-0.36**	-0.71***	0.13
Asp	0.12	-0.17	-0.34*	-0.15	0.12	0.37**	-0.07	-0.28*	0.20	0.02	-0.24	0.19
Arg	0.26	0.32*	-0.12	-0.09	0.20	0.44***	-0.04	0.13	0.37**	-0.07	-0.60***	0.14
Val	-0.01	0.01	-0.14	-0.07	0.05	0.43**	0.06	-0.13	0.33*	0.05	-0.43***	0.14
Lys	0.09	0.38**	0.11	-0.14	0.04	0.37**	-0.07	0.07	0.26	-0.16	-0.56***	-0.03
Phe	0.21	0.12	-0.10	-0.07	0.23	0.33*	0.00	-0.10	0.35**	0.09	-0.42***	0.18
Ser	0.03	0.30*	-0.07	-0.19	0.05	0.66***	-0.03	-0.22	0.17	-0.31*	-0.70***	0.05
Ile	0.16	0.16	0.00	0.14	0.30*	0.28*	0.03	0.12	0.37**	0.25	-0.24	0.18
Met	0.17	0.14	-0.07	-0.13	0.17	0.26	0.14	-0.04	0.35**	0.10	-0.40**	0.20
Thr	-0.09	-0.10	-0.20	-0.27	-0.11	0.33*	0.02	-0.35*	0.35*	-0.12	-0.35**	-0.06
Leu	0.20	0.45***	0.08	0.22	0.33*	0.37**	-0.09	0.27*	0.40**	0.01	-0.33*	0.09
Gly	-0.06	0.30*	-0.06	-0.24	-0.06	0.66***	-0.12	-0.18	-0.08	-0.53***	-0.70***	-0.04
Ala	0.07	0.52***	-0.02	-0.07	0.09	0.71***	-0.14	0.02	0.15	-0.47***	-0.76***	-0.06
Total AA	0.38**	0.19	-0.22	-0.03	0.28*	0.44***	-0.09	0.04	0.40**	0.01	-0.54***	0.25

*, **, *** show a significant correlation at P<0.05, P<0.01, and P<0.001, respectively
AA is abbreviation of free amino acids

The functions of Mo as a plant nutrient are related to the valency changes it undergoes as a metal component of enzymes (Marschner, 1995). Moreover, Mo is indicated to be involved in protein synthesis. The correlation between Mo and chlorogenic acid indicates involvement of Mo in the shikimate pathway which is a synthesis pathway of phenolic compounds such as chlorogenic acid, caffeic acid, and phenolic amino acids such as Phe, Tryp, and Tyr (Harborne, 1979).

Furthermore, Mo as well as Na have significant correlations with a number of free amino acids in potato tubers as shown in Table 24. The correlation of free amino acids with Mo and Na was antagonistic. Boghour et al. (2003) found a strong relationship in potato plants between Mo and nitrate reductase, which catalyzes the reduction of nitrate to nitrite, a fundamental process for the

conversion of mineral to organic N. In the winter wheat leaf, application of Mo increased the free amino acids in the vegetative growth stage and caused a decrease in their levels during the ear sprouting stage (Hu et al., 2002). In the potato tuber, an increase in Mo concentration led to a decrease in most of the free amino acids.

Contrary to Mo, Na had a positive significant relationship with free amino acids. Plants that are grown in soil with a high concentration of Na have high levels of free amino acids in their leaves (Di Martino et al., 2003) as well as in the grains of cereals (Simon-Sarkadi et al., 2002). In the potato tuber, an increase in Na was followed by an increase in free amino acids, particularly Ala, Glu, and Ser.

5.4 Conclusion

The treatments led to broad variations in Mo, Fe, Na, Zn, N, Cu, and Mn concentrations in the potato tubers as well as to wide variations in reducing sugars, chlorogenic acids, ascorbic acid, citric acid concentration and free amino acid composition. The relationships between Mo and Na and chemical compounds that were found in the potato tubers confirm the role of these nutrients in the metabolism of potato tubers.

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6 Effect of calcium fertilizer on color quality of potato chips

Abstract

In contrast to the effect of $CaCO_3$ application, gypsum application could improve the color quality of potato chips during long-term storage. The main contributors to the deterioration of color quality of potato chips were reducing sugars. Meanwhile, sucrose, ascorbic acid, chlorogenic acid, and free amino acids as well as Lys, Leu, Phe, and Met made small contributions to the darkening of potato chips.

6.1 Introduction

One of the most difficult problems in the potato chip industry is the maintenance of desirable chip color throughout the year. Dark color development at frying is a major constraint for consumer acceptance of potato chips. A dark chip has often undesirable flavor and taste, as a result of the Maillard reaction between reducing sugars and free amino acids (Bennett, 2001). Meanwhile, sucrose, ascorbic acid, and chlorogenic acid are reported to contribute to the darkening of chips (Leszkowiat et al., 1990; Rodriguez-Saona et al., 1997).

The accumulation of sugars as key substances in the Maillard reaction is the result of starch degradation during tuber storage. Sugar accumulation occurs due to the activity of phosphorolytic (Sowokinos, 2001) and amylolytic enzymes (Cottrell et al., 1993). The activity of phosphorolytic enzymes is influenced by several factors during planting and post-harvest treatment, such as stress and storage temperature (Sowokinos et al., 2000). Amylolytic enzymes are known as metallic enzymes because their activity is affected by the presence of metals, for example Ca (Belitz et al., 2004). Calcium is reported to play an important role in the structure of cell walls and membranes, cell elongation and cell division (Marschner, 1995), and may influence the tuber respiration (Recasens et al., 2004). Nevertheless, the effect of Ca on the storability of potato tubers in relation to the stability of color quality of potato chips is not

known. In this study, the effect of application of three different Ca sources on the color quality of potato chips in long-term storage was investigated.

6.2 Material and methods

Potato cv. Saturna was grown in sandy soil in Northern Germany in three years of field experiments from 2001 to 2003. The experiment was performed on plots which consisted of six rows 0.75 m apart and 8 m long according to randomized block design with six replications in 2001 and four replications in 2002 and 2003. Three Ca fertilizers were compared in this study: gypsum, $CaCO_3$, and basic slag with application rates of 450 and 900 kg Ca ha^{-1} in 2001 and 560, 1120, and 2240 kg Ca ha^{-1} in 2002 and 2003. All plots received 160, 44, 100, and 24 kg ha^{-1} of N, P, K, and Mg, respectively. The plants were planted in April and harvested in mid-September when the tubers achieved their physiological maturity. Investigations were performed on the freshly harvested tubers and after three, five and seven months of storage at 8°C, RH 98%.

Potato chips were produced according to the EAPR method (1974). Tubers were peeled with a hand peeler and sliced with a home slicer into regular slices of 1.2 – 1.3 mm thickness. The potato slices were fried in 2.5 kg of vegetable fat (Lidl, Germany) in an electrical household fryer at a temperature of 175 ± 5°C. The weight ratio of potato slices to fat was one to fifty. The chips were adequately fried when a water content of 2% was achieved, which was shown by absence of air bubbles in the frying oil. The frying oil was renewed after about 40 tests. The color quality of the potato chips was determined by measurement of the lightness of the potato chips with a chromameter CR-300 (Minolta, Japan) based on CIE 1976 (L*a*b) color parameters. The L* -value quantified the lightness where the low and the high values indicated dark and light color, respectively. The comparison between dark and light chip colors can be seen in .

Sugar, free amino acid and organic acid concentration in the tuber were determined. Sugars (D-glucose, D-fructose, and sucrose) were determined enzymatically by the spectrophotometric method of Roche catalogue no. 0139076. Free amino acids were extracted from freeze-dried potato flour with 6 M hydrochloric acid according to Anonymous (1993) and 16 amino acids were analyzed with RP-HPLC with the method described by Fisher et al. (2001). They are Tyr, Gln, Glu, Asp, Arg, Val, Lys, Phe, Ser, Ile, Met, Thr, Leu, Gly, Ala, and His. L-ascorbic acid was determined by titration with a solution of 2,6-dichlorophenol-indophenol (DIP) according to Diemair (1963). Chlorogenic acid was determined spectrophotometrically according to Griffith et al. (1992).

Analysis of variance and regression analysis were computed by using the software SAS release 8.02. The lightness of potato chips during storage is presented as a relative value of the untreated potato.

6.3 Results and discussion

6.3.1 Effect of calcium fertilization

Table 25 presents the analysis of variance of the color quality of the potato chips. Significant effects of rate and the interaction between location and storage were observed in all years. The effect of Ca sources and the interaction between Ca source and rate were very significant in 2002 and 2003, but not significant in 2001, which may have been due to a lower application rate in that year.

Between the years, the lightness of potato chips from planting season 2001 was the lowest and from 2003 it was the highest. The lightness pattern during storage in 2001 was different from that in 2002 and 2003. In 2001, the lightness worsened after three and seven months of storage and improved in the fifth month of storage. In 2002 and 2003, the lightness declined continuously during storage. The effect of location was very significant in 2003, where Hankensbuettel produced a higher lightness value than Gross Oesingen.

Table 25 Analysis of variance of lightness of potato chips from the planting seasons 2001, 2002, and 2003

Source	2001		2002		2003	
	df	MS	df	MS	df	MS
Location (L)	1	32.3*	1	3.9	1	116.3***
Ca source (F)	1	20.7	1	80.5***	2	13.8***
Rate (R)	2	23.2*	3	19.0***	3	5.1**
Storage (S)	3	1580.0***	3	86.2***	3	105.5***
L x F	1	14.9	1	3.0	2	3.1*
L x R	2	0.1	3	8.3*	3	6.6***
L x S	3	127.6***	3	61.9***	3	15.8***
F x R	2	10.8	3	44.4***	4	4.9***
F x S	3	4.4	3	6.0	6	0.3
R x S	6	8.1	9	13.6***	9	1.5
L x F x R	2	5.1	3	4.7	4	2.2
L x F x S	3	3.0	3	0.5	6	0.6
F x R x S	6	4.0	9	2.5	12	0.3
L x F x R x S	12	8.8	18	6.2**	21	1.1
Error	180	6.5	188	2.9	232	1.7

*, **, *** show a significant effect at P<0.05, P<0.01, and P<0.001, respectively
MS and df are mean square and degrees of freedom, respectively

The lightness alteration of potato chips from tubers fertilized with different rates of Ca during storage is presented in Figure 23, Figure 24, and Figure 25 as relative lightness. It is defined as the percentage of lightness alteration compared to the control in Figure 22. The results show that Ca fertilization mostly reduced the lightness of chips from the tubers that were not stored. Improvement of chip lightness could be seen after long-term of storage potato tubers, especially if the potatoes were fertilized with gypsum. Application of gypsum produced a better lightness in all locations and years with the exception of Hankensbuettel in 2003. On the other hand, application of $CaCO_3$ led to a worsening of chip lightness with the exception of Gross Oesingen in 2001. Gypsum application may result a better lightness of potato chips when compared to untreated potatoes or potatoes fertilized with $CaCO_3$ or basic slag.

L* = 80 **L* = 62**

Figure 21 Comparison between lightness values of light and dark potato chips

The tendency of increasing rates of gypsum application to improve the chip lightness was shown in Gross Oesingen in 2001 and 2003, and in both locations in 2002. However, the improvement of chip lightness was very small: about 2–8%, but it was important for the improvement of chip lightness. This is because if the percentage is converted to a lightness value it ranges between 1.4 and 8.5 lightness units. Comparison between the light and dark potato chips with their lightness unit can be seen in .

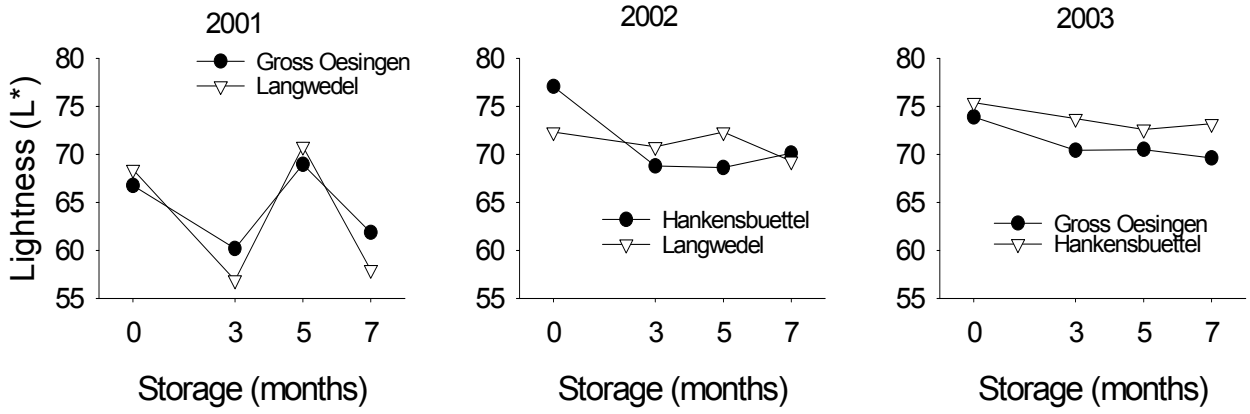

Figure 22 Change in lightness of potato chips from untreated potato tubers (control treatment) during storage

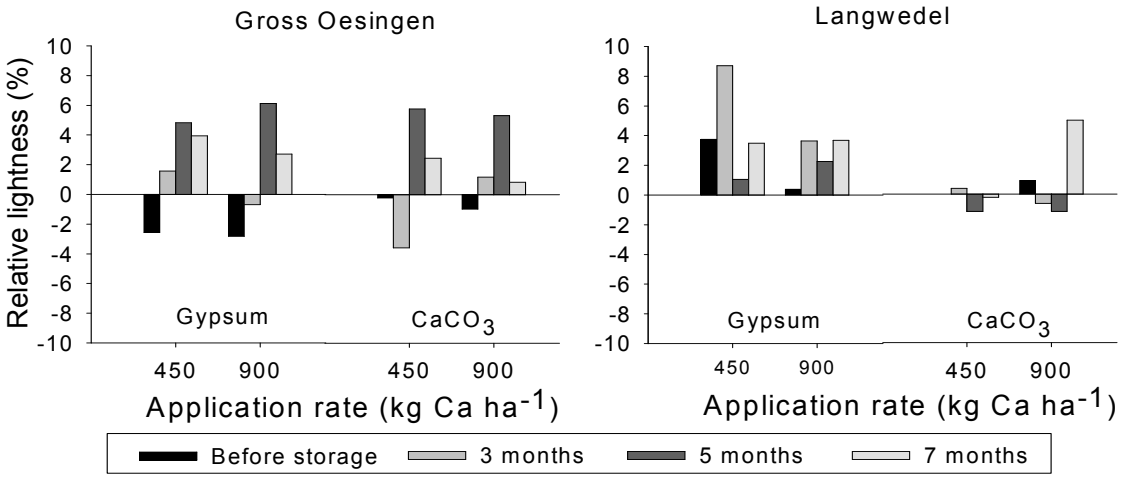

Figure 23 Relative change of lightness of potato chips made from fresh and stored potato tubers which were fertilized with gypsum and $CaCO_3$ in the planting season 2001

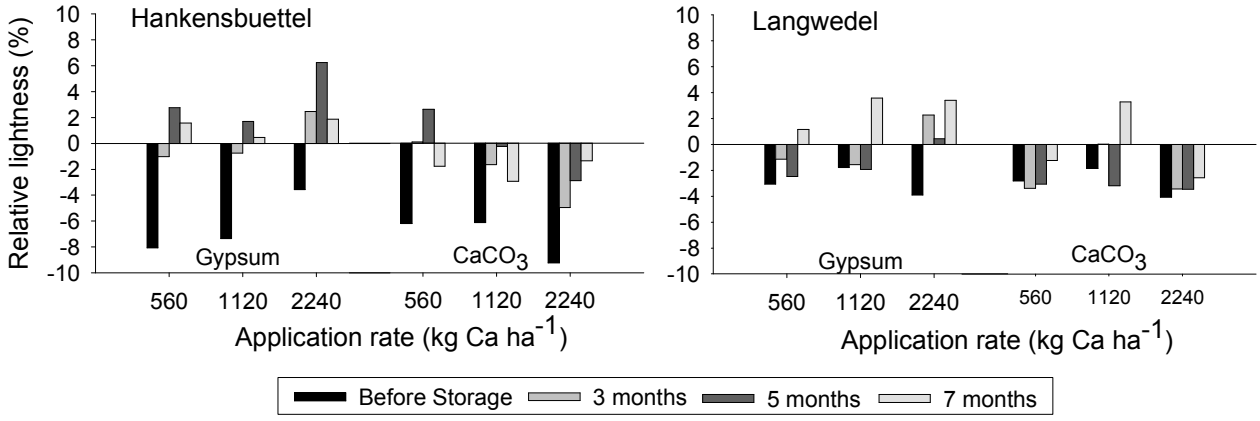

Figure 24 Relative change in lightness of potato chips made from fresh and stored potato tubers which were fertilized with gypsum and $CaCO_3$ in the planting season 2002

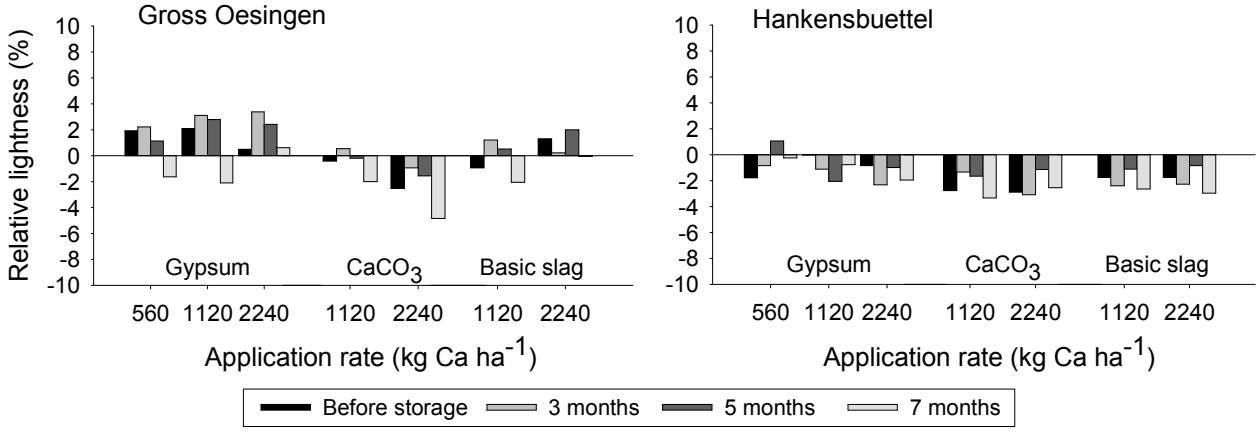

Figure 25 Relative change in lightness of potato chips made from fresh and stored potato tubers which were fertilized with gypsum and $CaCO_3$ in the planting season 2003

6.3.2 Factors affecting the color quality of chips

Figure 26 shows the correlation of sugars and organic acids with the lightness of potato chips. A highly significant correlation was observed between the lightness of potato chips and reducing sugars as well as organic acids ($P < 0.001$). Reducing sugars led to a decline in the lightness of potato chips. This is in agreement with Roe and Faulks (1991) because they are substances in the Maillard reaction. However, in this study, D-glucose and D-fructose explained the variability of the color quality of potato chips only about 37% and 29%, respectively.

A low correlation coefficient between reducing sugars and lightness was also found by Copp et al. (2000). The effect on decreasing the color quality of potato chips was similar between D-glucose and D-fructose, or half of the total reducing sugars (total sum of D-glucose and D-fructose content). An increase of 0.5 g kg^{-1} DM of single reducing sugars or 1.0 g kg^{-1} DM of total reducing sugars led to a reduction of one lightness of unit in the potato chip. The effect of total reducing sugars on decrease the lightness of potato chips was ten times lower than in the experiment by Rodriguez-Saona (1997) with leached potato slices. However, this result is in agreement with the finding that reducing sugars are major contributors to the deterioration of the color quality of potato chips.

The contribution of sucrose as well as ascorbic acid to the darkening of potato chips, which was found by Leszkowiat, et al. (1990), Rodriguez-Saona (1997) and Copp et al. (2000) is not supported by this study. The effects of sucrose, ascorbic acid, and chlorogenic acid on the change of chip lightness were considerably lower than the effect of reducing sugars.

In this study, the contribution of the total content of free amino acids on the reduction of chip lightness was not significant. Meanwhile, the contribution of single free amino acids, i.e. Leu and Lys, on the decline of chip lightness was significant, but they explained the variability only about 3 and 10%, re-

spectively (Figure 27). This result is in agreement with Roe and Faulks (1991), who found that Lys as well as γ-aminobutyric acid and Gly were the highest-browning amino acids. On contrary, Phe and Met contributed to the improvement of the lightness of potato chips but with low coefficient of correlations. However, the low correlation coefficient of free amino acids and the lightness of chips confirmed the conclusion of Roe et al. (1990) that the free amino acid concentration in the potato tuber was not a limiting factor for the color quality of potato chips.

The effect of Ca fertilizers on the color quality of potato chips in Table 25 was explained by the effect of Ca fertilizers on the reducing sugars accumulation in the potato tubers before and during storage (see Chapter 4). Reducing sugars content increased with a rise in rate of $CaCO_3$ application, while reducing sugars was not affected by gypsum application. During storage, reducing sugars content in the potato tubers increased and tubers applied with $CaCO_3$ contained more reducing sugars than tubers applied with gypsum and basic slag.

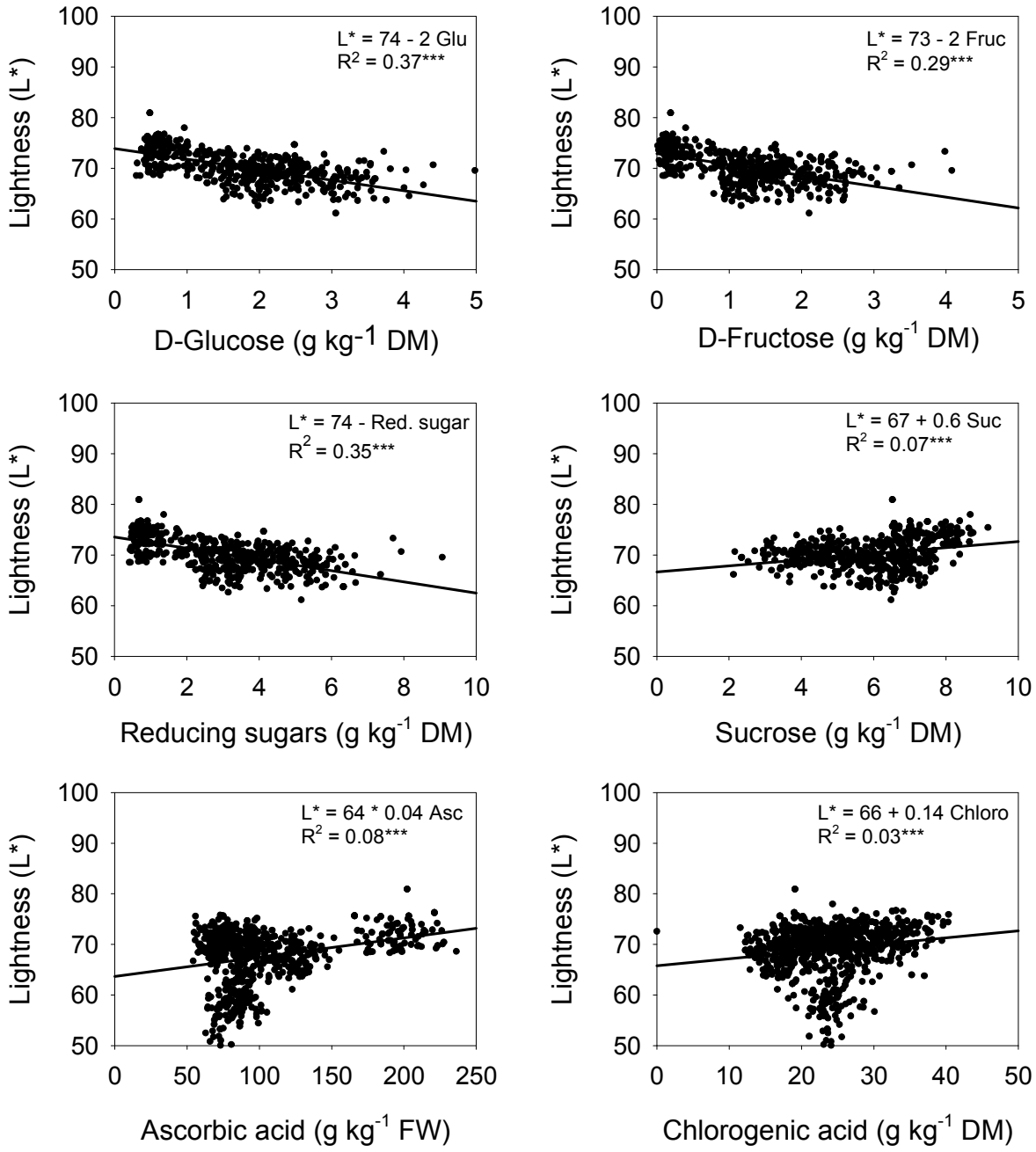

Figure 26 Correlation of D-glucose, D-fructose, reducing sugars (the sum of D-glucose and D-fructose), sucrose, ascorbic acid, and chlorogenic acid with lightness of potato chips

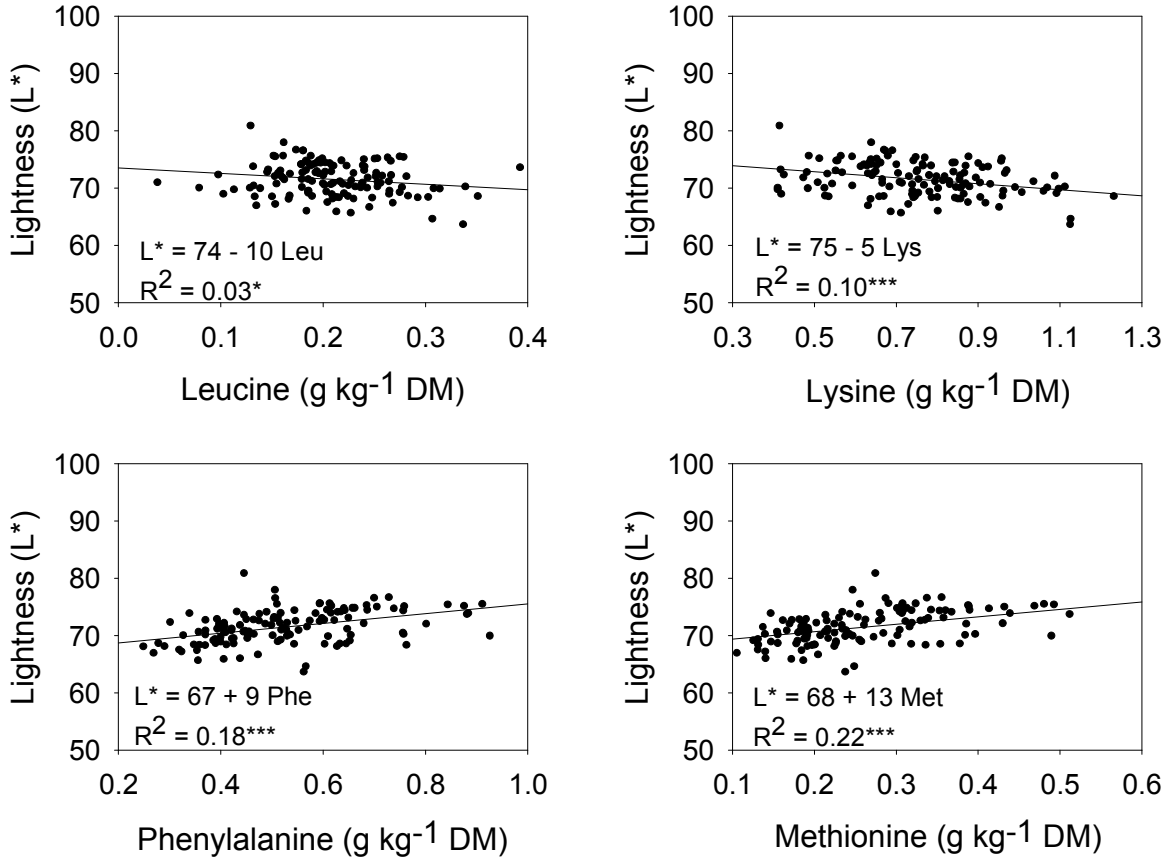

Figure 27 Correlation between free amino acids and lightness of potato chips

6.4 Conclusion

In contrast to the effect of $CaCO_3$ application, gypsum application could improve the color quality of potato chips during long-term storage. The primary contributors to the deterioration of color quality in potato chips were reducing sugars, while the contributions of sucrose, ascorbic acid, chlorogenic acid, and free amino acids as well as Lys, Leu to the darkening of potato chips were smaller. These results confirm that reducing sugars are a limiting factor for the color quality of potato chips.

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7 Pasting properties of potato flour and starch from potatoes fertilized with calcium

Abstract

Potato flour and starch from potato plants grown in sandy soil in two locations and fertilized with gypsum and lime at rates of 560, 1120, and 2240 kg Ca ha^{-1} were investigated to determine their pasting properties before and after 5 months storage. The results show that Ca fertilization had no effect on the pasting properties of potato flour and starch. The pasting properties were mainly affected by the distribution of the granule size of the starch, location and storage. Multiple regression analysis concluded that P, S, Ca, Na, Mn, Mo, and B in the tuber might have an influence on pasting properties of potato starch.

7.1 Introduction

Environmental factors during plant growth such as fertilization, temperature, location and annual climate have an effect on the factors that determine the physicochemical properties of starch such as P content, starch granule size, and amylose-amylopectin ratio (Haase and Plate, 1996; Tester et al., 1999; Morrison et al., 2000; Tester et al., 2004). In the starch, P is present in phosphate ester groups which are bound to amylopectin molecules. Phosphate ester groups have an impact on the starch properties, e.g. pasting temperature, water binding capacity, viscosity and clarity of starch (Sitohy et al., 2000). To improve the starch quality, it is important to increase the P concentration in the potato tuber.

According to Mengel and Kirkby (1982), P solubility in the soil is highly pH dependent. Soluble P increases with a rise in soil pH from 4 to 6.5. In acidic soil, the pH is commonly adjusted by liming with Ca in the form of oxide or carbonate. On the other hand, Ca is essential for the potato plants and tubers because increasing Ca in the tubers has been associated with increasing the resistance to nematode, bacterial and fungous disease during growth and tuber storage (Fatemy and Evans, 1986; McGuire and Kelman, 1986). Al-

though Ca in the plant's cells is not found in amyloplast (Oparka and Davies, 1988; White and Broadley, 2003) where the starch granules are stored, more than 90% of tuber Ca is in a physiologically active form (Davies and Millard, 1985). It is already known that Ca is an activator for the enzyme amylase which is responsible for starch degradation (Belitz et al., 2004). The effect of Ca and Ca fertilization on the starch quality of potato tubers is still not well investigated. This chapter aims to study the direct or indirect effect of Ca nutrition on the pasting properties of potato flour and potato starch.

7.2 Material and methods

Field experiment

Potato cv. Saturna was grown in loamy sandy soil in two locations, Hankensbuettel and Langwedel, Northern Germany, in 2002. The experiment was conducted according to randomized block design with four replications. Each plot consisted of six rows 0.75 apart and 8 m long. Two Ca fertilizers were compared in this experiment. Sieved $CaSO_4.2H_2O$ (gypsum) and granulate $CaCO_3$ were applied preplant broadcast by hand at rates of 560, 1120, and 2240 kg Ca ha^{-1}. All plots received 44, 100, 24, and 160 kg ha^{-1} of P, K, Mg and N, respectively.

The potatoes were planted in May and harvested in September 2002. After harvest, some tubers were directly used for investigation and some were stored for five months in a conditioning chamber at a temperature of 8°C and relative humidity of 92%.

Ten tubers were sampled from every treatment and then washed, peeled and cut longitudinally. One half was freeze-dried and the other was used for starch extraction. Freeze-dried potato was ground to produce potato flour and used for chemical analysis.

Starch isolation

Starch was isolated according to Jansen et al. (2001). Tubers were cut into large cubes and grated in a mixer. The juice was filtered through 70 µm gauze into a bowl with 250 mL distilled water. The water was decanted after sedimentation of the starch. The crude starch was separated from proteins by repeated washing with distilled water, sedimentation and decanting. Almost-dry starch was rubbed through a sieve and dried at room temperature.

Rapid visco analyzer (RVA) analysis

Pasting properties of potato flour and potato starch were evaluated using the Rapid Visco Analyzer (RVA-3 Newport Scientific, Australia) according to ICC standard no. 162 (1999). Two grams of potato flour or starch with moisture content of 14% were measured into the RVA canister followed by the addition of 25 mL distilled water. The suspension was analyzed under conditions of continuous shear (160 rpm) with heating and cooling cycles at temperatures of 50 to 95°C for 15 min. of testing. Pasting temperature, peak viscosity, time of peak viscosity, trough, breakdown, setback and final viscosity were recorded in a time, temperature and viscosity curve. Pasting temperature is the temperature at the onset of viscosity increase or at which the starch starts to gelatinize, as shown by swelling of the starch. Peak viscosity indicates the maximum viscosity that occurs at the equilibrium between swelling and amylose leaching. During a hold period at constant high temperature (95°C) the viscosity decreases after peak viscosity, which indicates an amylose leaching, a rupture of starch granules and polymer alignment. This period is accompanied by a breakdown in viscosity and indicates a hot paste viscosity or trough. Breakdown is derived mathematically by peak viscosity minus trough. After heating, the paste was cooled down and its viscosity normally increased. An increasing in viscosity indicates the re-association between starch molecules in an ordered structure and that retrogradation proceeded. The final viscosity indicates the ability of the material to form a viscous paste or gel after cooking and cool-

ing. It is used commonly as a quality parameter for processing products based on starch. Setback is calculated as final viscosity minus trough and is also known as degree of retrogradation.

Starch granule size

Particle size distribution was determined under microscope by measuring the diameter of more than 500 starch granules using the software Adobe Photoshop 5.5.

Analytical methods

The amylose and amylopectin ratio was determined by staining the starch with I_2 – KI and measuring the absorbance at 550 and 618 nm by spectrophotometer (Hewlett Packard 8453, Germany) according to Hovenkamp-Hermelink et al. (1988). Twenty-five milligrams of freeze-dried potato flour or potato starch was immersed in 0.5 mL 45% perchloric acid (Merck, Germany). After four minutes, 8 mL distilled water was added. I_2-KI staining was achieved by mixing 4 mL of the starch solution and 5 mL of a diluted (1:2 v/v) Lugol's solution (Merck, Germany). Lugol's solution was produced by mixing 2 g KI and 1 g I_2 in 300 mL distilled water. A standard curve was produced by measuring the pure potato amylose and amylopectin (Merck, Germany) with concentration gradients of 0.06, 0.13, and 0.30 mg mL^{-1} for amylase and 0.13, 0.26, 0.37, and 0.50 mg mL^{-1} for amylopectin.

Starch content was investigated polarimetrically by hydrolyzing the potato flour at 100 C for 15 min. according to the procedure of ICC-Standard no. 123 (Arbeitsgemeinschaft für Getreideforschung, 1994). The mineral content of the potato flour was digested with 65% nitric acid using a microwave (Mega 1200, Germany) according to Abu-Samra et al. (1975) and measured by using inductively coupled plasma-atomic emission spectroscopy (Perkin Elmer Optima 3000, USA).

Statistical analysis

Analyses of variance were computed by the general linear model procedure and the Pearson's correlation was generated by the regression procedure of statistical software SAS release 8.02.

7.3 Results and discussion

The effect of Ca application on starch concentration was more obvious in Hankensbuettel than in Langwedel (Figure 28); however, the trend was the same. After application of $CaCO_3$ at a rate of 560 kg ha^{-1} the highest starch content was achieved; thereafter, the starch concentration was not influenced by increasing the rate of $CaCO_3$ application. In contrast to $CaCO_3$, application of gypsum up to 1120 kg Ca ha^{-1} had a tendency to increase the starch content up to 30 g kg^{-1} DM.

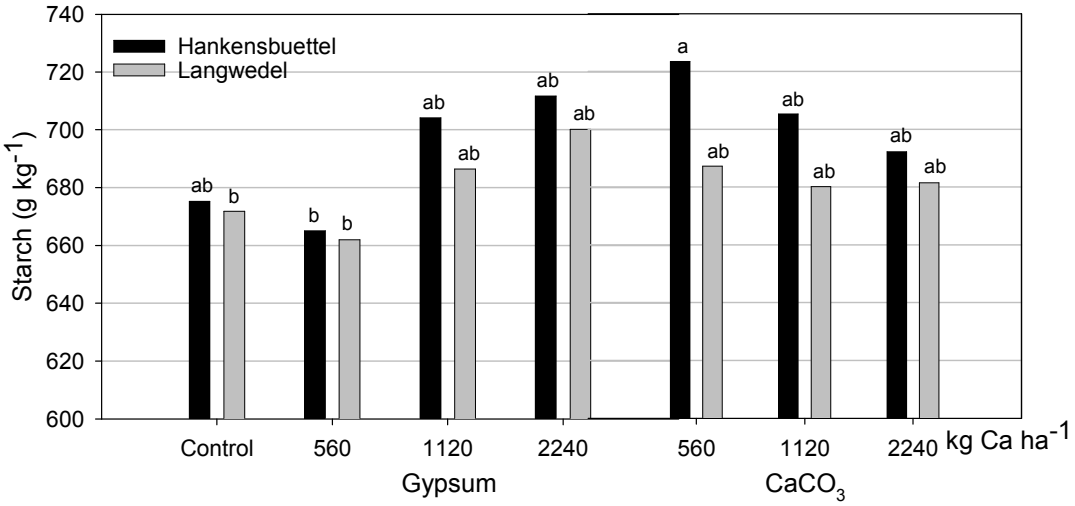

Figure 28 Effect of calcium fertilization on starch content of tubers grown in Langwedel and Hankensbuettel. Graphs with the same letters do not differ significantly.

Location had a strong effect on the starch content in the tubers ($P<0.001$). Starch concentration in Hankensbuettel was 13 g kg^{-1} DM higher than in Langwedel. Climate conditions were no factors for the variability of starch because the locations are near each other. The soil composition might be the reason for this, because Langwedel's soil is sandier than Hankensbuettel's. In sandy soil, the water-holding capacity of the soil is very low and can

cause water stress. Water stress reduces photosynthesis which leads to lower starch synthesis in the carbon cycle.

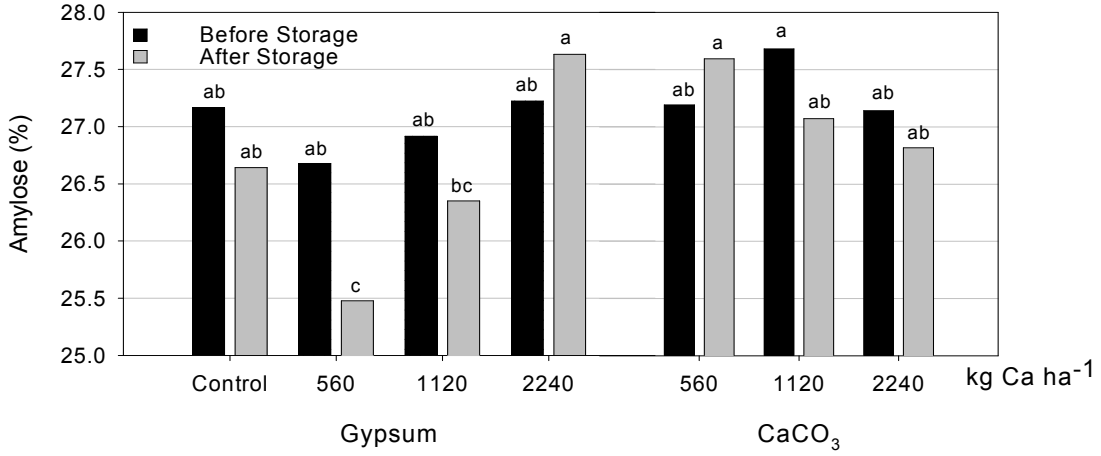

Figure 29 Effect of calcium fertilization on percentage of amylose in potato starch from potato tubers grown in Hankensbuettel. Graphs with the same letters do not differ significantly.

There was no indication of any effect of Ca fertilization on the amylose content of starch (Figure 29). Amylose content was affected mainly by the location. During storage the percentage of amylose significantly decreased up to 1.3 %.

The pasting properties of potato flour before storage were not affected either by the Ca fertilization or by the location (Table 26). The pasting properties of potato flour could not be compared with the pasting properties of potato starch. More chemical components in the potato flour made it more complex than potato starch. Potato flour contained not only 687 g starch kg^{-1} DM, but also fat, ash and dietary fiber. Protein, fatty acids, and some minerals can form a complex with the starch and change the pasting properties (Zobel, 1984).

The pasting properties of potato starch before storage were not affected by the Ca fertilizers (Table 27) but they were mainly affected by location ($P<0.001$). Moreover, peak viscosity and trough were affected slightly by the interaction of location x rate ($P<0.05$). The pasting properties of both locations

differed from each other. Tuber starch at location Langwedel had a higher peak viscosity but a lower pasting temperature and a shorter peak time than starch from Hankensbuettel. Pasting temperature and peak time are related to the energy required during paste production. Langwedel starch had a higher breakdown and resulted in a lower trough than did Hankensbuettel starch. This indicates that starch from location Hankensbuettel was more stable against heat and mechanical shear. However, starch from Langwedel has a higher setback than that from Hankensbuettel, but its final viscosity is lower.

Table 26 Effect of calcium fertilization on pasting properties of potato flour

Treatment	Application rate	Peak viscosity	Trough	Breakdown	Final viscosity	Setback	Peak time	Pasting temp.
	kg Ca ha^{-1}	RVU	RVU	RVU	RVU	RVU	min	^{o}C
Hankensbuettel								
Control		158	81	77	151	70	4.3	65.6
Gypsum	560	162	84	77	156	71	4.4	65.7
	1120	158	84	74	153	68	4.4	65.6
	2240	154	86	68	155	69	4.4	65.2
Lime	560	154	79	75	158	79	4.4	65.8
	1120	160	84	75	150	66	4.4	65.8
	2240	153	85	69	155	71	4.4	65.8
Average		157	83	74	154	71	4.4	65.6
Langwedel								
Control		157	83	74	154	71	4.4	65.8
Gypsum	560	158	81	78	149	69	4.6	66.2
	1120	156	78	78	149	71	4.5	65.2
	2240	157	78	79	152	74	4.4	65.4
Lime	560	156	79	77	150	71	4.3	65.2
	1120	155	82	74	152	70	4.3	65.6
	2240	160	80	80	153	73	4.5	65.0
Average		157	80	77	151	71	4.4	65.5
Significant		ns	ns	ns	ns	ns	ns	ns

ns shows a not significant F-test of the treatments

As reported by Jansen et al. (2001), pasting properties are affected mainly by the granule size of starch. Pasting properties of potato starch and granule size of starch were not affected by location in the study of Morrison et al. (2000), but these results show an effect of location on the granule size distribution of starch (Figure 30).

Table 27 Effect of calcium fertilization, rate of application, and location on pasting properties of potato starch from tubers before storage as well as analysis of variance F ratio and root mean square

Treatment	Application rate	Peak viscosity	Trough	Breakdown	Final viscosity	Setback	Peak time	Pasting temp.
	kg Ca ha^{-1}	RVU	RVU	RVU	RVU	RVU	min	^{o}C
Hankensbuettel								
Control		704	252	451	279	27	3.8	68.9
Gypsum	560	606	281	325	309	27	4.5	69.1
	1120	615	259	357	285	27	4.2	68.4
	2240	621	261	359	287	26	4.3	68.7
Lime	560	616	258	358	282	25	4.2	68.6
	1120	564	294	270	319	26	4.8	69.4
	2240	591	291	300	315	25	4.7	69.4
Average		617 [b]	271 [a]	346	297 [a]	26 [b]	4.4 [a]	69.9 [a]
Langwedel								
Control		701	193	508	227	34	3	66.9
Gypsum	560	688	192	496	219	27	3.1	66.3
	1120	670	194	476	226	32	3.2	67.4
	2240	695	184	511	215	31	3	67
Lime	560	693	177	516	209	32	2.9	66.7
	1120	705	185	520	214	29	3	67
	2240	717	199	518	227	28	3.1	67.1
Average		695 [a]	189 [b]	506 [a]	220 [b]	30 [a]	3 [b]	66.9 [b]
ANOVA	**df**							
Location (L)	1	25.64***	138.1***	66.07***	128.6***	17.02***	117.1***	145.44***
Fertilizer (F)	1	0.01	0.35	0.08	0.25	0.36	0.29	1.05
Rate (R)	3	4.29***	0.57	3.17	0.39	1.25	2.57	1.15
L x F	1	1.53	0.89	1.62	0.82	0.13	0.69	0.67
L x R	3	3.21*	0.98	2.88*	1.32	0.5	1.62	0.64
F x R	3	0.06	1.8	0.44	1.63	0.35	0.91	0.52
L x F x R	3	0.65	0.74	0.83	1.04	0.83	0.69	2.17
Root MSE	48	54.16	26.88	72.59	26.07	4.89	0.46	0.68

Values with the same letter in the same column do not differ significantly at P<0.05.
*, **, and *** are F-test significant at P<0.05, P<0.01, and P<0.001, respectively

The length of starch granules of potato tubers in this study is ranged from 15–105 µm. According to Whistler and BeMiller (1997), the granule size of potato starch ranges between 5–100 µm. Starch from location Langwedel had an average particle size of 38 µm, compared with the starch from Hankensbuettel which had an average particle size of 52 µm. Langwedel starch distributed more toward the small-size granules with starch granules size distribution <35, 35-65, and >65 µm of 50, 45, and 5%, respectively. In contrast, Hankensbuettel was distributed toward the large-size granules with the granule size distribution of 18, 59, and 23%, respectively.

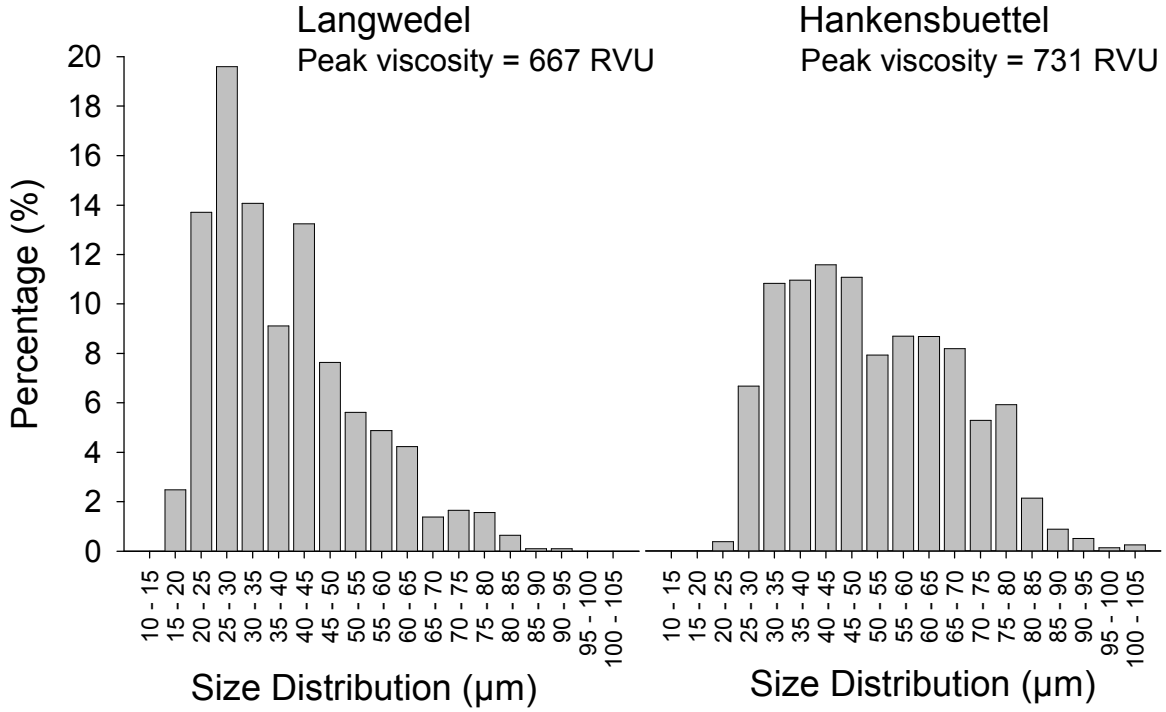

Figure 30 Size distribution of starch granules from potato tubers grown in Langwedel and Hankensbuettel

The relationship between amylose content and the pasting properties of starch from stored tubers was not found in this study, although some reports have suggested a relationship between amylose and peak viscosity (Sasaki et al., 2000; Varavinit et al., 2003; El-Khayat et al., 2003). This is perhaps due to small differences in percentages of amylose within treatments (Figure 29).

On other hand, the effect of storage was highly significant (P<0.001) on the peak viscosity, trough, breakdown, final viscosity, and peak time (Table 28). Nevertheless, there was no indication that Ca fertilization had any influence on the pasting properties after storage. An increase of about 17% in peak viscosity after storage occurred due to the decrease in amylose during storage (Figure 29). According to El-Khayat et al. (2003) and Varavinit et al. (2003), amylose has a negative correlation with peak viscosity and breakdown. This indicates that amylose content may have a greater affect on the swelling and disruption of the granule than on the subsequent realignment of the starch components during retrogradation. The increase of breakdown up to 42% after storage demonstrated that the disruption of starch granules after maximum swelling at the peak viscosity occurred more after storage than before storage. This may be related to starch degradation and alteration of the surface of starch granules as reported by Cottrell et al. (1993).

Table 28 Change in pasting properties of starch from stored potato tubers

Pasting Properties		Change (%)
Peak Viscosity (RVU)	732	+17 ***
Trough (RVU)	224	-17 ***
Breakdown (RVU)	508	+42 ***
Final Viscosity (RVU)	251	-15 ***
Set Back (RVU)	26	-4 ns
Peak Time (min)	3.4	-21 ***
Pasting Temperature (^{o}C)	67.9	+15 ns

ns and *** are F-test not significant and significant at P<0.001

While the peak time decreased significantly, the peak viscosity increased. This means that the starch is swelled more rapidly after storage. Moreover, final viscosity decrease 15% after storage; however, it was still higher than the final viscosity of Langwedel starch before storage (Table 28). The decrease in final viscosity indicated that the ability of starch to return to its

structure and form was decreased after storage. This correlates with amylose content (Sasaki et al, 2000).

Table 29 Correlation matrix between mineral nutrients of potato tubers and pasting properties of potato starch before storage

Nutrients	Pasting properties						
	Peak viscosity	Trough	Breakdown	Setback	Final viscosity	Peak time	Pasting temp.
P	-0.23 ns	0.62 ***	-0.42 ***	-0.35 ***	0.62 ***	0.55 ***	0.56 ***
K	-0.12 ns	0.18 ns	-0.15 ns	-0.15 ns	0.17 ns	0.17 ns	0.14 ns
Mg	0.31 *	-0.47 ***	0.41 ***	0.15 ns	-0.48 ***	-0.45 ***	-0.46 ***
S	-0.33 ***	0.39 ***	-0.38 ***	-0.21 ns	0.39 ***	0.41 ***	0.36 ***
Ca	-0.34 ***	0.27 *	-0.34 ***	-0.07 ns	0.28 *	0.31 **	0.19 ns
Na	-0.25 *	0.60 ***	-0.42 ***	-0.40 ***	0.58 ***	0.53 ***	0.58 ***
B	0.43 ***	-0.71 ***	0.59 ***	0.39 ***	-0.71 ***	-0.68 ***	-0.67 ***
Cu	-0.25 *	0.46 ***	-0.36 ***	-0.20 ns	0.46 ***	0.43 ***	0.44 ***
Fe	0.00 ns	-0.01 ns	0.00 ns	-0.20 ns	-0.03 ns	0.00 ns	-0.03 ns
Mn	0.27 *	-0.37 ***	0.34 ***	0.05 ns	-0.38 ***	-0.35 ***	-0.33 ***
Mo	-0.15 ns	0.29 **	-0.22 ns	-0.28 *	0.28 *	0.26 *	0.30 **
Zn	0.15 ns	-0.05 ns	0.12 ns	0.02 ns	-0.05 ns	-0.07 ns	0.03 ns

*, **, ***, and ns are F-test significant at $P<0.05$, $P<0.01$, $P<0.001$, and no significant different, respectively

This investigation attempted to find relationships between mineral compositions of tubers and pasting properties of potato starch. Although the mineral composition of potato tuber is not always the same as the composition of potato starch, at a minimum it can point out the important minerals that may affect the pasting quality of potato starch. Matrix correlation between the pasting properties and mineral content of potato tubers is presented in Table 29. Some minerals had a significant Pearson's correlation with the pasting properties of potato starch such as P, Mg, S, Ca, Na, B, Cu, and Na.

Phosphorus, which is present in amylopectin of potato starch as phosphate ester (Whistler and Daniel, 1984), correlated strongly with trough, final viscosity, peak time and pasting temperature, and correlated weakly with breakdown and setback. In this study, correlation of phosphorus with peak vis-

cosity was not supported, although this does not agree with the study of Hopkins and Gormley (2000). Their study shows a positive correlation of phosphorus with peak viscosity, setback, and trough. According to Whistler and BeMiller (1997), potato starch has due to its phosphate ester content a negative charge and a pKa of 3.7 which leads to a rapid swelling in warm water and to high viscosity, good clarity, and a low rate of retrogradation.

Monovalent cations, cations as K and Na and divalent cations, as Mg and Ca are present in salt formation and can associate with the monoester phosphate groups and form ionic bridges between the phosphate groups owing to suppression of swelling of starch granules (Whistler and BeMiller, 1997; Morrison et al., 2000). As a result of the cation presence, the peak viscosity is increased; on the other hand, peak time is decreased (Bergthaller et al., 1999).

Sulfur in plants is present bound in the amino acids Cys and Met, associated with lipids in membranes or polysaccharide as sulfolipids, and as sulfate (Marschner, 1995). The addition of sulfate dioxide in starch production has been reported to have an influence on the starch properties (Sriroth et al., 1998). Other microelements might have direct or indirect correlations with the pasting properties. There is no report about the role of microelements on the pasting properties. However, the microelements play a key role in photosynthesis and in catalyzing enzyme reactions (Marschner, 1995).

Multiple regression analysis was done to select the elements which have a correlation with the pasting properties and to analyze the interaction between elements (Table 30). In this study, Ca had a negative effect on the peak viscosity and breakdown, although this is not agreed with Bergthaller et al. (1999). Phosphorus affected positively the trough, final viscosity and pasting temperature. Setback was affected by Na in this study, but its correlation was very low.

Table 30 Stepwise multiple regression analysis of pasting properties of potato starch with mineral content of potato tubers

Pasting properties	n	R^2	Variables in Equation[1]
Peak viscosity	64	0.27	(Ca), B
Trough	64	0.61	P, (B)
Breakdown	64	0.43	(Ca), B
Setback	64	0.15	(Na)
Final viscosity	64	0.60	P, (B)
Peak time	64	0.63	S, (B), (Mn), Mo
Pasting temperature	64	0.52	P, (B)

[1]Significance level was 0.05 for variables to enter into equation and 0.025 to remove from equation; a variable in parentheses had a negative coefficient in the equation.

Boron was the only microelement in this study which might affect all pasting properties except the setback. There is less information about the effect of B on the pasting properties. In this study, B ranged between 3.3 and 5.0 mg kg^{-1} DM with an average of 4.1 mg kg^{-1} DM. In plants, B is present as boric acid and borate ester. Application of boric acid to the potato plant is accompanied by an increasing in photophosphorylation, activation of photosynthesis, an increase in the number of tubers, and is related to the phytohormone status of the tuber and leaves such as imidazoleacetic acid (IAA), abscisic acid (ABA) and cytokinin (Puzina, 2004). According to Marschner (1995), the role of B in carbohydrate metabolism must be focused on the synthesis of cell wall material and the transport of sugars. In the adhesive processing based on starch, B as sodium tetraborate (borax) was added commonly to increase tack or adhesiveness, viscosity and paste color (Kennedy and Fischer, 1984). Boron that was investigated in this study was the total B in the whole tuber and not in the starch.

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8 General conclusion

The current study showed the importance of Ca concentration in the soil solution during tuberization for Ca uptake of potato tubers. Through the method “soil solution displacement” of Adams (1974) it was possible to get informations about the Ca that is available for the tubers. It is different to the exchangeable Ca which gives informations about the quantity of Ca bound to the negative charge of soil. The exchangeable Ca must be released to the soil solution and the release depends on the pH and anion concentration in the soil (Brady and Weil, 2002). The comparison between the application of gypsum and $CaCO_3$ demonstrated that the exchangeable Ca could not explain the Ca status in the soil solution.

Calcium must be continuously available during tuber growth because potato tubers fulfill their Ca requirement by direct uptake through tuber roots, stolons and periderm surface, and not by the retranslocation from the shoot through the phloem system. In agreement with Simmons et al. (1988), Ca in the soil solution should be concentrated in that area where tubers and stolons are predominantly growing. Therefore, an efficient Ca fertilizer should have the following characteristics: (1) slow solubility to ensure that the Ca supply into the soil solution take place during tuber growth; (2) little effect on the soil pH because an increase of pH will reduce the release of Ca from the negative charge of soil. Moreover, watering or irrigation should be applied at night because Ca absorption by the tubers occurs at night when vapor-transpiration of the tubers is higher than that of the leaves (Krauss and Marschner, 1974).

In this study, it is important to differentiate between the effect of Ca concentration in the tuber and the effect of Ca fertilizer on the potato tuber quality. Although it is reported that Ca has several functions in the cell metabolism, in the current study, the Ca level had only slight effects on the analyzed biochemical parameters of the potato tuber. Calcium in the tuber was observed to have no effect on dry matter, starch, reducing sugars, ascorbic acid, chloro-

genic acid and free amino acids. The tuber Ca concentration had no effect on the color quality of potato chips and on the pasting properties of potato flour and starch. However, low concentration of tuber Ca together with low concentration of tuber K increased the occurrence of the internal tuber disorder “brown centre”. Quality parameters of potato tubers might have been affected more by other nutrients than by Ca. The Ca fertilizer that has influence on the soil pH may also affect the uptake of macro- and micronutrients by the plant (Tyler and Olsson, 2001). Several nutrients were observed to be affected by the Ca application. They are Mg, S, Na and Mo, B, Cu and Zn depending on the location. Different response at different locations showed a strong effect of the particular location on the nutrient composition in the potato tuber. Furthermore, some mineral nutrients in the potato tubers showed a significant relationship with the internal quality of potato tubers as well as pasting properties of flour and starch.

The reason for the effect of Ca from different sources on the color quality of potato tubers was still not clear. The application of all Ca fertilizers impaired the color quality of the chips produced from tubers directly after harvest. After storage, the application of $CaCO_3$ and gypsum showed an opposite effect. The treatment with $CaCO_3$ impaired, on the other hand, application of gypsum improved the color quality of the chips. Darkening of potato chips before and after storage was mainly caused by reducing sugars. It was not easy to asses the relationship between mineral nutrients and the concentration of reducing sugars because the concentration of reducing sugars was always changed during the storage. It might be an effect of Ca in the tuber because Ca has an effect on the activity of α-amylase. However, it could be notified that potato chips from tubers with higher Ca concentration as a result of gypsum application had a better color quality after storage than from tubers with lower Ca concentration.

Future study

It would be of interest to investigate the role of mineral nutrients in relation to the sugars metabolism in potato tuber during storage. Investigation on tuber Ca should be focused on the Ca uptake and Ca forms in the tuber tissue. Calcium fractions and their metabolism during storage had been studied by Davies and Maillard (1985). Their studies showed that the Ca fractions composition was changed during sprouting and only the physiological active forms may have effects on the tuber metabolism. The method to determine Ca forms in tuber tissue needs also to be improved to get accurate results. Knowledge about this may give an answer of the question: which Ca forms are predominately affected by an increase of Ca tuber concentration and what is their role on the quality of potato tubers during storage?

There are two opinions in the starch degradation resulting in the accumulation of reducing sugars in the potato tuber during storage. The study of Cottrell (1993) showed that the increase in reducing sugars during storage is a result of the activity of starch hydrolyzing enzymes while the study of Sowokinos (2001) showed that it was a result of phosphorolytic enzymes activity. Change of the sugars content during storage caused difficulties to find relationship between sugars and mineral nutrients in the tuber, although minerals have an influence on the activity of many enzymes. Therefore, it seems to be also important to investigate the relationships between sugars metabolism and mineral nutrients during storage.

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