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0144-8617(94)00064-6

Carbohydrate Polymers **26** (1995) 47–54

0144-8617/95/$09.50 + .00

Swelling behaviour of cell wall and starch in potato (*Solanum tuberosum* L.) tuber cells — I. Starch leakage and structure of single cells

Ilan Shomer

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

(Received 3 March 1994; revised version received 16 August 1994; accepted 16 August 1994)

Continuous starch leakage was observed during 120 min of boiling of potato tuber tissue. The leakage was higher in cortex than in pith tissue and in stored than in fresh potatoes, and it increased during boiling up to ~6 mg/g. Light microscope observations revealed that upon immersion of separated, boiled, dried and rehydrated cells (BDC) in water at room temperature or in an aqueous solution of up to 50% ethanol, the cell wall (CW) swelled to a higher average volume ($2{\cdot}5 \times 10^{-3}$mm^3) than the starch ($1{\cdot}2 \times 10^{-3}$mm^3). Above this ethanol level, the difference between the CW and the starch is diminished gradually until, in 100% ethanol, the BDCs were not swollen ($< 1 \times 10^{-3}$mm^3); below 50% ethanol, the starch swelling increased relative to the CW swelling, and at 100% water (at 70°C) the starch occupied the entire cell lumen (between 37 and 50×10^{-3}mm^3). The ultrastructure of gelatinized starch was seen as a swollen amorphous body, and extrusions of starch were seen between the main starch mass and the CW. At a high swelling pressure, cytoplasmic remnants and coagulated proteins were pressed compactly between the starch mass and the CW.

INTRODUCTION

The structural properties of heat-treated potato cells determine the textural and rheological features of potato products. Upon heat treatment, potato cells undergo two main structural changes: degradation of the pectic substances in the middle lamella and in the cell wall (CW) by β-elimination (Keijbets & Pilnik, 1974), and gelatinization of the starch. Potato cells contain starch which is produced within the amyloplasts and is seen as separated grains inside the protoplast (Banks & Greenwood, 1975). The starch granules include the crystalline part of amylopectin micelles. During heat gelatinization, the crystalline amylopectin regions of the starch are converted into swollen amorphous structures and lose their reaction to polarized light (Biliaderis, 1992; Swinkels, 1985). The structure of the gelatinized starch is determined by various factors such as plant origin, the relative proportions of amylose and amylopectin, the biosynthetic pathway, enzymatic effects, and the interactions undergone by proteins and glycoproteins. The structure of the potato starch, which determines its swelling properties, has been studied comprehensively (Banks & Greenwood, 1975; van Beynum & Roels, 1985). Several structural models have been suggested for the relationship between the behaviour of starch upon heat gelatinization and its texture (Sterling, 1965, 1974; Linehan *et al.*, 1968; Hoff, 1972; Gruber *et al.*, 1973; Swinkels, 1985).

Shomer *et al.* (1993) suggested that rheological and textural properties of potato cells, i.e. their elasticity, plasticity and mutual friction are determined by the swelling relationships between the CW and starch. It has been found that these parameters are sensitively affected by the swelling conditions such as temperature and CW strength. Although light microscope observations have been presented to explain the textural properties of cooked potatoes, there still exist controversies concerning the relationships between the structural and the textural behaviour (Briant *et al.*, 1945; Warren & Woodman, 1974; Fedec *et al.*, 1977; Davis & Gordon, 1984; Von Mica, 1985). However, the effects of the structural relationships between starch and the CW on the texture are not fully understood.

Upon heating, the swollen gelatinized starch is still entrapped within the cell while the CW remains intact (Davis & Gordon, 1984); it is reasonable to assume that the CW and the gelatinized starch confer synergistically the specific structural properties of the cells. The CW of edible parenchyma tissue is built of a cellulose micro-

fibrillar lattice, which is embedded in, bound to, and interwoven with an amorphous matrix that includes pectic substances, oligosaccharides, hemicelluloses, glycoproteins and proteins (Läuchli, 1976; Dey & Brinson, 1984; McNeil *et al.*, 1984; Cassab & Varner, 1988; Fry, 1988; Aldington *et al.*, 1991; Fischer & Bennet, 1991; Taiz & Zeiger, 1991). The ability of a given CW to expand is dependent on the distances between the insoluble crystalline cellulose units in the cross-linked micellar, microfibrillar and macrofibrillar cellulose lattice (Esau, 1965).

Potato tuber cells adhere to one another by means of middle lamellar pectin. Removal of the pectin either by heat (Keijbets *et al.*, 1976) or by enzymatic degradation (Shomer & Levy, 1988), resulted in maceration. In boiled macerate, starch gelatinization and CW pectin degradation are the main structural changes which determine the texture and rheology of the products (Shomer *et al.*, 1993). The texture and rheology of the macerated cells are affected by the structural properties of the CW and starch, which determine their swelling behaviour.

The swelling behaviour of non-starchy cells, such as macerate cells of tomato fruit pericarp, is determined solely by the CW structure and its ability to withstand collapse (Shomer *et al.*, 1984). Selective degradation of pectin allowed increased swelling as a result of the release of the systematic organized cellulose lattice from the pectin; while enzymatic cellulose degradation resulted in the disappearance of the CW structure and consequently, the loss of cell bulk (Shomer *et al.*, 1984). In potato tuber cells, although starch is quantitatively the major component, the cell bulkiness is determined by the structural properties of the very small amount of microfibrillar cellulose lattice material. This was demonstrated when enzymatic removal of the pectin resulted in degradation of the middle lamella, without a significant change in bulk, whereas enzymatic degradation of cellulose caused the CW structure to disappear, and the bulkiness was lost (Shomer & Levy, 1988).

The texture of boiled potato cells is dependent on the integrity of the CW and on the swelling capacity of the starch in the lumen, which inflates the cell (Shomer *et al.*, 1993). It was indicated that the structural swelling behaviour of the CW is determined by the diffuse electrical double layer (DEL) of the cellulose fibril surfaces, which is affected sensitively by some factors such as dielectric constant and electrolytes (Shomer *et al.*, 1991). The swelling behaviour of boiled macerate of starchy potato tuber cells in liquids of low dielectric constants (Shomer *et al.*, 1995), is similar to the behaviour of non-starchy cells (Shomer *et al.*, 1991). However, whereas increased temperature (between ambient and 80°C) did not result in increased swelling of non-starchy cells, in starchy potato tuber cells the swelling increased with increasing temperature (Shomer *et al.*, 1995). It has been suggested that these changes resulted from differences between the starch and the CW in their swelling capacity (Shomer *et al.*, 1993). In order to study the texture, it is necessary to understand the structural behaviour of the CW and starch under the various conditions.

The present study showed starch leakage during boiling, determined the dimensions, and characterized the structural behaviour of heat-gelatinized starch and CW in boiled, dried and rehydrated cells (BDC) under various swelling conditions.

EXPERIMENTAL

Measurements of cell volume

The volumes of BDC, which are usually oval in shape, were measured in suspensions of potato cells in aqueous (distilled water) solutions of 0, 25, 50 and 75% ethanol, and in 100% ethanol. One drop of agitated cell suspension was placed within a glass slide with a trough, filled entirely with the suspension solution and covered with a cover glass. The transverse (r_1) and the longitudinal (r_2) radii (in μm) of each single cell were measured at a $\times 160$ magnification (obtained by means of a $\times 16$ objective and $\times 10$ ocular lens), with a built-in 10 mm graticule divided into 100 units. At this magnification level, the cell volume (V) in mm^3 was calculated as:

$$V = \frac{4\pi r_1^2 r_2 \times 10^{-3}}{3 * 16^3}.$$

Leakage of starch

Starch leakage upon boiling was assayed for cortex and pith tissues. The tissues were cut into dice of about $3\,mm^3$, and were shaken strongly with distilled water several times until the washing water did not contain free starch grains. Leakage of starch from the cells was evaluated by determining the content of both soluble amylose and amylopectin in the boiling water as a function of time. The amylose/amylopectin content was assayed by colorimetric iodine method (Hovenkamp-Hermelink *et al.*, 1988; Landers *et al.*, 1991).

Light and electron microscopy

Individual heat-treated cells were examined under a light microscope, after rehydration of dried material with distilled water. CW was identified by polarized light and starch by iodine staining. 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, washed with 0·1 M phosphate buffer (pH 7), post fixed with 2% O_2O_4 in the phosphate buffer at 4°C for 2 h, washed several times with phosphate buffer and,

finally, washed with distilled water. The fixed macerate was dehydrated gradually with ethanol and embedded in 'Agar 100' resin (Agar Aids, Cambridge, UK). Ultrathin sections were stained with uranyl acetate and lead citrate. Starch staining was done by 1% periodic acid for 20 min at 20°C, followed by washing with distilled water, incubation in a solution of 1% thiosemicarbazide and 10% acetic acid, washing with distilled water, incubation in a solution of 1% silver proteinate for 30 min at 20°C in darkness, and washing again with distilled water. Observations were carried out with a Joel-100CX transmission electron microscope.

RESULTS AND DISCUSSION

Leakage of starch during boiling

The two main tissue zones of the potato tuber, the cortex and the pith, were studied in relation to leakage of starch upon boiling. Starch amylose was found to leak from both tissues, but leakage from the cortex was greater than that from the pith (Fig. 1). During 120 min of boiling, the rate of starch leakage increased with time. Leakage from tissues of stored potato tuber was remarkably greater than that from fresh potato tuber. This is the first demonstration of leakage of gelatinized starch from intact cells upon boiling; it can be hypothesized that starch leakage is one of the factors which determines the textural quality of potato products. As shown for permeation of proteins through BDCs (Shomer *et al.*, 1995), it can be considered that the CW pores allow leakage of starch molecules smaller than 600 kDa. It is well known that during cold storage, starch is degraded into sucrose and subsequently into reducing sugars (Burton, 1982). It is possible that the increased leakage of starch into the boiling water from

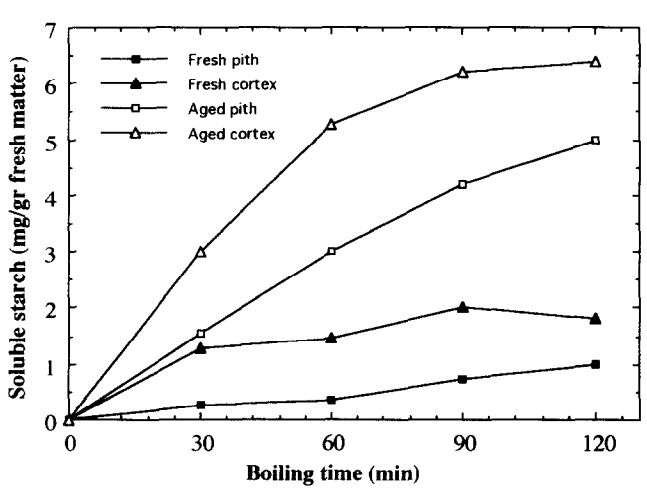

Fig. 1. Leakage of starch (mg/g fresh matter) in water of boiled potato cells. After each boiling cycle, the water with the soluble polysaccharides was separated from the cells and new water was added for the next boiling cycle.

stored potatoes is a result of changes in the starch polymers during storage. Two main reasons for this phenomenon may be: diminution of starch (amylose) molecular size during storage and augmentation of starch sensitivity to heat degradation. It appears that the higher leakage of heat-gelatinized starch from stored potatoes is a result of an increased amount of starch molecules smaller than CW pores which are <600 kDa proteins.

Microscopic observations showed that the CW remained intact during boiling (Fig. 3); therefore, leakage of soluble starch must have been by permeation through the intact porous lattice of the CW and not by disruption of the CW. Evidently, only a small portion of the gelatinized starch which became soluble in hot water, and had molecules smaller than the CW pores was available for diffusion through the CW. The observation that starch leakage increased with time suggests that the starch was continuously degraded during boiling. The total concentration of the starch inside the cell exceeds that of a saturated solution. Amylopectin has a molecular weight of ~2000 kDa or more, and its polymers are found as a mixture of soluble and gel fractions (Manners & Matheson, 1981). The amylose (with molecular weight of up to ~100 kDa) is interwoven with the amylopectin (Swinkels, 1985) and it may permeate the CW.

Microscopic determination of cell volumes

Light microscopy observations revealed that the cell swelling capacity significantly depended on the concentration of ethanol in water, and that the distribution of BDC volumes in water was within the range ~1·8–12×10^{-3} (Fig. 2). At room temperature the volume of the intracellular gelatinized starch was found to be somewhat smaller than that of the cell lumen, within the range $0{\cdot}55\text{–}11 \times 10^{-3}$ mm^3 (Figs 2, 3A and B). At temperatures higher than 70°C, the swollen gelatinized starch occupied the entire cell lumen and distended the surrounding CW (Fig. 3C and D). The BDCs' swelling level restrained with increasing ethanol concentration and at 100% ethanol the cell volume, as well as that of the gelatinized starch body, was less than $2{\cdot}5 \times 10^{-3}$ mm^3 (Fig. 2).

At ethanol concentrations exceeding 50%, cells were swollen only to a limited extent, and behaved similarly at both temperatures (Fig. 3C and D). The internal pressure exerted by the swollen starch (Briant *et al.*, 1945; Hoff, 1972) at 70°C, inflated the cells (Fig. 3C and D), and as a result the precipitate volume was higher than that obtained at room temperature (Shomer *et al.*, 1995). As suggested previously (Shomer *et al.*, 1993), it appears that differences between the swelling capability of the CW and that of the starch at various temperatures (Figs 2 and 3) affect the rheological properties of potato cells.

The swelling measurements (Fig. 2) and the light microscopy observations (Fig. 3) showed that the swelling properties of gelatinized starch are different from those of the CW. In non-starchy cells, such as tomato fruit pericarp cells, the extent of swelling is dependent on the existence and properties of the electrical diffused double layer of the CW fibrils, and it is affected by the nature of counter ions which neutralize the fibrillar fixed charges (Shomer *et al.*, 1991). This swelling behaviour depends on forces similar to the attractive and repulsive forces acting between charged suspended colloids (Jirgensons & Straumanis, 1962; Van Olphen, 1963). However, whereas suspended colloidal particles collide with each other, owing to Brownian movement, hydrated cross-linked CW fibrils may repel each other only within the limit range of the cross-linked lattice. In

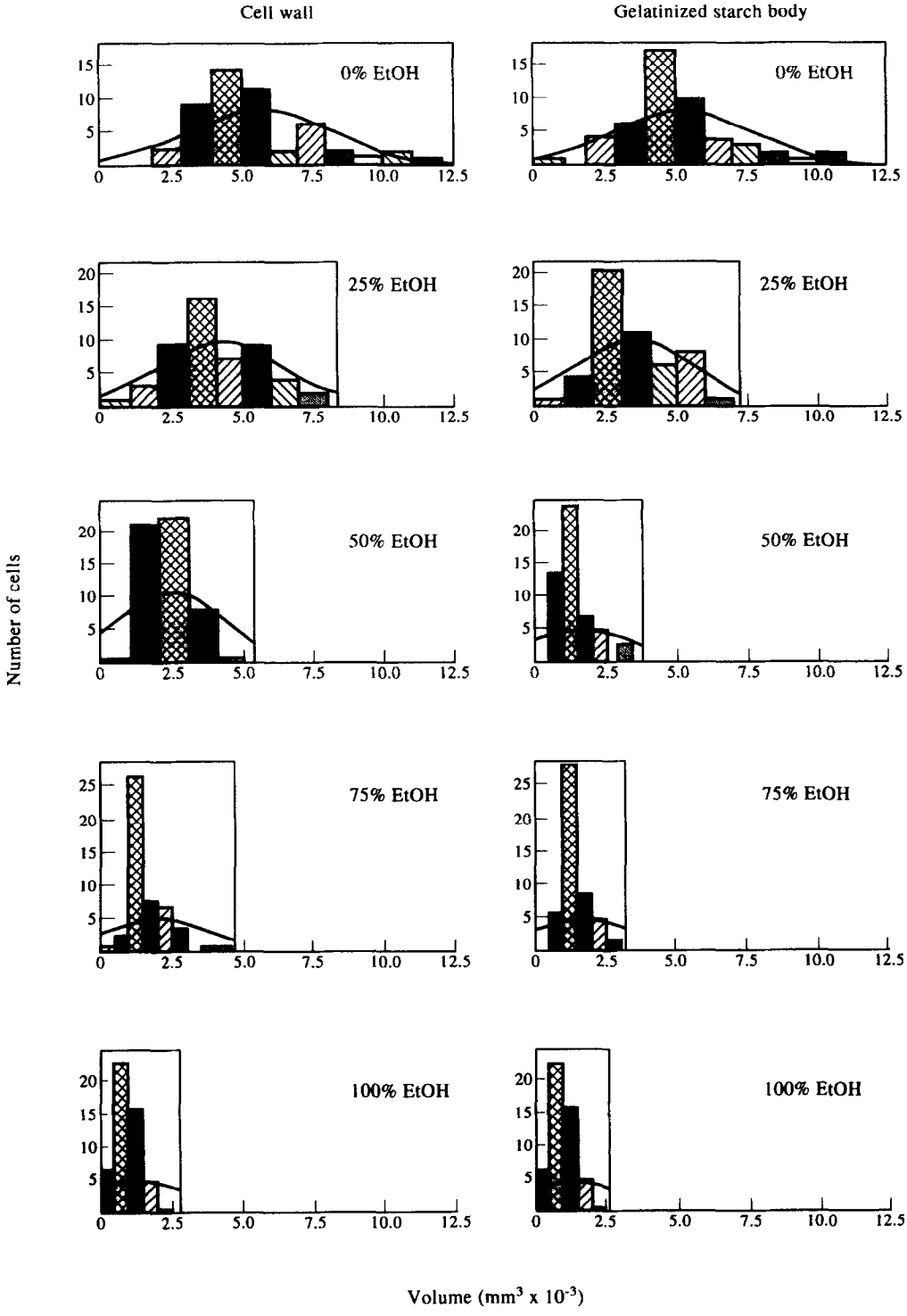

Fig. 2. Distribution of cell population according to volumes of both cells and gelatinized starch.

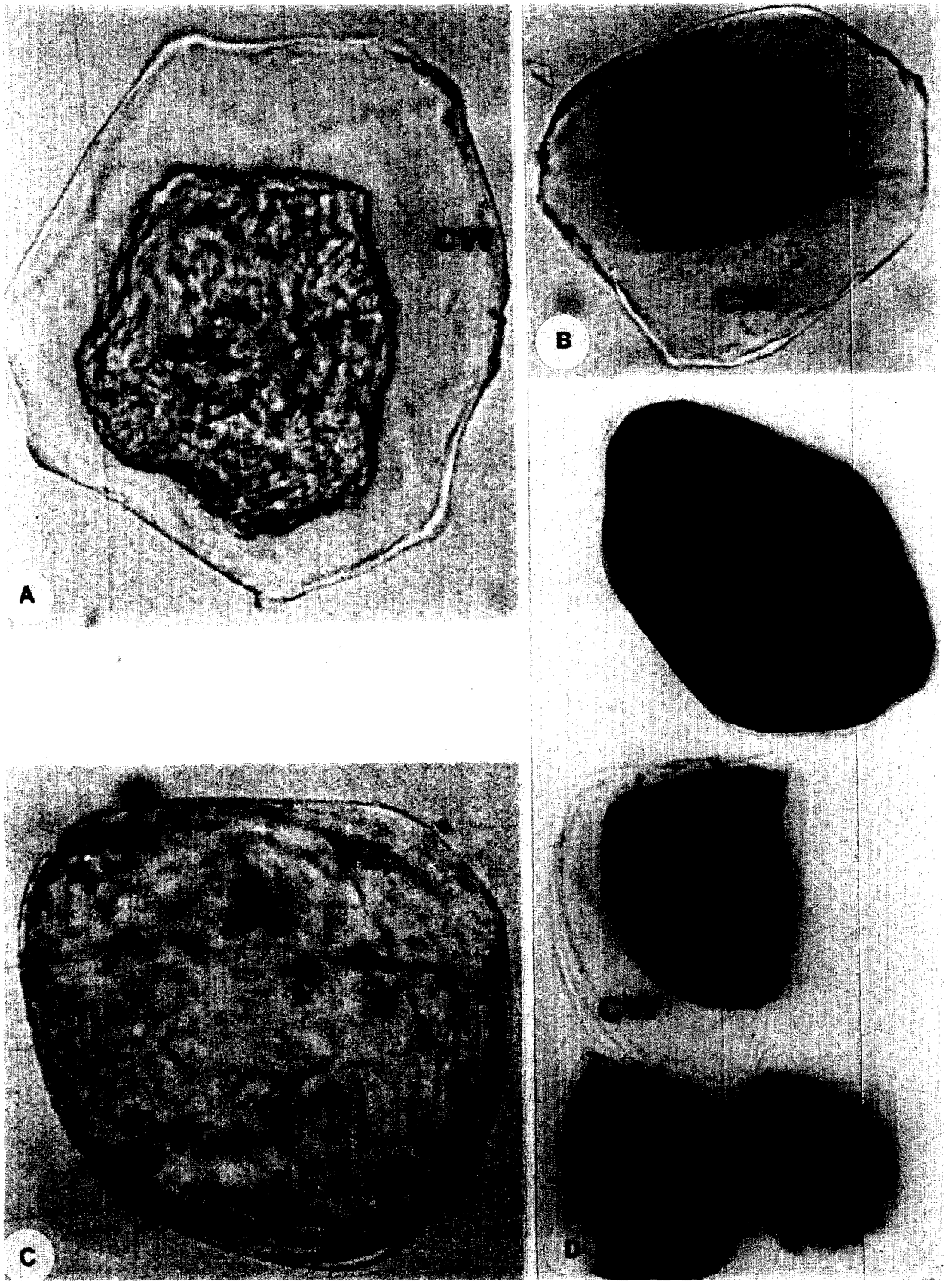

Fig. 3. Light micrographs of heat-gelatinized potato tuber cells observed under polarized light. (A) Representative rehydrated cell, swollen in water at 5°C or at room temperature within aqueous solution with 50% ethanol (×740); (B) same as (A), but stained with iodine (×650); (C) representative rehydrated cell, swollen in water at 70°C; the swollen gelatinized starch occupied the entire cell lumen (×980); (D) same as (C) but stained with iodine; in some cells the swollen starch did not occupy the lumen to capacity (×600).

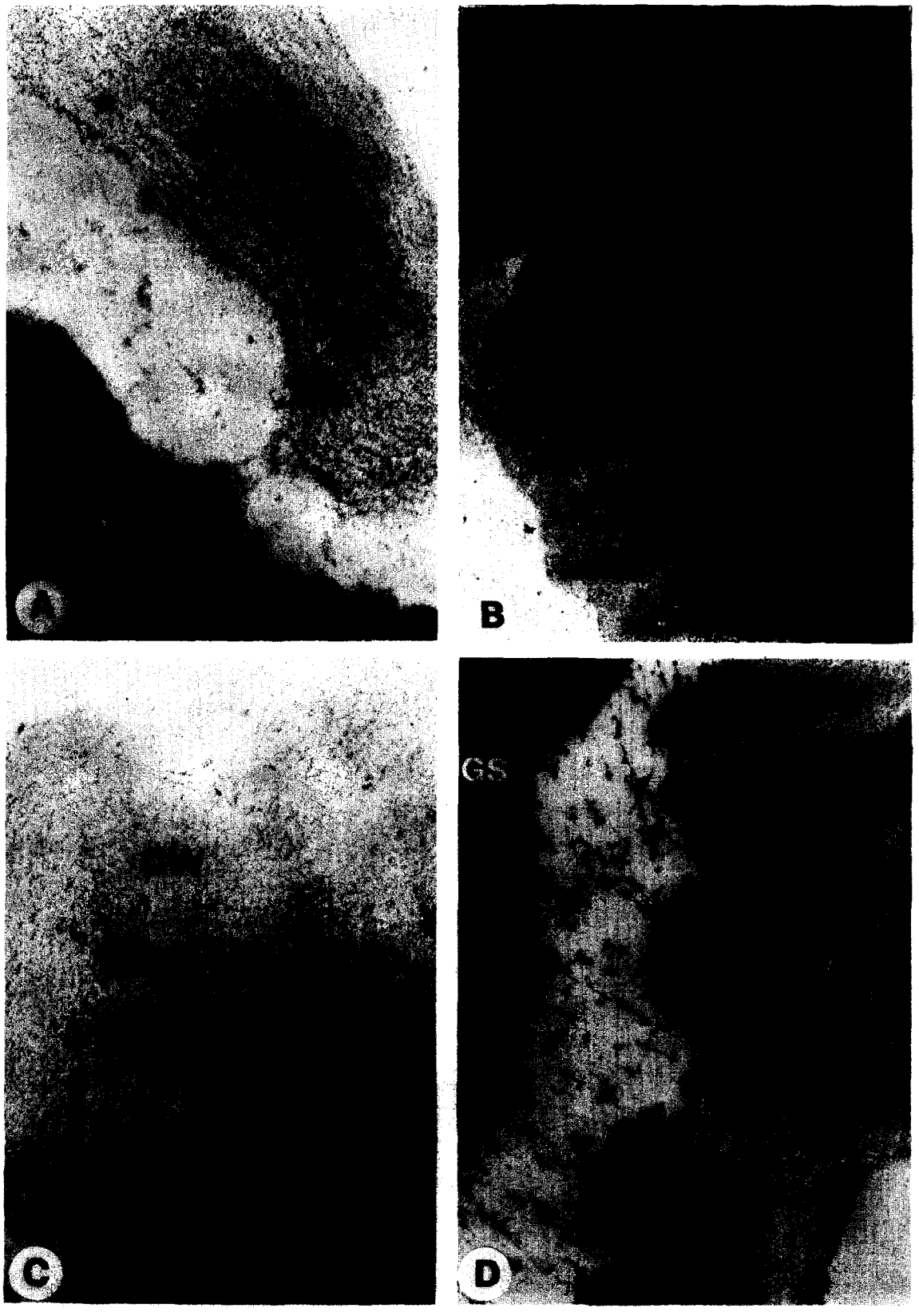

Fig. 4. Transmission electron micrographs of ultrathin sections of heat-gelatinized potato tuber cells. (A) Part of rehydrated cell, swollen in water at 50°C. Space is seen between the starch and the cell wall (×54,000); (B) part of rehydrated cell, swollen in water at 70°C. The starch is tightly attached to the CW; the outer region of the starch is presumed to be a mixture of soluble starch and proteinaceous matter (×28,000); (C) part of rehydrated cell, swollen in water at 50°C, stained with silver proteinate. Fragments of soluble starch mass are seen between the starch and the cell wall (×54,000); (D) part of rehydrated cell, swollen in water at 70°C. Thready patterns in the soluble starch mass are seen between the starch and the cell wall (×40,000).

this context, potato starch is known to be of exceptionally high swelling power even at relatively low temperatures, and especially high swelling pressure is exerted at high temperatures above 100°C (Whistler & Paschall, 1965).

The swelling power of potato starch is dependent on some factors such as amylose/amylopectin ratio, cleanliness, charged group etcetera. Two factors contribute to the swelling power of a given potato starch: hydrogen bonding through water bridges rather than direct molecular associations; and weak internal bonding due to the presence of ionizable esterified phosphate groups, which assist swelling by mutual electrical repulsion (Whistler & Paschall, 1965). The disruption of weak hydrogen bonds between the heat gelatinized starch molecules (Swinkels, 1985) enables the starch mass to be highly swollen inside the cell, while its expansion is limited by the surrounding CW; thus, in liquids of high dielectric constant (Shomer *et al.*, 1995) or when the starch solubility inside the cell is increased, the cells become inflated owing to the pressure exerted by the relatively immense quantity and high density of the swollen starch. These swelling effects may play a role in the textural properties of the cells (Shomer *et al.*, 1993).

Ultrastructure of heat-gelatinized cells

The difference in the internal pressure exerted by the swollen starch according to whether the swelling occurs at room temperature or at 70°C, results in a different ultrastructure of the internal face of the CW and the mass of the intracellular gelatinized starch (Fig. 4). Ultrastructural observations showed that the heat-gelatinized starch appears as an amorphous body in which single swollen starch grains are not identifiable, i.e., the heat-gelatinized starch body is seen as fused starch grains. When the starch was not fully swollen, a space was seen between the starch body and the CW (Fig. 4A). When the gelatinized starch was highly swollen and inflated the cell, heat-treated protoplasmic residues were trapped between the swollen gelatinized starch mass and the inner surface of the CW. In some sites a continuous osmiophilic layer was compressed compactly between the gelatinized starch body and the CW (Fig. 4B). In some cases osmiophilic inclusions were seen, with ultrastructure similar to that of heat-coagulated potato proteins (Shomer *et al.*, 1982). Silver proteinate staining revealed that, as extensions to the gelatinized starch body, filamentous elements of soluble starch occurred in the space between the starch body and the CW (Fig. 4C). In some sites, osmiophilic inclusions were seen adjacent to the inner surface of the CW beside the silver proteinate-stained filamentous starch structures. The ultrastructural observations indicated that the soluble starch had infiltrated into the CW (Fig. 4c) and exerted pressure which pressed protoplasmic residues and coagulated proteins against the inner surface of the CW.

The present study provides evidence that the structural swelling behaviour of BDCs depends on the conditions which determine the swelling relationships between the CW and the starch; as suggested previously (Shomer *et al.*, 1993), these swelling relationships affect the rheological and textural behaviour of potato cells.

ACKNOWLEDGEMENTS

Thanks are due to Rosa Vasiliver for the light and electron microscopy preparations and to Ms Limor Barak for her assistance in this study. The research was supported in part by Research Fellow Grant No. IS-1425-88R from BARD, the United States–Israel Binational Agricultural Research and Development Fund, and in part by the Chief Scientist's fund of the Ministry of Agriculture, Israel. Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel No. 1314-E series.

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