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FINAL REPORT

PROJECT NO. IS-1425-88

**Studies of Structure and Texture of Potatoes to
Enable Cultivar Selection and Development of
New and Improved Processed Products**

I. Shomer, M.C. Bourne

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Studies of structure and texture of potatoes to enable cultivarselection and development of new and improved processed products.Investigators' Names
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a. Abstract

This BARD project dealt with factors and mechanisms involved in textural quality of potatoes. The research was five fold: Permeability and swelling of heat-gelatinized potato cells; electrical response in relation to texture; rheology during temperature changes and enzymatic cell wall (CW) degradation; Texture of raw potatoes evaluated by puncture tests, considering morphology and specific gravity; and texture of heat-treated tissues determined by softening kinetics, puncture tests and sensory evaluations

Potato cell whose starch has been heat-gelatinized (HG-cells) were permeable to biopolymers of molecular size up to 700 kDa. Swelling was dependent on the dielectric constant, indicating the presence of an electrical double layer on CW fibrils and starch polymers. Induced electric field caused cell migration to the anode, indicating negative net charge dependent on cell size, density, ionic valence and concentration, and voltage. Cells included porous CW and a mass of swollen gelatinized starch, between which coagulated protein or cytoplasmatic remnants are seen.

Electrical measurements showed that the CW has a cation exchange response of Donnan potential and is very sensitive to electrolytes and pH. CW potential varied at rates of ~3, 25, and 840 mV/mM for Na^+ (or K^+), Ca^{++} and Al^{+++} , respectively. Na^+ , Mg^{++} and $\text{SO}_4^{=}$ induced higher potentials than did K^+ , Ca^{++} and $(2)\text{Cl}^-$, respectively.

Rheology of macerates of HG-cells was influenced by temperature because the latter affects swelling of CW and starch. Increased temperatures resulted in higher storage modulus, lower loss modulus, and lower complex viscosity. CW degradation decreased these values in apparent second-order behavior, resulting in a loss of cell structure, starch dispersion and liquefaction.

25 Raw potatoes of eleven cultivars were sorted according to specific gravity, and three were studied in detail by a puncture test at various sites of both cortex and pith. The cortex showed generally a significantly higher puncture force than the pith. With the exception of pith in Monona cultivar, puncture force increased with specific gravity. Dry matter content increased with the specific gravity; the cortex had 3-6% higher dry matter than the pith.

Heat-gelatinized tubers were tested by puncture force, at various sites of cortex and pith. Puncture force was increased with specific gravity in both raw and cooked tubers. Puncture force of the cortex was higher than in the pith raw tubers, and was the same in all regions of cooked tuber. Puncture force was correlated with sensory evaluations. Mealiness was specific to cultivar, but was neither correlated with hardness nor with specific gravity.

Thermal kinetics were studied by back-extrusion tests during the cooking in 4 cultivars (Atlantic, Chieftain, Monona, and Russet Burbank) each of which in two specific gravity levels. In all of the samples, the firmness versus cooking time exhibited three successive regions: I. Fast softening where ~95% of the initial firmness was lost, II. re-firming, and III. slow softening. The softening phases corresponded to first order kinetic behavior, but the behavior of the re-firming stage is not known.

d. Objectives of the Original Research Proposal

I. To study the cell-wall and its synergistic relationships with starch, solutes, protein and oil, in order to:

- a. understand the textural properties of raw and processed potato tissue with an emphasis on reconstituted products to obtain products having superior texture;
- b. investigate the involvement of oil with textural properties of fried and oily preserved products;
- c. provide knowledge on the liquefaction of potato tuber tissue.

II. To provide the knowledge needed to define and measure the textural and rheological properties of potato tissue and derived semi-fluid products in order to obtain better quality of existing and newly developed potato-based foods. Detailed objectives:

- a. To measure the rheological properties of potato puree and other semi-liquid potato products, showing how these properties are affected by changes in cell wall and starch, and relate them to sensory assessment of textural quality.
- b. To perform instrumental textural profile analysis on potato tissue and determine the effect of cultivar, heating regime and dehydration on textural parameters.
- c. To develop a system for measuring the physical properties of individual cells, cell-walls and the intercellular zone (middle lamella), and relate these measurements to microstructure of the cell wall and textural quality of the potato tissue.

25 In general, the research followed the objectives. Study of the behavior of potato tuber cells in oil was related to the effect of dielectric constant on swelling. The prominent phenomenon during substitution of water with organic liquid is a dramatic diminishing in the swelling ability of both cell wall and starch. Hence, swelling behavior under conditions of various dielectric constants was studied in aqueous solution of water-miscible organic solvent, including those with dielectric constants of edible oils. A comparative study showed that potato cells respond to swelling in edible oil in the same manner as in both water-miscible and water-non-miscible organic solvents; swelling was always related to the dielectric constant of the liquid, an exception being where the solvent dissolved starch (and this is not the case of edible oil). These findings show that the electric response of the polysaccharides play a role in swelling behavior; thus electric response was studied by several methods, e.g., cell motion under induced electric field, measurements of electric potential by micro electrodes, effects of electrolytes, and pH.

Experiments were carried out in order to examine the effect of starch gelatinization on endogenous protein coagulation and vice versa in relation to swelling and texture; at this stage, no indicative results have been found, and this subject will be studied more thoroughly in the future.

SWELLING, PERMEABILITY AND ELECTRICAL RESPONSE OF HEAT-STARCH-
GELATINIZED POTATO (*Solanum Tuberosum* L.) TUBER CELLS

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ABSTRACT

Permeability of heat-gelatinized potato tuber cells was determined by column chromatography of biopolymers (proteins and dextrans ranging from 6.5 to 2,000 kD) on potato cell matrix. Elution volumes were in accordance with molecular size, though partial adsorption as indicated by K_{av} values of 1.5-00.7; indicating cell wall (CW) pores within the range of several up to several hundred Ångstroms. Cell swelling in liquid media was found to be dependent on dielectric constants (within the range 25-109), indicating the presence of an electrical double layer upon both CW fibril surfaces and starch polymers. Solvents of high dielectric constant [formamide (109) and DMSO (45)] resulted in a marked increase in swelling, as compared with water. Cells migrated toward the anode in electric field at a velocity that was affected by cell size, electrolytes and voltage; thereby indicating their negative net charge. The CW demonstrating electrical potential, reached -70 mV and it decreased with an increase in concentration and valence of cations. These findings are in accordance with electrophoretic cell mobility and effect of dielectric constant on swelling. Heat-gelatinized potato cells include porous CW and a mass of gelatinized starch, between which coagulated protein or cytoplasmatic remnants were seen. Sometimes a space was seen between the starch and the CW, while in other cases starch was attached densely to the CW.

INTRODUCTION

Potato texture is determined by physical properties of the tuber cells and their mutual adhesion forces (Harris, 1982; Mullins et al., 1957). Preparation of potatoes for consumption entails heat treatment, that causes gelatinization of starch and solubilization of pectic substances in the middle lamella. These processes take place when converting the tissue into its edible form (Linehan et al., 1968). Generally, it is agreed that starch and cell wall are the structural components that make up and determine potato texture. (Gray and Hughes, 1982; Reeve, 1972).

Starch, the major constituent in the tuber cells, has been the focus of attention and controversy as to its function in potato texture (Gray and Hughes, 1982; Potter et al., 1959). Several structural models have been suggested for starch behavior upon heat gelatinization in relation to texture (Beynum and Roels, 1985; Gruber et al, 1973; Sterling, 1965; 1974; Swinkels, 1985). Potato starch consists of about 22-23% amylose and about 77-78% amylopectin (Hadziyev and Steel, 1979; Radiley, 1968; Van Beynum and Roels, 1985). Upon heating, the starch grains inside the cells become highly hydrated, swollen, gelatinized, fused, occupy the entire cell volume, and appear in a wide range of solid-gel-liquid states (Fedec et al., 1977). It has been suggested that gelatinized starch exerts swelling pressure on the cell wall (CW), and as a result leads to cell separation (Briant et al., 1945). Conversely, void space was found between gelatinized starch and the cell-wall (Fedec et al., 1977; Davis and Gordon, 1984). Warren and Woodman (1974) suggested that increased firmness is a function of increased viscosity rather than cell adhesion. On the other hand, Mica (1985) suggested that texture is affected more by physical properties of the starch than by its amount; however, no experimental evidence was provided to support the theory attributing potato texture mainly to starch (Hoff, 1972), and there is still some controversy on this subject.

In addition to starch, the cell-wall plays a role in potato texture, and some investigators consider it the most important factor (Burton, 1982; Gray and Hughes, 1982; Hadziyev and Steele, 1979). In this context, the CW and its middle lamella

confer tissue texture in edible (non-starchy) tissues (Fishman and Jen, 1986). The accepted approach to explain textural changes is based on changes in cell adhesion during ripening due to pectin degradation in the middle lamella (Huang and Bourne, 1983; Jen, 1989). In heat treated tissues, pectin degradation in the middle lamella is known to occur due to β -elimination; thus, cell adhesion is reduced or lost upon heating at boiling temperature. Under these conditions, texture and rheology are influenced less by cell adhesion and more by the physical properties of the cells themselves. In macerates, middle lamellar adhesion is absent; thus, the texture is affected by factors such as intercellular friction, density, CW elasticity, surface properties, intracellular solutes, protein coagulation, and starch gelatinization (Pitt, 1992). Composition of the cell-wall and changes taking place in it upon cooking are of major importance to the texture of processed potatoes (Reeve, 1967). The accepted approach explains the role of the CW is mainly by the solubilization of pectic substances in the middle lamella during heat treatment (Keijbets and Pilnik, 1974; Keijbets et al., 1976; Roberts and Proctor, 1955). Burton (1948), referring to investigations spanning 1897-1939, suggested that potato texture may be affected by pectic substances. This was explained to be a consequence of pectin transelimination, ion exchange, and hydrogen bond breakage (Keijbets et al., 1976; Sharma et al., 1959). From the point of view of the CW, the most convincing explanation is that potato texture is soften as a result of pectin degradation in the middle lamella (Hadziyev and Steele, 1979; Keijbets et al., 1976).

It has been suggested that the texture is influenced by the swelling level of the CW (Shomer et al., 1984). For this reason, attempts were made to remove specific fractions of pectin and/or cellulose and to examine the effect of the residual components on the CW swelling. Not only enzymatic pectin degradation not decrease swelling, it actually increased the CW swelling, while enzymatic cellulose degradation resulted in the loss of macerate bulkiness and texture (Shomer et al., 1984; Shomer and Levy, 1988). The CW swelling mechanism was attributed to attraction and repulsion

forces acting in a diffuse double layer of the CW fibrillar surfaces (Shomer et al., 1991).

Swelling properties of CW were found to play a role in the stability of macerates of edible plant tissues (Shomer et al., 1984). Although in quantitative terms potato cells contain mostly starch, the CW is the compartment that imparts the volumetric properties to the cells (Shomer and Levy, 1988). Study of the swelling mechanism has shown that the microfibrillar lattice of cellulose is the bulky skeleton and is affected by other factors such as pectin, electrolytes, and dielectric constant (Shomer et al., 1984; Shomer et al., 1991). Since the texture is affected by the CW swelling properties it is important to study the swelling behavior in potato cells, considering the involvement of both CW and starch.

Swelling properties determine, among others, the CW porosity. Two main approaches were applied to characterize CW porosity: 1. Permeability of fluorescent polysaccharides of known molecular weight into intact plasmolized cells, that are detectable by fluorescent microscopy (Baron-Eppel et al., 1988; Carpita et al., 1979; Carpita, 1982), and 2. elution of biopolymers through a column of CW fragments, and evaluation of the pore dimensions due to permeability of solutes with known molecular weight (Tepfer and Taylor, 1981). Regarding CW swelling, it is of importance to determine the porosity of potato tuber cells in relation to its water adsorption, hydraulic conductivity, and permeability of gelatinized starch.

The present study examined the swelling properties of heat-starch-gelatinized single potato cells in liquid series of various dielectric constants and temperatures. The porosity of the CW was characterized by its permeability to biopolymers of known molecular weight, leakage of heat gelatinized starch, and ultrastructure.

MATERIALS AND METHODS

The porosity of potato (*Solanum tuberosum* L., cv Desirée) tuber CW was determined by its permeability to soluble biopolymers of known molecular weights. Selected potato tubers were peeled, diced, and boiled for 40 min in distilled water; the soft cubes were mashed and passed first through a 2 mm mesh plastic network strainer and then through a 0.5 mm mesh strainer. The macerate was then rinsed and cleaned several times by suspending the whole separated cells with relatively large quantities of distilled water and allowing them to settle freely within the water column. Large cells and aggregates that settled quickly, and small cells that settled slowly were removed from the suspension, while cells of medium size were selected for the experiments. The retained fraction was examined by a light microscope to verify that it consisted of separated, whole, non-damaged cells. The macerated cells were packed in a glass column of LKB (1.5 cm dia. x 75 cm length) and were served as a chromatography matrix through which soluble biopolymers were eluted. The cells were kept throughout the preparation (straining, rinsing, separation, and collection) and column packaging in an aqueous medium with an excess of water in order to prevent undesirable changes such as shrinking, cross-linking, and deformations of starch and CW. About 5 mg of known protein were eluted by distilled water at a rate of 1 ml / min, and detected at 280 nm. Each elution was performed using tyrosine and dextran blue as reference for total volume and void volume respectively.

Swelling measurements were done according to Shomer et al. (1991) with modification in preparation of the cells. Macerated cells obtained as described above were dehydrated gradually with increased amounts of ethanol, dried by evaporation, and finally freeze-dried. Exact amount of 500 mg of dried cells mixed thoroughly were immersed and shaken gently overnight in 30 mL of the swelling liquid in 100 mL Erlenmeyer flask. The swollen cells with the liquid were transferred quantitatively into a tube of about 18 mL and left for 2 days to precipitate free at room temperature.

Leakage of starch and pectin from separated cells was evaluated by determining the content of amylose based on the assumption that amylose molecules of relatively low molecular weight permeate the cell wall, while amylopectin is retained inside the cells. The amylose was assayed by the iodine method. Ethanol was added to an aliquot of the starch solution to reach a solution of 70% ethanol; then, the alcohol insoluble solids were rinsed several times with 70% ethanol, separated from the alcohol, and redissolved in water. Pectin was analyzed according to Ahmed and Labavitch (1977), and starch as amylose was analyzed colorimetrically by 12.5 mM/L iodine in a 25 mM citrate buffer, pH 5 due to absorbency at 650 nm.

The electric responses of the CW were studied in two experimental systems: mobility of heat-inactivated cells in electric fields; and measurements of the electric potential of the CW in heat-inactivated potato cells. For the electrophoresis studies, single PCs were placed in NaCl or CaCl₂ solution in a vertical graduated tube (16 cm x 0.5 cm dia.) sealed with agar-covered sintered glass at the bottom. The upper and the lower chambers of the electrophoresis apparatus and the graduated tube were filled with the same electrolyte solution. A power supply was used to induce the electric field at voltage levels of up to 3000 V. Electrophoretic cell mobility was determined according to the time needed for an individual cell to climb 10 mm, as observed on calibrated tube (3 mm dia.) with a stereo microscope placed over an illuminated electrophoretic chamber. For electrical potential measurements, one drop of cell suspension was added to the aqueous medium in a 35x5x16 mm Plexiglas measurement chamber containing a fine sponge. The cells settled into the sponge cavities. Using a micromanipulator, a micropipette was inserted into the CW of a single potato cell, that was viewed through a horizontal microscope. The chamber was perfused with a constant electrolyte flow at 1 mL/sec. The electrolyte flow was required to keep the CW under constant and definite hydrodynamic conditions. This resulted in a thin, unstirred layer and minimized interfacial concentration gradients, leading to an accelerated response upon changing the electrolyte. CW potentials were measured for different electrolytes. All of the experiments began with CW

preparations bathed in 0.1mM NaCl solution. The electric potential under these conditions was somewhat different from one cell to another but was within the -60 to -80 mV range .

Heat-treated, individual cells were examined under a light microscope, after rehydration of dried material by distilled water. CW was identified by polarized light and starch by iodine staining for light microscope, and silver nitrate staining for electron microscopy, For electron microscopy, cell macerate was fixed in 5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7) at 4° C for 2 h. The macerate was then rinsed several times with the same buffer, followed by washing with 0.1 M phosphate buffer (pH 7), postfixing with 2% OsO₄ in the phosphate buffer at 4° C for 2h, and then washed several times with the phosphate buffer, followed by washing with distilled water. The fixed macerate was dehydrated gradually with ethanol and embedded in Agar 100 Resin (Agar Aid, Cambridge). Ultrathin sections were stained with uranyl acetate and lead citrate; starch staining was done by 1% periodic acid for 20 min at 20° C, then washing with distilled water, followed by incubation in a solution of 1% thiosemicarbazide and 10% acetic acid, washing with distilled water, then incubation in a solution of 1% silver proteinate for 30 min at 20° C in the dark, and rewashing with distilled water. Observations were made using a Jeol-100CX transmission electron microscope.

RESULTS AND DISCUSSION

Permeability and porosity of potato tuber cells: Boiled potato tuber cells are spherical oval bodies of about $25\text{-}125 \times 10^{-4} \text{ mm}^3$. The cell lumen is occupied to capacity with highly swollen gelatinized starch, that inflates the cell. The swollen gelatinized starch retains the cells as swollen bodies and prevents their collapse and compactness while using as column matrix. Fig. 1 is a representative chromatogram that shows elution of four representative solutes of known molecular weights: tyrosine, myoglobin, BSA, and dextran blue. Dextran blue, with a molecular weight of 2×10^6 , was used for determination of the void volume, while tyrosine was used to indicate the total elution volume. K_{av} represents the fraction of the stationary gel volume available for diffusion of a given solute, and was calculated according to the following equation:

$$K_{av} = (V_e - V_o) / (V_t - V_o)$$

where V_e is the elution volume of the examined material, V_o is the void volume and V_t is the total column volume. Distinct separation was obtained between the different eluted solutes.

Figure 2 shows K_{av} values for various proteins with various molecular weights, ranging from 6.5 to 669 kD, that were eluted through a column packed with potato cells. The various K_{av} values indicate a wide spectrum of CW pore dimensions within the range of several up to several hundred Ångstroms, that enables the separation of proteins due to their molecular weight. A previous study reported CW porosity based on determination of permeability through a column of fragments of disintegrated cells (Tepfer and Taylor, 1981). In this system the CW structure changed notably, including distribution and dimensions of pore size, thereby leading to changes in the CW permeability; hence, an evaluation of CW permeability into intact cell (Baron-Eppel et al, 1988; Carpita et al, 1979; Carpita, 1982) represents a more authentic state of CW porosity. Heat-gelatinized potato cells contained highly swollen starch where the CW was retained intact although some of its heat degradable components such as pectic substances removed. Under such conditions, CW porosity was mainly that of

the microfibrillar lattice of the cellulose and non-degraded hemicellulose and pectin. The permeability of proteins through the porous cells showed that the CW is permeable to molecules up to about 700 kD. As a significant portion of heat-gelatinized starch is trapped within the cells, this indicates that the molecular size is higher than the CW pores. In this context, the concentration of heat-gelatinized starch is relatively high, and in effect, the cells include saturated starch solution. The starch polymers are of a wide spectrum of molecular weights, and it is known that starch polymers may exceed 700 kD, which is about the highest pore size in heat-treated potato cell walls.

Despite the aforementioned, starch leaked from potato cells during boiling more or less continuously, while removed pectin decreased with time (Table 1). Microscopic observations showed that the CWs remained intact during the boiling treatments (Fig. 9), hence leakage of soluble starch infiltrates through the porous lattice of the CW. Evidently, some fraction of gelatinized starch is soluble in hot water and may permeate the CW, while other fractions are retained constantly within the cell. Potato starch includes about 21% amylose and 79% amylopectin each with a wide range of molecular sizes. Since starch is leaked from the cell continuously during boiling, it is possible that some portion of the starch is degraded during the boiling. According to the results of the potato cell column chromatography (Figs. 1, 2) it may be determined that soluble starch of a molecular weight higher than ~700 kD may not permeate the CW. It is possible that the concentration of the soluble starch inside the cell is higher than saturated solution. Under conditions of high concentration, amylopectin undergoes a retrogradation effect (Swinkels, 1985); in this context, it is reasonable to assume that some fraction of retrograded amylopectin and amylose are retained inside the cells. Amylopectin is known to be of a higher molecular weight (~2,000 kD or more), and its polymers are a mixture of soluble and gel fractions (Manners and Matheson, 1981). The molecular weight of amylose is about 3 kDa, and it is interwoven with the amylopectin (Swinkels, 1985). The heat-gelatinized starch inside the cell includes a mixture of highly swollen soluble molecules and gel fractions

packed mutually. Under these conditions, the gelatinized starch inside the cell is protected from free agitation and rinsing by water. Hence, leakage of soluble starch molecules is dependent (due to concentration gradient) upon both slow gradual diffusion and infiltration through the CW. Potato starch is known to be of exceptionally high swelling power already at relatively low temperatures, and particularly at high temperatures of 95-120° C (Whistler and Paschall, 1965). Two factors involved in the swelling power of potato starch: 1. Hydrogen bonding through water bridges rather than direct molecular associations (Leach et al., 1959), and 2. Weak internal bonding due to the presence of ionizable esterified phosphate groups (Whistler and Paschall, 1965), that assist swelling by mutual electrical repulsion.

Swelling behavior in liquids: Immersion of dried potato cells in various solutions of (water-miscible organic solvent such as) ethanol and water at ratios ranging from 0 to 100% ethanol (representing dielectric constants in the range of 25.00-80.37) resulted in different swelling levels (figs. 3, 4). These figures demonstrate that two factors affect cell swelling: 1. Temperature and 2. Ethanol:water ratio. As previous study showed that cell swelling in macerates is determined by the swelling properties of the CW either in (non-starchy) tomato cells (Shomer et al., 1984) or in (non-heat-gelatinized starchy) potato tuber cells (Shomer and Levy, 1988). Swelling of tomato cells without heat-gelatinized starch was affected directly by the dielectric constant of the liquid medium (Shomer et al., 1991). In potato cells, the swelling is affected by increasing temperature (Figs. 3, 4) and this may be explained by the swelling power of gelatinized starch (Whistler and Paschall, 1965; Swinkels, 1985); however, the effect of temperature on swelling decreases with the dielectric constant, and above 70% ethanol the swelling is not affected by temperature (Fig. 4). This indicates that the effect of dielectric constant is stronger than that of temperature.

Swelling of potato cells in a series of solutions ranging from 0 to 100% formamide in water showed that the swelling levels increased markedly with the formamide concentration (Fig. 5a). DMSO increased the swelling of potato cells to extremely high levels, while at 100% DMSO, the entire liquid column was occupied

by swollen potato cells Fig. 5b). While swelling of either non-starchy or starchy but non-gelatinized parenchyma cells is exclusively dependent on the CW swelling (Shomer and Levy, 1988; Shomer et al., 1984; 1991), swelling of potato tuber cells are affected greatly by the swelling power of the heat-gelatinized starch. In this context, heating is one way to affect the swelling pressure exerted by the gelatinized-starch, and another possibility is by increasing the solubility and/or the swelling of the starch itself, separately from the CW. Liquids with relatively high dielectric constant, such as formamide, affect CW swelling similarly to water (Shomer et al., 1991). Solvents of relatively high dielectric constant such as formamide (109) and DMSO (45) may be examined for comparisons between organic solvents of high dielectric constants and water. Comparisons between swelling levels in media of various liquids in terms of their dielectric constants, showed that the swelling increases with the dielectric constant, particularly above dielectric constant of 45 (fig. 6). The swelling level of non-starch tomato cells in formamide was somewhat lower than that of cells swollen in water (Shomer et al., 1991); in comparison, potato cells swelled markedly more in formamide and DMSO than in water.

The extent of swelling is directly dependent on the existence and properties of electrical diffused double layer (EDL) of the CW fibrils, and it is affected by counter ions that neutralize the fixed charges (Shomer et al., 1991). This swelling behavior is similar to the attractive and repulsive behavior of charged suspended colloids (van Olphen, 1963; Jirgensons and Straumanis, 1962). However, the swelling level of the CW is limited by physical forces of the cross-linked cellulose molecules; therefore, while suspended colloidal particles collide randomly with one another as a result of Brownian movement, hydrated CW fibrils exist within a defined lattice. According to this explanation, while the swollen CW is limited (due to the cross-linked cellulose microfibrils), the intracellular starch may be swollen to a relatively less limited extent [due to disruption of weak intermolecular hydrogen bonds (Swinkels, 1985)]. In addition to the above, the swelling power of the intracellular starch is much greater than that of the CW because of its relative immense amount and

density inside the cell. Formamide in ratios above 25% in water reduced the swelling level in tomato cells due to relatively strong hydrogen bonds between formamide molecules and water (Shomer et al., 1991). In potato cells, the increased swelling level in formamide (Fig. 5) may be attributed to the large amount of starch inside the cell and to the weak hydrogen bonds between the starch molecules (Swinkels, 1985); thus, high dielectric constant resulted in increased starch swelling, that inflated the cells. The cells were swollen in DMSO more than in water, although the former's dielectric constant is lower than that the later. This can be explained by the fact that the gelatinized starch inside the cell was dissolved by DMSO, thereby resulting in increased swelling; even though the high molecular weight starch may not infiltrates through the CW, the soluble molecules exert pressure on the CW envelope and inflate the cell.

Significant changes in cell dimensions were obtained by increasing the concentration of ethanol in water immersion solutions (Fig. 6). The cell population dispersion according to cell volume was within the $18\text{-}120 \times 10^{-4} \text{ mm}^3$ range; the volume of the intracellular starch mass was somewhat smaller, within the $5\text{-}110 \times 10^{-4} \text{ mm}^3$ range. The volume of the cells decreased gradually with an increase in ethanol concentration and at 100% ethanol, their volume was within the $0\text{-}<25 \times 10^{-4} \text{ mm}^3$ range. The swelling properties of the starch differ from that of the CW. In liquid medium of high dielectric constant, swollen gelatinized starch occupies the entire cell lumen and even inflates the surrounding CW. The CW tends to be swollen to higher levels than the starch as the ethanol content in the aqueous solution approaches 50%; above this percentage the volume of both the CW and the starch decreases; at 100% ethanol, the CW and the starch are fully shrunk.

Electrical response of potato cells: Monitoring the motion of single cells in a solution under induction of electric field revealed that potato cells move toward the anode, thus indicating their negative net charge (Fig. 7). Cell movement was affected by several factors, such as cell volume, type and concentration of electrolytes, and voltage. Cell mobility was found to decrease with increased concentration and valence

of the ions, such as Na^+ and Ca^{2+} . A lower concentration of positive charges resulted in less neutralized negative charges and in higher motion in under the induced electric field. The gelatinized intracellular starch weight impaired the ascending mobility of the cell toward the anode, indicating that the starch is not as negatively charged (if at all) as the CW. It is presumed that if one attributes the electrophoretic mobility to a diffuse double electric layer, it may also be attributed to the swelling pressure of the CW (as found by the relationship between dielectric constant and swelling). Hence, the electric potential of the CW affects the swelling of dried cells.

Electrical potential is exist in the cell wall: it reached -70 mV, and is affected by the electrolytic nature of the aqueous medium (Fig. 8). The CW potential decreased with an increase in the concentration and valence of cations in electrolyte solution. In solutions of 0.1 mM CaCl_2 the CW potential was approximately -46 mV, and in 0.1 mM CaSO_4 was -44 mV. Changing the medium solution to 0.1 mM K_2SO_4 or Na_2SO_4 , increased the CW potential to -60 and -62 mV respectively, and in 0.1 mM NaCl to -70 mV. Conversely, changing the aqueous medium to 0.1 mM $\text{Al}_2(\text{SO}_4)_3$ decreased the potential to +17 mV, that increased again by 0.1 mM Na_2SO_4 . These experiments showed the same trend of the CW behavior as that of the EDL. The concentration of positive counter charges found in a solution of 0.1 mM CaSO_4 cations is equal to that found in 0.1 mM CaCl_2 , and they have small difference in potential. When the electrolyte solution was changed to Na_2SO_4 or K_2SO_4 , the CW potential increased due to reduced affinity between the monovalent cations and the negative CW charges. In this context, 0.1 mM NaCl solution increased the CW potential due to a reduced number of the positive counter charges. In contrast, 0.1 mM $\text{Al}_2(\text{SO}_4)_3$ solution decreased the negative CW potential due to the higher affinity of the Al^{3+} cations to the negative CW charges, and to the increased number of positive charges in the solution.

Structure of heat-gelatinized cells: Under the conditions prevailing during the preparation procedure, the integrity of the CW as an envelope around the swollen gelatinized starch was retained (Fig. 9). The intracellular starch, found as an

amorphous mass, filled the entire cell lumen to capacity. Exertion of physical pressure on a swollen cell resulted in extrusion of gelatinized starch due to an explosion of the CW. Rehydration at low temperature resulted in rapid swelling of the CW, whereas the gelatinized starch swelled moderately. This situation led to the formation of a space between the starch mass and the CW. Upon heating to 70°C, the starch swelled, occupying the lumen and expanding the cell volume. This led to an increased volume of the cells precipitate.

Ultrastructural observations (Figs. 10a-d) showed that the CW is a porous body. At many sites the inner surface of the CW is adjacent to an osmiophilic layer, that was thought to consist of remnants of a proteinaceous cytoplasmatic substance, similar to the ultrastructure of coagulated proteins of potato tuber extracts (Shomer et al., 1982). Sometimes a space is found between the gelatinized starch and the CW, and sometimes the starch is attached in a compact mass to the CW. In many cases some spots were found inside the CW, and thready connections were seen between the gelatinized starch and the CW. The connections were stained with silver proteinate demonstrating their starchy nature.

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**Table 1: Leakage of starch and removed pectin ($\mu\text{g/ml}$) in water of boiled potato cells
(after each boiling cycle, the water with the soluble polysaccharides was separated from the cells and new water was added for the next boiling cycle).**

Boiling cycle <u>(min)</u>	<u>Starch</u>		<u>Pectin</u>	
	<u>Fraction</u>	<u>Accumulated</u>	<u>Fraction</u>	<u>Accumulated</u>
0 - 30	60	60	99	99
30 - 60	160	220	70	169
60 - 120	260	480	40	209
120 - 150	270	750	30	239

EXPLANATIONS OF FIGURES

Figure 1: Representative column chromatography of soluble biopolymers on a matrix of macerated heat-gelatinized potato tuber cells (BSA - bovine serum albumin; Myg. - myoglobin; Tyr. - tyrosine).

Figure 2: Kav Values vs. molecular weight (kDa) of various eluted soluble proteins.

Figure 3: Degree of swelling of precipitate of macerated potato tuber cells in various water-ethanol solutions.

Figure 4: Degree of swelling of precipitate of macerated potato tuber cells in various solutions of formamide-water and ethanol-dimethylsulfoxide.

Figure 5: Dispersion of cell populations according to volume of cells and gelatinized starch.

Figure 6: Relative swelling of potato in precipitate of macerated potato tuber cells for liquids with varying dielectric constants.

Figure 7: Electrophoretic mobility (cm/min) of a single potato cell in an electrolytic solution (V - volt).

Figure 8: Electrical potential of cell wall in various electrolyte solutions at concentrations of 0.1 M.

Figure 9 (a-d): Light micrographs of heat-gelatinized potato tuber cells observed under polarized light. **a** - Representative rehydrated cell, swollen in water at 5°C or at room temperature in an aqueous solution of 50% ethanol (x740); **b** - Same as (a), but stained with iodine (x650); **c** - Representative rehydrated cell, swollen in water at 70° C. The swollen gelatinized starch occupied the entire cell lumen (x980); **d** - Same as (c), but stained with iodine. In some cells, the swollen starch did not occupy the lumen to capacity (x600).

Figure 10 (a-d): Transmission electron micrographs of ultrathin sections of heat-gelatinized potato tuber cells. **a** - Part of rehydrated cell, swollen in water at 50° C. Space is seen between the starch and the cell wall (x54,000); **b** - Part of rehydrated cell, swollen in water at 70° C. The starch attached tightly to the CW, The outer region of the starch suppose to be a mixture of soluble starch

and proteinaceous matter(x28,000); c - Part of rehydrated cell, swollen in water at 50° C, stained with silver proteinate. Fragments of soluble starch mass seen between the starch and the cell wall (x54,000); d - Part of rehydrated cell, swollen in water at 70° C. Thready patterns in soluble starch mass seen between the starch and the cell wall (x40,000).

NUMBER OF CELLS

VOLUME RANGE ($\text{mm}^3 \times 10^{-4}$)

ELECTRIC POTENTIAL OF PLANT CELL WALL AND ITS REACTION TO ION CHANGES

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Direct electrical measurements provide evidence that the plant cell wall (CW) has electric potential in the absence of viable protoplasmic compartments. CW electric potential exhibits the typical cation exchange response of the Donnan potential. It is sensitive to electrolyte concentration and valence of the cations and pH. CW potential varies in rates of ~3, 25 and 840 mV/mM for Na⁺ or K⁺, Ca⁺⁺ and Al⁺⁺⁺, respectively. NaCl causes to slightly higher potentials than KCl, and MgCl₂ causes to higher potentials than CaCl₂. Lower potentials indicate slightly stronger attraction of the cation to fixed CW anionic groups according to the Donnan concept. Potentials measured in solutions with the same cation but with sulfate anion rather than chloride are significantly more positive, indicating a balance between negative and positive charges. Donnan potential increases with the pH, indicating the presence of carboxylic groups in the CW.

INTRODUCTION

The swollen cell wall (CW) is a protective envelope that prevents uncontrolled expansion of the protoplast, defines the cell's physical strength, and limits cell volume. Thus, CW determines the volumetric dimensions of the tissue and consequently, the yield of agricultural crops. In this context, the degree of CW swelling is an important factor in biological activities such as ion reservoir, hydraulic conductivity, nutrient uptake, transport of assimilates, secretion, growth regulation etc. Although cell expansion as a whole has been studied comprehensively (Taiz, 1984; Taiz and Zeiger, 1991), expansion characteristics of the CW itself still deserve further elucidation.

Cell wall swelling may be attributed to two main factors: 1. protoplast turgidity that inflates the cell lumen, thereby pushing the CW outwards, and 2. the dimensions of spaces within the CW that are determined both by the distances between the cellulose polymers (inside micelles and microfibrils and between the micro- and macrofibrils) and by the length of the cellulose molecules and their fibrils. Cellulose fibrillar lattice, the skeleton of the CW, is interwoven and interacts mutually with other CW components such as hemicellulose, pectin, proteins etc. (Bacic et al., 1988; Dey and Brinson, 1984; Fry, 1986; McNeil et al., 1984). The swelling phenomenon however, is based on attractive, repulsive and interweaving forces among the cellulose units. Cell bulk was shown to be related to the swelling properties of the CW (Shomer and Levy, 1988; Shomer et al., 1984). It was reported that CW swelling is influenced by electrolytes and dielectric constant, and was suggested that the extent of swelling depends directly on the existence and properties of electrical diffused double layer (EDL) of the CW fibrils (Shomer et al., 1991). CW swelling was attributed to the properties of counter ions which neutralize the CW fixed charges (Shomer et al., 1991) triggering attractive and repulsive

behavior similar to that of charged suspended colloids (Jirgensons and Straumanis, 1962; van Olphen, 1963). However, as the CW fibrils are cross-linked, attractive and repulsive forces among the CW fibrillar elements are affected not only by the EDL, but also by the physical forces cross-linking them. Furthermore, suspended colloidal particles collide randomly with each other as a result of Brownian movement, whereas hydrated CW fibrils are found within a defined lattice.

Numerous complementary studies have been carried out on CW composition, structure and biochemistry (Delmer and Stone, 1988; Fischer and Bennet, 1991; Fry, 1986; Fry, 1988). The CW is composed of a tri-dimensional network of various skeletal polysaccharides: cellulose, hemicellulose, pectin and neutral sugars (Albersheim, 1975; Taiz and Zeiger, 1991). Other constituents such as proteins, glycoproteins, lignin etc., are also found in the CW and are involved in CW function (Fischer and Bennet, 1991; Fry, 1988). The individual charge of each CW component and its chemical and biochemical changes contribute to the overall charge equilibrium that determines the net CW electric potential. As swelling was found to be affected by fibril charge, it is important to elucidate the electrical properties and behavior of the CW in relation to ions.

The CW is known to behave as an ion exchanger where fixed charges in the CW interact with exchange ions (Briggs and Robertson, 1957; Gillert and Lefèbvre, 1981). There are indications that the net charge of the CW is a result of dissociated weak acidic groups with pK values similar to that of polygalacturonic acid (Reichter and Dainty, 1989a; Reichtie and Larkum, 1982; Saftner and Raschke, 1981). CW potential has been studied indirectly by several methods such as potentiometric titration and analysis of the released

ions (Richter and Dainty, 1989b), chemical assays of ions, mass-action (Bush and McCole, 1987) and x-ray microanalysis (Grignon and Sentenac, 1991).

Regarding the Donnan theory, the CW bathing solution is divided into Donnan free space (DFS) and wall free space (WFS) (Briggs and Robertson, 1957; Dainty and Hope, 1961; Grignon and Sentenac, 1991; Richter and Dainty, 1989a, b). In proximity to the charged surfaces, cation concentrations of the DFS are higher and anion concentrations lower than those of the external solution; ion concentrations of the WFS are the same as those of the external solution (Richter and Dainty, 1989b). If the mean distance between the CW and the plasmalemma is not greater than the EDL, the ion concentration near the plasmalemma is affected by the electrostatic field of the CW.

There are indications that the CW has net negative potential (Richter and Dainty, 1989a, b; Richter and Dainty, 1990a, b; Saftner and Raschke, 1981; Shomer et al., 1991), but no clear evidence has been presented to distinguish it from plasma membrane potential. Previous studies investigating ion behavior examined separated CW matrix by ion extraction, potentiometric titration or in terms of the mass-action equation of soil science (Gillert and Lefèbre, 1981; Richter and Dainty, 1989a, b; Richter and Dainty, 1990a, b). These procedures may not eliminate absolutely effects of cytoplasmatic components. Electrophysiological studies of plant cells were related mostly to membrane potential in intact cells (Novacky et al., 1989; Taiz and Zeiger, 1991; Ullrich and Novacky, 1990; Ullrich-Eberius et al., 1990).

The present study was undertaken to provide direct evidence for the existence of EDL on fibrillar surfaces by direct measurement of CW electric potential in aqueous media employed for electrophysiological studies. This technique enabled measurements of CW potential of heat-killed cells, and assessed the ionic condition of the CW in real time.

MATERIALS AND METHODS

Potato (*Solanum tuberosum* L.) tuber, from which epidermal and subepidermal cell layers were removed, was used to separate cells for measurements of CW potential. Small tissue dices of $\sim 3 \text{ cm}^3$ were boiled for 20 minutes, mashed, and rinsed several times with distilled water in order to obtain single biologically inactivated cells. The inactivated macerate was passed through a strainer with 2 mm holes followed by straining through a 0.1 mm mesh sieve while rinsing with distilled water. The obtained inactivated macerate was rinsed and suspended in a column of distilled water. Large cells and cell aggregates that settled quickly, and small cells that settled slowly, were removed from the suspension while cells of medium size were retained as a cell suspension for potential measurements. Boiled potato cells are especially suitable for electrical measurements because gelatinized starch inflates the cell lumen, pushes the CW outwards, and enables stable contact with the microelectrode tip for several hours. CW potential in the ghost cells was not affected by intracellular constituents, since no marked response was obtained upon tip penetration into the cell lumen.

Cell wall potentials were measured with microelectrodes prepared from 1 mm borosilicate microcapillaries containing fiberglass fillamental (WPI, Inc.) pulled into a micropipette with a vertical pipette puller (D. Kopf). Tip diameter was $< 0.5 \mu\text{m}$ and tip impedance; tip potential -5 to -15 mV and resistance 5 to 15 M Ω . The micropipette was filled with 3 M KCl. A 3 M KCl / 2% aqua salt bridge was used as reference. Both salt bridges were connected via Ag/AgCl electrodes with electrometer amplifier (WPI Intra 767) and a line recorder (Pavlovkin et al., 1986; Ullrich and Novacky, 1990).

One drop of cell suspension was added to the aqueous medium in a 35x5x16 mm Plexiglas measurement chamber containing a fine sponge (Fig. 1).

The cells settled into the sponge cavities. Using a micromanipulator a micropipette was inserted into the CW of a single potato cell that was viewed through a horizontal microscope. The chamber was perfused with a constant electrolyte flow of 1 ml/sec. The electrolyte flow is required to maintain constant and definite hydrodynamic conditions of the CW. This creates a thin unstirred layer and minimizes interfacial concentration gradients, leading to an accelerated response upon changing the electrolyte. CW potentials were measured in different electrolytes. All of the experiments started with CW preparations bathed in 0.1mM NaCl solution. The electric potential under these conditions was somewhat different from cell to cell, but was within the -60 to -80 mV range.

RESULTS AND DISCUSSION

The electric potential measurements performed in this study showed that the CW of biologically inactive cells are still electrically active. Electrical charges within both living and heat-killed CWs originate from charged sites such as carboxylic groups, bound proteins and glycoproteins, and strongly bound multivalent ions.

The electric potential was sensitive to both electrolyte type and concentration. Since measurements were made at equilibrium, it can be safely assumed that no concentration gradients are present either in the outer bulk solution or within the CW. Thus, the potentials measured in this work are, in fact, of the Donnan type and can be expressed by the following formula (Helfferrich, 1962):

$$E_{\text{Donn.}} = \frac{RT}{Z_i F} \ln \frac{C_i F_i}{C_i \bar{f}_i}$$

where C_i , $\bar{C}_i$, f_i , $\bar{f}_i$ are the concentrations and activity coefficients of ion i in the solution and within the CW, respectively, R , the gas constant, T , the temperature, Z_i , the valence of ion i , and F the Faraday constant.

Since the outer solutions used in this study are very low in ion concentration, f_i can be considered as unity. In the absence of thermodynamic information regarding the CW interior, $\bar{f}_i$ will also be considered as unity, keeping in mind that all deviations from ideal Donnan should be accounted for this arbitrary selection behavior.

Potential responses with electrolyte concentration change: Potential variations caused by changes in NaCl concentration in the bathing solution are shown in Fig. 2. It is evident that CW potential moves towards more positive values (with respect to the bathing solution) as the concentration increases, becoming more negative with decreasing concentrations. No hysteresis is

apparent in the potential behavior of increasing and decreasing concentrations. Similar behavior is observed with KCl solutions (Fig. 3). Potential changes are close to those obtained for NaCl solutions, approximately 3 mV/mM within the range of 0.1 to 10 mM.

By comparison, cell wall potential is much more sensitive to concentration changes of salts of multivalent cations such as Ca^{2+} and Al^{3+} than of monovalent cations (Figs. 4 and 5). The rate of potential change in the presence of Ca^{2+} is 25 mV/mM within the range of 0.05 to 3 mM, and in the presence of Al^{3+} is 840 mV/mM within the range of 0.01 to 0.06 mM. It should be noted that due to the low AlCl_3 concentrations used throughout the experiments, no significant pH changes were observed and the potential variations were caused exclusively by changes in the salt concentration.

Calculated comparisons based on Helfferich formula: Assuming an ideal Donnan behavior, relationship according to equation 1, $Z_i F E_{\text{Donn}} / RT$ as a function of $\log (C)$ should be a linear and the lines for the different electrolytes should coincide. The lines of NaCl and KCl for both increasing and decreasing concentrations are similar within the experimental error (Fig. 6); however, the lines for AlCl_3 and CaCl_2 deviate significantly from these lines and from one another (Fig. 6). This behavior can be attributed to the specific interactions between the multivalent ions and ionic groups within the CW skeleton. As with synthetic ion exchangers (Helfferich, 1962), these interactions are expected to become stronger with multivalent ions or as the ionic radius increases. Under these circumstances, the concentration and consequently, the activity of free ions within the CW decreases. A close analysis of Eq. 1 and comparison with Fig. 6 reveals that the intersection of the individual lines with the abscissa provides values for the activity of the free ions within the CW. According to Fig. 6, these values indeed decrease significantly as the valence

of the cation increases. It is important to note that only the relative activity values for the different ions can be considered, since no information is absent as to either concentration of the CW fixed charges or the activity coefficients of the counter ions within the CW matrix.

The fact that the Donnan potential decreases more sharply with increasing concentration as the charge of the counter ions becomes higher can be interpreted via the Donnan exclusion (Helfferich, 1962) or the interfacial electrical double layer models. The Donnan exclusion is a unique feature of electrolyte sorption by ionic sorbents in which co-ions (or salt) are repelled by the charge of the sorbent. In this way, it prevents the co-ions concentration from exceeding a certain equilibrium value, that is usually much lower than its concentration in the external solution. The Donnan potential and the efficiency of electrolyte exclusion are reduced by more highly charged surface because the counter ions are more strongly associated with the fixed ionic groups within the CW. Thus, increased the external concentration results in a higher salt concentration in the CW that decreases further the measured potential.

In the interfacial electrical double layer model, strong association of the counter ions results in a more localized electrical double layer in the vicinity of CW charged components. This alone will induce a smaller repulsion of co-ions and a sharper potential reduction.

The role of the electrical double layer in CW swelling has been discussed previously (Shomer et al., 1991). It is well known that multivalent ions (such as Ca^{+2}) increase tissue firmness and tensile strength (Grignon and Sentenac, 1991) that limit the degree of swelling. It was suggested that this phenomenon is due to "bridges" formed by the ions between the cell charged components. In addition, swelling is also controlled through variations of the osmotic pressure,

by variations in the salt (co-ion) concentration within the CW. As discussed above, this is governed by the Donnan exclusion, that becomes weaker in the presence of multivalent ions. The measurements performed in this study provide additional insight into the dependence of swelling on the ionic composition of the surrounding solution.

Potential response to ionic changes: Electrical measurements performed on a single cell have the advantage of being devoid of small variations among different cells. Thus, these measurements provide important information on the *relative* Donnan potential variations when the composition of the outer solution is changed.

Some representative examples of these measurements are presented in Figs. 7-9 and in Table 1. Each measured potential in Table 1 is an average of at least three measurements made with the same electrolyte. The potentials measured in the presence of NaCl and KCl are similar within the standard deviation (Table 1); however, as a rule, changing the solution from NaCl to KCl was always accompanied by a slight decline in the potential and a slight increase when changing from KCl to NaCl (Figs. 7a, 7b). This change was more pronounced for salts of two monovalent cations in the molecule as obtained upon changing from Na_2SO_4 to K_2SO_4 (Figs. 6, 7). For a comparison between ionic vs. concentration changes, following an ionic change (from Na_2SO_4 to K_2SO_4 and from NaCl to KCl), 0.1 M of KCl was changed to 0.5 M KCl and vice versa, in the same cell (Figure 7b) The same pattern was observed when replacing MgCl_2 solution by a CaCl_2 solution (Fig. 8). According to the Donnan concept, this indicates that the interactions of K^+ and Ca^{++} and the fixed ionic groups in the CW matrix are slightly stronger than Na^+ and Mg^{++} . The stronger attraction can be attributed to the fact that K^+ and Ca^{++} are less hydrated than Na^+ and Mg^{++} (Robinson and Stokes, 1959), resulting in

decreased masking of their charges. Comparisons between CaCl_2 and CaSO_4 showed that CW potential responds to anion change with the same cation (Fig. 9).

The potential differences observed when the same CW was loaded intermittently with Na^+ and K^+ or with Ca^{++} and Mg^{++} are noteworthy, since they indicate a high sensitivity of the CW to slight differences among the interacting ions. The origin of this sensitivity is the interaction affinity between these ions and the charged matrix components.

It should be noted that the CW potential differs somewhat from one cell to another, whereas the relative potential changes in response to changes in electrolyte concentrations are the same for different cells. This may be attributed to variations in CW composition, structure density and perhaps also to small differences in the preparation procedure and micropipette properties. This results in a rather large distribution of potentials between different CWs under exactly the same condition, therefore such measurements can sometimes be misleading; for instance, in Fig. 6, the potentials for K^+ appear slightly higher than for Na^+ as a result of averaging.

As shown in Fig. 9 and Table 1, the potentials measured with sulfate solutions are significantly more positive than those measured with chloride solutions, provided that the comparison is made on the basis of the same cation and its molar concentration in the outer solution. Elucidation of this phenomenon is not straightforward, as a stronger Donnan exclusion expected for an anion carrying a higher charge should result in an opposite effect. However, it should be recalled that the negative charge of the CW results from a balance between both negatively and positively charged groups. The anion concentration within the CW should decline as a result of the higher exclusion rate and therefore, a smaller balancing degree of the positive charges is

expected. This will result in a more positive Donnan potential as found in the measurements. Although the explanation outlined above appears reasonable, there is no doubt that this phenomenon should be studied further.

Effect of pH on Donnan potential: Donnan potential is sensitive to changes in pH levels (Figs. 10a, 10b). Each pH change was followed by CW equilibration with 0.1 mM NaCl solution. The potentials measured for pH 5.24 and 6.87 are -67.5 and -80 mV, respectively. This response can be explained by the fact that, as already mentioned, the main origin of negative charges within the CW are the carboxylic groups of poly acids. Grignon and Sentenac (1991) concluded that polygalacturonic acids with an average pK of about 3 contribute most of the carboxylic acid groups. The dissociation degree of these groups increases with pH, thereby resulting in a higher density of negative charges and a more negative wall potential.

Richter and Dainty (1989a; 1989b) determined the magnitude of the Donnan free space (DFS) by direct measurement of Na^+ , Ca^{2+} , and Cl^- concentrations within the CW at different pH levels. Following the Manning (1969) theory, they showed that Ca^{2+} ions are condensable within the Donnan free space and that their internal concentration depends on the degree of dissociation of the fixed groups (Richter and Dainty, 1989b). Accordingly, the internal concentration of Na^+ and Cl^- was almost independent of the external pH. In this context, it can be assumed that direct Donnan potential measurements made under the same conditions should give some more valuable information on e.g., ion binding strength at different dissociation degrees.

According to Richter and Dainty (1989b), the CW bathing solution may be divided, in accordance with Donnan theory, into DFS and WFS. Most of the CW spaces' volume throughout the CW consist of electrolytic solution. The insoluble matrix occupies a marginal quantitative portion of the CW even

though the fibrillar, amorphous and soluble CW polymers are dispersed densely within the CW sphere. Since most of the CW solution is found in the spaces within the CW, the WFS should be the dominant solution with the DFS occupying relatively marginal volume. In such a situation, one would expect the CW to contain a relatively low amount of charges, that would be reflected by low electric potential. The microelectrode tip (about 0.5 μm in dia.) measures a representative area which includes both the surfaces of insoluble structures and intra spaces within the CW, i.e., WFS and DFS. Hence, the microelectrode measures the electric potential of a weighted average of both WFS and DFS. In our measurements, the electric potential responded very sensitively and sharply to any small ionic change, indicating that the DFS is the dominant CW solution in our system. The negative net charges are a result of several factors such as galacturonic acid, CW proteins, glycoproteins, and an unevenly distributed electronic atmosphere within the CW polymers (Cassab and Varner, 1988; Fry, 1986; Grignon and Sentenac, 1991; Hayashi, 1989; Shomer et al., 1991; Shomer and Levy, 1988). Furthermore, although the potato cells we examined had lost some of their CW components (such as pectic substances during boiling) their electric potential was very obvious. Based on the above it appears that the EDLs of the fibrillar surfaces overlap one another mutually, thereby creating a homogeneous DFS over the entire region within the CW.

CONCLUDING REMARKS

This study presents for the first time direct measurements of Donnan potentials across the CW of single potato cells. CW potentials measured under a wide variety of ionic compositions and concentrations in the outer solution show clearly a typical cation exchange response: the Donnan potential decreases with electrolyte concentration and at the same concentration, it becomes more positive as the valence of the cation increases. However, the response to solutions with different anionic compositions (with the same cation) indicates that there is also a considerable anion exchange capacity within the CW. This capacity has been ignored in most previous studies.

The sensitivity of the Donnan potential to ions such as Na^+ and K^+ or Ca^{2+} and Mg^{2+} is an interesting feature. This indicates that the CW components discriminate between ions that are chemically similar but have small differences in physical properties, such as ionic radius or hydration energy. This phenomenon should be given more attention.

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Table 1.: Donnan potentials of a single cell wall for different electrolytes.

Electrolyte	Concentration (mM)	Donnan Potential (mV) $\pm$ SD
KCl	0.1	66.3 $\pm$ 0.82
NaCl	0.1	66.8 $\pm$ 1.48
KCl	0.2	61.8 *
NaCl	0.2	62.3 *
Na ₂ SO ₄	0.1	60.6 $\pm$ 0.89
K ₂ SO ₄	0.1	55.3 $\pm$ 1.53
CaSO ₄	1.0	42.2 $\pm$ 0.29
CaCl ₂	1.0	45.0 $\pm$ 1.00
MgSO ₄	1.0	44.0 $\pm$ 0.01
MgCl ₂	1.0	45.0 $\pm$ 0.01

* Calculated from the values measured for 0.1mM and the slopes of the K⁺ and Na⁺ lines in Figure 6.

FIGURE LEGENDS

Figure 1. A schematic presentation of the experimental setup

Figure 2. Donnan potential of potato tuber cell wall in NaCl solutions of A. Increasing and, B. Decreasing concentrations (arrows indicate point of solution change)

Figure 3. Donnan potential of potato tuber cell wall in KCl solutions of A. Increasing and, B. Decreasing concentrations (arrows indicate point of solution change)

Figure 4. Donnan potential of potato tuber cell wall in CaCl_2 solutions of A. Increasing and, B. Decreasing concentrations (arrows indicate point of solution change)

Figure 5. Donnan potential of potato tuber cell wall in AlCl_3 solutions of increasing concentrations (arrows indicate point of solution change)

Figure 6. Normalized Donnan potential of potato tuber cell wall as a function of $\log (C)$ for different electrolytes

Figure 7. Donnan potential of a single potato tuber cell wall in 0.1 mM solutions of NaCl, KCl, Na_2SO_4 and K_2SO_4

Figure 8. Donnan potential of a single potato tuber cell wall in 1 mM solutions of CaCl_2 , MgCl_2 , CaSO_4 and MgSO_4

Figure 9. Donnan potential of a single potato tuber cell wall in NaCl and phosphate buffer solutions (concentrations and pH values as indicated)

Fig. 1 - Shomer et al.

Fig. 2 - Shomer et al.

Fig. 3 - Shomer et al.

Fig. 4 - Shomer et al.

Fig. 5 - Shomer et al.

Fig. 6 - Shomer et al.

Fig. 7a - Shomer et al.

Fig. 7b - Shomer et al.

Fig. 8 - Shomer et al.

Fig. 9 - Shomer et al.

Figure 10a - Shomer et al.

Figure 10b - Shomer et al.

f. Description of Cooperation

The Israeli group has concentrated on aspects related to relationships between structure, permeability of cell wall to biopolymers, swelling of cell wall/starch, and electrical properties of cell wall. The US group dealt with texture of raw tubers, texture of cooked tubers, and softening kinetics. Regular exchange of information was maintained between the Israeli and the US groups throughout along the research period. Collaboration was realized between both groups on rheology of cell suspensions; in Israel, during the visit of Malcolm C. Bourne - observations on cell suspensions and microscopy of macerated cells, and in US during the visit of Ilan Shomer - rheology measurements. This collaboration included the study of the effect of dielectric constant on cell swelling and the effect of temperature changes and enzymatic liquefaction rheology of cell macerates. Fruitful discussions and correspondence on the progress of research were conducted throughout duration of research particularly during the visit of Malcolm C. Bourne in Israel and the visit of Ilan Shomer in Geneva, New York.

g. Evaluation of the Research Achievements

The accepted approach(es) to the evaluation of the quality of potatoes for both fresh consumption and industrial processing, are based on several simple traditional methods. There is a lack of information on the mode of evaluation and determination for sorting and breeding of potato cultivars for various purposes; there is also a limited development of high quality industrial products. Hence, the present research dealt with the study of mechanisms involved in the creation and stability of potato texture, including the study of texture of both raw and heat-treated tuber tissue.

Study of swelling mechanisms in relation to texture: This research showed for the first time direct relationships between cell swelling and texture. This led us to study the swelling mechanisms of potato cells with regard to two main textural components, CW and starch, and their mutual effects as related to texture. The research included a detailed study of the textural properties of both raw potato tuber tissue and tissue and cells whose starch has been heat gelatinized heat-starch-gelatinized (HG-cells). Direct and indirect measurements showed for the first time that the swelling is determined by electrical properties of the cell wall and the starch, and is dependent on several factors such as electrolytes, dielectric constant, pH, biopolymers density, and variety.

Findings of the present research show the CW to be permeable to biopolymers, hence, heat-gelatinized starch is leaked from the cells upon thermal treatments above the gelatinization temperature. The tissue texture as well as rheology of purees and rehydrated cells affected by the swelling properties of the starch which inflates the cell. If these factors are specific to each cultivar, it is important to find the suitable quality factors for each consumption purpose and to determine the adapted cultivar accordingly. The swelling is dependent directly on the dielectric constant of the liquid in which the tissue and cells are immersed. In this context, liquids of low dielectric constant such as frying oils, caused dramatic textural changes as a result of reduced cell volume. This finding indicate the presence of an electric diffuse double layer upon the polysaccharide surfaces of both cell wall and starch. These findings represented an achievement contributing to an understanding of the mechanisms involved in the creation and retention of texture in potatoes.

Under induced electric field, single potato tuber cells migrated toward the anode at a velocity that was affected by cell size, electrolytes, and voltage, indicating their negative net charge. The CW demonstrating electrical potential,

reached -70 mV, and decreased with an increase in concentration and valence of cations. These findings are in accordance with electrophoretic cell mobility and effect of dielectric constant on swelling. Heat-gelatinized potato cells included porous CW and a mass of gelatinized starch, between which coagulated protein or cytoplasmic remnants were seen. Sometimes a space was seen between the starch and the CW, while in other cases, starch was attached densely to the CW.

This study provides for the first time direct measurements by microelectrodes of Donnan potentials across the CW of biologically inactivated single cells. The CW potentials measured at a wide variety of ionic compositions and concentrations in the outer solution show clearly a typical cation exchange response: the Donnan potential decrease with electrolyte concentration and at the same concentration, became more positive as the valence of the cation increased. The response to solutions differing in anionic compositions (having the same cation) indicates that there is also a considerable anion effect within the CW. This indicates that the CW components discriminate between ions that are chemically similar but differ slightly in terms of their physical properties, such as ionic radius or hydration energy. This phenomenon is of importance for a better understanding of cell wall swelling mechanisms.

Textural studies: 1. Dynamic rheological tests were suitable for detecting potato cell texture in terms of elasticity, plasticity, friction (apparent viscosity), and enzymatic degradation of the cell wall. The latter could be described by an apparent second order rate equation. This study is of importance for the evaluation of potatoes subjected to production of purees and powders for reconstituted products; in addition, these results may be applied for quality control of new products treated with enzymes. The enzymatic liquefaction may be useful for the development of new potato based products, such as potato milk, and improved the textural quality due to control of the inflation state of the cells.

In heat-gelatinized cells, the amylose is affected by retrogradation, and tends to be leaked from the cells. Amylopectin is the main component retained inside the cell, and it interweaves with amylose, thereby inhibiting starch leakage and affecting the cell texture. The porosity and strength of the CW determine the rate of starch permeability and the ability of the cell to withstand explosion as a result of exerted pressure of swollen starch. A better understanding of the texture upon starch-heat gelatinization has led to the awareness that the physical behavior of the intracellular starch plays an important role in potato texture in accordance with the CW behavior.

2. Differences between cortex and pith tissues within the tuber. Findings of the present research show that of the outer cortex ring of the potato is about 50% firmer than the inner pith tissue, and that the cortex is 3% to 6% higher in dry matter than the pith tissue. This knowledge of the great difference between the two tissues of the potato can be used to manipulate quality and functional properties of potato. For example, a plant breeder who wants to increase the dry matter content could select for potatoes with a higher proportion of cortex tissue and track separately the differences in dry matter content of the cortex and pith tissues. The considerable characteristic difference between cortex and pith can affect future scientific studies. Two publications have been resulted from this work.

3. Kinetics of thermal softening. Findings of the present research show for the first time that potatoes undergo three stages of textural changes during cooking. First, a rapid rate of softening that lasts for 10-15 minutes; secondly, a firming at moderate rate; and a third phase of slow softening where the firming decreased slightly. This is the first time a firming affect has been found in potatoes while cooking. Finally, there is a slow rate of softening for as long as the potatoes are cooked. Other vegetables show only two stages of softening - a fast rate of softening followed by a slow rate of softening but no firming stage. This work is being prepared for publication. The cause of the firming effect can be speculated on at this time. The positive proof of a firming stage opens many new questions about changes in the chemistry and structure of potato during cooking. This finding is of commercial importance, as the processed potato industry often encounters problems in the control of texture and mouthfeel of their products. An understanding of the three stages of firmness change during cooking provides a potentially better control of the process, and subsequently more uniform quality products. This work is being prepared for publication.

4. Single cell puncture. The results will give a better understanding of the nature of the cell wall and middle lamella and factors that affect their strength and hence the texture of potato. Once operating, the assembly can be used to study cell wall of other plant materials.

h. Publications Generated from the Project

Anzaldúa-Morales, M.C., Bourne, M.c. and Shomer, I. 1992. Cultivar, specific gravity and location in tuber affect puncture force of raw potatoes. *J. Food Sci.* **57**: 1353-1356.

Shomer, I. and Novacky, A. 1991. Electric response of cell wall and starch swelling as related to swelling of potato tuber cells. 8th World Congress of Food Sci. Technol. Toronto, Canada. Canadian Inst. Food Sci. Technol. (Abstract).

Shomer, I., Novacky, A. and Pike, S. 1993. Real time assesment of cell wall ion behavior and swelling by electrical gauging. *J. Cellular Biochem. Keystone Symp. Molecular & cellular Biol. Suppl.* **17A**: 22. (Abstract).

Anzaldúa-Morales, M.C., Bourne and Shomer, I. 1993. Cultivar, specific gravity, location in tuber and cooking method affect puncture force of cooked potatoes. (submitted to *J. Food Sci.*).

Shomer, I., Rao, M.A., Bourne, M.C. and Levy, D. 1993. Rheological behavior of potato tuber cell suspensions during temperature fluctuations and cellulase treatments. (submitted to *J. Sci. Food Agric.*)

Shomer, I., Novacky, A., Pike, S. and Oren Y. 1993. Electric potential of plant cell wall and its reaction to ion behavior. (will be submitted in the near future).

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Shomer, I., Novacky, A., Vasiliver, R. and Levy, D. 1993. Swelling, permeability and electrical response of heat-starch-gelatinized potato (*Solanum tuberosum* L.) tuber cells. (will be submitted in the near future).

Anzaldúa-Morales, M.C., Bourne. 1992. Differences in texture and solids content of the cortex and pith tissues of potato tubers. *Search÷Agriculture* New York State Agric. Exp. Station, Geneva, NY USA, **37**: 1-29.