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CRYO-SCANNING ELECTRON MICROSCOPY INVESTIGATION OF THE TEXTURE OF COOKED POTATOES

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Abstract

The texture of steam-cooked potatoes was investigated by examining the fracture planes of four different cultivars, using cryo-scanning electron microscopy (cryo-SEM), which yielded a good preservation of the hydrated structures in potato tissue. For all cultivars, fracturing after steam-cooking took place between cells preferentially alongside the cell walls. However, textural difference appeared from the degree of intercellular contact, the cell shape and the appearance of cell surfaces. Cells in the fracture planes of firm potatoes had large intercellular contacts. In this case, most of the cells were flat and cell surfaces showed folds and cracks. For mealy potatoes, it appeared that cells in the fracture planes had little intercellular contacts. The cells were round and turgid and had smooth surfaces. In conclusion, the structure of cell wall and middle lamella, and the starch content, appear to be important parameters to distinguish firm and mealy cultivars.

Key Words: Cell, cryo-scanning electron microscopy, potato, cultivar, steam-cooking, texture, fracture plane, pectin, starch, cell wall.

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Introduction

Texture is a quality parameter of cooked potatoes which varies from firm to mealy. The choice of a potato cultivar for particular processing may be based on textural properties of the potato tissue after cooking. Firm potatoes are suitable for processing into products in which pieces of tissue should be recognizable. For instance, in Germany this type of potatoes is used for the production of salads. On the other hand, a mealy texture is preferred for mashed potatoes. For this reason, mealy table potatoes are popular in the Netherlands. Knowledge of the parameters which determine the texture of potatoes may contribute to optimizing processing conditions and selection of suitable raw material.

Scanning electron microscopy (SEM) has previously been applied to study potatoes during processing. Generally, steam-cooked potato cells remained intact, but they had wrinkled surfaces (Fedec *et al.*, 1977; Moledina *et al.*, 1978). However, in these studies, tissue was pretreated by chemical fixation and dehydration. These techniques may alter the highly hydrated structures, cell walls and gelatinized starch, present in steam-cooked potato tissue (Robards and Sleytr, 1985; Sargent, 1988). Physical fixation by cryo-methods results in better preservation of hydrated structures, although it may also alter structures due to ice crystal formation. Recently, it has been shown that the combination of physical fixation by cryo-methods and freeze-drying in comparison with chemical fixation and dehydration resulted in less wrinkled cell walls (Huang *et al.*, 1990).

During cooking, pectin is degraded from the middle lamellas and cell walls, resulting in the separation of the potato cells (Burton, 1989). The degree of cell separation was not clearly visible in a section of cooked tissue composed of cross-sections of cells (Moledina *et al.*, 1978), but sections with cell surfaces revealed better images (Fedec *et al.*, 1977).

Additionally, the gelatinization of starch was clearly visualized. Heated cells were filled by gelatinized starch, which had a reticulated structure, and a void space was always left between cell walls and starch gel (Huang *et al.*, 1990).

In this study, cryo-SEM was used to distinguish

Table 1: Cooking classification of four potato cultivars (Parlevliet *et al.*, 1991).

cultivar	type of cooking behaviour			
	A	B	C	D
Nicola	------	--		
Eersteling	----	-----		
Irene			----	----
Eigenheimer		-	-------	--

- type A: an especially firm, non-mealy potato with a fine structure;
- type B: a firm, slightly mealy potato with a fine or rather fine structure;
- type C: a rather loose, mealy potato;
- type D: a loose, very mealy potato.

Table 2: The mean starch content on fresh weight of four potato cultivars assessed by weighing in water.

cultivar	% starch
Nicola	12.5 ± 2.1
Eersteling	15.3 ± 1.2
Irene	18.2 ± 2.0
Eigenheimer	18.8 ± 2.0

steam-cooked potato tissue of four cultivars based on the textural differences at the cellular level. To preserve the original hydrated structures of the tissues, we used cryo-fixation. The results revealed a relationship between cellular organization of the fracture planes and texture of the cultivars.

Materials and Methods

Four potato cultivars, Eersteling, Eigenheimer, Irene and Nicola, were grown in 1990 on clay soil at our experimental station in the North East Polder. Mature potatoes were harvested and potatoes with size 45/55 mm were stored at 6 °C and 90-95% relative humidity. According to the classification described by Parlevliet *et al.* (1991), the four cultivars represented two extreme forms of texture (Table 1). This classification was based upon the appearances of whole cooked potatoes. The term "fine structure" stands for a regular surface structure of the whole potato, without disturbances. Potatoes with a mealy structure have surfaces with bursts and loose cells.

Determination of starch

The starch content of the cultivars was assessed by weighing ten potatoes of each cultivar in water. A washed and dried unpeeled potato was weighed in air (dry weight = DW) and under water at 10°C (under water weight = UWW). By using the DW and UWW, we calculated the starch content of the tuber as described by Burton (1989).

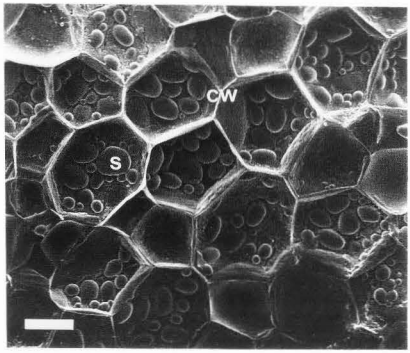


Figure 1. Fracture plane of non-cooked potato tissue of the cultivar Nicola. Bar = 0.1 mm. s = starch granule; cw = cell wall.

Cryo-scanning electron microscopy

For cryo-SEM, five hand-peeled (about 18% weight loss) potatoes of each cultivar were steam-cooked separately with demineralized water for 30 minutes (under normal pressure) and broken into two halves.

Fracture planes were obtained before freezing by cutting pieces of the internal phloem storage parenchyma tissue (2 x 3 x 5 mm^3) from the fracture planes of the two halves. The pieces of tissue, with the fracture plane (2 x 5 mm^2) up, were mounted on brass stubs using carbon cement. The stubs were immersed in nitrogen slush (about 60 °K), using a Hexland CT1000/CP2000 cryo-system. The samples were etched (20-30 minutes at about 0.1 Pa and 190 °K) in a Philips SEM 535, equipped with a cold stage. Sputtering with gold took place in the Hexland cryo-system (2 minutes at 6.5 Pa and 100 °K). Cross-sections were obtained after freezing by fracturing the frozen tissue with a cooled razor blade in the Hexland cryo-system before etching. The samples were examined in the Philips SEM 535 at 15 kV accelerating voltage. During etching, sputtering and examination the temperature of the anticontaminator was held at 90 °K (Robards and Sleytr, 1985; Sargent, 1988).

Results

Starch content

The starch content of the four cultivars was determined to investigate its correlation with texture (Linehan and Hughes, 1969). The results are given in Table 2. It appeared that the mealy-cooking cultivars, Irene and Eigenheimer, had a higher starch content in comparison with the firm-cooking cultivars, Nicola and Eersteling.

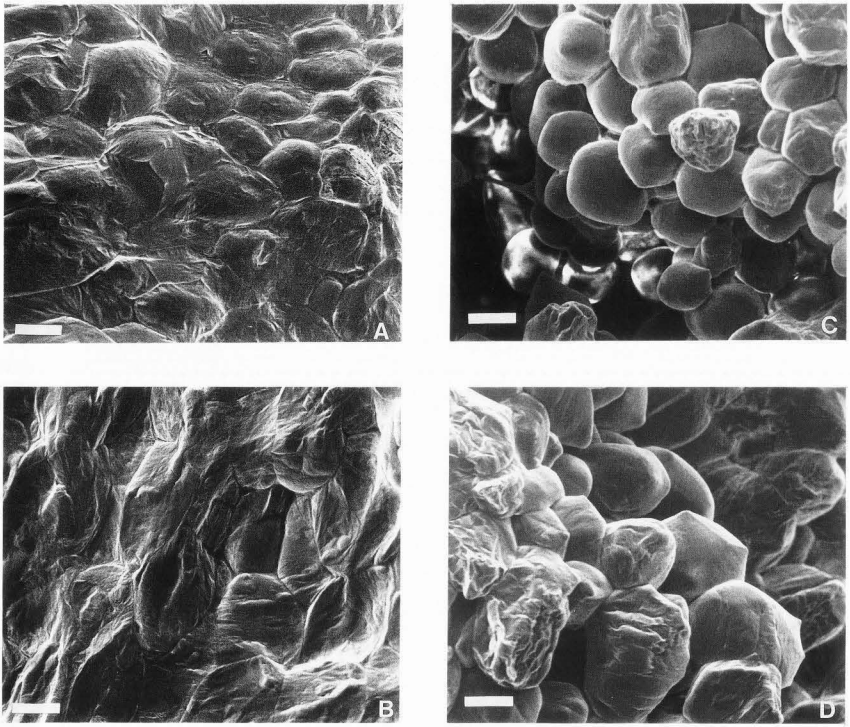


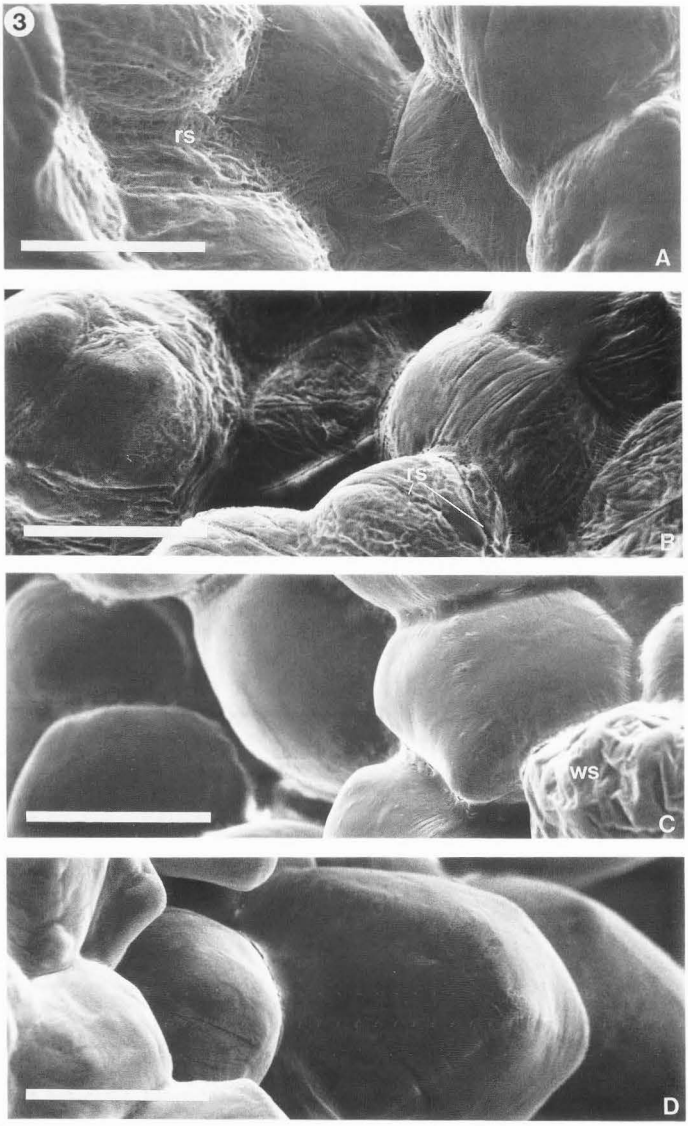
Figure 2. Fracture planes of steam-cooked potato tissue of four cultivars. The fracture planes of the cultivars Nicola (**A**) and Eersteling (**B**) have a flat appearance. Those of the cultivars Irene (**C**) and Eigenheimer (**D**) have a rough appearance. Bar = 0.1 mm.

Cryo-scanning electron microscopy

By using cryo-SEM, fracture planes of non-cooked and cooked potatoes were examined at the same magnification. For non-cooked potatoes, fracturing took place through cells leaving the starch granules intact. This way of fracturing appeared similar for all cultivars studied. Fig. 1 shows a fracture plane of the cultivar (cv.) Nicola. The parenchyma cells had large intercellular contacts and only a few intercellular spaces were present. The numerous starch granules present in non-cooked potato cells were clearly visible.

In contrast to non-cooked potatoes, fracturing of steam-cooked potatoes took place between cells preferentially alongside the cell walls (Fig. 2). Further, significant differences could be observed between fracture planes of the firm-cooking cultivars, Nicola and Eersteling, and the mealy-cooking cultivars, Irene and Eigenheimer. The cultivars Nicola and Eersteling had fracture planes with a generally flat appearance and large intercellular contacts were visible (Figs. 2A and 2B). In contrast to the firm-cooking cultivars, the fracture planes of the cultivars Irene and Eigenheimer appeared to be rougher and intercellular contacts were small (Figs. 2C and 2D).

Higher magnifications revealed more details concerning the cell surfaces which form the fracture planes.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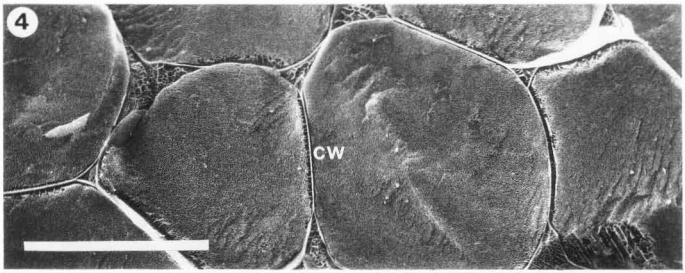

Figure 4 (above). Cells in a cross-section of steam-cooked potato tissue of the cultivar Nicola. The cell walls are visible as clear lines. Bar = 0.1 mm. cw = cell wall.

Figure 3 (facing page). Cells in the fracture planes of steam-cooked potato tissue of the four cultivars. The cultivars Nicola (**A**) and Eersteling (**B**) have cell surfaces showing cracks with reticulated structures. The cultivars Irene (**C**) and Eigenheimer (**D**) have round, turgid cells with smooth surfaces, although one cell shown has a wrinkled surface. Bar = 0.1 mm. rs = reticulated structure; ws = wrinkled surface.

For the firm-cooking cultivars, Nicola and Eersteling, most of the cell surfaces had little folds and cracks (Figs. 3A and 3B). However, some cell surfaces appeared round and possessed many cracks with a reticulated structure. For the mealy-cooking cultivars, Irene and Eigenheimer, cells appeared round and turgid. Frequently, remnants of the shape of the non-cooked cells were recognizable (Figs. 3C and 3D). Most of the cell surfaces were smooth, but some cells had wrinkled surfaces.

Although for cooked potatoes of all cultivars, fracturing took place between cells, the appearances of the cell surfaces were clearly different by comparing the firm-cooking with the mealy-cooking cultivars. To prove the fact that for firm-cooking potatoes fracturing really took place between cells, rather than through cells, fracture planes and cross-sections were compared for each piece of potato. In this case, the cross-sections revealed the structures, which would be visible if fracturing took place through cells. In Fig. 4, a cross-section of the cultivar Nicola is shown. The cell walls between adjacent cells were visible as clear lines. Furthermore, the individual starch granules, present in non-cooked cells, had disappeared and the cooked cells are filled up with gelatinized starch. For all the cultivars, the observed differences between cell surfaces and cross-sections were clear.

Discussion

According to Parlevliet *et al.* (1991) cooked potatoes of the cultivars Nicola and Eersteling were firm and those of the cultivars Irene and Eigenheimer were mealy. This study showed that these cultivars could similarly be classified by comparing their tissue structures by using cryo-SEM. Moreover, this technique revealed clear differences at the cellular level between the firm and mealy potatoes.

For mealy potatoes, it appeared that the intercellular contact was clearly diminished, resulting in cells with a round and turgid appearance. Occasionally, the straight edges and corners of the non-cooked cells were recognizable (Figs. 1, 2C and 2D). Most of the cells had smooth surfaces (Figs. 3C and 3D). In contrast, in firm potatoes large intercellular contacts were preserved and most of the cells were flattened with cracks and folds in the surfaces (Figs. 2A and 2B).

Comparing details of cell surfaces in the fracture planes of the cv. Nicola (Fig. 3A) with cross-sections of the same tissue at the same magnification (Fig. 4) revealed that the structures seen at the cell surfaces in the fracture planes were ruptured cell walls. These structures did not have the reticulated structure of the cell contents (Huang *et al.*, 1990; Pagani *et al.*, 1989) as was shown by cross-sections. Thus, in firm potatoes fracturing took place between cells and the cell walls appeared to be ruptured (Figs. 3A and 3B). In contrast, fracture planes of mealy potatoes showed smooth cell surfaces, which suggested that the cell walls remained intact.

Cells and cell walls visualized by cryo-SEM were less wrinkled in comparison with results of previous SEM-studies, in which chemical fixation and dehydration (Fedec *et al.*, 1977; Moledina *et al.*, 1978) or physical fixation (Huang *et al.*, 1990) followed by freeze-drying was applied as a preparation technique. The few cells with wrinkled surfaces in the fracture planes of mealy

potatoes probably collapsed during preparation of the samples. In comparison with previous studies (Moledina *et al.*, 1978; Huang *et al.*, 1990), little void space between cell walls and gelatinized starch was visible, indicating that cryo-SEM gave less shrinkage of the gelatinized starch.

Cell surfaces of the round cells of potatoes of the cultivars Nicola and Eersteling had cracks and reticulated structures (Figs. 3A and 3B). The nature of the reticulated structure was not clear. It might be composed of amylose, because leakage of amylose was also mentioned by Hoover (1981) and Fedec *et al.* (1977). Secondly, these structures might be formed during breakdown of the cell wall (Haydar *et al.*, 1980; Moledina *et al.*, 1978).

Mealy and firm potatoes differed clearly with respect to intercellular contact, cell shape and cell surface. These differences could be explained by different breakdown of (i) the middle lamella or (ii) the cell wall or (iii) by the starch content of the potatoes.

During cooking, the conditions in potato tissue cause the breakdown of pectin (Hughes *et al.*, 1975), the major component of the middle lamella. This process results in a decline of the intercellular adhesion (Burton, 1989). Therefore, the small intercellular contacts observed between cells in mealy potatoes might be the result of an almost complete breakdown of the middle lamella. Consequently, fracturing between cells could take place easily, resulting in intact cell surfaces. In the potatoes classified as firm, large parts of the middle lamella might still be present, explaining the cracks on the cell surfaces, which occurred upon fracturing. Sterling and Aldridge (1977) also mentioned this difference in intercellular contact after cooking, depending on texture.

Additionally, general cell wall degradation (Burton, 1989) will occur during cooking. The results of our study suggested that the cell walls of firm potatoes might be degraded more depending upon the original structure or on the reaction conditions in the tissue (for example the ionic strength) (Shu-l *et al.*, 1988). The observed cracks and loosening of the cell wall structures could explain the leakage of amylose (Fedec *et al.*, 1977). Further microscopic research (e.g., transmission electron microscopy) may give more detailed information about breakdown of cell walls and middle lamella.

Finally, the amount of starch may contribute to the difference in texture. A previous study indicated that starch concentrations up to 30% enhanced the rigidity of the starch gel (Ring, 1985). Since the mealy cultivars had a higher starch content than the firm cultivars (Table 2), one could imagine that in mealy potatoes the starch gel formed upon cooking was more rigid. This resulted in round cells with a more turgid appearance due to a higher starch swelling pressure (Jarvis *et al.*, 1992) and more resistance to forces exerted during fracturing.

We concluded that differences in texture of cooked potatoes were also visible on an ultrastructural level. The results of this study suggested that the structure of the cell wall, the middle lamella and the starch content are important parameters in determining firm and mealy texture after cooking. Further research will be aimed at the (bio-)chemical characterization of cell walls and middle lamellas of different cultivars.

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Discussion with Reviewers

D.J. Gallant: Your paper was limited by the use of SEM (topography). Considerable additional information (composition) can be obtained by also using the cyto-chemical methods associated with the light microscope. For instance, on the cooked samples one may determine: complementary results on starch granule swelling, amylose leaching, starch reticulation, protein denaturation, middle lamella solubilization, etc. Then, although it should be possible that Moledina *et al.* (1978) have not clearly seen the degree of cell separation on a section of cooked tissue, some methods, however, are able to show such details well.
Authors: Light microscopy in combination with cyto-chemical methods gives a lot of information. In fact, the idea to study fracture planes of cooked potatoes by cryo-SEM was born during an examination of fracture planes, after staining with Lugol and methylene blue, by stereo-microscopy. However till now, we have got more information by observing fracture planes than by cross-sections of cooked potatoes. We intend to do further microscopic research in order to get more detailed and specific information concerning breakdown of cell walls and middle lamellas and leakage of amylose during cooking of potatoes.

D.J. Gallant: A few words on the comparative physiology of the four cultivars should be welcome. What was their respective maturity time?
G. Mazza: Were all potatoes harvested at the same state of physiological maturity?
Authors: The four cultivars can be classified into four maturity types: Eersteling, first early; Eigenheimer, second early; Nicola, early maincrop; and Irene, late maincrop (Parlevliet *et al.*, 1991). This characterization is based on earliness of bulking. Although the time of maturity differs, in this study the four cultivars can be compared, because all potatoes were harvested after maturing.

G. Mazza: How long were the tubers stored at 6°C? Were the potatoes stressed in any way during harvest and storage?
Authors: The potatoes were stored for 9 months. For the different cultivars, fracture planes have been studied after 3, 4 and 8 months of storage. The features of the fracture planes stayed the same. The potatoes were not stressed during harvest and storage.

A.M. Hermansson: Not only chemical fixation and dehydration may alter the structure but also freezing due to ice crystal formation!
Authors: Freezing may alter structures due to ice crystal formation, but in comparison with chemical fixation and dehydration, cryo-methods give better results. The quality of a sample prepared by cryo-fixation depends on the medium and rate of cooling. Our specimens were cooled by plunging into nitrogen slush, which is preferred above liquid nitrogen. Furthermore, we are examining surface features and in that case, cooling rates are less critical (Sargent, 1988).

A.M. Hermansson: Why was such a high accelerating voltage as 15 kV used. The flat appearance with edge effects shown in Fig. 1 is probably due to the high accelerating voltage as well as the smooth surface structures shown at the higher magnifications. With regard to acceleration voltage a compromise has to be made between the high noise level and low electron emission at low accelerating voltages and the loss of surface resolution at high accelerating voltage.
Authors: An accelerating voltage between 10 and 20 kV is normally used for similar SEM investigations (Echlin *et al.*, 1981: Pagani *et al.*, 1989). The edge effects shown in Fig. 1 are the result of etching (at 190 °K): the upper layer of the cell contents is etched, while the cell walls remain, appearing as protruding edges. We agree with you that a compromise has to be made between the disadvantages of low and high accelerating voltages. At the Technical and Physical Engineering Research Service, we have had good experience with accelerating voltages between 10 and 15 kV.

D.J. Gallant: I think that you could remove the key word "fracture plane" because fracturing cannot be controlled in the Hexland system.
A.M. Hermansson: The way potatoes fracture is discussed as an important factor separating mealy and firm potatoes. However the fracture in this work was made in the frozen state. How did unfrozen potatoes fracture?
Authors: As stated in the text, during our research potatoes were broken after steam-cooking. A piece of tissue out of the fracture plane was mounted on a brass stub and the fracture plane was examined. The potato-pieces were not broken in the frozen state, except to look at the cross-section (Fig.4). The key word "fracture plane" is still mentioned, because fracture took place before freezing.

D.J. Gallant: You showed a fracture plane of an uncooked Nicola potato. In Huang *et al.* (1990), differences were shown in the same tuber between center, side, and end regions. Some differences may also exist between tubers of different sizes. In our laboratory, important differences are seen between tubers from sweet potato, yam and malanga. We can observe also some differences in cells around the vascular bundles, although not often described; and generally, on the side region, some cells without any starch granules can appear as protein-rich. Accordingly, are you sure that

nothing else than the firm (non-mealy) and loose (mealy) aspects of the parenchyma cells could be described? Therefore, I consider that only one micrograph given as a reference for the uncooked tubers is not enough. Your work would be particularly improved with a more complete study by comparing, in detail, all the blank samples.

W.M. Hess: One thing that worries me is that the authors refer to specific micrographs as if that is the only observation. Normally when SEM studies are conducted the micrographs shown are the average example of many observations. I assume that was the case with this study.

It is stated that five hand peeled potatoes of each cultivar were steam-cooked with demineralized water. How many were examined with the freeze-fracture SEM procedures?

Cell structure can be very different from different regions of tubers and at different depths. Freezing is very different at different depths. They indicated that a piece of potato from phloem storage parenchyma was mounted on a brass stub, where did it come from?.

Authors: Your assumption that the micrographs shown are the average example of many observations is correct. The tubers used were taken at different periods during storage. The observations of each cultivar appeared very similar and representative micrographs are shown.

We used cryo-SEM to reveal whether there is a relationship between texture and cellular structure of cooked potatoes. With respect to intercellular contact, two extreme forms of texture showed a clear difference in cellular structure of the cooked tissue. The cellular organization of the non-cooked tissue with respect to the intercellular contact was comparable for the four cultivars. So, only one figure of fractured non-cooked potato tissue is shown to illustrate the difference in intercellular organization between non-cooked and cooked tissue.

For each cultivar many potatoes have been processed for different purposes. So, we have a good idea of the variations in texture for each cultivar and representative cooked potatoes were examined by cryo-SEM. Furthermore, we have also studied the fracture planes of cooked potatoes by stereo-microscopy, after staining with Lugol and methylene blue. This technique gave the same type of information about the three-dimensional cellular organization. So, our conclusions are not based on only a few SEM observations.

Potatoes are not homogeneous and the cellular organization may differ with respect to the different tissues. The largest part of potato tissue consists of the internal phloem storage parenchyma tissue. We decided to use this well-defined tissue region for a comparative microscopic and biochemical study between cultivars. For cryo-SEM, the piece of phloem storage parenchyma was always taken from the middle region in the potato. We agree with you that other tissues also may be of interest.

In this study, fracturing took place before freezing, thus the surface features of the frozen tissue are examined. So, the effect that freezing is different at different depths is not under discussion.

G. Mazza: As the degree of cooking was not determined, is it possible that the perceived textural differences are related to differences in degree of cooking or "doneness"? The authors suggest three possible reasons for the observed difference between the mealy and firm potatoes with respect to intercellular contact, cell shape and cell surface. These are: structure of cell wall, the middle lamella and the starch content. With respect to cell wall, it is generally accepted that the cell wall degenerates during cooking. Thus, for the texture of cooked potatoes to be different, either the cell wall of mealy and firm potatoes has to be different or the rate of cooking for the two types of potatoes has to be different. Since the cooking time used in this study was the same (30 minutes), is it possible that the observed textural differences reflect differences in cooking rate of the two types of potatoes? Similarly for the middle lamella, why would the middle lamella of mealy potatoes break down more completely than for firm potatoes?

Authors: The perceived textural differences cannot be explained by a difference in degree of cooking or cooking rate. For example, when potatoes of the cv. Nicola are steam-cooked for one hour or more, they do not get the texture of mealy-cooking potatoes.

A difference in breakdown of the middle lamella may be related to texture. This may be the result of a difference in reaction conditions (pH, ionic strength) in the tissue during pectin breakdown or of a difference in structure of the pectin. Chemical characterization of cell wall and middle lamella will prove whether our hypothesis is correct. These results will be dealt with in a separate paper.

D.J. Gallant: On Figs. 2C and 2D, it is evident that fracturing occurred alongside the cell walls, particularly because of the solubilization of the middle lamella. On Figs. 2A and 2B, it is clear that the middle lamella remained. Consequently, structure was harder and my own interpretation of your photographs is, on the contrary, that fracturing occurred through the cells!

Authors: Evidence for the fact that in firm-cooking potatoes fracturing also took place between cells rather than through cells, is given in Fig. 4. Potato tissue broken before freezing showed cell surfaces with little folds and cracks after etching (Fig. 3A). The same tissue broken after freezing showed cellular contents (reticulated structures), while the cell walls between adjacent cells were visible as clear lines (Fig.4).

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