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Comparison of Different Microscopic Methods for the Study of Starch and Other Components within Potato Cells

Malin E. Sjoo[1*] • Ann-Charlotte Eliasson[1] • Karin Autio[2]

[1] Division of Food Technology, Department of Food Technology, Engineering and Nutrition, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden
[2] VTT Biotechnology, P.O. Box 1500, FIN-02044 VTT, Finland

Corresponding author: * **Malin.Sjoo@food.lth.se**

ABSTRACT

The microstructure of potato was studied by different microscopic techniques. The methods used were bright field (BFM), fluorescence (FM), confocal laser scanning (CLSM), scanning electron (SEM), and transmission electron (TEM) microscopy. The focus was on starch and structure of cells before and after cooking. Also components such as cell walls, protein, and pectin were identified by using different staining procedures. The use of different methods improved the interpretation of results and facilitated the identification of possible artifacts. The distribution of starch and proteins differed between tuber parts but also between adjacent cells. The amount of starch in individual cells clearly affected the shape of cells in cooked potatoes. Starch and cell shape were clearly visualised by all methods but TEM, which due to the high magnifications was better suited for studying cell walls and heat promoted separation of cells. CLSM was the preferred method for studying starch with minimal impact on the cell structure since artificial effects could be induced during preparation.

Keywords: cell wall, cooked, microscopic techniques, microstructure, raw, tuber

INTRODUCTION

The potato is an important staple food worldwide and is sold to the consumer either as raw tubers or in a processed form. Since starch is the major component on the basis of dry matter, and has great contribution to the texture of a cooked potato, it is important to study starch behaviour in raw and cooked potato tissue. However, also other components such as proteins and cell walls are of interest when comparing raw and cooked potato tissue. To study effects of processing on starch in potatoes, thermal analysis methods as differential scanning calorimetry (DSC), rheological methods, x-ray diffraction, and several other methods could be used. Such methods providing numerical data may productively be complemented with imaging techniques. The means by which cell structure and different tuber components affects textural and thermal properties of potato samples is still not fully understood.

Numerous microscopic techniques, including instruments as well as preparations and stains, have been used to study botanical tissues and starches. An overview of microscopic techniques for food structure analysis has been presented by Kaláb *et al*. (1995). For the study of cereal foods, untreated as well as heat treated, starch, proteins and cell walls have been displayed by different methods (Yiu 1993; Autio and Salmenkallio-Marttila 2001). Regarding potatoes, the work generally has been focused either on the tissue or on starch, which has been thoroughly investigated in the isolated form. Some basic works on potato tissue (Reeve *et al*. 1969; Fedec *et al*. 1977), although mainly describing uncooked tubers, have shown that the dry matter content, and concurrently the starch content, is increasing from the centre of the tuber to the outer parts. This was also confirmed by Karlsson and Eliasson (2003). Several microscopic studies concerning cooking behaviour of potatoes have also been performed, evaluating the cooking method (Huang *et al*. 1990; van Marle *et al*. 1992, 1997; Thybo *et al*. 1998; Lamberti *et al*. 2004), or other characteristics of the potatoes used (McComber *et al*. 1994; Shomer 1995). Most of these previous studies focus on cell walls and shape of cells, whereas in this study the main focus is on starch as a cell component. Furthermore, in most studies one or few different microscopic techniques were used. Here well-known and complementing methods were used to study the distribution of starch and proteins in raw potatoes, and the impact of gelatinised starch on cells in cooked potatoes, along with other components within potato tissue.

Techniques such as bright field microscopy (BFM) and fluorescence microscopy (FM) are commonly used for studying plant tissues, since they allow selective staining of different chemical components. These methods require the same pre preparations. The main preparation techniques for BFM and FM are fixation, dehydration and plastic embedding, or freezing, with or without chemical fixation. Efficient stains for starch, protein and cell walls have been identified for BFM (Flint 1988) and FM (Fulcher *et al*. 1989), respectively. The use of confocal laser scanning microscopy (CLSM) in food research has been described previously (Heertje *et al*. 1987; Blonk and Aalst 1993; Vodovotz *et al*. 1996; Ferrando and Spiess 2000; Dürrenberger *et al*. 2001). Several different stains are available for the study of starch and tissue, the most widely used are Safranin O for visualising cell walls and starch (Gray *et al*. 1999; Dürrenberger *et al*. 2001; van de Velde *et al*. 2002), Acridine Orange for starch, cell walls and protein (Francisco 1989; Adler *et al*. 1994; Alvarez *et al*. 2000; Suutarinen *et al*. 2000), and Acid Fuchsin, a suitable stain for protein (Dürrenberger *et al*. 2001; Lamberti 2003).

Scanning electron microscopy (SEM) normally requires dry samples. For SEM investigations of tissue samples containing starch, cryo fixation has been recommended above chemical fixation (Uwins *et al*. 1993; Kaláb *et al*. 1995). Preparation of potatoes for SEM may include freezing in liquid freon and freeze drying (Huang *et al*. 1990), or freeze-substitution followed by critical point drying (CPD) (Uwins *et al*. 1993) or freeze drying (Fedec *et al*. 1977). Alternatively, also chemical fixation with glutaraldehyde (GA) followed by dehydration and CPD has been used (Uwins *et al*.

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1993; Valetudie *et al*. 1995; Thybo *et al*. 1998; Valetudie *et al*. 1999). Also cryo-SEM (van Marle *et al*. 1992) and environmental SEM (Uwins *et al*. 1993) have been used for the study of potato tissue. For transmission electron microscopy (TEM) the normal preparation procedure is fixation with GA, post fixation with osmium tetroxide, dehydration and plastic embedding (Shomer 1995; van Marle *et al*. 1997).

The microstructural effect of cooking and general structure of potato cells was evaluated using a number of different sample preparation and microscopy techniques. The objective of this work was not to make a full evaluation of available methods, but to compare raw and cooked potato tissue samples, and some different microscopic methods. Preparation methods have mainly been selected to preserve starch, even though different staining systems were used to also visualise components other than starch. The use of supplementary methods was designed to yield a better understanding of the tissue structure before and after starch gelatinisation.

MATERIALS AND METHODS

Potato material

The firm cooking variety Asterix and the rather firm cooking variety Bintje were grown under similar conditions and harvested in October years 1999 (SEM), 2000 (BFM, FM and CLSM) and 2001 (TEM, only Asterix). Tubers were stored 4-6 months at 6°C before being studied by the selected microscopic methods. Potato tubers were supplied by Solanum AB, Kävlinge, Sweden. Samples from raw and cooked tubers were analysed. Cooking procedures were selected for practical reasons and differed slightly. For SEM and TEM (performed in Lund, Sweden) potatoes were boiled soft in water, the consistency was checked mechanically with a metal stick and the cooking time was approximately 20 min. Tubers used for BFM, FM and CLSM (performed in Espoo, Finland) were steam cooked to a center temperature of 80°C. This temperature has previously been shown by DSC to completely gelatinise starch within potato tissue samples, and furthermore the water available within cells was proven to be enough for complete gelatinisation (Karlsson and Eliasson 2003).

Stem end storage parenchyma has previously been reported to be more representative for different varieties than bud ends (McComber *et al*. 1987) and was therefore selected for the first study (SEM). To be able to compare different tissue zones before and after cooking, samples from four different tissue zones were examined by BFM and FM, namely pith, cortex and storage parenchyma from stem and bud ends, respectively. Based on these results pith and cortex tissue were subsequently selected for CLSM. Finally, since the dry matter content was largest in cortex tissue and the variation between cultivation years and individual tubers also was smallest for this tissue zone as has been reported previously (Karlsson and Eliasson 2003), cortex was selected for TEM experiments. The high dry matter content was considered to be advantageous for the high magnifications used with TEM. The methods used within this work can only be compared in general and not in detail, since the different cultivars, growing seasons and tissue parts may have an impact on the results. However, the information achieved provides a basis for method selection for the study of potato tissue.

Bright field microscopy (BFM)

Samples from selected tissue zones were extracted with a cork borer and cut into 5 mm cubes. The cubes were fixed in GA, 1 g/100 ml 0.1 M phosphate buffer (pH 7), and dehydrated in a graded ethanol series prior to infiltration and polymerization using a Historesin Embedding Kit (Jung, Germany). Thin sections, 4 µm, were cut (Leica 2055 Autocut Rotary Microtome) and stained. An alternative preparation was performed by freezing in liquid nitrogen after fixation in GA. Samples were then conditioned at –40°C for 10 min and stored over night at –18°C before cryo sectioning to 7 µm. Samples for BFM were stained with Lugol's iodine (I_2/KI) solution, Light Green (0.01 g/100 ml), or Ruthenium Red (0.2 g/100 ml), staining starch blue, protein green, and pectin pink, respectively. An Olympus BX-50 microscope (Olympus, Japan) was used together with AnalySIS Pro 3.1 software (Soft Imaging System GmbH, Germany). Four samples from each tissue zone in four different raw and cooked tubers were analysed.

Fluorescence microscopy (FM)

Preparation and sectioning of samples were performed as for BFM with the same material. The stains used for FM were Calcofluor White (0.01 g/100 ml) and Acid Fuchsin (0.1 g/100 ml), staining cell walls blue and protein orange, respectively. An Olympus BX-50 microscope (Olympus, Japan) was used together with AnalySIS Pro 3.1 software (Soft Imaging System GmbH, Germany).

Confocal laser scanning microscopy (CLSM)

The same sample material, raw and cooked, was used as for BFM. Slices of 2 mm height with the sides 10 × 10 mm were cut from pith and cortex tissues of the tubers. These were stained with Acridine Orange, 0.1 g/100 ml 0.1 M phosphate buffer (pH 7). A BIO RAD RadiancePlus CLSM with Nikon Eclipse E800 microscope was used and samples were excited by an Argon laser beam at 488 nm. Emitted light was selected by two filters, detecting starch at 500-560 nm (green) and cell walls and proteins at >600 nm (red).

Scanning electron microscopy (SEM)

From storage parenchyma tissue cylinders with a diameter of 3.5 mm were punched and cut into 1-3 mm thick slices. The samples were frozen in liquid nitrogen and freeze-dried over night. Samples were stored in a desiccator before analysis. Both broken and cut samples were mounted onto aluminum specimen stubs, coated with Au/Pd in a BIORAD Polaron E5400 High resolution sputter coater (Polaron Range, East Grinstead, U.K.) and examined in a JEOL JSM-T 330 Scanning microscope (JEOL, Tokyo, Japan) at 10 kV. Four samples from each of two raw and cooked tubers were prepared.

Transmission electron microscopy (TEM)

From cortex tissue samples were punched and cut into cubes with the side 1 mm. The cubes were immediately immersed in GA 5 g/100 ml 0.1 M cacodylate buffer (pH 7.2). Post fixation was carried out in osmium tetroxide and samples were dehydrated in a graded ethanol series. Uranyl acetate was added prior to infiltration and polymerization using Epon epoxy resin. After ultra-thin sectioning, samples were stained with uranyl acetate and lead citrate. The sections were examined in a JEOL 1200 EX transmission electron microscope (JEOL, Tokyo, Japan) at 80 kV. Four samples from each of two raw and cooked tubers were prepared.

RESULTS AND DISCUSSION

General

Most starch and protein were located in the outer parts, cortex, of the tuber, whereas the centre, pith, contained the lowest amounts of these components, and the parenchyma tissue from stem and bud ends mostly resembled cortex tissue (**Fig. 1**). This was seen by BFM and FM, and has also been confirmed by measuring the dry matter contents of these tuber parts (Karlsson and Eliasson 2003). The differences between pith and cortex were also clearly detected by CLSM. The two varieties Asterix and Bintje did generally not differ in this aspect.

Only small differences were detected between the two varieties (**Table 1**). Most noteworthy was that Asterix cells contained more starch in all tissue parts, whereas the protein content tended to be higher in Bintje. Furthermore, starch in Asterix pith tissue was poorly gelatinised in some tubers; although in Bintje poorly gelatinised starch was only occasionally found. Starch in other parts of the tuber was completely gelatinised in both varieties.

A rather limited amount of tubers and samples were used for the analyses. However, samples of the same bat-

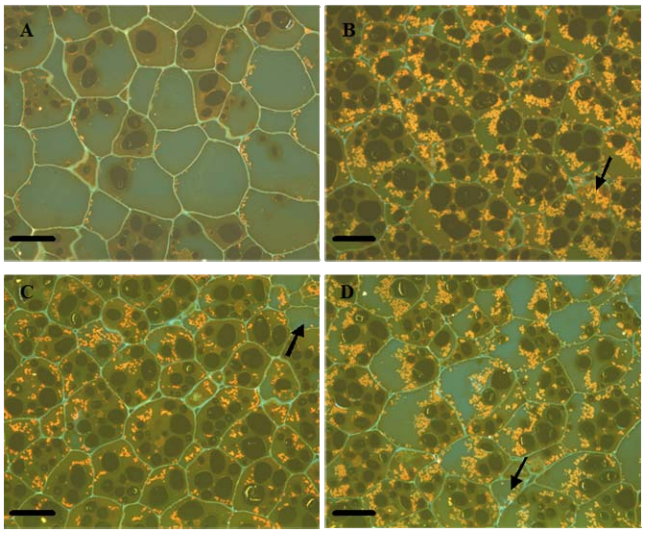

Fig. 1 Overview of different tissue parts in Asterix potato as viewed by FM. Thin lines represent cell walls, starch granules are seen as dark particles, and protein are seen as small light particles. (**A**) pith, (**B**) cortex, (**C**) stem end storage parenchyma, (**D**) bud end storage parenchyma. Arrows point at starch deficient cells. Scale bars: 100 µm.

Table 1 Properties found for the two varieties Asterix and Bintje. Results are estimated from micrographs and not numerical determinations, and are valid for both raw and cooked samples and in all tissue parts if not stated otherwise. Both + (positive) and – (negative) results as referring to the statement in the left column are scaled. To point out clear differences, especially regarding amounts, ++ or +++ is used, results that are merely a weak tendency are marked by a parenthesis ().

	Asterix	Bintje
Presence of starch deficient cells	+	+ +
Amount of starch in cells	+ + +	+ +
Presence of small starch granules	+	+ +
Presence of large starch granules	+ +	+
Complete gelatinisation of starch (pith)[a]	- - + [d]	- + + [d]
Complete gelatinisation of starch (other tissue parts)[b]	+ + +	+ + +
Amount of protein	+ +	+ + +
Cell size (pith)[a]	(++)	(+)
Cell size (other tissue parts)[b]	(+)	(++)
Decreased intensity of cell wall stain after cooking[c]	nd[e]	(+)
Cell separation after cooking	(+ +)	(+)
Equal heterogeneous pectin distribution	+	+

[a] valid for pith tissue only, [b] parenchyma tissue from stem end, bud end, and cortex, [c] decreasing from inner to outer parts, [d] both positive and negative results obtained, [e] nd -not detected

ches have been analysed by other methods, mainly DSC, as reported elsewhere (Karlsson and Eliasson 2003). Since these previously reported results strongly support the present study, the results presented for the varieties (**Table 1**) were regarded as valid for these batches, and the comparison of methods (**Table 2**) was relevant.

Starch granules in raw tubers

Starch granules were unevenly distributed both within and between cells (**Fig. 1**), and not all cells contained starch, a result found regardless of the method used. The presence of starch deficient cells was not considered as an artifact from preparations, since this was observed by all methods used, including CLSM where water was not removed and samples were optically sectioned, i.e. cells were still closed by cell walls. Therefore, it could be concluded that there are cells in all tissue parts that do not contain any starch granules, although most starch deficient cells definitely are found in pith tissue. Nevertheless, some of these empty cells may be results of preparation, CLSM and plastic embedding being the methods best preserving starch within the cell structure, whereas freeze drying for SEM, BFM and FM introduced loss of starch granules during cutting (micrographs not shown).

The different methods gave supplementing information about the raw granules (**Fig. 2**). By iodine staining and BFM, the growth rings of the starch were visualised. Considerably sharper growth rings were revealed by starch granules in pith compared to other tissue parts and furthermore, pith starch was stained more purple by iodine whereas bluer in other parts (**Fig. 3**). These effects were seen in both varieties and indicate higher amylopectin content in pith starch. With CLSM also starch granules covered by cell walls were seen (**Fig. 2E**). Starch granule surfaces as studied by SEM mostly displayed a smooth surface, although occasionally material seemed to be attached to the granule surface (**Fig. 2G**). This could be an artifact, although rest material from the amyloplast membrane or from ruptured cell walls is a more plausible explanation.

Starch and cell separation in cooked potatoes

After cooking, the uneven distribution caused gelatinised starch to fill some cells completely, whereas in other cells, the gelatinised starch formed an entity distanced from the cell walls (**Figs. 2, 4**). This finding was in line with previous works (Fedec *et al*. 1977; McComber *et al*. 1994). As expected, a comparable number of starch deficient cells were detected after cooking as in raw samples. Potato starch granules are known to possess a high swelling capacity (Swinkels 1985) leading to close packing of gelatinised starch in filled cells. In other terms, the gelatinised starch in individual filled cells could be described as analogous to rather concentrated heated potato starch dispersions, whereas in non-filled cells being more diluted. The appearance of gelatinised starch stained by iodine in potato cells equals the appearance of potato starch pastes as described by Hermansson *et al*. (1996). Amylose, stained blue, is present in the middle of and between swollen granule structures composed of mainly amylopectin and stained purple.

No breakage of cell walls caused by cooking was detected by any microscopic method and generally no leaked

Table 2 The different methods applied and their usefulness for microstructure analysis of potato tissue samples relative to each other on a scale from + + + (excellent) to – (poor) for microscopic techniques, and from * * * (excellent) to – (poor) for pre preparations.

	BFM	FM	CLSM	SEM	TEM	Plastic sections	Cryo sections[a]
Smallest resolvable size, nm	10^2	10^2	10^2	10^1	10^{-1}	na[b]	na[b]
Overall structure	+	+ +	+ + +	+	-	* *	*
Starch distribution	+	+	+ + +	+	-	*	-
Starch granules	+ + +	+	+ + +	+ ++	-	* *	-
Starch gel	+ + +	+	+ +	+	+	* *	*
Cell shape	+ +	+ +	+ + +	+	-	* *	*
Cell wall separation	+	+	+	+ +	+ + +	* * *	*
Cell walls	+	+	+	+ +	+ + +	*	-
Protein distribution	+ +	+ + +	+ + +	-	+	*	-
Proteins	+	+	+	-	+	* *	*
Other organelles	-	-	-	-	+	-	-

[a] only cocked samples, [b] not applicable

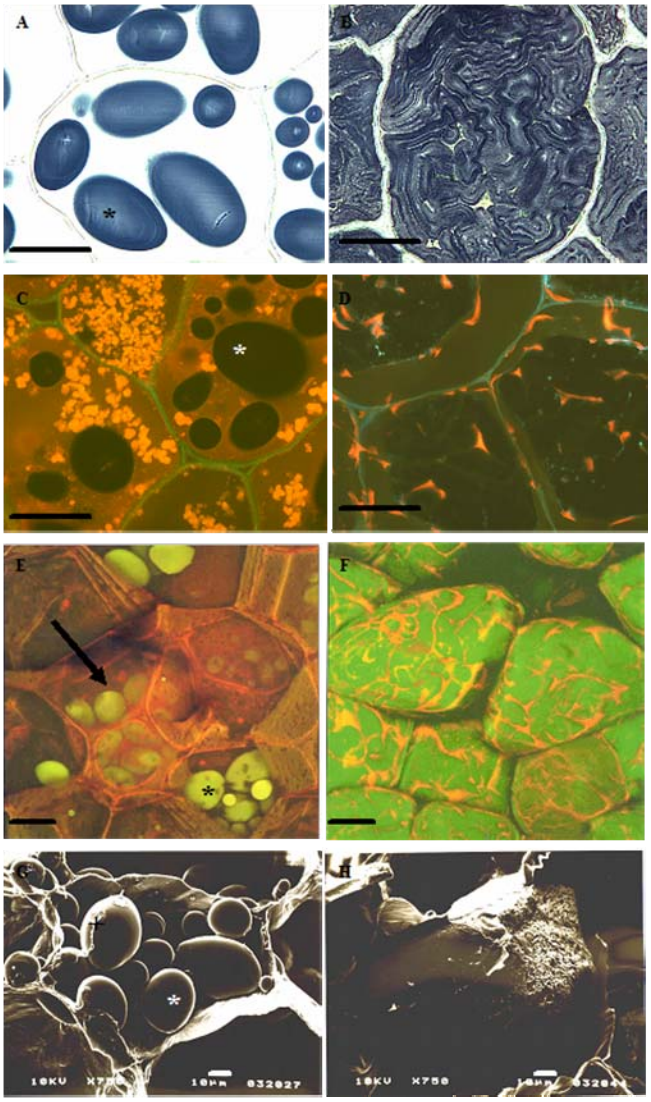

Fig. 2 Cells with starch in raw (A, C, E, G) and cooked (B, D, F, H) potatoes, respectively. Starch granules are marked *. In cooked potatoes the disrupted starch granules are seen as a matrix surrounding protein (**B, D, F**) or as a sponge-like structure under a ruptured cell wall (**H**). The arrow mark an intact (not cut or disrupted) cell (**E**). Rest material attached to the granule surface (probably from the amyloplast membrane or from ruptured cell walls) is marked + (**G**). (**A, B**) BFM, (**C, D**) FM, (**E, F**) CLSM, (**G, H**) SEM. Scale bars: 50 µm (**A-F**), 10 µm (**G, H**).

starch was found between cells. It was clear that the gelatinised starch affected the shape and stabilised the structure of filled cooked cells (**Fig. 2**). The intercellular contacts between cells were reduced by cooking. Cell walls in cooked samples were more separated and swollen as compared with raw samples (**Fig. 5**). The separation of cells after cooking was clear and best analysed by TEM, although also seen by the other methods (micrographs not shown). As a reason for cell separation a swelling pressure exerted by gelatinised starch (Jarvis *et al*. 1992) has been proposed. In filled cells this pressure may have an impact; however, cell separation is seen also between non-filled cells (micrograph not

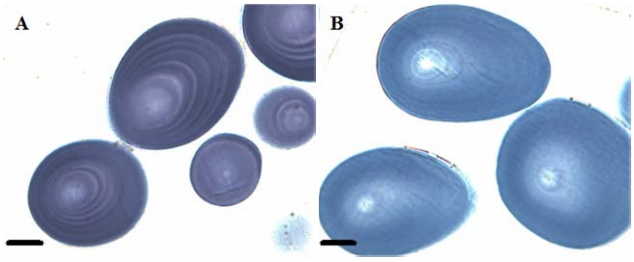

Fig. 3 Starch granules in different parts of raw Bintje potato as viewed by BFM. (**A**) pith, (**B**) cortex. Scale bars: 10 µm.

shown). A more plausible explanation is that the rounder shape of cells after cooking is influenced by the gelatinised starch although being enabled by cell separation. This is in line with previously presented explanations (Warren and Woodman 1974; Reeve 1977).

Cell walls in raw and cooked tubers

Cell walls displayed an irregularly shaped surface both in raw and cooked samples as seen with TEM (**Fig. 5**). In both filled and non-filled cells shrinkage of cell walls in plastic sections of cooked samples was sometimes detected (**Figs. 2, 4**). These effects were probably introduced during dehydration. Conversely, the freeze preparation for SEM caused cell walls of non-filled cells to collapse around the content (**Fig. 4C**). These collapsed cells were not present in raw potatoes or among filled cooked cells, hence, it is probably a combined effect of pectin breakdown facilitating separation of cell walls, the amount of gelatinised starch in cells and the freeze preparation technique.

A further change that was detected within the cell wall was the less distinct staining with Calcofluor for FM after cooking (**Table 1**). The effect was more pronounced in the outer part of the tubers, which was logic considering heat transfer when the whole tubers were cooked. Furthermore, Bintje was more affected than Asterix (not shown), indicating chemical differences within the cell walls due to the variety. Degradation of cell walls during cooking was also indicated by CLSM, since cell walls in raw potato tissue were exhibited clearly red (>600 nm), whereas in cooked potatoes, cell walls were either not stained by Acridine Orange or detected in the region 500-560 nm (green) (**Fig. 2E, 2F**).

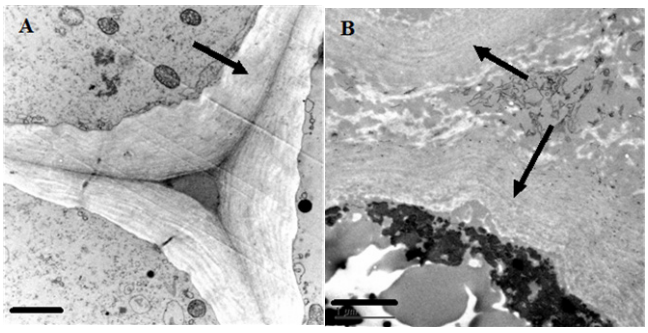

Fig. 5 Cell walls indicated by arrows in (A) raw and (B) cooked potato as viewed by TEM. Scale bars: 1 µm.

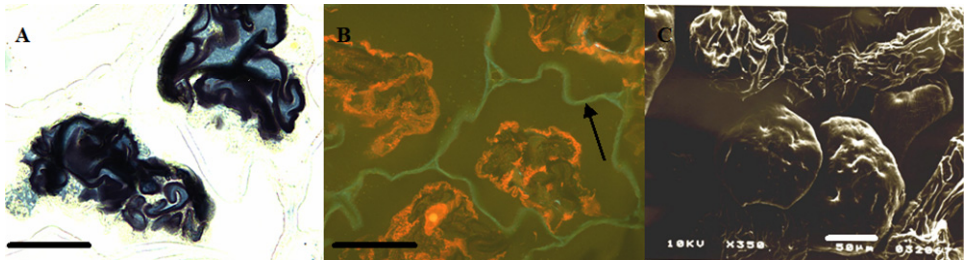

Fig. 4 Gelatinised starch in cells with low starch content. The arrow indicates cell wall shrinkage due to preparation. (**A**) BFM, (**B**) FM, (**C**) SEM. Scale bars: 50 µm.

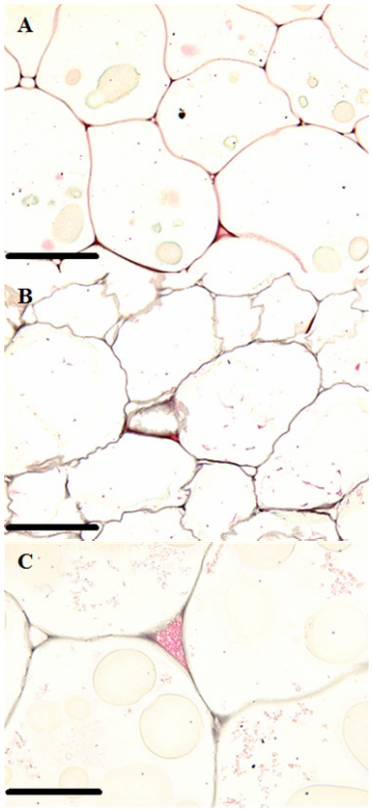

Fig. 6 Pectin seen as darker areas of cell walls in raw (A, C) and cooked (B) potato as viewed by BFM. Scale bars: 100 µm (**A, B**), 50 µm (**C**).

Between cell walls, in the middle lamellae, pectin is located as seen both before and after cooking (**Fig. 6**). It was not evenly distributed within the samples, and the total amount of pectin seemed not to differ between tissues. However, only unesterified pectin is stained by Ruthenium Red (Sabba and Lulai 2002) and cell wall pectin remains unstained. The study of pectin was not a main topic of this work, but rather a complement to define the different structures within the potato tissue.

Protein distribution

In raw potatoes, protein was mainly located close to cell walls, whereas in cooked potatoes the majority of the protein was found within or adjacent to the starch gel matrix, mainly to the leached amylose fraction (**Figs. 2, 4**). Protein staining with Acid Fuchsin for FM correlated to BFM staining with Light Green, confirming this was protein, and the same structures were also found with CLSM and TEM. The most abundant protein in potatoes is patatin, a group of glycoproteins stored in vacuoles in raw tuber cells (Sonnewald *et al*. 1989). The sugar portions of glycoproteins are stained by Rutenium Red (**Fig. 6C**) revealing that protein detected by other methods includes patatin. In some occasions, protein was also detected in some intercellular spaces, which was previously found also for strawberry tissue (Suutarinen *et al*. 2000). Protein can also be studied by SEM (Nuss and Hadziyev 1980), although this requires chemical fixation of samples.

The fact that protein is located within the starch gel matrix indicates that interactions between starch and protein could occur. These potential interactions may explain effects on starch gelatinisation in the presence of potato protein as determined by DSC (Suzuki and Hizukuri 1979; McComber *et al*. 1988).

Further methodological aspects

1. Overview of used methods

From **Table 2** it is clear that the most useful method for studying potato tissue samples is CLSM, being practical or even superior for most structures examined. CLSM, BFM and FM are important methods for potato samples, since they allow examination of a large area. Overview micrographs are important due to the huge variations between tissue parts and individual tubers making potato a difficult object to analyse. These methods also can provide details, although for high magnifications SEM or TEM is required. SEM and TEM complement each other well, SEM being more suitable for analysing starch whereas TEM better visualises smaller components, and for studies of cell walls both supplement the other methods used. BFM and FM are equally good; the difference between these methods is mainly the stains that are used to study different components. Of the two preparation methods used for BFM and FM, plastic embedding introduced least artifacts and was valuable for both raw and cooked samples. Cryo sections were useful for the study of cooked sampled, in particular for gelatinised starch.

2. Preparation for BFM and FM

Cryo sections displayed extensively more damaged cell walls as compared with plastic sections, when viewed by BFM and FM (**Figs. 2, 7A**), even though occasionally shrinkage of cell walls in plastic sections occurred. In addition, gelatinised starch appeared to be somewhat smeared, principally in cryo sections of non filled cells. These artifacts may result from mechanical and thermal stress during cryo sectioning. The effects were most pronounced in raw samples and non filled cells in cooked samples, since these structures were not compact. Nevertheless, when samples are prepared in the same way useful information could be achieved. Results from cryo sections confirmed results regarding starch distribution and gelatinisation.

3. Preparation for SEM

Gelatinised starch has been reported to produce a sponge-like structure (McComber *et al*. 1994; Valetudie *et al*. 1999). This structure was seen also in this investigation (**Fig. 2H**), although in some cells the structure was markedly different (**Fig. 7B**). It could be concluded that some of these structures resulted from ice crystals formed during freezing of samples. The degree of ice-crystal damage differed between samples; **Fig. 7B** shows a seriously damaged starch gel. Raw potatoes did not display these ice crystal damages, revealing that gelatinised starch was more susceptible to freeze injuries. Being aware of potential ice crystal damage, cooked samples with gelatinised starch could be analyzed by SEM.

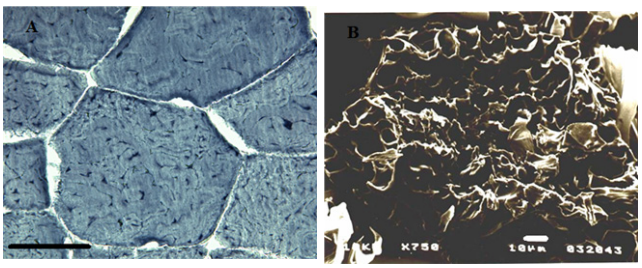

Fig. 7 Artifacts from different methods. (**A**) smeared cell walls and gelatinised starch in cryosection for BFM, (**B**) gelatinised starch damaged by ice crystal formation in a SEM micrograph. Scale bars: 50 µm (**A**), 10 µm (**B**).

CONCLUSIONS

Informative micrographs revealing different aspects of potato tissue were obtained by all methods used as seen in **Table 1**. A three dimensional effect was provided with CLSM and SEM, whereas the other methods offered two dimensional images. BFM provided clear views of starch, and also of protein and pectin. The FM investigation mainly confirmed results from BFM, although cell walls and protein were better visualised by this method. Starch granules, cell walls and protein were clearly displayed by CLSM. Cell walls and starch were also seen in the SEM micrographs. The high magnification of TEM enabled various cell components to be identified. However, the focus of interest was on starch and cell walls.

Increased knowledge about cell separation, starch distribution and filling of cells can be obtained by using these methods and may aid interpretations of texture measurements of potato samples. Furthermore, starch granule morphology along with distributions of starch and other components, mainly protein, and the contact between them, can help explain results obtained from thermal analysis.

The smallest resolvable size for the methods described range from 0.2 to 300 nm (Davis and Gordon 1984). A method not mentioned here is atomic force microscopy (AFM), which can be used to measure topography at the nanometer scale (Baker *et al*. 2001). This method is advantageous for analysing specific details such as starch granule structure or cell walls, but not to for the study of the tissue on a larger scale.

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