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Swelling behaviour of cell wall and starch in potato (*Solanum tuberosum* L.) tuber cells—II. Permeability and swelling in macerates

Ilan Shomer*, Rosa Vasiliver & Pinhas Lindner

Department of Food Science, Agricultural Research Organization, The Volcani Center, PO Box 6, Bet Dagan, 50250 Israel

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The permeability and swelling behaviour in macerates of potato tuber cells were studied, because of their role in determining the texture of potato products. Chromatography of globular proteins on a column of macerate of boiled potato cells indicated cell wall (CW) pore dimensions smaller than the protein molecular size of 600 kDa. Swelling of boiled and dried potato tuber cells, resuspended in various liquids, was dependent on the dielectric constant of the liquid (2·5–109), indicating the presence of an electrical double layer upon fibril surfaces of CW and starch polymers. Solvents of high dielectric constant such as formamide and dimethylsulphoxide showed remarkably high swelling capacity; this was attributed to the behaviour of the starch which behaves as an inflating agent of the cell owing to its solubility and expansion capability in solvents of high dielectric constant.

INTRODUCTION

Texture is the main quality factor of the potato tuber (Burton, 1982; Gray & Hughes, 1982), and it has been related to two dramatic changes occurring during heat treatment: (1) starch gelatinization and swelling (Potter *et al.*, 1959; Linehan *et al.*, 1968; Gray & Hughes, 1982; van Beynum & Roels, 1985); and (2) loosening of the middle lamella (Hadziyev & Steele, 1979; Keijbets & Pilnik, 1974; Keijbets *et al.*, 1976; Roberts & Proctor, 1955; Shomer *et al.*, 1990) and cell wall swelling (Shomer *et al.*, 1991, 1993).

Starch, which is the major intracellular solid component, focused attention and aroused controversies as to its function in potato texture (Potter *et al.*, 1959; Linehan *et al.*, 1968; Gray & Hughes, 1982). Briant *et al.* (1945) and Hoff (1972) discussed aspects of starch swelling and considered it to exert pressure which causes rupture of the cell wall or a rounding-out of the cell surface, resulting in cell separation. In other cases, void space was observed between gelatinized starch and the CW, indicating that the swollen starch did not occupy the entire cell lumen (Fedec *et al.*, 1977; Davis & Gordon, 1984). These controversial findings may be understood in terms of differences in structural behaviour between the CW and starch under various swelling conditions, taking into account diversity within a given cell population (Shomer, 1995). The elasticity, plasticity and friction of potato cells determine the texture, and are affected by the ability of the swollen starch to inflate the cells and by starch leakage from the cells (Shomer, 1995; Shomer *et al.*, 1993). It can be supposed that these properties are determined by the amount and the size of the starch molecules, and by the amylose/amylopectin ratio.

Regarding the role of the CW in the texture, it is known that textural changes of edible plant tissues are ascribed to loosening in the cell adhesion during ripening, owing to enzymatic degradation of the middle lamellar pectin (Huang & Bourne, 1983; Jen, 1989). In heat-treated edible plant tissues, including potato tuber tissue, the textural changes have also been related mainly to stabilization of the pectic substances in the middle lamella due to β-elimination (Doesburg, 1965; Fishman & Jen, 1986; Hadziyev & Steele, 1979; Huang & Bourne, 1983; Jen, 1989; Keijbets & Pilnik, 1974; Keijbets *et al.*, 1976; Shomer *et al.*, 1990).

However, in the heated tissue where the pectic substances have been dissolved or degraded, the texture is determined by the non-degraded CW and its degree of

*Author to whom correspondence should be addressed.

swelling (Shomer *et al.*, 1984, 1993; Shomer & Levy, 1988). In heated potato tuber tissue, the cell swelling has been suggested to be dependent on two main factors: (1) attractive and repulsive forces acting in the diffuse electrical double layer at the CW fibril surfaces; and (2) inflation of the cell by heat-gelatinized starch (Shomer, 1995; Shomer *et al.*, 1991, 1993). These studies also show that the texture and the rheology are affected by temperature and wholeness of the cellulose lattice in the CW.

The above description indicates the mutual role of both starch and CW in conferring textural properties to potato cells. In this context, it seems that permeability of the CW to starch is involved in determination of the texture and rheology of the cells. Diffusion of starch from potato tissue during cooking had been ascribed to cell rupture (Hoff, 1972). However, starch leakage has been found to occur during boiling of potato tissue, even when the CW remained intact (Shomer, 1995); the leakage was higher from the cortex than from the pith and higher from stored than from fresh potatoes, and it increased with the boiling time. The heat-gelatinized starch is found in various sol–gel states, but only the soluble molecules have been found to leak into the boiling water; therefore, it is important to characterize the CW porosity in relation to starch which may leak from the cells. CW porosity was determined by observing infusion of known biopolymer into intact cells (Carpita *et al.*, 1979; Carpita, 1982; Baron-Eppel *et al.*, 1988) or by elution of biopolymers through a column of CW fragments (Tepfer & Taylor, 1981).

The present investigation examined the permeability of heat-treated potato cells by measuring their capacity to fractionate proteins of known molecular weights, and studied the swelling behaviour of the cells in a series of liquids of various dielectric constants.

EXPERIMENTAL

Cell permeability to proteins

The porosity of the potato (*Solanum tuberosum* L., cv Desirée) tuber CW was determined by measuring the permeability of a column of boiled cell macerate, to soluble proteins of known molecular weights. Selected potato tubers were peeled, diced and boiled for 40 min in distilled water; the soft dice were mashed and passed through a 2 mm mesh plastic strainer and then through a 0·5 mm mesh strainer. The macerate was then rinsed and sorted several times by suspending the separated whole cells in 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 suspensions; cells of medium size (within the range $4–6 \times 10^{-3}$ mm^3 (Shomer, 1995)) were selected for the experiments. The retained fraction was examined by means of a light microscope to verify that it consisted of separated, whole, undamaged cells (Shomer, 1995). The macerated cells were packed within a glass column (76 cm in length and 1·6 cm in diameter) and were used as a gel-permeation bed for chromatography, on which standard proteins were fractionated. Throughout the preparation (straining, rinsing, separation and cell sorting) and column packing, the cells were kept within the aqueous medium, with excess water, in order to prevent shrinking and other undesirable effects of drying. The column was loaded with about 5 mg of each of the following proteins and eluted: tyrosine (0·18 kDa), alcohol dehydrogenase; aprotinin (6·5 kDa), cytochrome (12·4 kDa), myoglobin (17 kDa), carbonic anhydrase (29 kDa), bovine serum albumin (66 kDa), apoferitin (443 kDa) and tyroglobulin (669 kDa). The proteins were eluted with distilled water at a rate of 1 ml/min and detected by absorbance at 280 nm in a spectrophotometer (*2138 UNICORD S—'LKB'*) equipped with a continuous flow cell. The total volume (V_t) was taken as the volume of the potato cells bed, and the void volume (V_0) was determined by measuring the elution volume of dextran blue (2000 kDa). The K_{av} of each eluted protein was calculated from the elution volume (V_e) by equation:

$$K_{av} = \frac{V_e - V_0}{V_t - V_0}.$$

Swelling determinations

Swelling measurements of cell macerates were carried out according to Shomer *et al.* (1991), with modifications in the preparation of the potato tuber cells. The maceration of boiled potato tuber tissue and the preparation of the cells were carried out as described above. For the swelling determinations, the boiled macerated cells were gradually dehydrated with ethanol, which was later evaporated and the cells freeze-dried. A sample of 500 mg boiled and dried potato tuber cells (BDC) was immersed in 30 ml of the swelling liquid in a tightly closed 100 ml flask. The solvated BDCs, with the liquid, were quantitatively transferred into a test tube (14·5 × 1·2 cm) and left to precipitate freely for 2 days at room temperature, and the volumes of the precipitates obtained in the various media were compared.

RESULTS AND DISCUSSION

Permeability and porosity of potato tuber cells

Chromatography of proteins through potato cell column

Boiled potato tuber cells are elongated spheroidal bodies of volume within the range, $2·5–12·5 \times 10^{-3}$ mm^3 (Shomer, 1995). The cell lumen is filled with highly

swollen gelatinized starch, which inflates the cells, preventing their collapse and compaction when they are used as a chromatographic matrix. The elution patterns of four solutes of known molecular weights, tyrosine, myoglobin, bovine serum albumin (BSA) and dextran blue are shown in Fig. 1. Distinct separations were obtained among the different solutes.

The K_{av} values for various proteins in the range 6·5–699 kDa, eluted through a column packed with boiled potato cells, are shown in Fig. 2; these results indicate that a bed of boiled potato cells can be used to separate globular proteins in the molecular weight range < 600 kDa by a mechanism based mainly on gel filtration. The size of the largest CW pores is about the size of an apoferitin molecule, i.e., 600 kDa. The mechanism of separation of globular proteins by boiled potato cells is not solely by gel permeation. Thus, for low molecular weight substances, tyrosine and aprotinin $K_{av} > 1$ was obtained (Fig. 2). This was probably due to weak adsorption by the BDCs which was operative in addition to the gel filtration process.

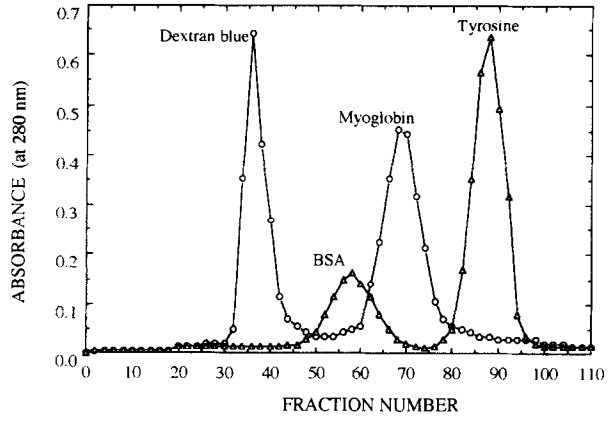

Fig. 1. Column chromatography of soluble proteins on a matrix of heat-treated potato tuber cells (BDA, bovine serum albumin).

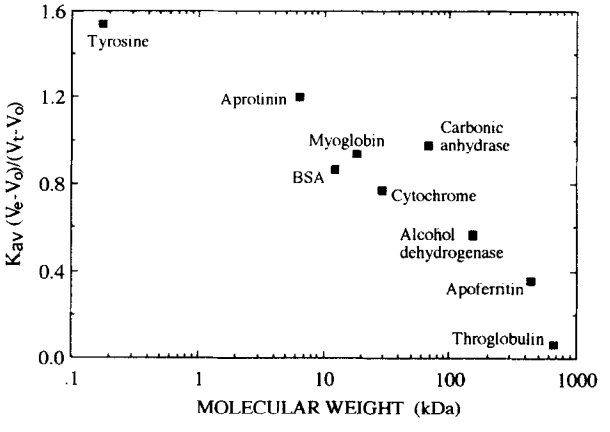

Fig. 2. Values of K_{av} vs molecular weight of various eluted soluble proteins.

A previous study of CW porosity was based on determination of permeability through a column of fragments of disintegrated cells (Tepfer & Taylor, 1981); in such a system, the CW disruption may lead to erroneous results. Evaluation of the permeability of biopolymers through CW of intact cells (Carpita *et al.*, 1979; Carpita, 1982; Baron-Eppel *et al.*, 1988) provides a more realistic evaluation of CW porosity. In the present study, the boiled potato cells contained highly swollen starch while the CWs remained intact (Shomer, 1995), although some of the heat-degradable CW components, such as pectic substances, were removed. Under these conditions, the CW porosity is determined mainly by the microfibrillar lattice of cellulose and the non-degraded hemicellulose and pectin. It is not known whether the heat-gelatinized starch affects the protein elution, but the CW pores are undoubtedly smaller than the intracellular starch molecules. However, removal of the CW resulted in dispersion of the starch (Shomer, 1995). This suggests that the starch is not highly associated inside the cell, and presumably will not affect the distribution of the eluted molecules between the intra- and intercellular spaces.

Swelling in aqueous and non-aqueous media

The swelling capacity of BDCs immersed in aqueous ethanol solutions (dielectric constants in the range 2·5–80·37) was dependent on the ethanol concentration (Fig. 3). In addition, the swelling significantly increased with temperature. However, the effect of temperature decreased with decreasing dielectric constant so that, above 70% ethanol, swelling was not affected by the temperature (Fig. 4).

Previous studies have shown that cell swelling in macerates of tomato pericarp and fresh potato tuber is mainly determined by the physical properties of the CW (Shomer *et al.*, 1984; Shomer & Levy, 1991). The swelling of BDCs was found to depend on both dielectric constant and temperature (Fig. 3); this can be ascribed to the swelling properties of the gelatinized starch (Whistler & Paschall, 1965; Hoff, 1972), and its structural behaviour as an inflating agent under conditions of high swelling pressure (Shomer, 1995).

The swelling of BDCs in a series of aqueous solutions of formamide was found to increase remarkably with formamide concentration (Fig. 4). Dimethylsulphoxide (DMSO) increased the swelling of the BDCs to extremely high levels; thus, in 10% DMSO the whole liquid column was filled with swollen potato BDCs (Fig. 4).

Whereas in non-heated starchy potato tuber cells or in non-starchy tomato parenchyma cells the swelling is exclusively dependent on the physical properties of the CW (Shomer *et al.*, 1984, 1991; Shomer & Levy, 1988), in BDCs the extent of swelling is also dependent on the swelling capacity of the gelatinized starch (Shomer *et al.*, 1993; Shomer, 1995). The swelling pressure of the

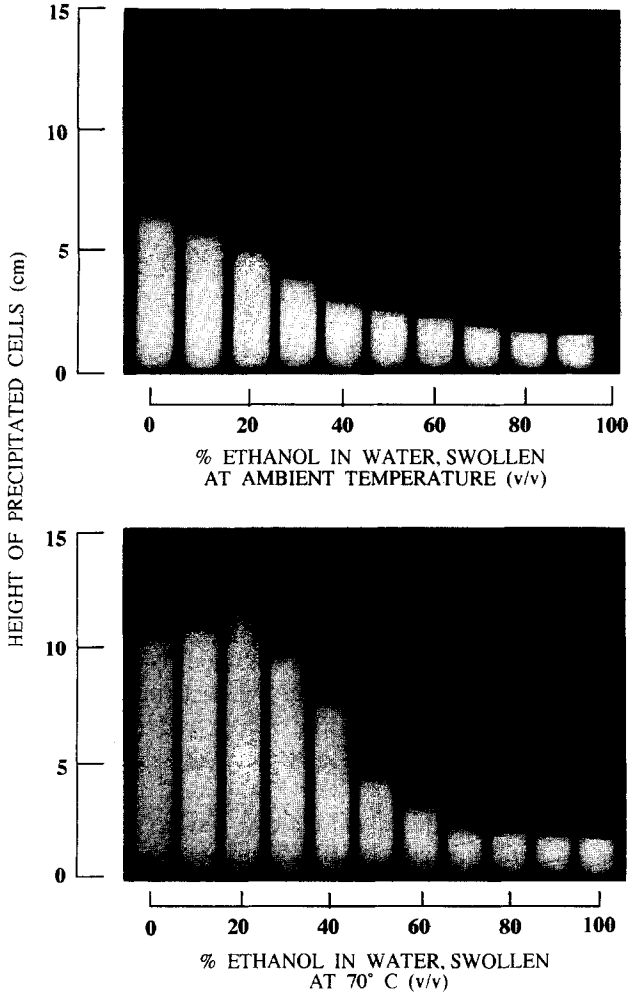

Fig. 3. Photographs of standard tubes showing the degree of swelling of macerates of boiled and dried potato tuber cells in various water–ethanol solutions at room temperature (upper part) and at 70°C (lower part).

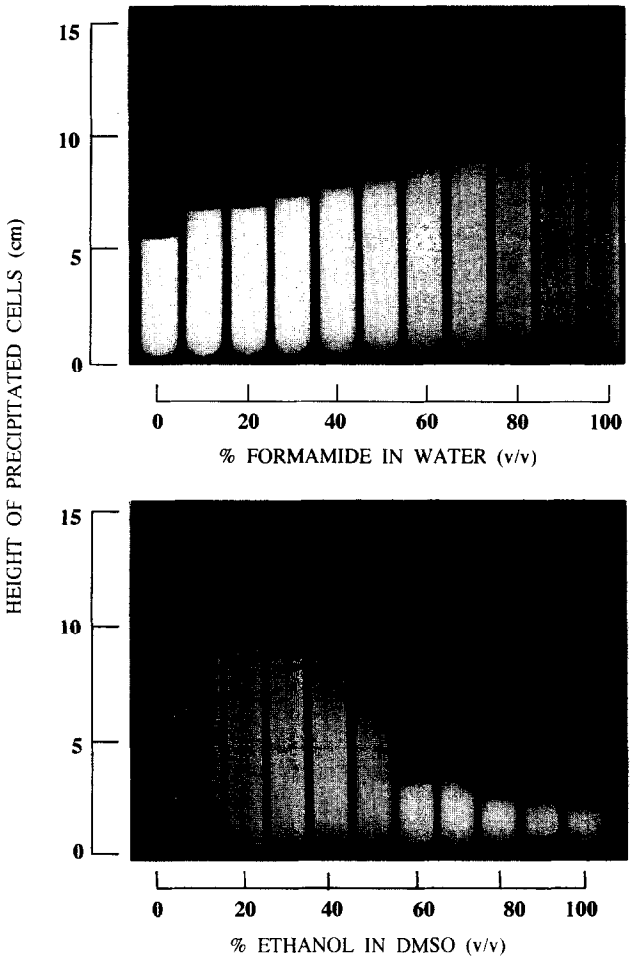

Fig. 4. Photographs of standard tubes showing the degree of swelling of macerates of boiled and dried potato tuber cells in various solutions of formamide–water (upper part) and ethanol–dimethylsulphoxide (lower part).

intracellular gelatinized starch (Hoff, 1972) is increased by the dielectric constant of the liquid (by starch solubilization and heating) (Fig. 3).

The swelling of BDCs was very limited in liquids of low dielectric constants within the range of 25·0–33·6 (Fig. 5). Exceptional swelling behaviour was obtained in DMSO, although its dielectric constant is lower than that of water. The abnormally high swelling in DMSO can be attributed mainly to solubilization of starch (Banks & Greenwood, 1975). The soluble high molecular weight starch, which may not infiltrate through the CW, exerts pressure on the CW and inflates the cell due to the starch expandability in solvents of high dielectric constant.

Liquids with relatively high dielectric constant, such as formamide, affect the CW swelling of non-starchy cells similarly to water (Shomer *et al.*, 1991), but the starchy potato cells swelled in formamide to a significantly greater extent than in water. Formamide in water, at concentrations exceeding 25%, reduced the

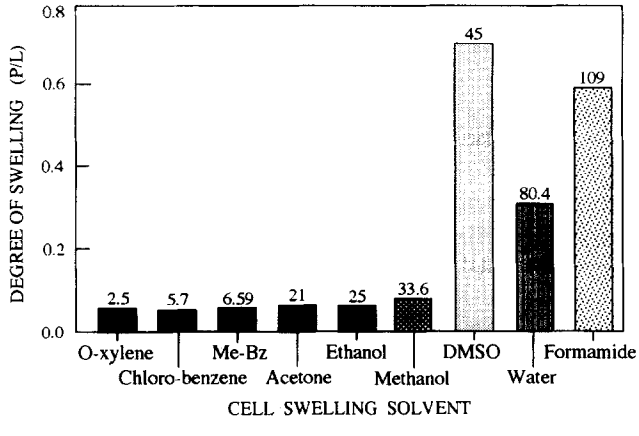

Fig. 5. Degree of swelling of a precipitate of macerated potato tuber cells in various media of pure solvents with different dielectric constants (number mentioned above each column). DMSO, dimethylsulphoxide; L, height of the liquid column; Me-Bz, methyl benzoate; P, height of the precipitate.

swelling level in tomato cells, owing to relatively strong hydrogen bonds between formamide molecules and water (Shomer *et al.*, 1991). In BDCs, the increased swelling level in formamide (Figs 4, 5) may be attributed to the large amount of starch inside the cell; the swelling of this starch is greater in formamide than in water.

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