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The influence of starch swelling on the material properties of cooked potatoes

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Cooked potatoes have a wide range of food applications, but the mechanism by which softening occurs on heating is not clearly understood. Heating potato parenchyma tissue results in two independent, concurrent events; weakening of the binding between cells and swelling of intra-cellular starch. Potato plants containing starches with a range of high amylose contents and reduced swelling properties were available. This provided the opportunity to separate cooking effects of inter-cellular pectin from swelling of intra-cellular starch. Their individual contribution to the separation of cells and the softening of cooked potato tissue was established by studying the influence of heat on the material properties of a range of starch-modified potatoes. For all potato lines studied, the strength of the heated tissue decreased markedly following 30 minutes at 80°C or 5 minutes at 100°C. Microscopy of the line in which there was minimal starch swelling, indicated that the cells of the cooked tissue principally contained fluid, in contrast to the controls in which the cells were filled with swollen starch on cooking. Since all the lines followed the same trend with regard to the thermal weakening of the tissue, we conclude that weakening of potato tissue on cooking is primarily controlled by thermal degradation of the middle lamella.

1. Introduction

Once cooked, potatoes have a wide number of applications in foods. A major effect of cooking is to cause softening of the tissue to a more palatable and processable texture. However, the mechanism by which softening occurs on heating is not clearly understood. The cells in the potato tissue are bound together by a pectin-rich region known as the middle lamella [1] and in raw potato, the cells contain numerous partially crystalline starch granules. The material properties of raw potato [2] can be understood in terms of a combination of cell turgor pressure and cell wall mechanics. On heating, turgor pressure is readily lost [3]. In addition to the disruption of membranes that results in turgor pressure loss, the application of heat to potato tuber parenchyma tissue results in two independent events; a weakening of the binding between the cells and gelatinisation and swelling of the intracellular starch [4]. The characteristic softened texture of cooked potato tissue (and many other edible plant tissues [5]) is caused by the easy separation of the cells on the application of mechanical stress [6]. The swollen starch typically fills the cells during thermal processing and could act to force the cells apart [7, 8], whilst the thermal degradation of the pectin in the middle lamella, by β-elimination, could weaken the intercellular binding [5, 9, 10]. However, the concurrent nature of these two processes has meant that in the past it has not been possible to establish their individual contribution to the separation of cells in the cooked tissue.

Potato starch is composed of amylose and amylopectin. In current varieties, the amylose content ranges from 23–29%, with the starch typically swelling at between 62°C and 69°C [11]. Potato plants containing starches with a range of increased amylose contents up to about 90%, which consequently show reduced swelling properties, have been developed by antisense inhibition of starch branching enzymes of both type A and type B [12]. As this series of materials was generated by specific genetic modification of a single starch synthesizing activity, pectin and cell wall structures are expected to be conserved. These modified starch compositions and properties therefore provide the opportunity to separate cooking effects of the middle lamella pectin from the swelling of the starch, by studying the influence of heat on the material properties of a range of starch-modified transgenic potatoes.

The degree of softening of the potato tissue following heating was analysed using a compressive mechanical test, with the amount of fluid expressed from the tissue during the compression being measured. Light microscopy was used to examine the tissue structure, primarily with regard to the extent of starch swelling and the failure mechanism of the tissue. On occasion, crossed-polars indicated whether the starch had remained in a partly crystalline state or had gelatinised on heating [13].

2. Experimental methods

2.1. Sample preparation

The potato lines were transformed and grown in the field according to the procedure described previously [12], with the background variety being Desiree. The transgenic lines were generated by retransformation of antisense SBE B lines [14] with a 1.2 kb antisense SBE A construct [15]. Line 208 [12] was derived from line 15, whilst lines 150 and 161 were both derived from line 17 [14]. The control line was wild-type Desiree. The amylose content of the extracted starch was measured by a colourimetric technique [16], whilst the swelling temperature and extent of swelling was determined by rapid viscometric analysis [17].

Cylinders of potato parenchyma tissue (1 cm diameter & length) were removed from the raw tuber using a cork borer and cut to length using a microtome blade. The cylinders were placed in a water bath at a pre-set temperature for a specified time. The heating conditions ranged from 60–100°C for 5 and 30 minutes. The cylinders were then removed and placed in an ambient water bath to cool prior to further analysis.

2.2. Mechanical testing

A flat plate compression test was performed on the individual cylinders to 80% strain, with a crosshead speed of 100 mm/min, using an Instron Universal Testing Machine. The cylinders were weighed before and after testing, with any expressed fluid being removed from the surface of the compressed material. This was repeated for 10 cylinders and the weight loss due to fluid expression calculated for each cylinder. Representative stress/strain curves for each time/temperature regime were selected for presentation.

2.3. Light microscopy

Three of the heated cylinders were subjected to a standard histological preparation procedure, prior to examination by light microscopy. Initially, the cylinders were placed in fixative (formal acetic acid) for 10 days. A series of increasing alcohol steps then dehydrated the tissue, before the alcohol was removed using toluene and the tissue was impregnated with wax. The samples were embedded into moulds and 5 μm sections were cut, before incubation overnight at 56°C. The wax was then removed from the sections using xylene, which were then hydrated using a series of decreasing alcohol steps. The sections were then stained with saturated Saffranine-0 and 0.2% iodine, air dried and mounted in a resinous mountant. Light microscopy examination was then performed in order to assess primarily the degree of swelling of the starch granules within the cells and also observe the tissue microstructure. The crystalline or gelatinised nature of the starch was examined using crossed-polars.

3. Results

3.1. Composition of extracted starch and swelling behaviour

Four lines of potato were studied, with the amylose contents and swelling temperatures of the extracted starch shown in Table 1. The degree of swelling of the extracted starch was as follows:- control > 150 > 161 > 208.

3.2. Mechanics

The relatively high stiffness and strength of the raw potato tissue from the control line was retained following heating to temperatures ranging from 60°C to 80°C for 5 minutes (Fig. 1). A significant reduction in the strength of the tissue, and less so the stiffness, was only observed when the tissue was subjected to 100°C for 5 minutes or 80°C for 30 minutes.

TABLE I Amylose content and swelling temperatures from the extracted starch [18]

Transgenic line	% Amylose content	Swelling temp (°C)
Control	25	64
150	38	76
161	52	84
208	68	>95

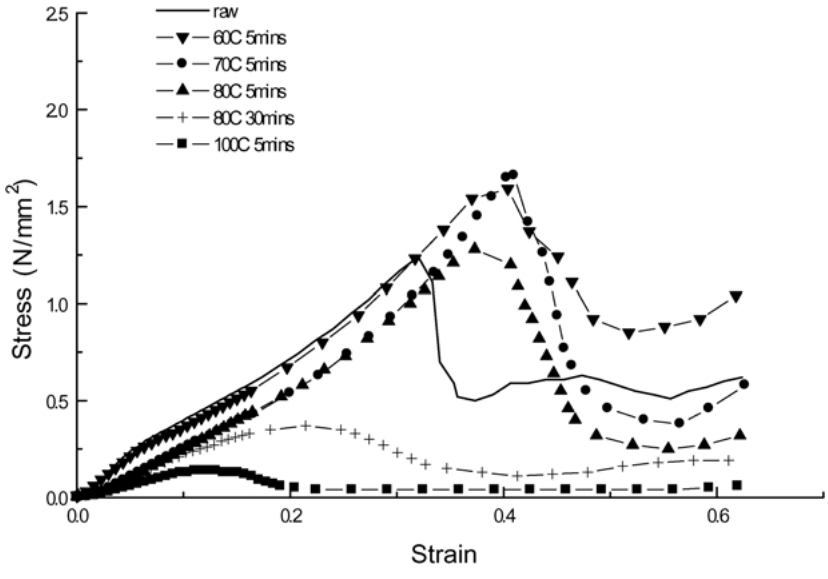

Figure 1 The compressive mechanical behaviour of potato tissue from the control line, following a range of thermal treatments.

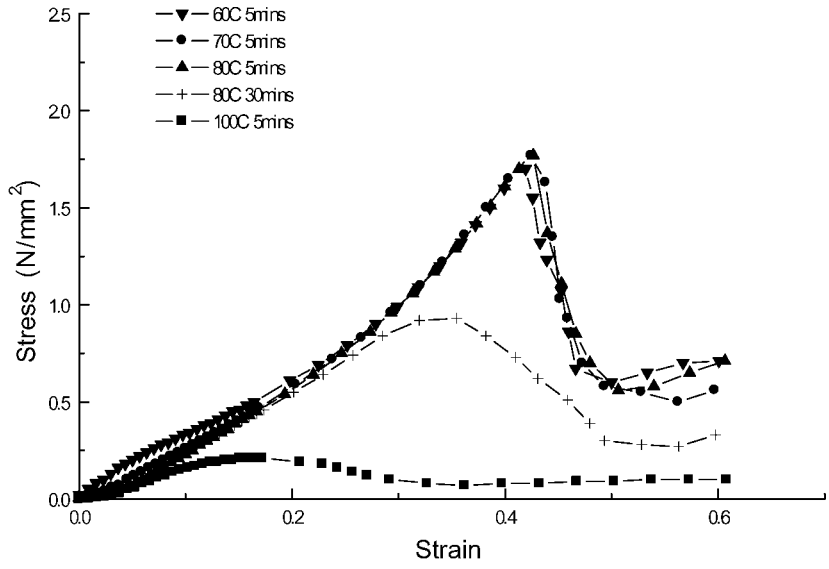

Figure 2 The compressive mechanical behaviour of potato tissue from line 208 (high amylose), following a range of thermal treatments.

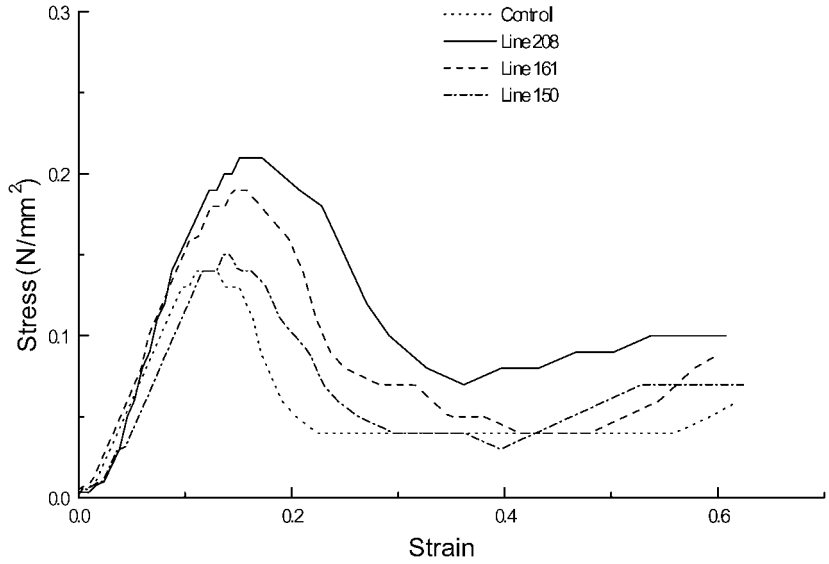

Figure 3 The compressive mechanical behaviour of all the potato lines following heating to 100°C for 5 minutes.

The same trend of tissue strength and stiffness only lowering significantly following heating at 80°C for 30 minutes or 100°C for 5 minutes was observed for the starch-modified transgenic potato lines, for example line 208 (Fig. 2).

After heating at 100°C for 5 minutes, the tissues of the starch-modified lines, although notably weakened, are all slightly stronger than the control tissue (Fig. 3). The line with the highest amylose content (208) is the strongest, with the strength of the tissue decreasing with decreasing amylose content, following this heating regime. This trend was also observed following heating at 80°C for 30 minutes.

3.3. Microstructure

The extent of starch swelling in the tissue of the various lines, following a range of heat treatments, is shown in Fig. 4. The starch of the control potato line (Fig. 4a) swells dramatically on heating to 70°C, or above, for 5 minutes. At 60°C, the starch is still partly crystalline as indicated by observation of the characteristic Maltese cross pattern under crossed polars [13] (data not shown) and the cells are predominantly filled with fluid. By contrast, at 70°C the starch had lost the Maltese cross pattern and swollen to such an extent that it filled the cells, incorporating most, if not all, the cellular fluid within the starch gel.

The starch of line 208 (Fig. 4c), with the highest amylose content, showed limited swelling even on heating to 100°C for 5 minutes. Crossed polars indicated that at 70°C the starch granules were still crystalline, whilst at 100°C the granules had gelatinised, although little swelling had occurred. Consequently for all the heating regimes applied to line 208, the cells were primarily filled with cellular fluid. The starch swelling in lines 150 and 161 (Fig. 4b) was intermediate in nature, compared to the control and 208 lines. In line 150, significant swelling was only observed at 80°C and above, whilst in line 161 some swelling was observed at the higher temperatures.

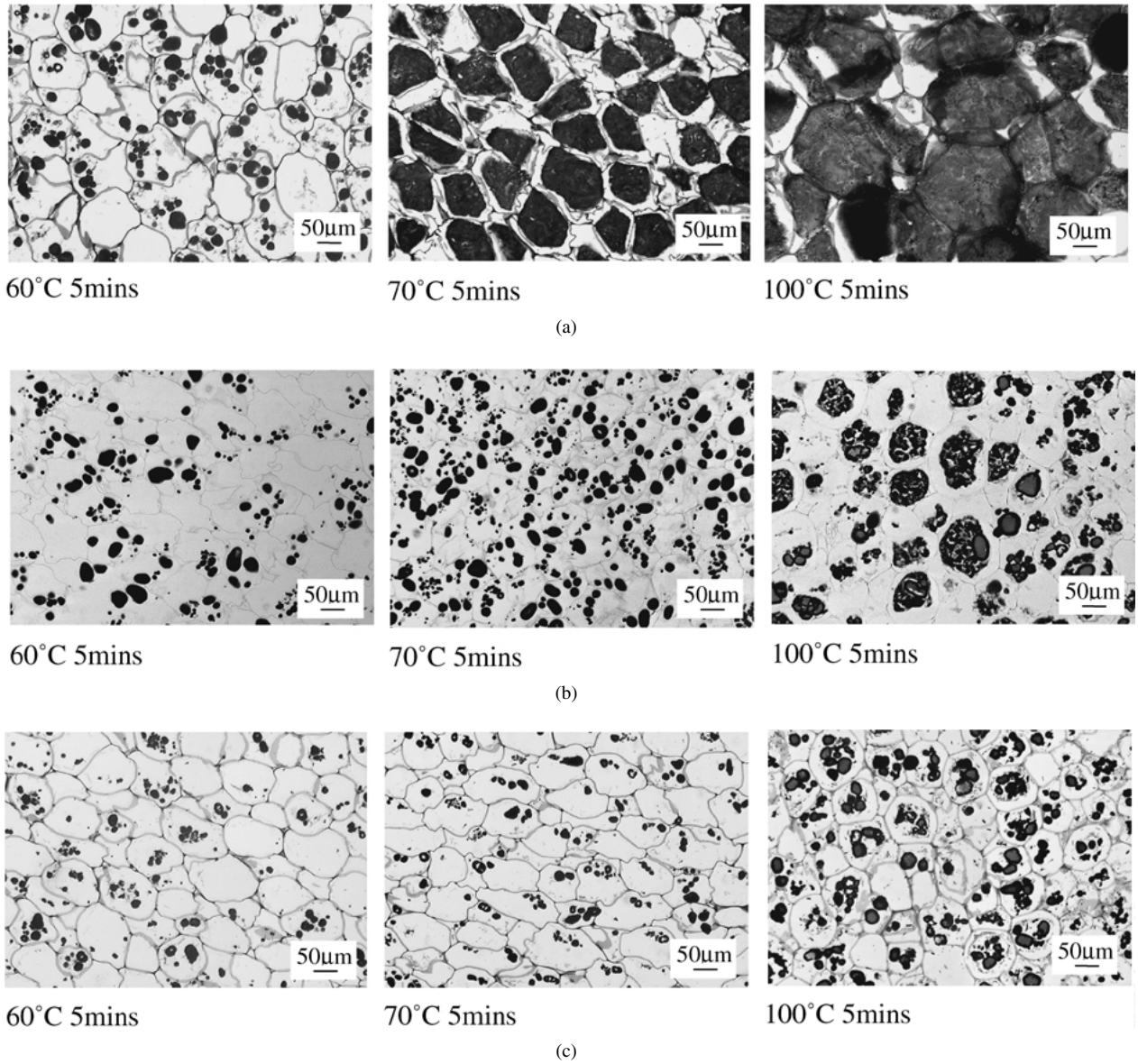

Figure 4 The microstructures of the control, 161 and 208 lines, following heating for 5 minutes at a range of temperatures, showing the extent of starch swelling within the cells. (a) Control; (b) 161 line; (c) 208 line.

3.4. Failure mechanisms

The failure mechanisms of the potato tissue were examined at the macro- and micro-structure level, however due to limited available material only the failure mechanisms of the control line could be studied. Following treatment at 60°C for 5 minutes, the control line, at 50% strain, shows a shear failure characteristic of a brittle fracture, whilst the tissue subjected to 100°C for 5 minutes exhibits more, slower fractures, even after 30% strain (Fig. 5), apparently producing more damaged regions to the macro-structure. The higher temperature also causes a change in the failure mechanism of the cellular microstructure (Fig. 6). At 60°C the tissue fails by the apparent brittle fracture propagating through the cells, whilst at 100°C the slower fracture propagates primarily between the cells.

3.5. Fluid expression

The variation with temperature in the amount of fluid expressed from the potato tissue during the compression is shown in Fig. 7. At 60°C, all the potato lines expressed a similar, relatively large amount of fluid on compression. The fluid expressed from the controls is significantly less at 70°C and remains low for the subsequently higher temperatures. Line 150 behaves in a similar manner, though the reduction in fluid expression occurs only above 70°C. The fluid expression from line 208 is relatively large at all temperatures. There is a slight initial increase in expressed fluid and then a small reduction at 100°C. The fluid expression versus temperature profile for line 161 is intermediate between lines 150 & 208.

4. Discussion

4.1. Softening of potatoes on cooking is primarily due to weakening of inter-cellular binding

The strength and stiffness of the potato tissue from the control line only decreases significantly on heating to 80°C for 30 minutes or 100°C for 5 minutes (Fig. 1). This change in the mechanical properties of the tissue is equivalent to the well known softening that occurs

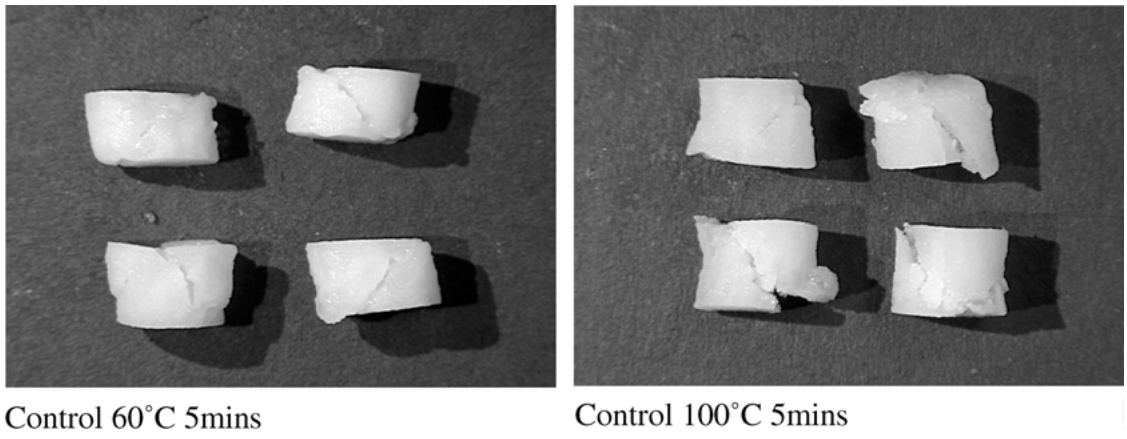

Figure 5 Macroscopic images of tissue failure at 60°C (50% applied strain) and 100°C (30% applied strain).

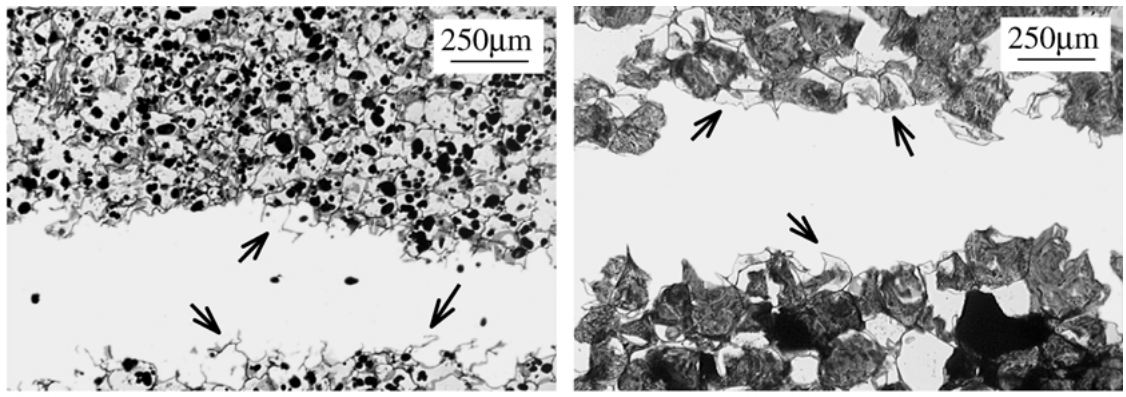

Figure 6 Tissue microstructure upon failure at 60°C (50% applied strain) and 100°C (30% applied strain).

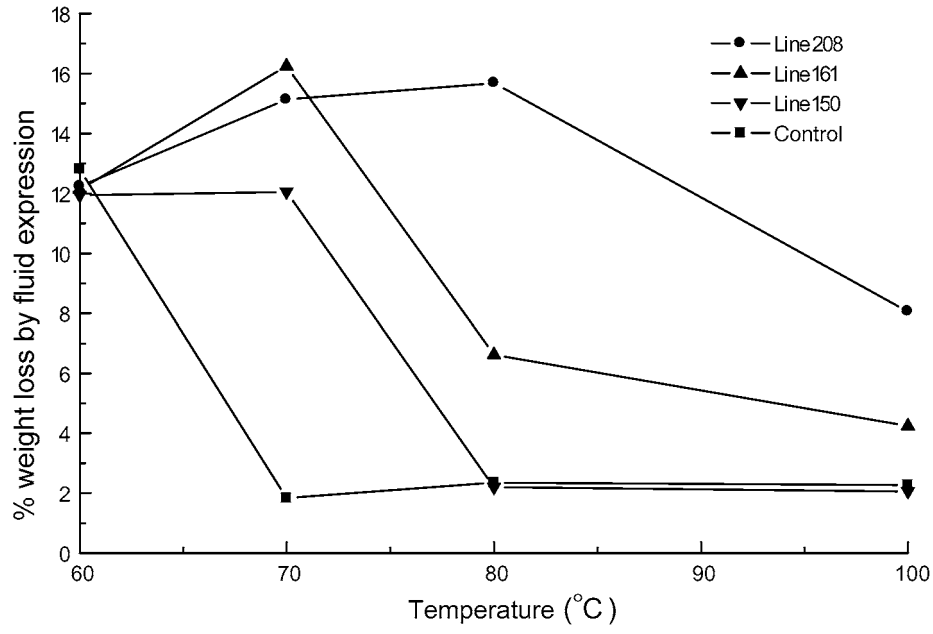

Figure 7 The percentage weight loss by fluid expression, during compression, for all the potato lines, following heating to a range of temperatures for 5 minutes.

in potatoes on cooking. The weakening of the tissue is caused by the cells becoming easier to separate and as a result the softening is accompanied by a change in failure mechanism of the tissue from cell rupture to cell separation (Fig. 6). From this data alone, it is not possible to deduce whether the change in failure mechanism is caused by the pectin in the middle lamella being degraded by β-elimination at high temperature [9, 10] or by the swollen starch exerting an internal cellular pressure [7, 8]. At temperatures of 70°C or

above, the starch is gelatinised and swollen to such an extent that the cellular fluid is incorporated within a starch gel (Fig. 4). The swollen starch could therefore exert pressure within the cells causing them to be more rounded and consequently less adhered, which would lead to a weakening of the tissue.

The starch-modified transgenic potato lines demonstrated the same trend as the controls regarding the change in mechanical properties with temperature. As a result, weakening of the tissue was only observed when the tissue was subjected to 80°C for 30 minutes or 100°C for 5 minutes (Fig. 2; line 208). The starch swelling in line 208 was minimal, even at 100°C (Fig. 4), due to the high amylose content of the starch, and yet the degree of softening was comparable with that of the control line (Fig. 1). Therefore, potato tissue can soften on heating without the occurrence of starch swelling, indicating that changes to the cell walls must be the primary factor causing the cells to separate more easily and the tissue to soften. The high temperatures at which softening occurs also correlates with temperatures at which pectin degradation by β-elimination can occur [19]. When tissue softening occurs, for example at 100°C, although all the potato lines are significantly weaker, it is apparent that the control line is the weakest (Fig. 3). The transgenic lines are all slightly stronger, with the tissue strength increasing with increased amylose content and corresponding reduced starch swelling. This suggests that although changes to the cell walls are the primary cause of tissue softening, the starch swelling is having a minor, secondary effect. The larger degree of starch swelling in the control line could lead to further weakening of the inter-cellular binding and hence the control line is marginally the weakest, with line 208 the strongest, due to its limited swelling.

4.2. Starch swelling and failure mechanisms determine fluid expression

The swelling of the starch in the tissue (Fig. 4) followed the same trend as that previously observed for the extracted starch, from these lines [18] ie. control > 150 > 161 > 208. The amount of fluid expressed from the tissue during compression varied dramatically depending on the line and the temperature it had been subjected to (Fig. 7). This can be accounted for by the degree of starch swelling within the cells. For all the lines at 60°C, the fluid expression is relatively high. At this temperature, all the lines contain unswollen starch, hence the cells are primarily filled with cellular fluid, which is released from the tissue during breakdown as compression occurs. At 70°C, the starch of the control line has swollen such that the cellular fluid is seemingly completely incorporated within the starch gel. Consequently, the amount of fluid released on compression of the tissue heated to 70°C (or above) is significantly lower. For the 70°C treatment, following the mechanical test, the fragmented tissue is sticky, rather than wet or moist as at 60°C, due to the starch gel being released from the ruptured cells upon breakdown.

Similar behaviour is observed in the tissue of line 150, albeit at higher temperatures. At 70°C, microscopy indicated that the starch had not swollen, so the fluid expression is comparable to that at 60°C. Significant swelling in line 150 occurred at a higher temperature than the controls and could clearly be seen at 80°C, hence the lower fluid expression at this and higher temperatures.

In line 161, some swelling occurred at higher temperatures, hence the decrease in the fluid expression. However, even at 100°C the microscopy indicated that the amount of swelling, although extensive, was less than that observed in the control or 150 lines at that temperature. Accordingly, the fluid expression at 100°C is slightly greater from line 161.

The least swelling was observed in line 208 and as a result, at all temperatures, the cells are predominantly filled with "free", expressable fluid. This accounts for the fluid expression from this line remaining relatively high as the temperature increases. The slight decrease at the higher temperatures, is probably due to a change in the failure mechanism of the tissue on compression, since the extent of starch swelling is still limited. As the temperature increases, the tissue will be more prone to cell separation, potentially resulting in less fluid being released, due to encapsulation within separating cells. However following 100°C for 5 minutes, comparable to cooking the potato, the fluid expression of line 208 is still relatively high compared to the controls.

4.3. Consequences for sensorial texture of cooked vegetables

The amount of fluid expressed during the mechanical compression test can be primarily accounted for by the extent of starch swelling that has occurred at a particular temperature. The regime of 100°C for 5 minutes applied to the cylinders is similar to conventional cooking of potato tissue. When standard quality assessment tests were applied to the cooked whole potatoes, the high amylose lines, and particularly line 208, were notably different compared to the controls [20]. On mashing the boiled potato from the control line with a fork, the tissue had a "dry, softened" appearance, which is typical of cooked potato. By contrast, the boiled potato from line 208 was similarly softened, but expressed fluid during cutting and mashing.

By using experimental raw materials to advance mechanistic understanding, this study suggests that there are two principal factors that affect the sensorial properties (firmness, juiciness) of cooked vegetable tissue. One is the transition from cell rupture to cell separation caused by thermal degradation of pectin and the second is the extent to which intra-cellular volumes are filled by gelatinised starch. Control of these two features by raw material and process combinations provides much of the known range of cooked vegetable texture.

5. Conclusions

On heating potato tissue, significant weakening still occurs when there is limited or no starch swelling,

indicating that the weakening is primarily controlled by the thermal degradation of the middle lamella. The elevated temperatures cause a change in failure mechanism from cell rupture to cell separation. Swelling of the starch may be a secondary factor, since there were slight decreases in the tissue strength as the degree of swelling increased, possibly due to the internal swelling pressure of the starch. The reduced swelling of the high amylose starches resulted in essentially "free" fluid being present within the cells of the cooked tissue and hence the amount of fluid released from the cooked tissue on compression was measurably greater for the starch-modified lines, which resulted in novel, cooked potato textures.

This study has also highlighted the utility of genetically modified lines in providing raw materials with precisely defined compositional differences in otherwise identical backgrounds. The fact that individual transformants give rise to different strengths of transgenic effect (in this case amylose content and hence granule swelling temperature) provides an homologous series of potato tubers. In such complex materials this allows effects of individual components to be studied without invasive processing.

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