Physicocemical Properties and Processing of Tuber and Cereal Crops

イモ類および穀類の物理化学的特性と高度加工利用に関する研究

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Chapter 1. Literature Review

1.1. Polysaccharides in Plant-based Foods

Polysaccharides are the main constituent of the plant-based foods consisting of fruits, vegetables, and other plant sources. These can be divided into two categories; storage polysaccharides and cell wall polysaccharides. Starch is one of the dominant storage polysaccharides in plant, while cell wall polysaccharides include a wide range of compounds such as cellulose, hemicellulose and pectic polysaccharides. These polysaccharides have an important role to provide daily energy for human as well as determine quality characteristics of plant-based foods including texture. In this review, the plant polysaccharides are discussed in relation to the texture characteristics of plant-based foods.

1.2. Cell Wall Polysaccharides

The plant cell walls are comprised of relatively stiff cellulose microfibrils embedded in a hydrated matrix of pectins, hemicellulose, and structural protein (Cosgrove 1997). The plant cell wall is a key determinant of texture in fruit and vegetables; its properties influence the way in which plant tissues undergo mechanical deformation and failure during mastication. Processes such as cooking, and physiological events such as ripening can reduce the strength of cell adhesion in many vegetables and fruit through depolymerization of pectic polysaccharides (Waldron et al. 1997). The structural features of cell wall polysaccharides are summarized as follows;

Cellulose; The primary structure of cellulose is an unbranched (1,4)-linked β-D-glucan. Numerous linear glucan chains compose cellulose microfibril that is synthesized in parallel

by protein complexes embedded in the plasma membrane (Cosgrove 2014).

Hemicellulose; Xyloglucan and arabinoxylan are two of the most abundant hemicelluloses. Details of their structure vary slightly among plant species. Xyloglucan has a backbone that is similar to that of cellulose, but it is decorated with xylose branches on 3 out of 4 glucose residues. Xylose can also be serially appended with galactose and fucose residues. Arabinoxylan consists of a (1,4)-linked β-D-xylan backbone decorated with arabinose branches. Other residues, such as glucuronic acid and ferulic acid esters (FAE), are also attached in arabinoxylan that are particularly abundant in cereal grasses (Cosgrove 2005).

Pectic polysaccharides; Rhamnogalacturan I consists of alternating residues of galacturonic acid and rhamnose, and probably has side branches that contain other pectin domains. Homogalacturonan comprises a linear chain of galacturonic acid residues, whereas xylogalacturonan is modified by the addition of xylose branches. The carboxyl groups of homogalacturonan and xylogalacturonan are often methyl esterified, a modification that 'blocks' the acidic groups and reduces their ability to form gels. Rhamnogalacturonan II is a complex pectin domain that contains 11 different sugar residues and forms dimers through borate esters (Cosgrove 2005).

1.3. Starch

Starch is the most abundant storage reserve carbohydrate in plants. It is found in many different plant organs, including seeds, fruits, tubers and roots, where it is used as a source of energy during periods of dormancy and regrowth. Starch is a relatively simple polymer composed of glucose molecules that are linked together in two different forms. Amylose, which makes up 20–30% of normal starch, is an essentially linear molecule in which the

glucose units are joined end-to-end by α(1-4) linkages. Amylopectin is the major component of starch (comprising 70–80%) and is a much larger branched molecule in which about 5% of the glucose units are joined by α(1-6) linkages (Jobling 2004).

1.4. Calcium in Plat-based Foods

Calcium is an essential nutrient for plants and animals, with key structural and signaling roles, and its deficiency in plants can result in poor biotic and abiotic stress tolerance, reduced crop quality and yield (Dayod et al. 2010). In the case of texture of plant-based foods, the effects of Ca^{2+} have been widely investigated for the roles on the tissue structural rigidity, but the cation also has been studied on the effects on the physical properties of plant-based suspensions. In this study, we focused on the French fries and corn starches as the model plant-based foods with the possible textural improvement and alteration resulting from the presence of Ca^{2+}. The background of each plant-based food and the correspond effects of Ca^{2+} are discussed in the following sections.

1.5. Production of French Fries

Potato (*Solanum tuberosum* L.) is the fourth most important food crop in the world after rice, wheat and maize (Ezekiel et al. 2013). The reasons for its worldwide popularity are its high adaptability for a wide range of environments and a high nutritional value (Lutaladio and Castaldi 2009; De Jong 2016). In addition, potato is a very efficient food crop and produces more dry matter, protein and minerals per unit area in comparison with cereals (Ezekiel et al. 2013). Therefore, potato has been recognized as a food security crop worldwide (Lutaladio and Castaldi 2009). Indeed, potato production in the world still has increased from 336 million tons in 2004 to 382 million tons in 2014 (FAOSTAT 2016).

In the world, almost 10% of the harvested potato is converted into consumer

products. For the cases in developed countries, 1/3-2/3 of the potato is processed into the products, and French fries accounts for the majority among them (Keijbets 2008). Frozen French fries, a semi-processed food product that is a common form of French fries, are produced from raw potato generally by washing, peeling, cutting, blanching, drying, par-frying and freezing (Gould 1999). Those unit operations are performed to maximize final product quality for meeting a consumer demand. Important sensory characteristics for consumer preference of French fries involve appearance, odor, mouthfeel, taste, flavor and after effects (Teruel et al. 2015).

1.6. Texture of French Fries and Its Control

Texture of foods is defined by Bourne (2002) as a group of physical characteristics that: i) arise from the structural elements of the food; ii) are sensed by the feeling of touch; iii) are related to the deformation, disintegration and flow under a force, and; iv) are measured objectively by functions of mass, time and distance. Among the foods, plant-based foods have particular structure of cell wall, and its mechanical properties are major factors determining the texture (Van Dijk et al. 2002; Waldron et al. 2003; Colle, et al. 2010; Zhao et al. 2016). The same can be said of French fries; textural change of potato is dominantly determined by degradation of the middle lamella during cooking, accompanied by smaller contribution of inter-cellular pressure caused by swelling of gelatinized starch (Alvarez et al. 2001; Ormerod et al. 2002; Fuentes et al. 2014). Pectin is an integral component of plant cell walls, particularly in the middle lamella, and forms an interconnected network structure, independent of the cellulose-xyloglucan network (Talbott and Ray 1992; Carpita and Gibeaut 1993; Morris et al. 2009), and thereby plays important role in maintaining cell wall integrity and cell-cell cohesion (Parker et al. 2001; Aghdam et al. 2012; Daher and Braybrook 2015).

A number of studies, therefore, has been attempted to improve stability of the pectin

network in potato tuber tissue during cooking, due to depolymerization and structural changes of the pectin have shown as a determinative factor on texture of plant-based foods (Van Dijk, et al. 2002; Sila et al. 2006; Phothiset and Charoenrein 2014; Zhao et al. 2016). In terms of this attempts, a well-established phenomenon widely taken into consideration is that Ca^{2+} cross-links antiparallel homogalacturonan chains with negatively charged carboxyl groups to form structures called "egg-box" and tightening the cell walls (Braccini and Pérez 2001; Willats et al. 2006; Cybulska et al. 2011). Based on this phenomenon, two major methods aiming to improve the pectin stability using plant-inherent characteristics have been suggested as (i) controlling Ca^{2+} availability in cell wall matrix, and (ii) increasing the specific sites for cross-linking within pectin chains for Ca^{2+}. For example, soaking/blanching potato strips in an aqueous solution containing Ca^{2+} has demonstrated to retain texture of French fries even after frying (Khalil 1999; Tajner-Czopek 2003). In these cases, Ca^{2+} was simply infiltrated into the plant cell wall for improving cross-linker availability within pectin chains. On the other hand, low-temperature blanching is recognized as the another method to controlling texture of plant-based foods by increasing non-esterified homogalacturonan blocks in pectin molecules, specific sites for formation of the egg-box within pectin chains, through activation of the pectin methylesterase (Canet et al. 2005; Sila et al. 2005; Sila et al. 2006). Pectin methylesterase catalyzes the demethylesterification of methylesterified-homogalacturonan and then leaving free carboxylic groups that reacting with Ca^{2+} (Micheli 2001). The demethylation may also reduce susceptibility for heat induced β-elimination, responsible for the depolymerization of pectin chains (Canet et al. 2005; Moelants et al. 2014). Indeed, Aguilar et al. (1997) reported that low temperature blanching prior to frying increases hardness of French fries, without significant contribution of specific gravity.

1.7. Calcium fertilization during Potato Cultivation

Significance of calcium nutrient for potato tuber quality has been investigated from the late 1920s. However, effective application method of calcium fertilizer for potato tuber had been unclear until systematic study was conducted by group of J. Palta in University of Wisconsin-Madison. Series of researches revealed that specific path way of Ca^{2+} into potato tuber during growth and its influences on the tuber qualities. At first, they reported that roots on the stolons and roots growing directly from the tuber transported water to the tuber under field condition, by examining the transport of water soluble dye from different type of roots (Kratzke and Palta 1985). After 19 years, they used a radioactive isotope of calcium, ^{45}Ca, to show direct evidence for the calcium transport pathway to potato tubers. As a result, they confirmed that the roots on the stolon associated with the tuber supply water and calcium to the developing tuber (Busse and Palta 2006). In addition, the achievements by his group were not only a finding of the Ca^{2+} path way but also investigation of the effect of calcium concentration on potato tuber qualities. Specifically, enhancing calcium concentration of potato tuber through targeted fertilizer application contributes to reducing soft rot incidence (Kleinhenz et al. 1995), internal brown spot and hollow heart (Kleinhenz and Palta 1995; Kleinhenz et al. 1999) and blackspot bruise injury (Karlsson et al. 2006). The mechanisms involved in improving effects of calcium fertilization on the tuber qualities are still complex, but formation of the cross-links between pectin chains via Ca^{2+} is likely to be responsible for the improvement (Palta 2010), i.e., calcium fertilization possibly increases the cross-links within pectin chains via Ca^{2+}.

1.8. Perspective of Calcium Fertilization during Potato Cultivation toward Controlling Texture of French Fries

Texture of French fries is possibly controlled by enhancing Ca^{2+} availability in the cell wall matrix by soaking/blanching potato strips in calcium salt solution during a manufacturing process, which increases Ca^{2+} mediated cross-linking within pectin chains. However, the calcium salt solutions were used to achieving the enhancement of Ca^{2+} availability, although demand for processed foods without chemical additives has been increased among consumers. Meanwhile, targeted calcium fertilization may increase Ca^{2+} availability in cell wall matrix, possibly accompanied with the potato tuber production with lower physiological disorders. Thus, in this study, we attempted to control texture of French fries through enhancing Ca^{2+} availability by calcium fertilization to demonstrate production of both high quality potato tuber and its final product with desired characteristics.

1.9. Corn Starch with Calcium Salts

The composition and structure of starch granules vary considerably between different plants, affecting the properties and functions of starches from different crops. Among these, corn starch makes up more than 80% of the world market for starch (Jobling 2004). Those starches are used in food industries with the presence other food ingredients including calcium salts. As antibrowning agent, antioxidant, coagulant, preservative and pH regulator, calcium salts may add in to various food products along with the starches. Recently, it has been reported that coexistence of various calcium salts influences on pasting, texture and rheological properties of starches varying in botanical sources (Zhou et al. 2014; Chuang et al. 2015). Thus, the evaluation of the effects of the Ca^{2+} in the suspension composed of plant-based foods should be carried out to produce the final product with desired texture.

1.10. Summary

The texture of plant-based foods, i.e., French fries and corn starch suspension, could be

controlled/ altered by the Ca^{2+}. Thus, in this study, the effects of Ca^{2+} in the plant-based foods were investigated to provide insights into further development and improvement of the plant-based foods.

Chapter 2. Effects of Calcium Concentration in Potato Tuber Cells on the Formation of Cross-Links between Pectin Molecules by Ca^{2+}

2.1. Introduction

The texture of cooked and processed potato is an important quality attribute contributing consumer acceptance and preference (Burton 1989). As cell-cell adhesion in plant tissue is regulated through middle lamella (Jarvis et al. 2003), textural change of potato tuber is dominantly determined by degradation of the middle lamella during cooking, accompanied by smaller contribution of inter-cellular pressure caused by swelling of gelatinized starch (Alvarez et al. 2001; Ormerod et al. 2002; Fuentes et al. 2014). Pectin is a major constituent of potato parenchymal middle lamella (Caffall and Mohnen 2009). The galacturonic acid of potato pectin is partially methyl- or acetyl-esterified (Ishii 1997; MacKinnon et al. 2002), and non-esterified homogalacturonan blocks in pectin molecules are the sites for cross-linking polymeric chains through the site specific interaction with Ca^{2+} (Grant et al. 1973; Braccini and Pérez 2001). Thus, the cross-linking of pectin via Ca^{2+} is known as a key factor for determining processing properties of potato and its products, due to an increase in the thermal stability of Ca^{2+} cross-linked pectic polysaccharides, which are involved in cell-cell adhesion (Van Marle et al. 1997; Ng and Waldron 1997; Khalil 1999; Matsuura-Endo et al. 2002; Tajner-Czopek 2003; Ross et al. 2011). In addition, availability of Ca^{2+} was suggested as a limiting factor, regarding formation of the cross-linked pectin molecules within cell wall of potato tuber (Ng and Waldron 1997). Indeed, soaking or blanching of potato strips into $CaCl_2$ solution resulted in significant increase in hardness and reduction of oil absorption of French

fries (Khalil 1999; Tajner-Czopek 2003). Therefore, increased availability of Ca^{2+} in the potato tuber middle lamella during growth might also contribute to higher processing properties of potato and quality of its products.

An increased application of calcium fertilizer, targeted to the roots on the stolon associated with tuber, has been demonstrated to increase calcium concentration in the tubers (Kratzke and Palta 1986; Kleinhenz et al. 1999; Ozgen et al. 2003). Recently, we found a significant effect of calcium fertilization on hardening of the center and bud-end of French fries after 6 months of frozen storage. We hypothesized that this improvement of frozen storability of French fries made from calcium fortified tubers may be due to the formation of cross-linkages in the middle lamella pectin via Ca^{2+} (Murayama et al. 2016). However, the mechanisms involved in the role of calcium transported into potato tuber for the formation of Ca^{2+} cross-linking in the middle lamella pectin are not yet clearly investigated. Therefore, the objective of the present study was to investigate whether calcium absorbed by potato tuber concomitantly increases calcium concentration in cell wall and how calcium concentration influences on the formation of cross-linking of pectin via Ca^{2+}. In agreement with previously published results, a wide variation in calcium concentration among tubers harvested from any plot was observed (Kleinhenz et al. 1999). This variation is expected because the calcium is transported from soil surrounding the tuber area via roots closely associated with tubers (Kratzke and Palta 1985; Kratzke and Palta 1986). Thus, for the purpose of this study, each tuber was individually analyzed. Their properties were compared between the low-calcium and high-calcium tubers on the three harvest dates, where tubers having calcium concentration less than or equal to the median calcium concentration among tested tubers were classified as the low-calcium tubers, while tubers having calcium concentration higher than the median concentration among tested tubers were classified as the high-calcium tubers.

2.2. Materials and Methods

2.2.1. Plant Material and Field Experimental Design

Potato tubers used in this study were grown in a field in Memuro, Tokachi, Hokkaido, Japan, in which soil type is Andosol (Fueki et al. 2011). Toyoshiro, an early maturing variety for processing, was planted on May 7, 2015 and grown in a commercial field using conventional fertilization commonly practiced by commercial farmers in Tokachi, according to the fertilizer application guide by Hokkaido Prefecture (Hokkaido Department for Agropolicy 2010). Fertilizer application in this area includes ammonium sulfate, ammonium phosphate, potassium sulfate and magnesium sulfate to give 60, 88, 91 and 24 kg ha-1 of N, P, K and Mg, respectively. On July 3, 2015, calcium was applied as $CaCl_2$ solution on the top of the hill to give 135 kg ha-1 of calcium. In one row, five plants were applied with $CaCl_2$ solution and adjacent five plants were used as a control without calcium application. This treatment was repeated on three rows, with one guard row being left untreated between the experimental rows. Tubers were hand harvested from the center three plants of the experimental rows on 78, 99 and 120 days after planting (DAP). Those harvest times correspond to 3, 6 and 9 weeks after the calcium treatment. At each harvest, at least 19 medium size tubers were collected and analyzed. The average tuber size was 67.8, 96.3 and 103.1 g on 78, 99 and 120 DAP, respectively. Rainfall during cultivation was as follows: May 7 to July 3 with 84.0 mm; July 3 to 78 DAP with 35.5 mm; 78 to 99 DAP with 72.5 mm and 99 to 120 DAP with 57.5 mm.

2.2.2. Preparation of Freeze-dried Tuber Sample and Cell Wall Material (CWM)

After harvest, all tubers were stored in a storage chamber at 4 °C and used for preparation of CWM within 96 h as follows. For this purpose, a potato tuber was peeled and diced into $0.5 \times 0.5 \times 0.5$ mm^3, approximately 25 g of diced tuber was used for preparation of CWM and residual diced tuber was used for freeze-dried tuber sample. The freeze dried sample was stored at 4 °C until analysis of calcium concentration. CWM was prepared based on the method reported by Hoff and Castro (1969) with slight modification. Approximately 25 g of diced tuber was homogenized three times in 50 ml of cold deionized water (< 4 °C), using an Osterizer blender (Sunbeam Products, Inc., US) for 30 sec at maximum speed. Additionally, 150 ml of cold deionized water was added into the blender and homogenized again for 10 sec at maximum speed. Resultant slurry was sieved through a 106 μm sieve and washed with 500 ml of cold deionized water. The residue was suspended in 20 ml of cold deionized water and transferred to a 50 ml Falcon tube. The slurry was sonicated for 3 min at 200 W using Ultrasonic Processor (VC-505, SONICS & MATERIALS, Inc., US) with ice around the sample. The slurry was sieved through a 106 μm sieve and washed with 1000 ml of chilled deionized water. Residual CWM was transferred into a 30 ml vial, freeze-dried and stored at 4 °C until analysis. The temperature of CWM slurry was kept lower than 10 °C throughout preparation using chilled deionized water and ice-cooling to minimize degradation and demethylation of pectin molecule (Moledina et al. 1981; Andersson et al. 1994).

2.2.3. Determination of Calcium Concentration of Freeze-dried Tuber Sample and CWM

One hundred milligram of freeze dried potato tuber sample and 50 mg of CWM were wet-digested with H_2SO_4/H_2O_2 mixture (2:1, v/v), and resultant solution was diluted to 50 ml using deionized water. The solution obtained was subjected to calcium quantification using

an inductively coupled plasma spectrometer (ICPS-8100, Shimadzu Co. Ltd., Japan) with triplicate measurements.

2.2.4. Sequential Extraction of Pectin Fractions

Pectin fractions were extracted using a modified procedure based on the methods reported by Marle and Recourt (1997), Van Dijk et al. (2002) and Vicente et al. (2005). For this purpose, 50 mg of CWM was transferred into a 50 ml plastic test tube containing 15 ml of deionized water and then stirred with a magnetic stirrer for 8 h at 20 °C. The suspension was filtered through a filter paper (Advantec No. 5A), and the residue was washed with deionized water. The filtrate was diluted to a final volume of 50 ml, and was encoded as water soluble pectin fraction. The residue was then re-suspended in 15 ml of 0.05 M cyclohexane diamine tetra acetic acid (CDTA, pH 6.5), and stirred for 16 h at 20 °C. The suspension was filtered through a filter paper, and the residue was washed with deionized water. This filtrate was diluted to a final volume of 50 ml, and was encoded as chelator soluble pectin fraction. Subsequently, the residue was hydrolyzed by 72% (v/v) sulfuric acid for 3 h at 20 °C. Residual hydrolysate was diluted to 1 M sulfuric acid and then hydrolyzed further for 1 h at 100 °C (Selvendran and O'Neill 1987). Residual hydrolysate was neutralized with sodium hydroxide solution. After cooling, the suspension was filtered through a filter paper. The residue was washed with deionized water and filtrate was diluted to final volume of 100 ml. This was encoded as insoluble pectin fraction. Duplicate determinations were performed for each CWM samples.

2.2.5. Quantification of Pectin

The galacturonic acid content in the pectin fractions were measured by the *m*-hydroxydiphenyl method, as described by Blumenkrantz and Asboe-Hansen (1973) with the modifications proposed by Filisetti-Cozzi and Carpita (1991). Absorbance at 525nm was read by spectrophotometer (U-5100, Hitachi Ltd., Japan), 20 min after addition of m-hydroxydiphenyl solution. Glucuronic acid exists only in trace amount in potato cell walls (Jarvis et al. 1981). Thus, the glucuronic acid content was considered to be negligible. Duplicate measurements were performed for each pectin fraction.

2.2.6. Determination of Degree of Methylesterification

Methanol was released by saponifying 4 mg of CWM and quantified by colorimetrically, based on the method described by Wood and Siddiqui (1971) with the modifications proposed by Kim and Carpita (1992). Percent methylation was calculated as a molar ratio between methanol released and total galacturonic acid. The total galacturonic acid content was calculated as a sum of water soluble, chelator soluble and insoluble pectin contents. Triplicate measurements were performed for each CWM sample.

2.2.7. Microstructure Analysis of CWM Prepared from Mature Potato Tuber

CWMs prepared from mature low- and high-calcium tubers harvested on 120 DAP were analyzed using atomic force microscopy (AFM). Each CWM was suspended in 20 mM HEPES buffer (pH 7.0) and stirred at 20 °C for 90 min. Part of CWM prepared from low-calcium tuber was subjected to pectinase treatment by suspending it in 20 mM HEPES buffer containing 0.1 % (w/v) pectinase (Pectolyase Y-23, Kikkoman Co., Tokyo, Japan) and

incubating at 20 °C for 90 min with stirring. The suspension was deposited on a poly-L-lysine coated glass substrate, and was air-dried at room temperature for at least 15 min after carefully removing excess liquid by blotting. AFM imaging was performed in air using a Multimode 8 microscope (Bruker, Santa Barbara, US) operated in peak-force tapping mode at a scan rate of 1 Hz and in a scan area of $10 \times 10\ \mu m^2$. Imaging of CWMs originating from the vascular tissue was avoided by visual observation under an optical microscope that enabled to distinguish parenchyma cell walls from vascular cell walls. AFM images were analyzed using NanoScope Analysis software version 1.50 (Bruker, Santa Barbara, US).

2.2.8. Statistical Analysis

Statistical analyses were conducted using SPSS for Windows (ver. 17.0). Two-tailed *t*-tests were conducted between the low- and high-calcium tubers on calcium concentration, chelator soluble and insoluble pectin contents and degree of methylation for each of the three harvest dates. One-way analysis of variance was carried out within the three harvest dates for all data sets. When significant differences were observed ($p < 0.05$), Tukey's multiple range test was performed. The data obtained from individual tubers and corresponding CWMs were subjected to correlational analysis.

2.3. Results

2.3.1. Changes in Calcium Concentration of Potato Medulla Tissue and Cell Wall during Tuber Growth

Individual potato tubers contained a wide range of calcium from 99 to 221, 98 to 266 and 99

to 221 μg g^{-1} tuber on dry weight (dry wt) on 78, 99 and 120 DAP, respectively. Calcium concentrations of potato tubers, expressed as the mean value of all tubers harvested, were not significantly different within the three sampling dates (Fig. 2.1A). On the other hand, a significant increase in calcium concentration of CWM was observed in mature tubers at 120 DAP as compared with CWM prepared from tubers harvested at 78 and 99 DAP ($p < 0.05$; Fig. 2.1A). Comparisons were made between the low- and high-calcium tubers on calcium concentrations of tubers and CWMs at the each three growth dates (Fig. 2.1B and 2.1C). The ranges of calcium concentrations of the low- and high-calcium tubers, for instance, on 120 DAP of mature potato were 98.88 to 157.29 and 158.27 to 220.83 μg g^{-1} tuber on dry wt, respectively. The high-calcium tuber contained significantly higher amount of calcium in the tuber tissue than the low-calcium tuber throughout the three harvest dates ($p < 0.05$; Fig. 2.1B), whereas significantly higher calcium concentration of CWM prepared from the high-calcium group was observed on 120 DAP ($p < 0.05$; Fig. 2.1C).

2.3.2. Effect of Calcium Concentration of Potato Tuber on Pectin Composition and Degree of Methylation

Chelator soluble and insoluble pectin contents and degree of methylation for CWMs prepared from the low- and high-calcium tubers harvested on the three harvest dates are presented in Table 2. The high-calcium tuber, harvested on 120 DAP, showed significantly higher chelator soluble pectin content as compared with that of the corresponding low-calcium tuber ($p < 0.05$). For insoluble pectin content and degree of methylation, there were no significant differences between the low- and high-calcium tubers on all of the three harvest dates ($p < 0.05$). On the other hand, potato tuber harvested on 120 DAP showed significantly higher chelator soluble and insoluble pectin contents as compared with those of the tubers harvested

on 78 and 99 DAP ($p < 0.05$). Degree of methylation was significantly decreased with the growth of the tubers ($p < 0.05$).

2.3.3. Role of Calcium Concentration on Formation of Cross-Linking of Pectin Molecules via Ca^{2+} in Cell Wall of Potato Tuber

Correlation analysis was carried out to elucidate the effect of calcium concentration of cell wall of potato tuber on the formation of the cross-linking of pectin molecules via Ca^{2+}. Significantly positive correlations were observed between calcium concentrations of tuber tissue and CWM on 99 and 120 DAP as shown in Fig. 2.2A and 2.2B (r = 0.594 with $p < 0.01$ and r = 0.512 with $p < 0.05$, respectively). However, the significant correlation was not found on 78 DAP. Figure 2.3 indicates that calcium concentration of CWM was significantly and positively correlated with chelator soluble pectin content on the three harvest dates with r = 0.459 to 0.759 ($p < 0.01$ for 78 and 120 DAP, $p < 0.05$ for 99 DAP). In contrast, no significant correlation was observed between calcium concentration of CWM and insoluble pectin content on the three harvest dates with r = -0.230 to 0.240 ($p > 0.05$). No significant correlation was found between degree of methylation and chelator soluble pectin content on the three harvest dates (r = -0.212 to -0.008, $p > 0.05$).

2.3.4. Microstructure of CWM Prepared from Low- and High-calcium Potato Tubers

CWMs prepared from the low- and high-calcium tubers harvested on 120 DAP were subjected to AFM studies after incubation in 20 mM HEPES buffer with or without containing 0.1% of pectinase at 20 °C for 90 min. Figure 2.4A shows a topographical image

of CWM prepared from the low-calcium tubers and then treated with pectinase. The CWM is revealed as a laminated fibrous structure. These fibrous structures were considered to represent cellulose microfibrils since pectin was completely removed by the enzymatic treatment. Figure 2.4B shows a topographical image of CWM prepared from the low-calcium tubers but untreated with pectinase. The image shows the presence of two types of branched fibrous structures differing distinctively in thickness as indicated by black and white arrows. Thicker fibrils appear to be fairly straight, indicating a certain mechanical rigidity of these fibrils. In contrast, thinner fibrils appear to be more deformable. It has been reported that pectin and hemicelluloses are flexible and deform upon contact with an AFM probe (Morris et al. 1997; Cárdenas-Pérez et al. 2016). Some ends of thinner fibrils can be seen in the image. The presence of these ends can be explained if these fibrils are parts of three-dimensional networks and an end is observed where the height of a network decreases too abruptly for an AFM probe to reach the network. Figure 2.4C shows a topographical image of CWM prepared from the high-calcium tubers but untreated with pectinase. The image reveals the presence of thick and thin fibrils similar to the image of the low-calcium CWM. However, the AFM image of the CWM prepared from the high-calcium tubers (Fig. 2.4C) appears to be more blurred than that prepared from the low-calcium tubers (Fig. 2.4B), which may indicate the presence of a greater amount of deformable polysaccharides such as pectin and hemicellulose in the high-calcium CWM. Circular depressions having diameters of approximately 3 μm observed in Fig. 2.4B and 2.4C were considered to represent primary pit-fields, thinner areas with several plasmodesmata, on the primary cell wall (Kirby et al. 1996; Orfila and Knox 2000; Ding and Himmel 2006).

2.4. Discussion

2.4.1. Increase in Calcium Concentration of Tuber Tissue Leads to Increase in Cell Wall Bound Calcium in Mature Potato Tuber

In the present study, it was shown that calcium absorbed by potato tuber was distributed within and bound to its cell wall as the water insoluble form on 99 DAP or later (Fig. 2.2A and 2.2B). Busse and Palta (2006) reported that calcium is transported to potato tuber parenchyma tissue along with water via the stolon roots closely associated with the tuber, without direct uptake across the periderm. Furthermore, Subramanian et al. (2011) suggested that the direct uptake of minerals, including calcium, across living epidermis prior to the full development of the periderm would be possible in a developing tuber. Therefore, significantly positive linear correlation between calcium concentrations of the mature potato tuber and its CWM indicates that calcium transported through either living epidermis or stolon roots increases the calcium concentration of both tuber tissue and cell wall. This observation is in accordance with McGuire and Kelman (1983).

2.4.2. Calcium Availability in Tuber Cell Wall Influences Formation of the Cross-Linking of Pectin Molecules via Ca^{2+}

With an increase in the distributed calcium concentration of the cell wall, chelator soluble pectin content increased throughout tuber bulking and maturation stages (Fig. 2.3). The formation of Ca^{2+} cross-linkages within pectin molecules is regulated through both intrinsic and extrinsic parameters such as the amount and distribution of methyl esters, chain length, pectin side chains, the amount of Ca^{2+} and pectin content (Fraeye et al. 2010). Chelating-agent is widely used to solubilize the pectin molecules forming the "egg-box structure" in plant cell wall, and chelator soluble pectin is referred to as the pectin held in the

cell wall by ionic bonds (Jarvis 1982). Therefore, significantly positive correlation between the amounts of calcium and chelator soluble pectin contained in cell wall indicates that an increase in calcium concentration of tuber cell wall induces the formation of the Ca^{2+} cross-linkages of pectin. Indeed, Sriamornsak (2003) reported that the formation rate of the cross-linkages possibly increased by a given amount of Ca^{2+} in the presence of a sufficient amount of non-esterified pectin. Furthermore, $R = 2[Ca^{2+}]/[COO-]$ is the stoichiometric ratio of Ca^{2+} concentration to the amount of carboxyl group of non-esterified galacturonic acid (Fraeye et al. 2010), and, in the present study, approximately 10-times higher amount of calcium, as compared with calcium present in CWM, is required to fully saturate all non-methylesterified galacturonic acid residues in the mature tuber. Thus, despite that there are other factors involved in the formation of the cross-linkage, this great difference between the amount of existing calcium in cell wall and the required Ca^{2+} for the saturation may support the hypothesis that the availability of Ca^{2+} is the limiting factor for the formation of the Ca-pectin cross-linkages.

2.4.3. Insoluble Pectin Content is not altered by Increase in Calcium Concentration of Cell Wall

There were no significant differences in insoluble pectin contents between the low- and high-calcium tubers as well as no significant correlation between the amount of insoluble pectin and the calcium concentration of cell wall, throughout tuber bulking and maturation stages (Table 2.1). The insoluble pectin may be mainly consisted of covalently bonded pectin molecules among homogalacturonan, rhamnogalacturonan-I and -II and neutral polysaccharides such as xyloglucan (Caffall and Mohnen 2009). Meanwhile, significant increase in insoluble pectin content during growth of the tuber may be due to accumulation of

pectin in middle lamella and primary cell wall (Bush et al. 2001). Therefore, calcium accumulated into cell wall significantly affects the formation of Ca^{2+} cross-linkages of pectin rather than the increase in insoluble pectin content.

2.4.4. Degree of Methylation of Pectin Molecules is not the Limiting Factor on Formation of the Calcium-Pectin Interaction

Non-significant correlation between the amount of chelator soluble pectin and the degree of methylation further suggests that there are enough carboxyl groups of galacturonic acid available for cross-linking via Ca^{2+}, although higher degree of methylation contribute to gradual decrease in the affinity of Ca^{2+} for pectin molecules (Thakur et al. 1997; Tibbits et al. 1998). Pectins are secreted from Golgi apparatus into cell wall as highly methyl-esterified forms, and subsequently they can be demethylated by pectin methyl-esterase (Micheli 2001). Indeed, Bush et al. (2001) reported that a ratio of monoclonal antibody of JIM5, which stains homogalacturonan methyl-esterified up to 40%, is increased in potato parenchymal cell walls during tuberization. Similarly, significant decrease in the degree of methylation with growth of the tuber was observed in this study (Table 2.1). It is therefore assumed that there were enough chelation sites within pectin chains for the cross-linking with Ca^{2+} at the conventional harvest time, even though Toyoshiro which vegetation period is shorter than other middle or late maturing potato varieties.

2.4.5. Calcium Concentration in Mature Potato Tuber Influences Cell Wall Structure

The cell wall in the parenchyma tissue consists of a primary cell wall and middle lamella, but

lacks lignified secondary wall layers (Gibson 2012). In this study, we observed the parenchyma cell wall prepared from potato tubers after removing the plasma membrane (Ding and Himmel 2006). The pectinase treatment resulted in observation of laminated structures of cellulose microfibrils (Fig. 2.4A), similar to those observed in previous AFM studies(Kirby et al. 2006; Cybulska et al. 2013; He et al. 2015). In contrast, CWMs that were not treated with pectinase revealed the presence of two distinct types of fibrous structures. The thicker fibrils may represent cellulose macrofibrils, diameters of which have been reported to range from 50 to 250 nm (Ding and Himmel 2006). The thinner fibrils appear to be deformable and form three-dimensional networks with fairly large mesh sizes. These structural features do not resemble those that can be seen in the reported AFM images of cell walls (Kirby et al. 1996; Kirby et al. 2006; Thimm et al. 2009; He et al. 2015). HEPES buffer used in this study solubilizes a water soluble fraction of pectin among other pectin fractions (Melton and Smith 2001; van Dijk et al. 2002). Pectin molecules cross-linked by Ca^{2+} were considered not to have been solubilized but to have existed in the CWM subjected to AFM imaging. In the AFM image of CWM prepared from the low-calcium tubers (Fig. 2.4B), thin and thick fibrils were more clearly revealed as compared with those prepared from the high-calcium tubers (Fig. 2.4C). Pectin and hemicelluloses are known to be difficult to be visualized using AFM, since they are flexible and deform upon contact with an AFM probe (Morris et al. 1997; Cárdenas-Pérez et al. 2016). Therefore, it is likely that a limited solubilization of pectic polysaccharides in the high-calcium tubers, due to an enhanced formation of cross-links between pectin molecules via Ca^{2+}, has limited the exposure of network structures in their CWM and, consequently, contributed to an enhanced formation of pectin-calcium networks in the cell wall.

2.5. Conclusion

The results of the present study using a variety of Toyoshiro indicated that an increased absorption of calcium by a potato tuber concomitantly increased the calcium concentration in the cell wall 99 DAP or later, and that there were linear correlations between the calcium concentration in the cell wall and the formation of cross-links between pectin molecules via Ca^{2+} throughout tuber bulking and maturation stages. The degree of methylation was not found to be a limiting factor on the formation of cross-links between pectin molecules. Furthermore, a higher calcium concentration of a mature potato tuber contributed to the enhancement of resistance of pectin-calcium networks in the parenchyma cell wall against water and HEPES buffer washing.

Table 2.1 Effect of calcium concentration of potato tuber on amount of chelator soluble pectin, insoluble pectin and degree of methylation at 78, 99 and 120 days after planting.

Days after planting	Chelator soluble pectin in cell wall (mg g^{-1}, db)			Insoluble pectin in cell wall (mg g^{-1}, db)			Degree of methylation (%)		
	Low-Ca tuber	High-Ca tuber	Average	Low-Ca tuber	High-Ca tuber	Average	Low-Ca tuber	High-Ca tuber	Average
78	9.78	10.69	10.22 a	159.31	138.43	149.32 a	57.68	60.68	59.11 b
99	11.37	10.92	11.14 a	147.09	131.28	139.19 a	53.48	52.78	53.13 ab
120	12.89	19.39 *	15.97 b	216.68	212.86	214.87 b	49.71	46.14	48.02 a

* indicates significant differences between low-calcium tuber and high-calcium tuber at $p < 0.05$. The mean values within columns followed by the same letters are not significantly different at $p < 0.05$.

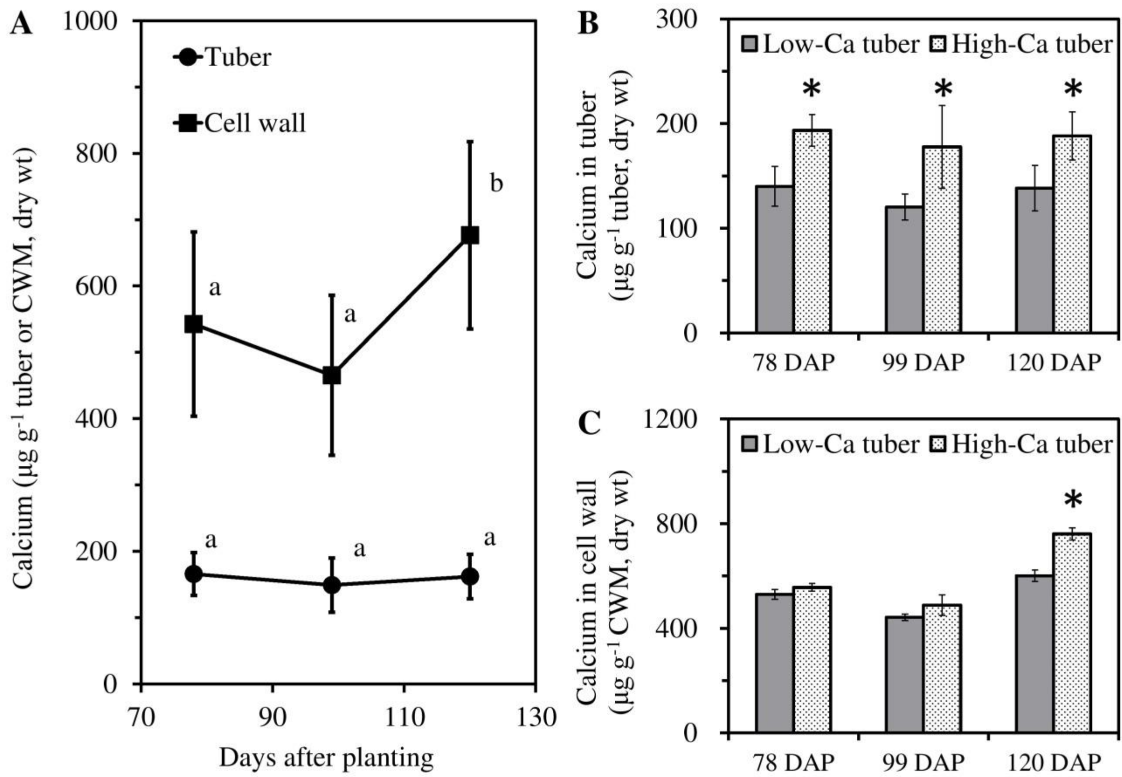


Figure 2.1 Changes in calcium concentration of potato tuber and cell wall material at 78, 99 and 120 days after planting (DAP).

The mean values and standard deviations were calculated from at least 19 samples for A and 9 samples for B and C at the each time point. Different letters shown in A indicate significant difference at $p < 0.05$. * shown in B and C indicate significant difference at $p < 0.05$.

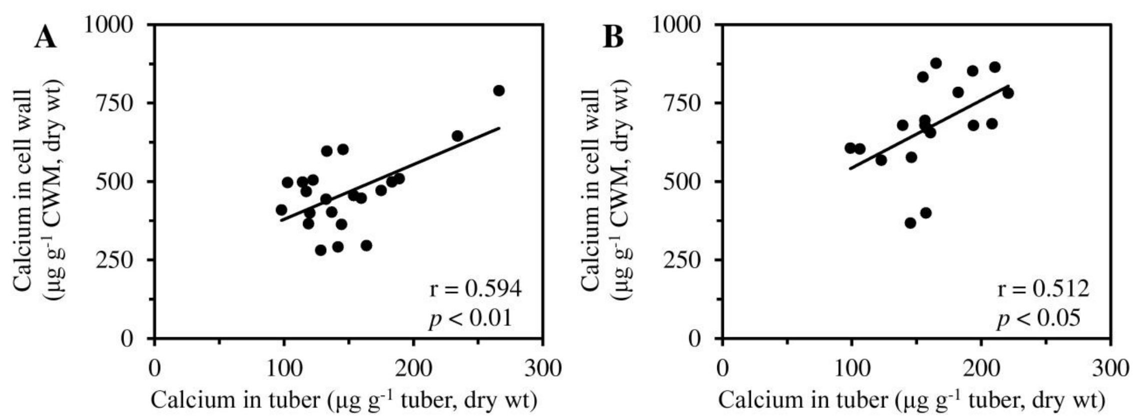


Figure 2.2 Linear relationships between calcium concentrations of tuber and cell wall at 99 (A) and 120 (B) days after planting.

Each point represents the mean value of individual potato tubers and corresponding CWMs.

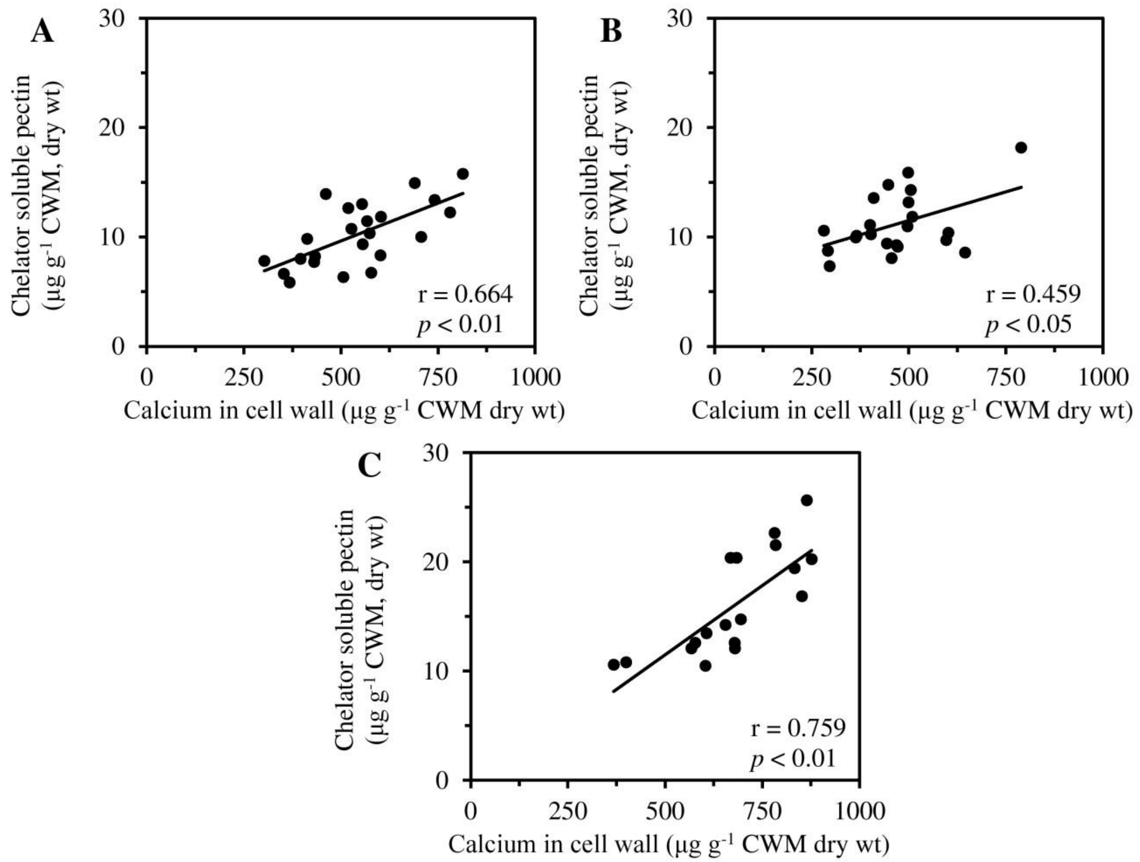


Figure 2.3 Linear relationships between calcium concentration and chelator soluble pectin content of cell wall material at 78 (A), 99 (B) and 120 (C) days after planting.

Each point represents the mean value of individual CWMs prepared from potato tubers.

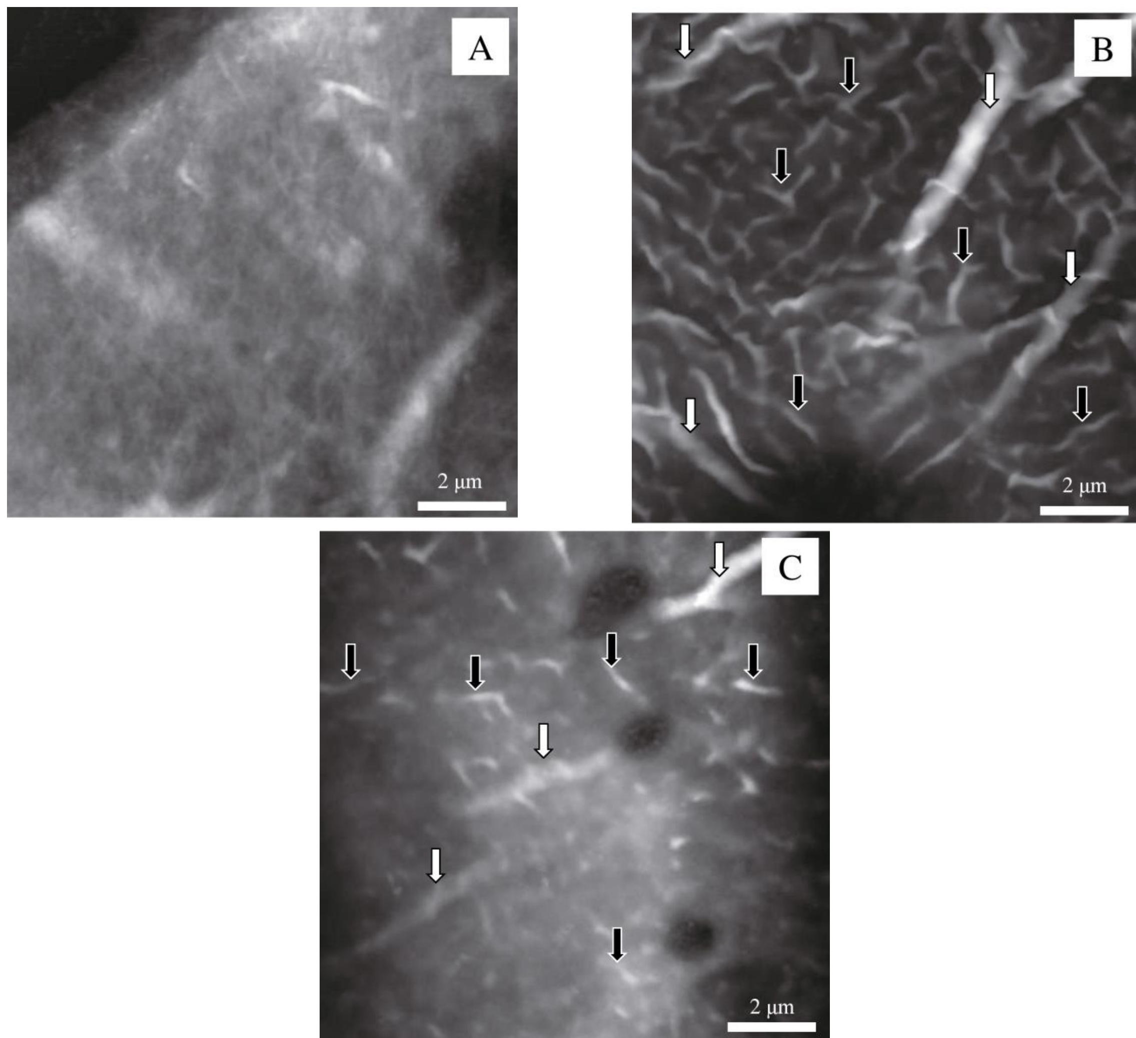


Figure 2.4 Topographical AFM images of CWMs prepared from low-calcium (A, B) or high-calcium (C) potato tubers harvested 120 days after planting.

CWMs were suspended in 20 mM HEPES buffer with (A) or without (B, C) containing 0.1% pectolyase, incubated at 20 °C for 90 min, deposited onto glass substrates, and air-dried prior to imaging. Black and white arrows indicate thin and thick fibrils, respectively. Bars represent 10 µm.

Chapter 3. Effect of Calcium Fertilization on Processing Properties and Storability of Frozen French Fries

3.1. Introduction

French fries are a popular food world-wide because of their pleasant taste, which is created by the combination of a crispy crust, soft inside and typical fried potato flavor (Van Loon et al. 2005). Frozen French fries, a semi-processed food product that is a common form of French fries, are produced from raw potato generally by washing, peeling, cutting, blanching, drying, par-frying and freezing (Gould 1999). The frozen French fries are final-fried by consumers, e.g., households and restaurants, prior to serving. During frozen storage of French fries, however, the growth of ice crystals, known as an ice recrystallization, is promoted by prolonged periods of frozen storage (Sutton et al. 1996; Hagiwara et al. 2002; Pronk et al. 2005), and the presence of large ice crystals within the tuber tissue can result in physical damage, drip loss and a consequent reduction in product quality (Sun and Li 2003).

Texture is a major factor determining consumers' acceptance of French fries, and depends on both the characteristics of the raw material and the processing history (Du Pont et al. 1992). In addition, oil content is another important quality characteristic of French fries that contributes to consumer acceptability (Romani et al. 2008), notable because of the demand to reduce the oil content of fried foods in response to health concerns (Moreira and Barrufet 1998). According to various studies, the tuber, starch and pectin properties of potato contribute to the texture as well as the oil uptake of French fries (Kirkpatrick 1956; Johnston et al. 1970; Sayre et al. 1975; Aguilar et al. 1997; Khalil 1999; Tajner-Czopek 2003; Tajner-czopek and Figiel 2003; Golubowska 2005). For example, Johnston et al. (1970)

reported that the texture of French fries was positively correlated with starch content, dry matter content and specific gravity of raw potato tubers; and higher viscosity and swelling power of starches isolated from raw potatoes were associated with lower hardness of French fries. Moreover, blanching of potato strips in 0.7% $CaCl_2$ solution reduced oil penetration by up to 14% in French fries due to the formation of Ca-pectates (Khalil 1999).

The starch and pectin properties of potato can be modified by mineral fertilization. For instance, in vitro experiments demonstrated calcium (Ca) fertilization enhanced the Ca concentration of the potato tuber cell wall and increased its galacturonic acid content (McGuire and Kelman 1986). Ca fertilization of potato is also known to improve resistance against internal and external defects (Tawfik and Palta 1992; Karlsson et al. 2006; Ozgen et al. 2006), due to the formation of Ca^{2+} bridges between pectin molecules in the middle lamella, which provides cell wall rigidity (Palta 2010). In addition, a study assessing the effect of Ca fertilization on the pasting properties of potato starch revealed significant correlations between the Ca content of potato tuber and pasting properties, such as peak viscosity, breakdown and final viscosity, of isolated starches (Bogucka 2014). Furthermore, varietal and genetic differences affect the starch and pectin properties of potatoes, even if the tubers are grown under an identical fertilizer application regime (Clough 1994; Haase and Plate 1996; van Marle et al. 1997; Van Dijk et al. 2002; Park et al. 2005; Karlsson et al. 2006).

Thus, the objectives of the present study were to evaluate the effect of Ca fertilization on the tuber, starch and pectin properties of three different varieties of processing-type potatoes, and their influence on the processing properties and storability of frozen French fries.

3.2. Materials and Methods

3.2.1. Potato Varieties

Three processing varieties of potatoes (Solanum tuberosum L.) were used in the present study, namely Toyoshiro (TS), Kitahime (KH) and Snowden (SD). TS is an early variety, while KH and SD are late varieties.

3.2.2. Potato Production

Potatoes were cultivated from May to September 2014 under conditions commonly practiced by commercial farmers in Tokachi Subprefecture and based on the fertilizer application guide of Hokkaido Prefecture (Hokkaido Department for Agropolicy 2010). The experimental field was divided into two sections, where conventional and calcium fertilizers were applied at 0 and 147 kg-Ca/ha, respectively, using calcium sulfate.

3.2.3. Raw Materials

Approximately 50 kg of each harvested potato variety was stored, after harvesting on respective harvest dates, in a storage chamber at 4 °C for 1 month, and then these potatoes were further stored at 14 °C for 1 month for conditioning.

3.2.4. Determination of Specific Gravity and Dry Matter Content of Potato Tuber

Specific gravity was determined by the method reported by Nzaramba et al. (2013). To measure dry matter content, 3 g of diced potato tuber was vacuum dried at 60 °C and 30 cmHg to constant weight using a vacuum drying oven (DPF-41; Yamato Scientific Co., Ltd.,

Tokyo, Japan).

3.2.5. Preparation of Starch and Cell Wall Material (CWM) from Potato Tuber

Starch and CWM were prepared from potato tuber based on the method described by Noda et al. (2004) with slight modification. In addition, this procedure was designed to prevent β-elimination of pectin by maintaining a temperature lower than 80 °C throughout preparation (Sila et al. 2009). One kilogram of potato tubers was washed using deionized water (DW), cut into 1 × 1 cm cubes, homogenized with 10 L of DW using a blender (JMM-1020-WY; YAMAZEN Co., Osaka, Japan) and filtrated through 710, 106 and 75 μm metal sieves. The residue was suspended in 3 L of DW, blended and sieved again, and this procedure was repeated twice more. The residue remaining on the sieves was dried in a hot air oven (WFO-700; TOKYO RIKAKIKI Co., Ltd., Tokyo, Japan) at 55 °C for 16 h and the resultant material was used as CWM. The obtained starch slurry was filtrated through a glass filter (26G2; ASAHI GLASS Co., Ltd., Tokyo, Japan), and then the residual starch was suspended in 3 L of DW and stirred overnight. The starch slurry was again filtrated through a glass filter, and the washing procedure was repeated twice more. The obtained starch was dried in the hot air oven at 55 °C for 4.5 h.

3.2.6. Analytical Methods for Measuring Physical Properties of Starch

Mean particle size was determined using a compact laser diffraction particle size analyzer (LA-300; HORIBA Co., Ltd., Kyoto, Japan). The method reported by Noda et al. (2004) was employed to determine pasting properties, where a 4% starch slurry (on a dry weight basis)

was subjected to analysis using a Rapid Visco Analyzer (RVA-4; Newport Scientific, Inc., Warriewood, Australia).

3.2.7. Analytical Methods for Chemical Composition of CWM

Moisture content was determined using the standard methods of the Association of Official Analytical Chemists (AOAC) (AOAC International 2005). Ca content was determined by an inductively coupled plasma mass spectrometry (ICPS-8100; Shimadzu Co., Ltd., Kyoto, Japan) based on the method reported by George et al. (2004) with slight modification. CWM in a porcelain crucible was dry-ashed using a muffle oven (FM-35; Yamato Scientific Co., Ltd.) at 550 °C for 8 h. The resultant ash was dissolved in 2N-HCl, and the solution obtained was subjected to mineral analysis by ICP. Chelating-agent soluble pectin (CSP) content was determined by extracting alcohol insoluble solid (AIS) from CWM based on the method described by Sabir et al. (1976) and Kato et al. (1997) with slight modification. AIS was prepared by washing CWM with 95% (v/v) ethanol, 99% (v/v) ethanol, acetone and diethyl ether. AIS (100 mg) was extracted with 50 ml of DW at room temperature overnight to remove the water-soluble pectin. The suspension was filtrated, and the residue was washed with DW. The residue was then re-suspended in 50 ml of 0.4% sodium hexametaphosphate and extracted for 1 h at 90 °C. The suspension was filtrated, and the residue was washed with DW. The supernatant and washings were combined, and termed CSP. An aliquot of the CSP fraction was saponified with 0.2 M NaOH, and then subjected to the m-hydroxydiphenyl method described by Blumenkrantz and Asboe-Hansen (1973) to quantify galacturonic acid. Glucuronic acid exists only in trace amounts in the potato cell wall (Jarvis et al. 1981), and thus the glucuronic acid contribution was ignored.

3.2.8. Preparation of French Fries

French fries were prepared based on the procedure described by Agblor and Scanlon (2000). Potato tubers were washed and peeled using a commercial peeler. Strips (1.0 cm × 1.0 cm × length of tuber) were cut along the apical to basal axis from the pith of the parenchyma region of the tubers using a commercial French fries cutter. The strips were rinsed with tap water immediately after cutting. Subsequently, the strips were blanched at 85±3 °C for 2 min using tap water, and dried at 100 °C for 25 min by a forced-air drier (Minimini DX II; TAIKISANGYO, CO., Ltd., Okayama, Japan). Dried strips were parfried at 182±5 °C for 1 min using an electric fryer (TEFL-45N; TANICO Corp., Osaka, Japan) and then frozen by a blast freezer (FFB-092FMD6-N; Fukushima Industries Corp., Fukushima, Japan) at -40 °C for 30 min. Frozen French fries were packed into polyethylene freezer bags and stored at -20 °C in a deep freezer (SRR-K1583C2; Panasonic Corp., Tokyo, Japan) for 24 weeks. Parfried and frozen French fries were finally fried at 166±5 °C for 2.5 min and then cooled for 30 min at room temperature, without the French fries overlapping each other, prior to analysis.

3.2.9. Analytical Methods of Processing Properties of French Fries

Moisture content was determined by the vacuum drying method, where 2 g of French fries was vacuum dried at 60 °C and 30 cmHg to constant weight. The Soxhlet extraction method was applied to measure oil content according to the method described in the AOAC standard method (AOAC International 2005). Hardness of French fries was evaluated using a texture analyzer (TA-HDi; Stable Micro Systems Ltd., Surrey, UK) at three measurement points, i.e., stem-end, center and bud-end of potato strips, as shown in Fig. 3.1. The rupture force as

hardness was measured by penetrating with a 2 mm diameter flat-ended cylindrical probe (P/2; Stable Micro Systems Ltd.) into French fries at a 1-mm/sec compression rate.

3.2.10. Statistical Analysis

Statistical analyses were conducted using SPSS for Windows (ver. 17.0). *t*-test and two-way analysis of variance (ANOVA) were carried out to assess the effect of Ca fertilization, variety and frozen storage on the physicochemical properties of raw tuber, starch and CWM, as well as processing properties and storability of French fries.

3.3. Results and Discussion

3.3.1. Effect of Ca Fertilization on Tuber, Starch and CWM Properties

Table 3.1 indicates specific gravities and dry matter (DM) contents of different potato tubers with conventional or Ca fertilizer application. Specific gravities and DM contents of potato tubers did not change significantly with Ca application ($p < 0.05$); similar observations were reported by Silva et al. 1991, Locascio et al. 1992 and Clough 1994.

Table 3.2 summarizes mean particle sizes and pasting properties of starches isolated from three different varieties of processing type potato tubers. Ca fertilization significantly decreased the mean particle size of starches isolated from TS and KH ($p < 0.05$). The effect of mineral fertilization on starch granule morphology has not been reported sufficiently for discussion. For example, Bogucka (2014) reported that foliar application of fertilizers containing manganese or boron did not result in significant changes in potato starch

morphology. The present study demonstrated that Ca fertilization significantly decreased the mean starch granule sizes of the processing-type potatoes TS and KH. Among pasting properties, starch isolated from TS and SD grown with Ca fertilization showed significantly lower peak viscosity than the non-Ca applied samples ($p < 0.05$). Similarly, breakdown of starch isolated from all potato varieties grown with Ca fertilization was significantly lower than the corresponding non-Ca treated counterparts ($p < 0.05$). On the other hand, Sulaiman (2005) reported that Ca fertilization had no effect on the pasting properties of starches isolated from potato cv. Saturna. Therefore, the effect of Ca fertilization on the pasting properties of potato starch may depend on varietal or genetic differences.

Table 3.3 presents Ca and CSP contents of CWM extracted from TS, KH and SD. Ca fertilization significantly increased Ca contents of CWM extracted from TS, KH and SD ($p < 0.05$). Ca fertilization also increased CSP contents of TS ($p < 0.05$) and SD. McGuire and Kelman (1986) reported that an increase in the Ca concentration of soil leads to a higher Ca content of CWM and a concomitant increase of galacturonic acid contents *in vitro*. Sriamornsak (2003) reported an increased probability for the formation of cross-linkages by a given amount of Ca^{2+} in the presence of a sufficient amount of low esterified pectin. Indeed, Ng and Waldron (1997) reported that the availability of Ca^{2+} might be a limiting factor in the crosslinking of pectic polysaccharides in potato tissue. In addition, CSP mainly contains ionically cross-linked pectin (Sila et al. 2009). Thus, the significant increase in CSP contents of CWM extracted from TS may result from the formation of Ca^{2+} bridges between pectin molecules. On the other hand, even though KH and SD showed a significant increase in Ca contents of CWM, significant changes in CSP concentrations were not observed. Quantification of CSP was conducted by sequential extraction using a chelating-agent, and the possibility exists that the chelating-agent was not sufficient to fully solubilize the Ca-pectate in potato tuber tissue (Zykwinska et al. 2005). In addition, the maturation of

potato tuber has a significant effect on the degree of esterification of its periderm pectin (Sabba and Lulai 2002), i.e., the growth period may affect the degree of methyl/acetyl esterification of potato pectin, which consequently contributes to the formation of Ca-pectate.

3.3.2. Effect of Ca Fertilization on Oil Uptake in Frozen French Fries

Oil contents of French fries before and after 24 weeks of frozen storage are shown in Fig. 3.2. Significant differences were not observed between the oil content of French fries prepared from potatoes with/without Ca application within the same variety ($p < 0.05$). In the case of French fries, oil penetration occurs mainly after the removal of potato strips from the frying medium, via pores in the cellular structure created by vapor pressure and heat degradation (Mellema 2003; Dana and Saguy 2006). Although Ca fertilization did not have a significant effect on the oil absorption of French fries in this study, Khalil (1999) reported that blanching of potato strips in $CaCl_2$ solution reduced the amount of oil penetration into French fries because of the formation of Ca-pectates, which increase middle lamella-cell wall rigidity and resistance to degradation by the frying process. The blanching of potato strips using $CaCl_2$ solution appeared to be more effective for the formation of Ca-pectates compared to Ca fertilization. Thus, even though significant increases in Ca contents of CWM by Ca fertilization were observed (Table 3.3), the increase in Ca contents may have been insufficient to reduce oil absorption.

3.3.3. Effect of Ca Fertilization on the Texture of Frozen French Fries

The hardness of French fries at the stem-end, center and bud-end before and after frozen storage is summarized in Table 3.4. Before frozen storage, French fries prepared from TS

grown with Ca fertilization showed significantly greater hardness at the stem-end than the corresponding non-Ca application ($p < 0.05$). Ca fertilization significantly increased hardness at the center and bud-end of French fries prepared from TS and KH, respectively, after 24 weeks of frozen storage ($p < 0.05$).

French fries texture is characterized by both a crispy outer crust and a soft mealy interior (Agblor and Scanlon 2000), and the interior texture is similar to that of cooked potatoes (Van Loon et al. 2007). The crust thickness was about 1 mm for all French fries samples (data not shown), and a similar observation was reported by (Lima and Singh 2001). Since peak rupture force was detected at a position deeper than 1 mm from the French fries surface, the force required to rupture the interior was considered as hardness in this study. Thus, it is assumed that the texture of French fries is affected by a combination of tuber properties, such as specific gravity and DM content (Barrios et al. 1963; Jaswal 1970), starch properties as swelling behavior and granule size (Whittenberger 1951; Barrios et al. 1963; Johnston et al. 1970), and pectin properties of quality and quantity (Khalil 1999; Tajner-Czopek 2003; Ross et al. 2011)..

Significantly higher Ca and/or CSP contents of CWM extracted from TS and KH may contribute to higher hardness of those frozen French fries, as a result of the formation of Ca-pectate in the middle lamella. It is known that Ca-pectate contributes to firm cohesion among contiguous non-esterified galacturonic acids and consequently provides rigid middle lamella-cell wall linkages (Grant et al. 1973), as well as firmer cell-cell adhesion (Knox 1992; Parker et al. 2001). Ormerod et al. (2002) reported that weakening of potato tissue on cooking is primarily controlled by thermal degradation of the middle lamella, and Tajner-Czopek (2003) revealed that soaking of potato strips in 0.4% $CaCl_2$ solution results in texture increase in French fries. Therefore, the formation of Ca-pectate induced by Ca

fertilization in the middle lamella may have increased the hardness of French fries owing to improved resistance against degradation of the tuber tissue structure during the frying process and frozen storage.

On the other hand, tuber properties, such as specific gravity and DM content, and starch properties, such as granule morphology and pasting properties, seemed to have relatively little influence on the texture of French fries. For TS and KH, there were significant differences in hardness between French fries grown with and without Ca fertilization, although there were no significant differences in specific gravities and DM contents. This limited contribution of tuber density in cooked potato texture is in agreement with Van Dijk et al. (2002) and Hejlová & Blahovec (2008). In addition, Johnston et al. (1970) reported that the higher viscosity and swelling power of starches isolated from raw potatoes were associated with the lower hardness of French fries. This is in accordance with the observation in French fries prepared from TS in the present study.

Furthermore, a significant decrease in the hardness of frozen French fries at the stem-end, center and bud-end after frozen storage was observed in all varieties ($p < 0.05$). This is may be due to the formation of ice crystals and recrystallization, which causes rupture of the cell structure in potato tuber tissue (Alvarez and Canet 2000; Ullah et al. 2014). The rigid and firm potato tissue structure, resulting from the formation of Ca^{2+} bridges between pectin molecules in the middle lamella and cell wall, therefore, may provide greater resistance against structural damage by the frying process as well as expansion by the growth of ice crystals during prolonged frozen storage. In addition, since French fries were stored at -20 °C for 24 weeks in the present study, lower temperature storage, such as -50 °C, may suppress ice crystal growth (Hagiwara *et al*. 2005).

Meanwhile, in SD as a late variety, a significant effect of Ca fertilization on French fries

texture was not observed, even though the Ca contents of CWM was significantly increased by Ca application. Karlsson et al. (2006) reported that the incidence of blackspot bruising of potato tuber is dramatically reduced when the tuber Ca concentration approaches approximately 250 ppm. Therefore, at Ca lower than 250 ppm or variety-specific concentration, sufficient modification of the tuber tissue structure to contribute to textural improvement of French fries might not be achieved.

3.3.4. Two-way ANOVA for Evaluation of the Effect of Variety and Ca Fertilization on Storability of Frozen French Fries

Table 3.5 summarizes the result of two-way ANOVA to assess the effect of Ca fertilization and variety on the physicochemical properties of raw tuber, starch and CWM as well as processing properties of French fries. From the two-way ANOVA, both variety and fertilization significantly affected the Ca content of CWM (p <0.05 and <0.001, respectively). Thus, it was confirmed that Ca fertilization significantly increased the Ca content of the middle lamella and/or cell wall. On the other hand, there was no significant effect of Ca fertilization on the CSP content (p = 0.181), although significant varietal differences were observed in the CSP content of CWM (p < 0.001). Thus, it was inferred that even if the Ca content is significantly increased by Ca fertilization, other factors, such as degree of methyl/acetyl esterification, may contribute to the formation of Ca^{2+} bridges between pectin molecules in the middle lamella. Furthermore, significant effects of Ca fertilization on hardness of French fries were observed at the center and bud-end after 24 weeks of frozen storage (p = 0.042 and 0.006, respectively), and significant interaction of *Variety* × *Fertilization* was shown in hardness at center after 24 weeks of frozen storage (p = 0.003). Hence, at the bud-end, a significant effect of Ca fertilization was observed on hardness. In the

present study, hardness of French fries was evaluated at three measurement points, i.e., stem-end, center and bud-end of potato strips, due to differences in the distribution of DM and chemical components such as Ca (Park et al. 2005; LeRiche et al. 2009; Ross et al. 2010 and Subramanian et al. 2011). Moreover, LeRiche et al. (2009) reported that the Ca content decreases from the stem-end to bud-end of potato tubers. Therefore, the effect of Ca fertilization on the hardness of French fries may be greater than that at regions of relatively lower Ca content in potato tuber, i.e., the bud-end.

Meanwhile, in this study, higher Ca or CSP contents of CWM did not consistently result in higher hardness of French fries. Potato tubers are reported to vary in chemical composition, and such variations were observed within tubers as well as plants grown under identical conditions (Kleinkopf et al. 1987; Dijk et al. 2002; Busse and Palta, 2006). Thus, variability among the sample tubers might be a reason for the unsystematic results of the present study, and careful investigation of individual tuber characteristics is necessary.

3.4. Conclusion

The present study demonstrated considerable improvement of the hardness of French fries prepared from three processing-types of potatoes, as a result of Ca fertilizer application. French fries prepared from TS and KH grown with Ca fertilization showed significantly greater hardness as compared with non-Ca treated counterparts even after frozen storage ($p < 0.05$). In addition, the significant effect of Ca fertilization in hardness of French fries was confirmed by two-way ANOVA. Therefore, results of this study suggest that Ca fertilization may be an effective method to improve the processing properties and storability of French fries made from processing-type potato varieties.

Table 3.1 Tuber properties of three potato varieties.

Variety	Ca fertilizer	Specific gravity (g/cm^3)	Dry matter (%)	Starch content (%)
TS	-	1.087 ± 0.002	25.80 ± 0.30	15.49 ± 0.43
	+	1.087 ± 0.002	25.35 ± 0.39	15.46 ± 0.36
KH	-	1.072 ± 0.001	23.95 ± 1.01	12.23 ± 0.18
	+	1.072 ± 0.003	20.88 ± 1.63	12.21 ± 0.69
SD	-	1.085 ± 0.004	25.38 ± 0.98	14.99 ± 0.96
	+	1.087 ± 0.003	24.31 ± 2.14	15.38 ± 0.59

Abbreviations: TS, Toyoshiro; KH, Kitahime; SD, Snowden

n=4, specific gravity; n=3, dry matter content

Table 3.2 Mean particle sizes and pasting properties of starch isolated from potato tubers.

Variety	Ca fertilizer	Mean particle size (μm)	Peak viscosity (cP)	Break down	Setback	Pasting temperature (℃)
TS	-	43.34 ± 1.28 *	2672.67 ± 12.58 *	1570.67 ± 18.72 *	146.67 ± 9.87	66.97 ± 0.08
	+	39.98 ± 0.82	2564.33 ± 11.15	1347.00 ± 7.55	145.33 ± 8.08	67.53 ± 0.46
KH	-	44.81 ± 0.34 *	4030.33 ± 6.66	2623.33 ± 33.86 *	143.00 ± 40.78	68.85 ± 0.52
	+	41.97 ± 0.71	4082.67 ± 27.47	2483.67 ± 50.77	119.67 ± 12.01	68.03 ± 0.53
SD	-	39.47 ± 0.01	3198.33 ± 10.60 *	2072.33 ± 13.58 *	145.00 ± 17.78	66.95 ± 0.05
	+	39.44 ± 0.05	3065.50 ± 3.54	1809.00 ± 72.27	140.00 ± 7.94	67.77 ± 0.10 *

Mean values in the same column with * are significantly higher than that of the corresponding same potato variety with different Ca fertilization ($p < 0.05$).

n=3

Table 3.3 Ca concentration and CSP contents of CWM extracted from three potato varieties.

Variety	Ca fertilizer	Ca (mg/100g, db)	CSP
TS	-	28.80 ± 0.37	523.33 ± 41.70
	+	32.04 ± 0.36 *	605.43 ± 10.80 *
KH	-	32.76 ± 1.24	855.10 ± 7.37
	+	36.29 ± 0.47 *	842.74 ± 7.70
SD	-	28.69 ± 1.29	824.54 ± 78.08
	+	34.95 ± 1.99 *	891.15 ± 90.53

Mean values in the same column with * are significantly higher than that of the corresponding same potato variety with different Ca fertilization ($p < 0.05$).

n=3

Table 3.4 Hardness of French fries at the stem-end, center and bud-end prepared from six different potato tubers before and after 24 weeks of frozen storage.

Variety	Ca fertilizer	Hardness (N)					
		Stem-end		Center		Bud-end	
		0 week	24 weeks	0 week	24 weeks	0 week	24 weeks
TS	-	1.53 ± 0.57	1.23 ± 0.42	1.49 ± 0.68 †	0.79 ± 0.28	2.02 ± 0.74 †	1.21 ± 0.50
	+	2.41 ± 0.69 * †	1.52 ± 0.55	1.55 ± 0.53	1.28 ± 0.49 *	1.77 ± 0.65	1.44 ± 0.47
KH	-	1.68 ± 0.37 †	0.89 ± 0.26	1.15 ± 0.31 †	0.54 ± 0.19	1.27 ± 0.76 †	0.58 ± 0.20
	+	1.49 ± 0.37 †	1.13 ± 0.50	1.27 ± 0.41 †	0.50 ± 0.11	0.91 ± 0.17	0.78 ± 0.25 *
SD	-	2.25 ± 0.92 †	1.43 ± 0.60	1.48 ± 0.64 †	0.91 ± 0.42	1.40 ± 0.43 †	1.05 ± 0.41
	+	2.16 ± 0.62 †	1.44 ± 0.62	1.40 ± 0.42 †	0.89 ± 0.32	1.49 ± 0.33	1.32 ± 0.43

Mean values in the same column with * are significantly higher than that of the corresponding same potato variety with different Ca fertilization ($p < 0.05$).

Mean values in the same row with † are significantly higher than that of the corresponding French fries under frozen storage for 24 weeks among stem-end, center or bud-end ($p < 0.05$). n=15

Table 3.5 Two-way ANOVA to assess the effect of variety and Ca fertilization on the storability of frozen French fries

		Variety	Fertilization	Variety × Fertilization
		p value		
Tuber properties				
Specific gravity		0.609	0.920	**0.025**
Dry matter		**0.002**	**0.024**	0.211
Starch properties				
Mean particle size		**<0.001**	**<0.001**	**0.004**
Peak viscosity		**<0.001**	**<0.001**	**<0.001**
Break down		**<0.001**	**<0.001**	0.054
Setback		0.433	0.310	0.601
Pasting temperature		**<0.001**	0.291	0.004
CWM properties				
Ca		**0.002**	**<0.001**	0.150
CSP		**<0.001**	0.254	0.172
French Fries properties				
Crude fat	0	**<0.001**	0.491	**0.042**
	24th	**<0.001**	0.558	**0.044**
Hardness (Stem-end)	0	**0.001**	0.137	**0.002**
	24th	**0.003**	0.100	0.553
Hardness (Center)	0	0.063	0.733	0.731
	24th	**<0.001**	**0.042**	**0.003**
Hardness (Bud-end)	0	**<0.001**	0.136	0.272
	24th	**<0.001**	**0.006**	0.932

Values of p in bold are significant ($p < 0.05$)

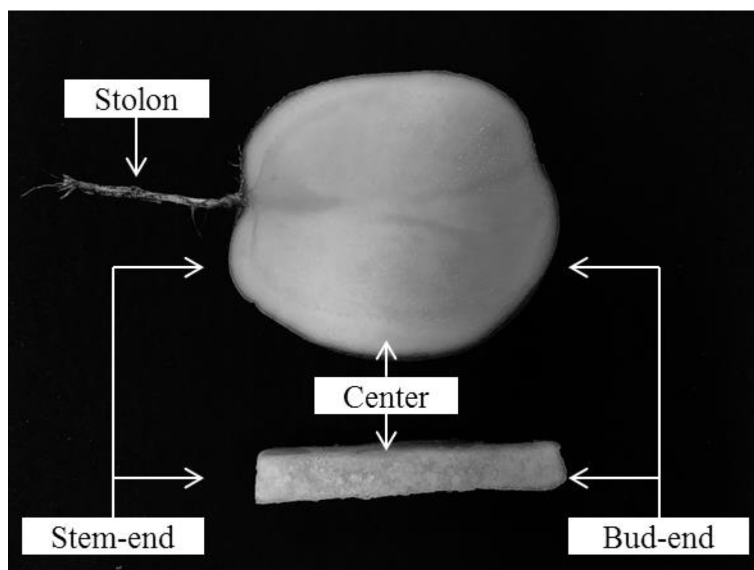


Figure 3.1 Photograph of potato tuber and French fries.

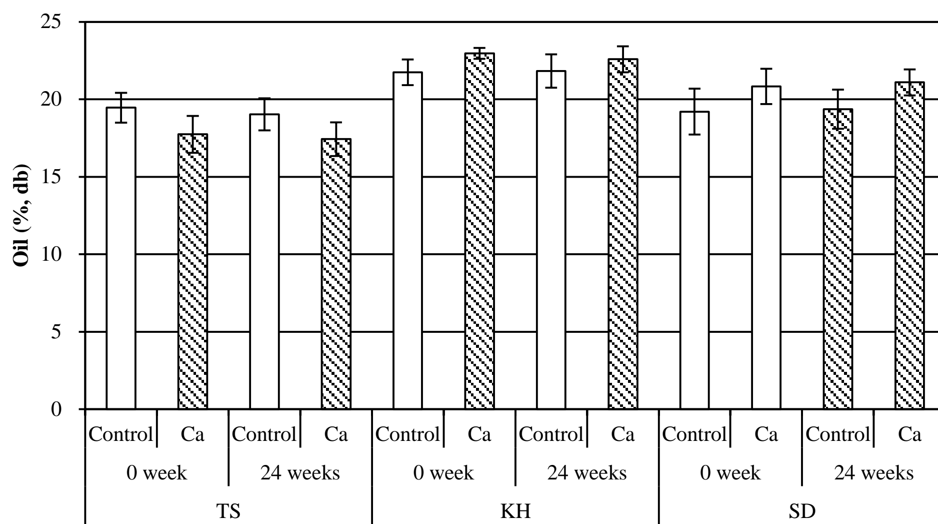


Figure 3.2 Oil content of French fries prepared from before and after frozen storage.

Chapter 4. Controlling Texture of French Fries through Effective Use of Endogenous Calcium and Pectin Methylesterase in Potato Tuber

4.1. Introduction

Texture of plant-based foods is important quality attribute for consumer acceptance, and it is mainly controlled by mechanical properties of plant cell walls (Van Dijk et al. 2002; Waldron et al. 2003; Fraeye et al. 2010; Zhao et al. 2016). Pectin is an integral component of plant cell walls and forms an interconnected network structure, independent of the cellulose-xyloglucan network (Talbott and Ray 1992; Carpita and Gibeaut 1993; Morris et al. 2009), and thereby plays an important role in maintaining cell wall integrity and cell-cell cohesion (Parker et al. 2001; Aghdam et al. 2012; Daher and Braybrook 2015).

A number of studies, therefore, have been attempted to improve stability of the pectin network in plant tissue during cooking, due to depolymerization and structural changes of the pectin have been shown as determinative factor on texture of plant-based foods (Van Dijk et al. 2002; Sila et al. 2006; Phothiset and Charoenrein 2014; Zhao et al. 2016). For example, soaking fruits and vegetables in an aqueous solution containing Ca^{2+} has demonstrated to retain texture of plant-based foods after frying, boiling and canning (Tajner-Czopek 2003; Manganaris et al. 2005; Zhao et al. 2016). In these cases, Ca^{2+} was simply supplied to plant cell wall for improving cross-linker availability within pectin chains, based on a phenomenon that Ca^{2+} cross-links antiparallel homogalacturonan chains with negatively charged carboxyl groups to form structures called “egg-box” and tightening the cell walls (Braccini and Pérez 2001; Willats et al. 2006; Cybulska et al. 2011). For the same

purpose, on the other hand, we focused on calcium fertilization during cultivation of potato as a novel method to increase Ca^{2+} availability in the cell wall for inducing the cross-links within the pectin molecules and improving texture of French fries. From our previous studies, we found a significant effect of calcium fertilization on hardening of the French fries even after 6 months of frozen storage (Murayama et al. 2016). In addition, we also showed that an increased calcium concentration of potato tuber is accompanied by an enhancement of Ca^{2+} availability in the cell wall and influenced on the formation of cross-links between pectin molecules via Ca^{2+} (Murayama et al. 2017).

Low-temperature blanching (LTB) is recognized as one of the method to controlling texture of plant-based foods by increasing non-esterified homogalacturonan blocks in pectin molecules, which are known as specific sites for formation of the egg-box within pectin chains, through activation of the pectin methylesterase (PME) (Canet et al. 2005; Sila et al. 2005; Sila et al. 2006). PME catalyzes demethylesterification of homogalacturonan (Micheli 2001), and demethylation may also reduce susceptibility for heat induced β-elimination, responsible for depolymerization of pectin chains (Canet et al. 2005; Moelants et al. 2014).

Thus, a combination of calcium fertilization and LTB may be the promising way to control the texture of plant-based foods by effectively utilizing endogenous calcium absorbed naturally during cultivation as well as PME in the cell wall. The objective of the present study was therefore to evaluate effects of calcium fertilization and LTB on hardness of French fries with possible modification in cell wall pectin characteristics.

4.2. Materials and Methods

4.2.1. Plant Material and Design of Field Experiment

Potato tubers used in this study were grown in a field in Memuro, Tokachi, Hokkaido, Japan, in which soil type is Andosol (Fueki et al. 2011). Toyoshiro, an early maturing variety for processing, was planted on May 3rd, 2016 and grown in a field with conventional fertilization, according to the fertilizer application guide by Hokkaido Prefecture (Hokkaido Department for Agropolicy 2010). Fertilizer application in this area includes ammonium sulfate, ammonium phosphate, potassium sulfate and magnesium sulfate to give 60, 88, 91 and 24 kg/ha of N, P, K and Mg, respectively. After flowering on July 3rd, calcium was applied three times as 150 mM $CaCl_2$ solution on the top of the hill to give 114 kg/ha of calcium at the each application on July 7th, 25th and August 12th. Thus, the total amount of calcium applied was 342 kg/ha. In one row, ten plants were applied with $CaCl_2$ solution and another ten plants in a row next to the row applied with calcium were used as a control, with one guard row being left untreated between the experimental rows. This application was repeated on three sets of rows. Tubers were hand-harvested on August 25th. All tubers were stored in a storage chamber at 14°C for 2 months, and 24 tubers with weight ranged from 147 to 195 g and specific gravity ranged 1.0942 ± 0.0112 were individually and randomly selected for further analysis.

4.2.2. Preparation of French fries

Potato tuber was washed and peeled using a commercial peeler, and at least 1 cm of stem-end was cut and removed. Nine strips (1.0 cm × 1.0 cm × length of tuber) were cut along the apical to basal axis from the pith of the parenchyma region of the tubers using a commercial French fries cutter. Length of potato strips was adjusted into 6 cm using kitchen knife. One potato strip from central region of the tuber was immediately cut into 0.5 mm^3 using kitchen knife, frozen using liquid nitrogen, freeze-dried and powdered using mortar and pestle for

quantification of calcium in raw potato tuber. Remaining eight strips were immediately blanched by following conditions; non-blanching by soaking in deionized water for 1 min; low-temperature blanching (LTB) by soaking for 1 min and then blanching for 30 min at 60°C using deionized water (Aguilar et al. 1997); high-temperature blanching (HTB) by soaking for 1 min and then blanching for 4 min at 85°C (Gupta et al. 2000). Blanched samples were cooled for 5 min at room temperature. Excess water was removed by paper towel. Three out of eight strips were fried at 185°C for 4.5 min using a table top electric fryer with an oil capacity of 1 L (Alvarez et al. 2000). A constant potato strips to oil ratio of 0.05 was maintained to ensure uniform frying conditions for all replications. After frying, French fries were allowed to cool for 5 seconds on the frying basket and shaken five times to remove the excess oil (Sandhu and Takhar 2015). Samples were then kept on shallow tray with rack for 20 min and immediately subjected for texture measurement. The remaining five strips after blanching treatments were cut into 0.5 mm^3 using kitchen knife and then frozen using liquid nitrogen. Approximately 35 g of the frozen cube were stored at -35°C until preparations of cell wall material (CWM) and sample for microscopic analysis, while the residual cubes were freeze-dried and powdered for calcium and PME analysis. For each treatment, 4 tubers cultivated under control or calcium fertilizations were individually subjected for preparation of the samples.

4.2.3. Isolation of CWM

CWM was prepared from the frozen blanched potato strips based on the method proposed by Huang et al. (2016, 2017) with slight modification. The frozen potato cubes (25 g) were mixed with 50 ml of chilled sodium acetate buffer (0.2 M, pH 5.2) and immediately homogenized for 1.5 min using a Waring blender (Model 700JBB, Osaka Chemical Co. Ltd.,

Japan) equipped with chilled glass container. Additional 25 ml of chilled sodium acetate buffer was added and immediately homogenized for 1.5 min, followed by addition of further 50 ml of chilled sodium acetate buffer and homogenized for 2 min. This homogenization step was carried out at 4°C. The resultant slurry was transferred into a beaker, heated to 80 °C and held for 30 min in a water bath with continuous mixing at 325 rpm. The samples were then transferred into a conical flask and then incubated at 40°C for 3 h with shaking at 100 rpm in a thermostatic shaker, with α-amylase from *Bacillus licheniformis* (1000 U, Sigma-Aldrich) and amyloglucosidase from *Rhizopus* mould (150 U, Megazyme). Subsequently, cell wall polysaccharides were precipitated by adding ethanol to a final concentration of 70% (v/v). After centrifugation for 15 min at 10,000 *g* and 4 °C, the pellet obtained was resuspended in the buffer and subjected to the hominization for 2 min. The resultant slurry was once again subjected to the heating and the enzymatic degradation procedure, followed by precipitation of cell wall polysaccharides by the adding ethanol. After centrifugation, the final pellet was washed with 70% ethanol until no more sugars were present in the supernatant. The washed pellet was lyophilized for yielding CWM.

4.2.4. Calcium Determination of Raw Potato Tuber, Blanched Potato Strip and CWM

Based on the previous study (Murayama et al., 2017), 100 mg each of freeze dried raw potato tuber and blanched potato strip, and 50 mg of CWM were wet-digested with H_2SO_4/H_2O_2 mixture (2:1, v/v), and the resultant solution was diluted to 25 ml using deionized water. The solution obtained was subjected to calcium quantification using an inductively coupled plasma spectrometer (ICPS-8100, Shimadzu Co. Ltd., Japan). Duplicate measurements were made.

4.2.5. PME Activity after Blanching

PME activity was measured according to the method reported by Abu-Ghannam and Crowley (2006). Thirty milligrams of freeze dried blanched strips was suspended in 1 ml of ice-cold water and shaken at 200 rpm for 1 h at 4°C. The sample was centrifuged for 5 min at 17,600 *g* and the supernatant was removed. The residue was resuspended in 1 ml of ice-cold 1M NaCl and shaken at 200 rpm for further 1 h at 4°C. The sample was then centrifuged for 5 min at 17,600 *g*. The assay was carried out by mixing 2.9 ml pectin assay solution with 100 µl of the supernatant recovered. Absorbance was monitored at 616 nm for 1 min at 20 °C. The activity value was expressed as µmol of COO^- produced per min from 1 g of the sample. Duplicate measurements were made.

4.2.6. Sequential Extraction of Pectin Fractions

Pectin fractions were extracted using a modified procedure based on the methods used in the previous study (Murayama et al. 2017). Fifty milligrams of CWM was transferred into a 50 ml plastic test tube containing 15 ml of deionized water and then stirred with a magnetic stirrer for 8 h at 20 °C. The suspension was filtered through a nylon mesh (47 µm), and the residue was washed with deionized water. The filtrate was diluted to a final volume of 50 ml, and was encoded as water soluble pectin fraction. The residue was then re-suspended in 15 ml of 0.05 M cyclohexane diamine tetra acetic acid (CDTA) solution (pH 6) in 0.05 M sodium acetate buffer, and stirred for 16 h at 20°C. The suspension was filtered through a nylon mesh, and the residue was washed with deionized water. This filtrate was diluted to a final volume of 50 ml, and was encoded as chelator soluble pectin fraction. Duplicate extractions were performed for each CWM sample. For quantification of total pectin content,

25 mg of CWM was hydrolyzed by 1 ml of 72% (v/v) sulfuric acid for 3 h at 20°C in 2 ml plastic tube. Resultant hydrolysate was diluted to 1 M sulfuric acid by deionized water and then hydrolyzed further for 1 h at 100°C (Selvendran and O'Neill 1987). Hydrolyzate was neutralized with NaOH solution. After cooling, the suspension was filtrated through a filter paper (Advantech No. 5A). The residue was washed with deionized water and filtrate was diluted to a final volume of 100 ml. This was encoded as total pectin fraction. Duplicate extractions were performed for each CWM sample.

4.2.7. Quantification of Pectin

The galacturonic acid content in the pectin fractions were measured by the *m*-hydroxydiphenyl method, as described by Blumenkrantz and Asboe-Hansen (1973) with the modifications proposed by Filisetticozzi and Carpita (1991). Absorbance at 525nm was read by a spectrophotometer (U-5100, Hitachi Ltd., Japan), 20 min after addition of *m*-hydroxydiphenyl solution. Glucuronic acid exists only in trace amount in potato cell walls (Jarvis et al. 1981). Thus, the glucuronic acid content was considered to be negligible. Duplicate measurements were performed for each pectin fraction.

4.2.8. Atomic Force Microscopy

Surface morphology and cell wall stiffness was evaluated by the method proposed by Zdunek et al. (2016). Each CWM was suspended in deionized water as 1 mg/ml and stirred at 20 °C for 60 min. The suspension was dropped on a microscope glass slide and then air-dried. Ten minutes before tests, deionized water was added to swell samples. AFM imaging was performed in air using a Multimode 8 microscope (Bruker, Santa Barbara, US) operated in

peak-force tapping mode at a scan rate of 1 Hz and in a scan area of 10×10 μm^2. AFM images were analyzed using NanoScope Analysis software version 1.50 (Bruker, Santa Barbara, US).

4.2.9. Hardness Measurement of French Fries

Texture of French fries was evaluated using a texture analyzer (TA-HDi; Stable Micro Systems Ltd., Surrey, UK) at three measurement points, i.e., stem-end, center and bud-end of potato strips (Murayama et al. 2016). Texture of stem-end and bud-end were measured at approximately 10 mm away from each end of the strip. The rupture force as hardness was measured by penetrating with a 2 mm diameter flat-ended cylindrical probe (P/2; Stable Micro Systems Ltd.) into French fries at a 1 mm/sec compression rate.

4.2.10. Statistical Analysis

All data are presented as the means ± standard deviation (means ± SD). Statistical analyses were conducted using SPSS for Windows (ver. 17.0). Two-way analysis of variance (ANOVA) was used to assess significance of the main effects of fertilization and blanching method as well as the interactions at 5% significance level. Three-way ANOVA was employed for hardness of French fries to evaluate the main effects of fertilization, blanching method and positions of French fries strips as well as the interactions at 5% significance level. As a post-hoc test, Tukey's multiple range tests were performed within the blanching methods and, strips positions for hardness of French fries at 5% significance level. The data obtained from individual tubers, corresponding blanched potato strips and CWMs were subjected for Pearson's correlation analysis.

4.3. Results and Discussion

4.3.1. Calcium Concentration

Calcium concentrations in raw potato tubers, blanched strips and CWMs are summarized in Table 4.1. Raw potato tubers cultivated under calcium fertilizer application showed significantly 56% higher concentration of calcium than that of the samples without calcium fertilization ($p < 0.05$). After blanching treatments, 22.4, 26.6 and 20.7% of calcium in blanched strips were lost from corresponding raw potatoes for control, LTB and HTB, respectively. Blanched strips and corresponding CWMs prepared from potato tubers applied with calcium fertilizer retained significantly higher concentrations of calcium than samples without calcium fertilization ($p < 0.05$). Since there was no significant effect of the different blanching treatments in reduction of calcium concentrations in the blanched strips and CWMs ($p > 0.05$), all data for the calcium concentrations were subjected to correlational analysis. Figure 4.1A indicates correlationship between calcium concentrations in raw potato tubers and their corresponding blanched strips, and significant positive correlation was observed with r = 0.816 ($p < 0.01$). Furthermore, calcium concentrations in the blanched strips and their corresponding CWMs were also significantly and positively correlated as shown in Figure 4.1B (r = 0.689, $p < 0.01$). The cell wall contains 60 to 75% of Ca^{2+} within the total plant tissue (Demarty et al. 1984), and the majority of Ca^{2+} in the plant cell wall binds to negatively charged carboxylic groups of galacturonic acids within the apoplast (Sattelmacher 2001). Thus, increased calcium concentration of potato tuber by the targeted calcium fertilization may have enhanced Ca^{2+} bound to pectin molecules in the cell wall of potato tissue even after blanching treatments.

4.3.2. PME Activity

PME activities in potato strips after different blanching treatments are shown in Fig. 4.2. There was no significant difference in PME activities between control and LTB samples, and thus PME probably had catalyzed demethylesterification of pectin molecules during 30 min of the blanching at 60°. On the other hand, only trace PME activity was detected in the potato strips treated with HTB. This is in accordance with Anthon and Barrett (2002), who reported that inactivation temperature of potato PME ranged from 65 to 70 °C.

4.3.3. Pectin Composition

Total pectin contents in CWMs were significantly decreased by LTB and HTB as compared with control ($p < 0.05$), while significant difference in total pectin contents between samples with and without calcium fertilization was not observed (Fig. 4.3A). Thus, amounts of water and chelator soluble pectins were expressed as % (w/w) in total pectin content for making comparisons among three different blanching treatments (Fig. 4.3B, 4.3C). For water soluble pectin, significant effect of calcium fertilizer application was not found ($p > 0.05$). Significant increase in water soluble pectin content was observed by HTB as compared with control sample ($p < 0.05$). In comparison between samples with and without calcium fertilization, chelator soluble pectin contents were observed with 35.0, 42.6 and 24.5% higher values in the calcium-fertilized samples for control, LTB and HTB, respectively. This may indicate that enhanced Ca^{2+} in potato tuber cell wall by calcium fertilization is responsible for the formation of the cross-links within pectin chains, in accordance with reports on post-harvest calcium application in fruits (Chong et al. 2015). Chelating-agent is widely used to solubilize pectin molecules forming the “egg-box structure” in plant cell wall,

and chelator soluble pectin is referred to as pectin held in the cell wall by ionic bonds (Jarvis 1982). Furthermore, significant differences in chelator soluble pectin contents among blanching methods were observed in the following order; HTB < non-blanching < LTB ($p < 0.05$). This may be attributable to increase in the specific sites for cross-links within pectin chains for Ca^{2+} (Zhao et al. 2016). The combination of calcium fertilization and LTB resulted in the significantly highest chelator soluble pectin content within all samples. This suggests that calcium fertilization and LTB concurrently influences the formation of cross-links within pectin chains from a different mechanism, i.e., enhancing the Ca^{2+} concentration and its affinity in the pectin matrix.

4.3.4. Atomic Force Microscopy

In this study, we most likely imaged and indented primary cell wall but not middle lamella during the analysis, as reported in a previous study (Zdunek et al. 2016). Figure 4.4A indicates a representative image of a CWM isolated from potato strips neither applied calcium fertilizer nor treated by the blanching. Two types of fibrous structures differing distinctively in thickness were observed and indicated by black and white arrows in the image of Fig. 4.4A. These fibrils were found in all samples (Fig. 4.4A-F), as in the previous study (Murayama et al. 2017). Thinner fibrils indicated by white arrows are referred to as microfibrils, and thicker one with black arrow is macrofibril in the present study. Since obvious differences in the surface morphology were difficult to observe, measurement of diameters of micro- and macrofibrils was conducted and these results are summarized in Table 4.2. Diameters of microfibrils and macrofibrils were ranged 1.5 to 2.6 nm and 21.6 to 61.4 nm, respectively. Bidhendi and Geitmann (2016) reported that some microfibrils in the higher plant cell wall (< 3 nm) form aggregates and exist in the form of bundles with possibly

higher than 200 nm in diameter. For diameters of micro- and macrofibrils in CWMs, significant decrease in the diameter was found by HTB as compared with control condition ($p < 0.05$), while LTB didn't alter the diameters significantly ($p > 0.05$). These changes may be consequences of partial removal of pectin in the cell wall and its limitation by the formation of cross-links between pectin chains via Ca^{2+}, as complemented by the result of pectin quantification (Fig. 4.3). Indeed, selective extraction of pectic polysaccharides has been reported to cause decrease in diameters of fibrinous structures in CWMs isolated from potato (Kirby et al. 2006). In the case of diameters of macrofibrils, the diameters in CWMs isolated from calcium-fertilized samples were significantly larger than those cultivated without calcium application ($p < 0.05$). The bundles of fibrils in the cell wall may be interconnected through xyloglucan as well as pectic polysaccharides (Wang et al. 2012), and Sze et al. (2016) suggested that pectin increases the fibril diameters as it coats the fibrils but its primary function is to fill up the gaps between cellulose fibrils. Thus, induced formation of the pectin-Ca^{2+} cross-links may have contributed the thickening of the macrofibrils by filling up the gaps by the more rigid network. Young's moduli of CWMs are also shown in Table 4.2. Based on one-way ANOVA, significant differences within all CWM samples were not found ($p > 0.05$). Average Young's moduli of CWMs treated by control condition, LTB and HTB were 35.29, 41.95, and 32.46 GPa, respectively. Among the moduli, significant difference was detected between the CWMs treated with LTB and HTB ($p < 0.05$), indicating that CWMs were slightly firmed by LTB and softened by HTB. Taken together, the atomic force microscopic analysis including measurements of the fibril diameters and Young's moduli may support that structural reinforcement in the cell wall of potato strips treated with LTB and prepared from potato tubers applied with calcium fertilization.

4.3.5. Hardness of French Fries

Figure 4.5 indicates hardness at stem-end, center and bud-end of French fries. Hardness of French fries were evaluated at the three different points at the potato strips owing to heterogeneous distribution of calcium and dry matter content in a potato tuber (Subramanian et al. 2011). Based on the three-way ANOVA for the hardness of French fries, significant effects of calcium fertilization, blanching method and measured part were observed with *p* values lower than 0.001 and significant differences in post-hoc tests as the following orders ($p < 0.05$); fertilization (control < calcium), blanching method (non-blanching = HTB < LTB) and measured part (bud-end < center < stem-end). Any significant interactions among the main effects were not found in the three-way ANOVA.

The significant increase observed in hardness of French fries clearly showed that effective utilization of endogenous calcium in potato tuber alters the quality of final products. By application of calcium fertilizer, French fries were hardened through increased Ca^{2+} concentration within pectin matrix, with the results similar to those shown by the direct infiltration of Ca^{2+} into potato strips using calcium salt solution (Khalil 1999; Tajner-Czopek 2003). Furthermore, targeted calcium fertilization for potato led to reduction of the incidence of physiological disorders (Ozgen et al. 2006). Thus, controlling texture of French fries by means of calcium fertilization may contribute to improving qualities of not only final products but also raw materials through endogenous calcium. LTB further increased the hardness of French fries but through a mechanism different from the calcium fertilization, i.e., activation of PME for catalyzing demethylesterification of methylesterified-pectin molecules. Aguilar et al. (1997) reported that the hardening effect of LTB for French fries is resulted from stronger intercellular bonds due to a modification of pectic substances by the PME. In this study, this modification is shown as the increase in chelator soluble pectin content by LTB (Fig. 4.3C). Demethylation of the pectin molecules by PME may also reduce

susceptibility for the heat induced β-elimination (Moelants, Cardinaels, Van Buggenhout, et al. 2014), and consequently may have inhibited excessive softening of the French fries.

4.4. Conclusion

The present study provides a basic concept for controlling texture of French fries by effectively utilizing endogenous calcium in potato tuber. Calcium fertilization enhanced Ca^{2+} concentration within pectin chains for formation of the cross-links, while LTB increased affinity of the pectin chains for Ca^{2+} by facilitating demethylesterification of pectin molecules. The combination use of calcium fertilization and LTB achieved the highest hardness of French fries among all samples. The results of PME activity measurement, pectin composition analysis and atomic force microscopy complemented the hardening effect of calcium fertilization and LTB in French fries. Controlling texture of French fries through effective use of endogenous Ca^{2+} in potato tuber was successfully achieved.

Table 4.1 Calcium concentrations in raw potato tubers, blanched potato strips and CWMs.

Blanching	Fertilization	Raw tuber		Blanched strip		CWM	
		Calcium concentration (μg/g, dry wt.)					
Control	Ca-	121.23 ± 19.60	ab	93.11 ± 10.70	ab	740.47 ± 54.94	ab
	Ca+	159.41 ± 33.50	ab	120.61 ± 21.82	a	853.71 ± 45.27	a
Low-temp.	Ca-	100.02 ± 21.65	b	79.71 ± 4.67	b	665.51 ± 27.64	ab
blanching	Ca+	187.61 ± 42.68	ab	121.60 ± 32.40	a	820.61 ± 185.69	a
High-temp.	Ca-	109.64 ± 28.41	b	86.06 ± 8.16	ab	577.04 ± 33.42	b
blanching	Ca+	168.69 ± 38.13	a	125.64 ± 14.21	a	798.67 ± 102.51	a

The data represent the mean ± SD of four individual samples with duplicate measurements. Different small letters in the same column indicate significant differences ($p < 0.05$).

Table 4.2 Diameters of micro- and macrofibrils and Young's moduli observed in CWMs isolated from potato strips treated either without blanching treatment or with LTB and

Blanching	Fertilization	Microfibril	Macrofibril	Young's modulus
		Diameter (nm)		(GPa)
Control	Ca-	2.6 ± 0.2 a	38.4 ± 11.8 bc	39.08 ± 8.57 a
	Ca+	2.2 ± 0.4 ab	61.4 ± 5.9 a	31.50 ± 4.90 a
Low-temp.	Ca-	2.3 ± 0.1 ab	44.2 ± 6.3 ab	43.86 ± 8.57 a
blanching	Ca+	2.1 ± 0.5 ab	57.6 ± 7.4 ab	40.05 ± 5.73 a
High-temp.	Ca-	1.5 ± 0.3 b	21.6 ± 2.4 c	34.82 ± 2.45 a
blanching	Ca+	2.0 ± 0.4 ab	42.2 ± 13.2 ab	30.10 ± 7.80 a

The data represent the mean ± SD of four individual samples with 10 replicates. Different small letters in the same column indicate significant differences ($p < 0.05$).

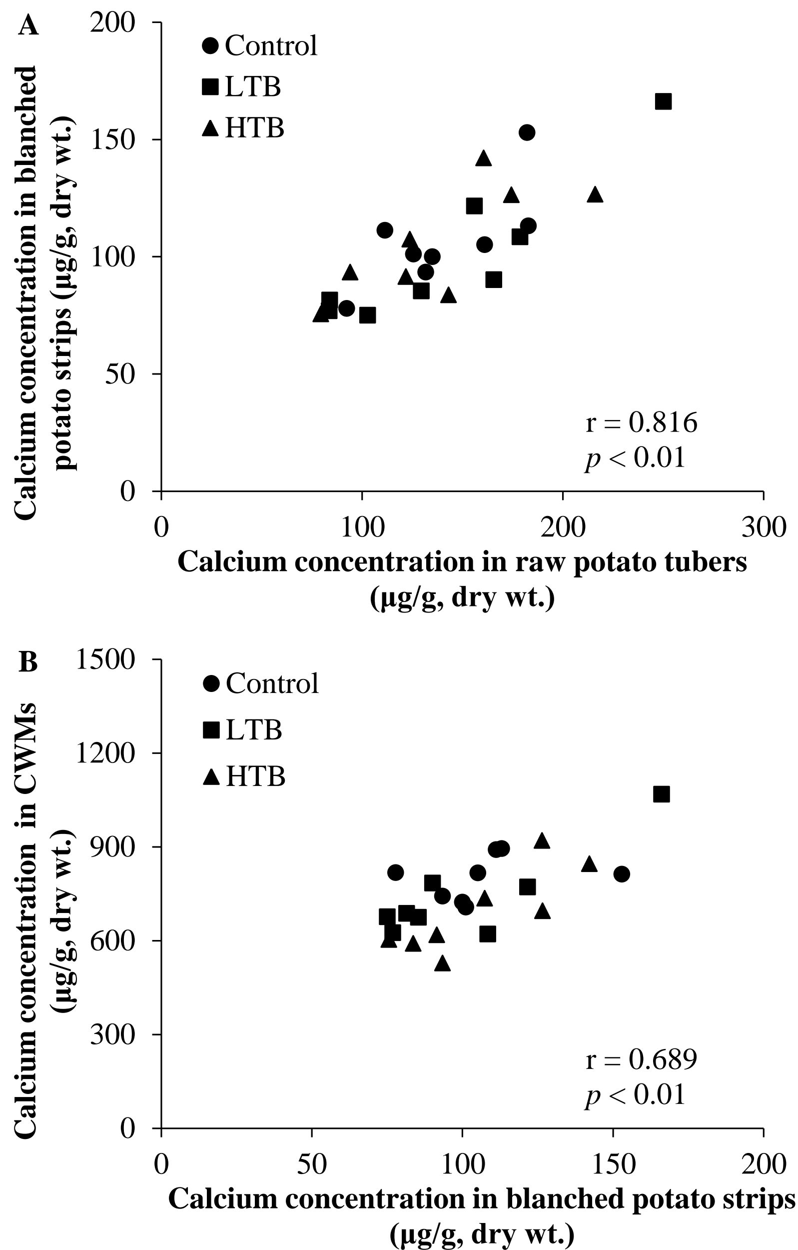


Figure 4.1 Relationship between calcium concentrations in (A) raw potato tubers and the corresponding blanched strips and (B) the blanched potato strips and theire corresponding CWMs.

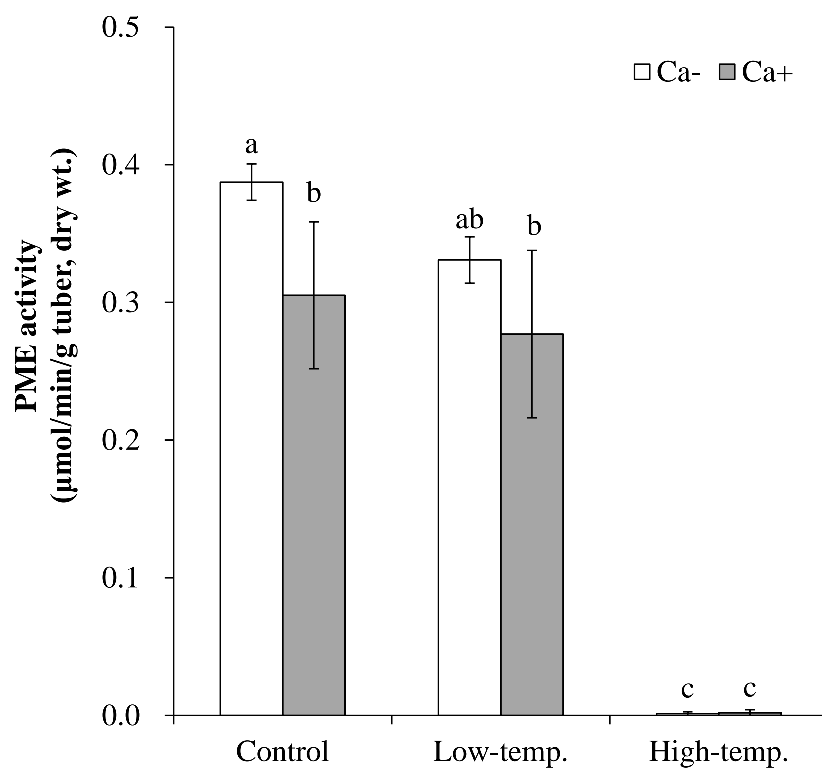


Figure 4.2 PME activity in potato strips after different blanching treatments.

Each bar represent the mean ± SD from four individual blanched potato strips with duplicate measurements. Different small letters indicate significant differences ($p < 0.05$)

1

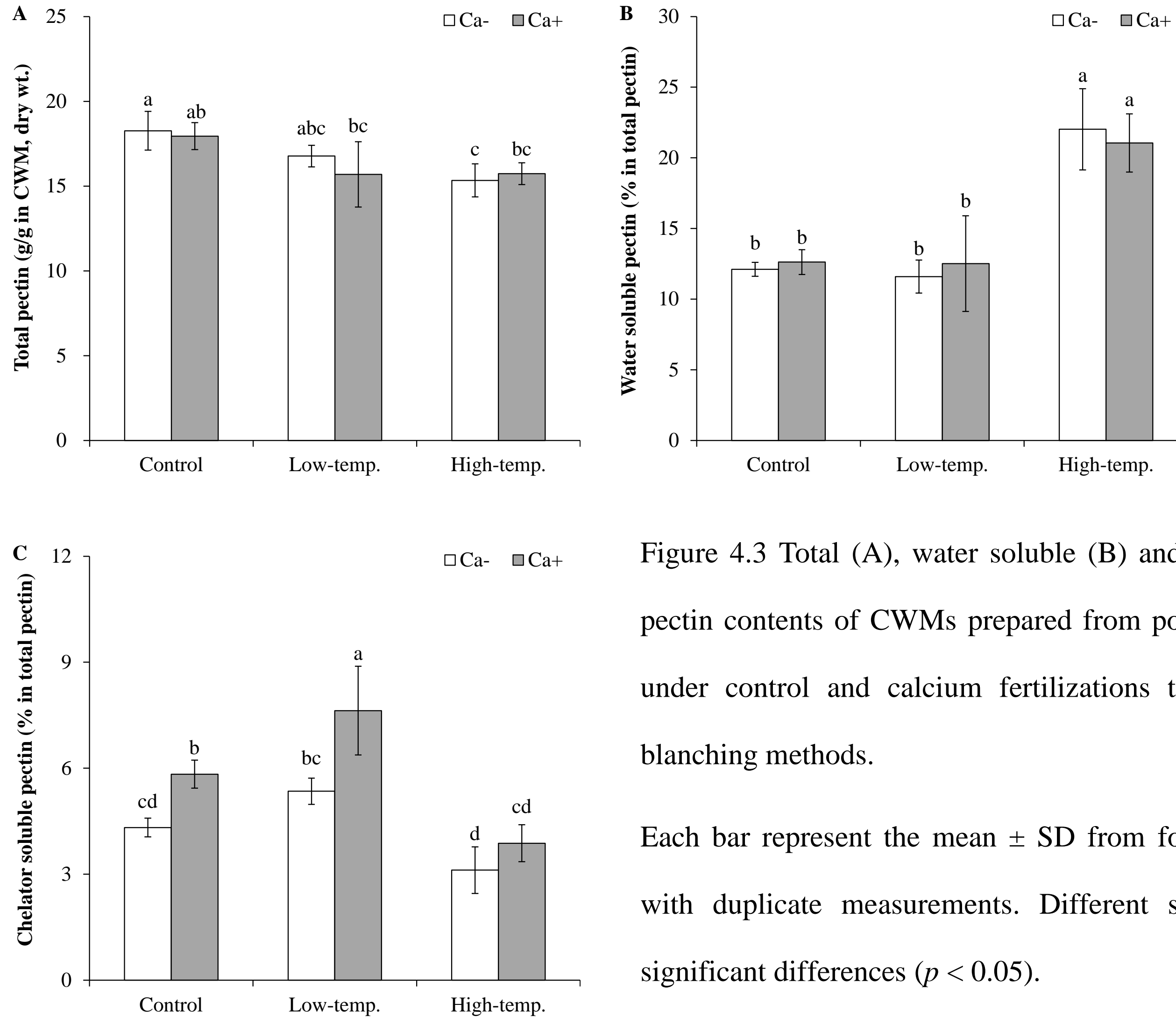
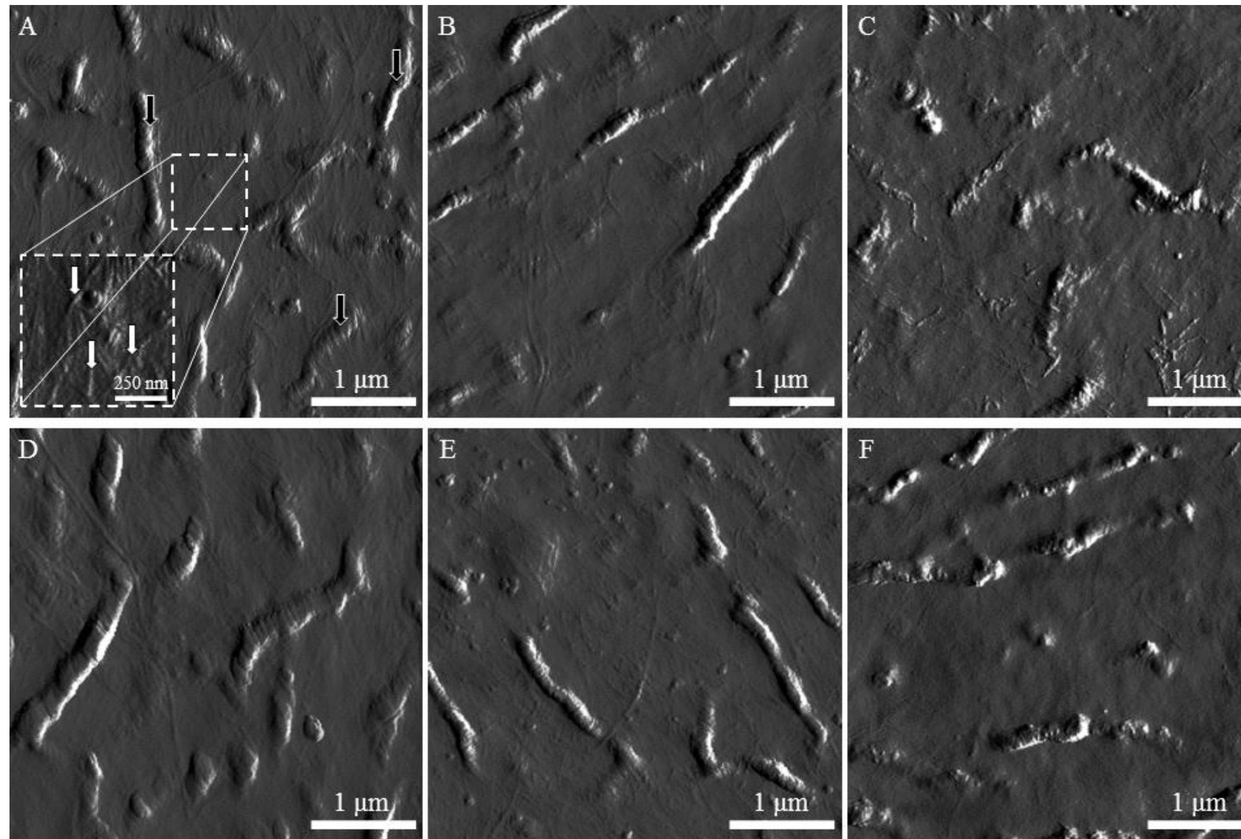


Figure 4.3 Total (A), water soluble (B) and chelator soluble (C) pectin contents of CWMs prepared from potato tubers cultivated under control and calcium fertilizations treated with different blanching methods.

Each bar represent the mean ± SD from four individual CWMs with duplicate measurements. Different small letters indicate significant differences ($p < 0.05$).



2 Figure 4.4 AFM peak force error images of CWMs prepared from potato strips witout blanching treatment (A, D) and treated with LTB (B, E)

3 and HTB (C, F). Potato tubers used for the sample preparation was cultivated with (D, E, F) or withou (A, B, C) calcium fertilizer application.

4 CWMs were suspended in deionized water at room temperature for 1 h, deposited onto glass substrates, and air-dried overnight prior to imaging.

5 Black and white arrows indicate micro- and macrofibrils, respectively. Bars represent 1 µm.

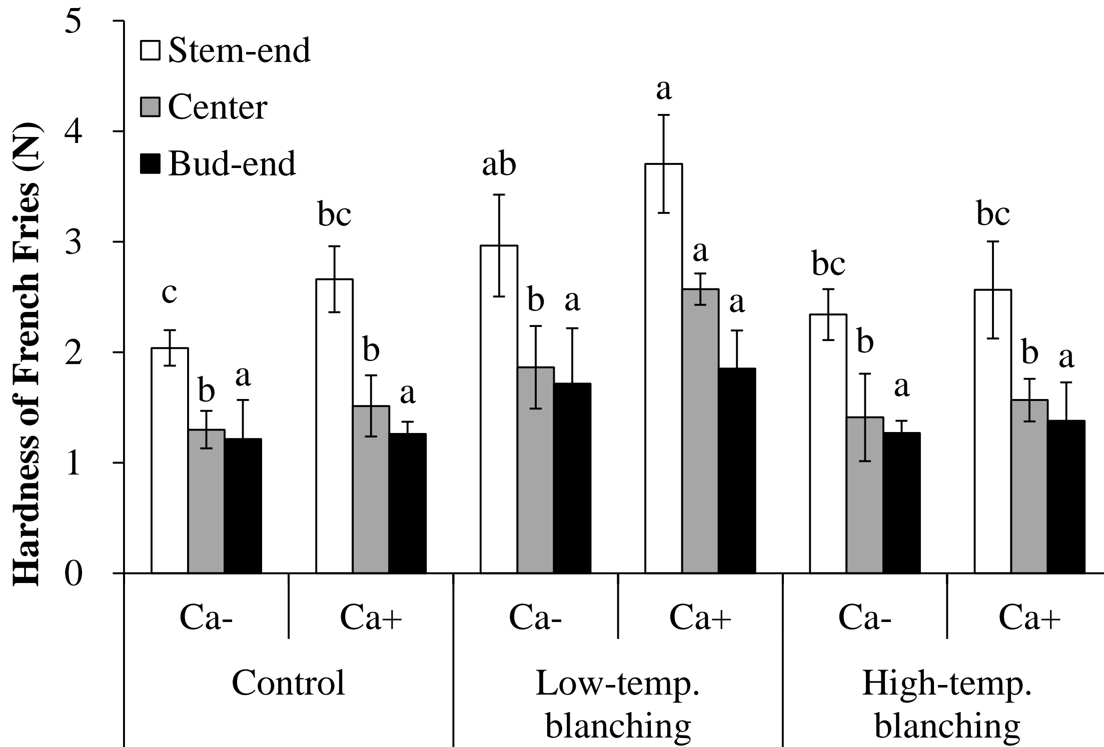


Figure 4.5 Hardness at stem-end, center and bud-end of French fries prepared from potato tubers cultivated under the control and calcium fertilizations with different blanching methods.

Each bar represent the mean ± SD from four individual samples with triplicate deteminations. Different small letters indicate significant differences within either stem-end, center or bud-end ($p < 0.05$).

Chapter 5. Effects of Calcium Salts on Physicochemical Properties of Hydroxypropylated Starches from Normal and Waxy Corns

5.1. Introduction

Among all kinds of starches, corn starch is the most widely used one in industry, making up more than 80% of the world starch market. In its native form, however, starch has a limited number of uses, mainly as a thickener or binder. Thus, chemical modification of starch granules as well as mutation and genetic modification have been achieved for controlling those physicochemical properties (Jobling 2004). For example, hydroxypropylation of starch imparts some useful physicochemical properties, such as lower pasting temperature and retrogradation tendency as well as higher freeze–thaw stability, by rendering a hydrophilic character (Wurzburg 1986; Pal et al. 2002). Meanwhile, mutation of the Waxy locus creates a starch that has no amylose, and only waxy maize is grown on a commercial scale among others. The lack of amylose in waxy corn starch means that it gelatinizes easily, yielding clear pastes that will not gel (Jobling 2004). Those starches are used in food industries with the presence other food ingredients including calcium salts. As antibrowning agent, antioxidant, coagulant, preservative and pH regulator, calcium salts may add in to various food products along with the starches. Recently, it has been reported that coexistence of various calcium salts influences on pasting, texture and rheological properties of starches varying in botanical sources (Zhou et al. 2014; Chuang et al. 2015). Therefore, in this study, the effects of calcium salts addition were examined for major two different types of corn starches with and without

hydroxypropylation to further evaluate starchy foods with desirable characteristics on physicochemical properties.

5.2. Materials and Methods

5.2.1. Starch Samples and Calcium Salts Solutions

Starch samples were supplied by Matsutani Chemistry Industry Co,. Ltd. (Hyogo, Japan). Samples included normal and waxy corn starches with and without hydroxypropylation. Molar substitutions of hydroxypropylated starches were 8.5 and 7.5% for normal and waxy corn starches, respectively, according to information provided by the company. Calcium chloride ($CaCl_2$) and calcium lactate ($C_6H_{10}CaO_6$) were used for all experiments after dissolved in deionized water at concentrations of 0.1 M. Deionized water was subjected as a control.

5.2.2. Pasting Properties

The method reported by Liu et al. (1999) was used to determine pasting properties. A 10.7% (dry basis, db) starch slurry was subjected to analysis using a Rapid Visco Analyzer (RVA-4; Newport Scientific, Inc., Warriewood, Australia) with a paddle rotating at 960 rpm for the first 10 sec and then at a fixed speed of 160 rpm. The slurry (28 g) was heated from 50 to 95°C at a rate of 12°C/min, held at 95°C for 2.5 min, cooled to 50°C at a rate of 12°C /min, and then kept at 50°C for 2 min. The pasting properties determined included peak viscosity (the highest viscosity observed during heating), hot paste viscosity (the viscosity at the end of the 95°C period), final viscosity (the viscosity at the end of the period at 50°C, breakdown

(peak viscosity minus hot paste viscosity), setback (final viscosity minus hot paste viscosity) and pasting temperature (Limpisut and Jindal 2002; Yadav et al. 2006).

5.2.3. Thermal Properties

Evaluation of thermal properties of the starch samples was conducted using a differential scanning calorimeter (DSC; DSC7000X, Hitachi High-Tech Science Corp., Tokyo, Japan). A sample of 10 mg (dry wt.) was weighed in a DSC pan, and deionized water or the calcium salts solutions were added to give a suspension of 30% dry wt.. The pan was sealed and allowed to stand at least 1 h at 20°C. The scanning temperature range was set at 30 to 95°C and the heating rate was 1.5°C /min. Deionized water was used as a reference.

5.2.4. Statistical Analysis

All data are presented as the means ± standard deviation (means ± SD). Statistical analyses were conducted using SPSS for Windows (ver. 17.0), focusing on the effects of different calcium salts addition on the pasting and thermal properties. Firstly, three-way analysis of variance (ANOVA) was carried out with “starch type”, “hydroxypropylation” and “calcium salt” as dependent variables at 5% significance level for both of the properties. When significant interactions relating to the effects of calcium salt were detected by three-way ANOVA ($p < 0.05$), two-way ANOVAs was subsequently conducted using two sets of dependent variables at 5% significance level. At first set, dependent variables were “starch type” and “calcium salt” among a sub-group of either native starch or hydroxypropylated starch. For a second set, dependent variables were “hydroxypropylation” and “calcium salt” among a sub-group of either normal starch or waxy starch. If still significant interactions

were observed in the two-way ANOVA, one-way ANOVA was further carried out within native normal, hydroxypropylated normal, native waxy and hydroxypropylated waxy starches at 5% significance level. As a post-hoc test, Tukey's multiple range tests were performed within three different calcium salts at 5% significance level.

5.3. Results

5.3.1. Pasting Properties

Table 5.1 summarizes results of pasting properties of four different starch slurries containing two types of calcium salts, and those pasting curves are shown in Fig. 5.1 for starches isolated from normal corn and Fig. 5.1 for starches isolated from waxy corns. Peak viscosities were varied from 3623.0 cP for native normal cornstarch with $CaCl_2$ to 6363.7 cP for hydroxypropylated waxy cornstarch with $CaCl_2$. In three-way ANOVA, significant three-way interactions were detected in the all parameters of pasting properties as shown in Table 5.3 ($p < 0.05$). Thus, two-way ANOVA was further carried out after separating all data into the two sets as indicated in the statistical analysis section. However, as in Table 5.4 and 5.5, significant interactions were still found in the all pasting properties ($p < 0.05$). Therefore, significant differences in the pasting parameters were compared based on the one-way ANOVA for within native normal, hydroxypropylated normal, native waxy and hydroxypropylated waxy corn starches as shown in Table 5.1. As shown by the small letters indicating significant differences among same corn starch slurries under control, $CaCl_2$ and $C_6H_{10}CaO_6$ treatments, the effects of calcium salt additions were completely different within the sample starches. For instance, slurries prepared from native normal corn starch showed

significantly different peak viscosity as following order; $CaCl_2$ < control < $C_6H_{10}CaO_6$ ($p <$ 0.05). On the other hand, the slurries prepared from native waxy corn starch were significantly different following order; $C_6H_{10}CaO_6$ < $CaCl_2$< control ($p < 0.05$). Those complicated and non-consistent effects of the addition of calcium salts in the corn starch slurries were observed through peak viscosity, breakdown, final viscosity and setback. On the contrary, additions of two different calcium salts similarly lead to increase in pasting temperatures. Except for the slurries prepared from hydroxypropylated waxy corn starch, significant increase in the pasting temperatures were achieved by the addition of the calcium salts ($p < 0.05$).

5.3.2. Thermal Properties

Results of thermal analysis for the totally 12 different samples are summarized in Table 5.2. As compared with the pasting properties, any significant three-way interactions were not observed in the three-way ANOVA for all parameters of thermal properties as shown in Table 5.3 ($p > 0.05$). In addition, any significant interactions relating to the dependent variable of "calcium salt" were not found in the all three-way ANOVAs ($p > 0.05$). For T_o, the addition of both $CaCl_2$ and $C_6H_{10}CaO_6$ significantly increased the average values from 62.2 °C of control to 67.2 and 66.6 °C, respectively ($p < 0.05$). While significant difference between the two different calcium salts in T_o was not found ($p > 0.05$). On the other hand, significant increase in T_p was observed with following order throughout all corn starch samples; control < $C_6H_{10}CaO_6$ < $CaCl_2$ ($p < 0.05$). Similarly, the same effect of $C_6H_{10}CaO_6$ and $CaCl_2$ additions in the corn starch slurries were observed in T_c ($p < 0.05$). The additions of both calcium salts resulted in significant increase in ΔH as compared with the control ($p < 0.05$), without significant difference between the two calcium salts ($p > 0.05$).

5.4. Discussion

5.4.1. Addition of Calcium Salts Alters Pasting Properties of Corn Starches Depending on Combinations of Botanical Source and Hydroxypropylation

In this study, the effects of presence of calcium salts in the slurries prepared from four different corn starches were evaluated from pasting properties and thermal properties. For the pasting properties, any consistent effects of the calcium salt additions into the corn starch slurries were not observed in all parameters even by two-way ANOVA (Table 5.1, 5.4 and 5.5). Peak viscosity, for instance, was reported to be decreased by CaCl2 addition in potato and cassava starches (BeMiller 1997; Jyothi et al. 2005), while the increase effect was shown in rice and corn starches (BeMiller 1997; Viturawong et al. 2008). However, in the present study, significant but slight decrease in peak viscosity was observed by $CaCl_2$ addition in native normal corn starch by -1.7%, while the $C_6H_{10}CaO_6$ addition resulted in the significant increase in peak viscosity by 28.7% (Table 5.3). The effects of salts on pasting properties of starches have been reported in several literatures, but the effect depends on the characteristics and concentration of salts (Oosten 1983; Bircan and Barringer 1998; Jyothi et al. 2005; Viturawong et al. 2008). Indeed, different concentrations of $CaCl_2$ can cause either an elevation or a depression of the gelatinization temperature of corn starch (Oosten 1983). Furthermore, chemical modification of starch also has influences on the pasting properties with the presence of salts. White and Johnson (2003) reported that the addition of salts to hydroxypropylated corn starch solutions caused a decrease in viscosity by reducing granule swelling. The increase in peak viscosity by addition of calcium salts in different starch

samples could be attributed to the starch/salt interactions which reduced mobility of the starch granules, leading to higher viscosity (Bircan and Barringer 1998). Meanwhile, Jane (1993) suggested that the mechanism of starch gelatinization in salt solutions can be attributed to: (1) structure-making and structure-breaking effects on water and (2) electrostatic interactions between salts and hydroxyl groups of starch. Thus, the opposite result, a decrease in peak viscosity, by the addition of calcium salts may be explained by the effect of salts on the structure-making on water with relatively lower interactions between salts and hydroxyl groups in starches.

5.4.2. Addition of Calcium Salts Alters Thermal Properties of Corn Starch without Influences of Botanical Source and Hydroxypropylation

In contrast with the pasting properties, clear effects of the addition of calcium salts on thermal properties were observed in thermal properties, i.e., To, Tp, Tc and ΔH were increased by the calcium salts additions as confirmed by the three-way ANOVA. Jane (1993) reported that presence of $CaCl_2$ at concentration of lower than 1 M increased Tp of corn starch. It seems that the influence of salts on the gelatinization properties of starch can be attributed to various factors, especially the influence on polymer–solvent interaction, the effects on water structure and the electrostatic interaction between starch and the ions (Viturawong et al. 2008). As similar to the mechanism in the decrease in peak viscosity, in the present study, the addition of calcium salts may have contributed to the structural stabilization of starch, and therefore led to the increase in thermal properties (Jane 1993). This influence was not differ between normal and waxy corn starches as well as hydroxypropylated or not, indicating that structural characteristics of starches were not contribute to the structural stabilization by the calcium salts.

5.5. Conclusion

In the present study, the effects of presence of $CaCl_2$ and $C_6H_{10}CaO_6$ on the pasting and thermal properties were evaluated in four different corn starches. The starches subjected in this study was different in botanical source, normal and waxy corns, and chemical modification, native and hydroxypropylated. For pasting properties, the effects of the calcium salt additions depended on the type of the salt as well as hydroxypropylated or not. On the other hand, To, Tp, Tc and ΔH were increased by the calcium salts additions regardless the salt type, starch botanical source and chemical modification.

Table 5.1 Pasting properties of starch slurries prepared from normal and waxy corn starches treated with/without hydroxypropylation.

Starch type	Modification	Calcium Salt	Peak viscosity (cP)		Breakdown		Final viscosity		Setback		Pasting temperature (°C)	
Normal	Native	Control	3686.7 ± 12.5	b	1671.7 ± 10.6	b	3474.7 ± 11.59	b	1459.7 ± 2.5	b	75.4 ± 0.4	c
		$CaCl_2$	3623.0 ± 13.7	c	1503.7 ± 9.6	c	3324.0 ± 19.67	c	1204.7 ± 2.3	c	79.9 ± 0.1	a
		$C_6H_{10}CaO_6$	3890.0 ± 22.3	a	2077.0 ± 13.9	a	3385.3 ± 10.97	a	1572.3 ± 2.5	a	79.1 ± 0.1	b
	Hydroxypropylation	Control	4730.3 ± 30.7	c	1621.7 ± 11.7	c	6520.0 ± 44.91	a	3411.3 ± 25.5	a	71.7 ± 0.1	b
		$CaCl_2$	5085.3 ± 31.5	a	1760.3 ± 6.4	b	6497.0 ± 50.59	a	3172.0 ± 25.2	b	73.1 ± 0.5	a
		$C_6H_{10}CaO_6$	4846.7 ± 20.2	b	2000.3 ± 44.5	a	5678.3 ± 41.74	b	2832.0 ± 71.6	c	72.9 ± 0.5	a
Waxy	Native	Control	4574.7 ± 22.4	a	3049.3 ± 53.5	a	2014.0 ± 8.89	b	488.7 ± 39.8	a	71.8 ± 0.1	b
		$CaCl_2$	4178.0 ± 21.9	b	2693.7 ± 54.6	b	2001.3 ± 26.35	b	517.0 ± 68.7	a	76.9 ± 0.5	a
		$C_6H_{10}CaO_6$	4013.0 ± 22.6	c	2323.3 ± 24.0	c	2148.3 ± 55.16	a	458.7 ± 53.5	a	76.7 ± 0.0	a
	Hydroxypropylation	Control	6133.0 ± 63.0	b	663.7 ± 18.0	b	9588.3 ± 111.24	b	4119.0 ± 33.5	b	68.8 ± 0.5	c
		$CaCl_2$	6363.7 ± 63.4	a	625.3 ± 36.3	b	10494.0 ± 71.58	a	4755.7 ± 40.1	a	73.5 ± 0.0	a
		$C_6H_{10}CaO_6$	6311.7 ± 72.5	a	1599.0 ± 247.8	a	9469.3 ± 45.37	b	4756.7 ± 131.2	a	72.7 ± 0.0	b

Different small letters in same row indicate significant differences among same starch samples at 5% significance level.

Table 5.2 Thermal properties of starch slurries prepared from normal and waxy corn starches treated with/without hydroxypropylation.

Starch type	Modification	Calcium Salt	T_o (°C)		T_p		T_c		ΔH (J/g, dw)	
Normal	Native	Control	67.3 ± 0.5	b	73.5 ± 0.4	c	80.3 ± 0.3	c	16.4 ± 0.9	a
		$CaCl_2$	72.8 ± 0.5	a	78.6 ± 0.2	a	85.2 ± 0.3	a	17.2 ± 0.8	a
		$C_6H_{10}CaO_6$	71.8 ± 0.2	a	77.8 ± 0.1	b	84.1 ± 0.2	b	17.2 ± 0.3	a
	Hydroxypropylation	Control	53.5 ± 1.3	b	59.1 ± 0.2	b	64.5 ± 0.3	b	5.6 ± 0.5	b
		$CaCl_2$	57.8 ± 0.4	a	64.7 ± 1.3	a	70.0 ± 0.6	a	6.5 ± 0.5	ab
		$C_6H_{10}CaO_6$	58.1 ± 1.0	a	63.4 ± 0.2	a	68.4 ± 1.5	a	7.1 ± 0.1	a
Waxy	Native	Control	67.5 ± 0.4	c	73.9 ± 0.4	c	82.5 ± 0.3	c	19.4 ± 1.1	b
		$CaCl_2$	72.8 ± 0.1	a	78.8 ± 0.2	a	87.2 ± 0.0	a	21.0 ± 0.2	ab
		$C_6H_{10}CaO_6$	72.0 ± 0.1	b	78.1 ± 0.1	b	86.2 ± 0.1	b	21.1 ± 0.2	a
	Hydroxypropylation	Control	60.5 ± 0.2	b	66.2 ± 0.3	c	73.5 ± 0.3	b	16.0 ± 1.1	a
		$CaCl_2$	65.3 ± 1.0	a	70.4 ± 0.1	a	77.2 ± 1.7	a	17.7 ± 0.7	a
		$C_6H_{10}CaO_6$	64.6 ± 0.1	a	69.8 ± 0.0	b	77.1 ± 0.1	a	17.1 ± 0.2	a

Different small letters in same row indicate significant differences among same starch samples at 5% significance level.

Table 5.3 Results of three-way ANOVA for pasting and thermal properties with starch type, modification and calcium salt as dependent variables.

	Starch type (S)	Modification (M)	Calcium salt (C)	S × M	S × C	M × C	S × M × C
	p value						
Pasting properties							
Peak viscosity	**< 0.001**	**< 0.001**	**0.020**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Breakdown	0.050	**< 0.001**	**< 0.001**	**< 0.001**	**0.001**	**< 0.001**	**< 0.001**
Final viscosity	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Setback	**< 0.001**	**< 0.001**	0.144	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Pasting temperature	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Thermal properties							
T_o	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	0.687	0.196	0.573
T_p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	0.072	0.727	0.284
T_c	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	0.190	0.941	0.307
ΔH	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	0.293	0.956	0.359

P values in bold indicate significance at 5% significance level.

Table 5.4 Results of two-way ANOVA for pasting and thermal properties of native and hydroxypropylated corn starches with starch type and calcium salt as dependent variables.

Native	Starch type (S)	Calcium salt (C)	S × C
	p value		
Pasting properties			
Peak viscosity	**< 0.001**	**< 0.001**	**< 0.001**
Breakdown	**< 0.001**	**< 0.001**	**< 0.001**
Final viscosity	**< 0.001**	**< 0.001**	**< 0.001**
Setback	**< 0.001**	**< 0.001**	**< 0.001**
Pasting temperature	**< 0.001**	**< 0.001**	**0.013**
Thermal properties			
T_o	0.385	**< 0.001**	0.894
T_p	**0.041**	**< 0.001**	0.667
T_c	**< 0.001**	**< 0.001**	0.774
ΔH	**< 0.001**	**0.011**	0.455

Hydroxypropylated	Starch type (S)	Calcium salt (C)	S × C
	p value		
Pasting properties			
Peak viscosity	**< 0.001**	**< 0.001**	**0.023**
Breakdown	**< 0.001**	**< 0.001**	**< 0.001**
Final viscosity	**< 0.001**	**< 0.001**	**< 0.001**
Setback	**< 0.001**	**< 0.001**	**< 0.001**
Pasting temperature	**< 0.001**	**< 0.001**	**< 0.001**
Thermal properties			
T_o	**< 0.001**	**< 0.001**	0.595
T_p	**< 0.001**	**< 0.001**	0.119
T_c	**< 0.001**	**< 0.001**	0.246
ΔH	**< 0.001**	**0.004**	0.239

P values in bold indicate significance at 5% significance level.

Table 5.5 Results of two-way ANOVA for pasting and thermal properties of normal and waxy corn starches with modification and calcium salt as dependent variables.

Normal	Modification (M)	Calcium salt (C)	M × C
	p value		
Pasting properties			
Peak viscosity	< **0.001**	< **0.001**	< **0.001**
Breakdown	**0.001**	< **0.001**	< **0.001**
Final viscosity	< **0.001**	< **0.001**	< **0.001**
Setback	< **0.001**	< **0.001**	< **0.001**
Pasting temperature	< **0.001**	< **0.001**	< **0.001**
Thermal properties			
T_o	< **0.001**	< **0.001**	0.288
T_p	< **0.001**	< **0.001**	0.747
T_c	< **0.001**	< **0.001**	0.679
ΔH	< **0.001**	**0.012**	0.601

Waxy	Modification (M)	Calcium salt (C)	M × C
	p value		
Pasting properties			
Peak viscosity	< **0.001**	< **0.001**	< **0.001**
Breakdown	< **0.001**	**0.001**	< **0.001**
Final viscosity	< **0.001**	< **0.001**	< **0.001**
Setback	< **0.001**	< **0.001**	< **0.001**
Pasting temperature	< **0.001**	< **0.001**	**0.039**
Thermal properties			
T_o	< **0.001**	< **0.001**	0.565
T_p	< **0.001**	< **0.001**	0.050
T_c	< **0.001**	< **0.001**	0.441
ΔH	< **0.001**	**0.003**	0.579

P values in bold indicate significance at 5% significance level.

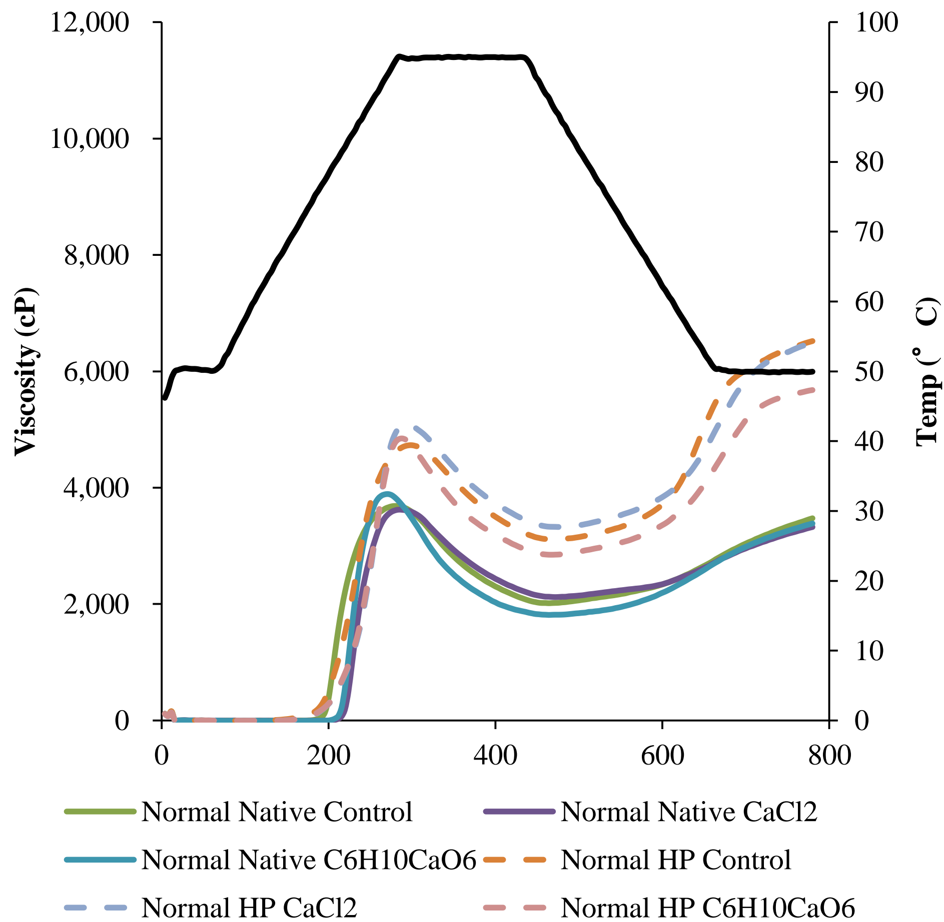


Figure 5.1 Pasting curves of slurries prepared from normal corn starches treated with/without hydroxypropylation.

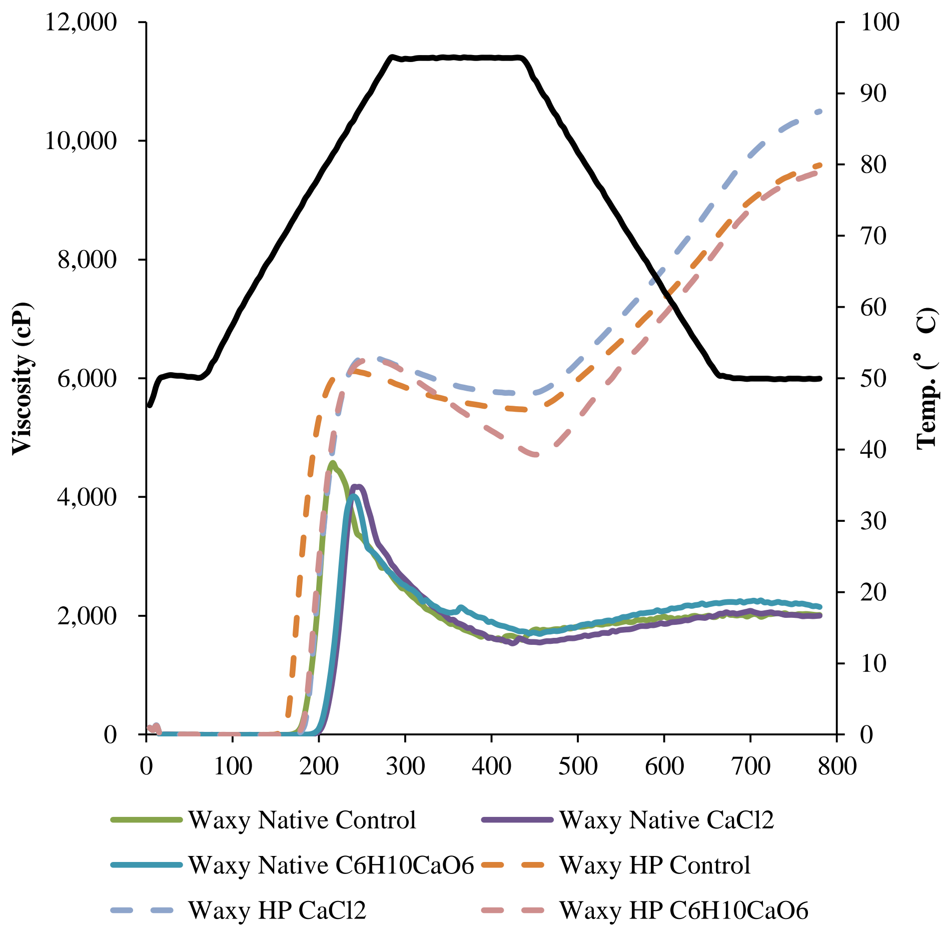


Figure 5.2 Pasting curves of slurries prepared from waxy corn starches treated with/without hydroxypropylation.

Chapter 6. Summary

Both projects included in this thesis focused on the effects of Ca^{2+} on texture of the plant-based foods, i.e. French fries and corn starches, and these effects were investigated to provide insights into further development and improvement of the plant-based foods.

The objective of the study in Chapter 2 was to investigate whether calcium absorbed by potato tuber concomitantly increases calcium concentration in cell wall and how calcium concentration influences on the formation of cross-linking of pectin via Ca^{2+}. Thus, for the purpose of this study, each tuber was individually analyzed. Their properties were compared between the low-calcium and high-calcium tubers on the three harvest dates, from immature to mature stages. The results indicated that an increased absorption of calcium by a potato tuber concomitantly increased the calcium concentration in the cell wall on 99 days after planting or later, and that there were linear correlations between the calcium concentration in the cell wall and the formation of cross-links between pectin molecules via Ca^{2+} throughout tuber bulking and maturation stages. The degree of methylation was not found to be a limiting factor on the formation of cross-links between pectin molecules. Furthermore, a higher calcium concentration of a mature potato tuber contributed to the enhancement of resistance of pectin-calcium networks in the parenchyma cell wall against water and HEPES buffer washing.

The study in Chapter 3 was conducted to evaluate the effect of calcium fertilization on the tuber, starch and pectin properties of three different varieties of processing-type potatoes, and their influence on the processing properties and storability of frozen French fries. For this purpose, three processing varieties of potatoes, namely Toyoshiro, Kitahime and Snowden, were cultivated either with or without calcium fertilizer application. Harvested

tubers were subjected for French fry preparation and isolation of starch and cell wall. The results showed that considerable improvement of the hardness of French fries prepared from three processing-types of potatoes, as a result of calcium fertilizer application. French fries prepared from Toyoshiro and Kitahime grown with calcium fertilization showed significantly greater hardness as compared with non-calcium treated counterparts even after frozen storage ($p < 0.05$). In addition, the significant effect of calcium fertilization in hardness of French fries was confirmed by two-way ANOVA. Calcium fertilization significantly increased the calcium contents of cell wall materials of all varieties, while a significant increase in chelating-agent soluble pectin content was observed in Toyoshiro ($p < 0.05$). The oil content of French fries was not significantly affected by calcium fertilization. Therefore, results of this study suggest that calcium fertilization may be an effective method to improve the processing properties and storability of French fries made from processing-type potato varieties.

A combined use of calcium fertilization and low-temperature blanching may be the promising way to control the texture of plant-based foods by effectively utilizing endogenous calcium absorbed naturally during cultivation as well as pectin methylesterase in the cell wall. Thus, in Chapter 4, the objective was to evaluate effects of calcium fertilization and low-temperature blanching on hardness of French fries with possible modification in cell wall pectin characteristics. In order to achieve this, potato samples were cultivated under the fertilizer application with or without calcium during tuber bulking period, and two different blanching methods of low-temperature longer time and high-temperature shorter time was employed during French fry preparation with comparing with control condition as without blanching treatment. Calcium fertilization enhanced Ca^{2+} availability within pectin chains for formation of the cross-links, while low-temperature blanching increased Ca^{2+} affinity of pectin chains for the cross-links by facilitating demethylesterification of pectin molecules.

Combination of calcium fertilization and low-temperature blanching showed the highest increase in the hardness as compared with French fries prepared from potato tuber cultivated under the an ordinary fertilization without blanching treatment. The significant changes in the pectic polysaccharides as well as the cell wall were confirmed by pectin methylesterase activity measurement, pectin composition analysis and atomic force microscopy for complementing the hardening effect of calcium fertilization and low-temperature blanching in French fries. Thus, controlling texture of French fries through effective use of endogenous Ca^{2+} and pectin methylesterase in potato tuber improves the final product quality.

In Chapter 5, the effects of calcium salts addition were examined for two major types of corn starches with and without hydroxypropylation to further evaluate starchy foods with desirable characteristics of physicochemical properties. The starches subjected in this study were different in botanical source such as normal and waxy corns, and chemical modification such as native and hydroxypropylated. For pasting properties, the effects of the calcium salt additions depended on the type of the salt as well as hydroxypropylated or not. On the other hand, T_o, T_p, T_c and ΔH were increased by the calcium salts additions regardless the salt type, starch botanical source and chemical modification.

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