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X-ray scattering study on potato (*Solanum tuberosum* L.) cultivars during winter storage

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Abstract

The nanometer range structure of potato (*Solanum tuberosum* L.) tubers was examined by wide-angle, small-angle and ultra small-angle X-ray scattering methods. The crystallinity of starch, the lattice constants of the hexagonal lattice of amylopectin, the average crystallite size in the direction [100], the lamellar distance and the thickness of lamella stacks were determined from the data. A new achievement presented in this paper is that reasonable results for these parameters of potato starch were obtained by carrying out experiments on slices and mashes of raw potato tubers. The effects of sample preparation were also investigated by doing experiments on air-dried and re-hydrated potato samples, and on isolated potato starch as well. Changes in the structure of three different cultivars grown in Finland (*S. tuberosum* cv. Satu, Saturna and Lady Rosetta) were studied monthly from August to May. The physiological ageing caused changes in the crystallinity and in the crystal structure. The mean values (± SD) were determined from the data measured between September and January (30 samples). The lattice constants $a = 18.4 \pm 0.06$ and $c = 10.4 \pm 0.04$ Å, the crystallinity of starch 24 ± 2% and the crystallite size 118 ± 10 Å were obtained. The lamellar distance was 97 ± 3 Å and the thickness of lamella stacks 513 ± 6 Å. The structural parameters did not vary significantly between Satu, Saturna and Lady Rosetta. For comparison, two cultivars grown in the Netherlands were studied in December. The Dutch cultivars showed the same structural parameters as the Finnish cultivars.
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Keywords: Potato; Starch; X-ray scattering

1. Introduction

The potato (*Solanum tuberosum* L.) is one of the staples in human diet throughout the world, and it is an important raw material for starch industry as well. As a result of wide breeding programs, various cultivars are available for food applications. Growing conditions together with genetic origins have a great effect on the tuber development. Furthermore, ageing during the storage affect the potato quality in processing (Cottrel, Duffus, Paterson, & Mackay, 1995; Liu, Weber, Currie, & Yada, 2003; Svegmark et al., 2002; Tester, Debon, Davies, & Gidley, 1999).

Potato tubers contain mainly water (70–80%) and starch (10–25%). The potato starch (B-starch) consists of linear amylose and branched amylopectin, which are polymers of glucose units linked through α-D-(1 → 4) glucosidic bonds, amylopectin having also α-D-(1 → 6) linkages. The starch is in the form of granules 15–100 μm in diameter. Various models of the starch granule structure on the basis of electron microscopic studies have been proposed (Gallant, Bouchet, & Baldwin, 1997; Tamaki, Teranishi, Hisamatsu, & Yamada, 1997). The semicrystalline granules have internal lamellae with a distance of 9 nm, where side-chains of branched amylopectin are suggested to form crystallites, and amylose (~20%) is the main amorphous component (Imberty, Buleon, Tran, & Perez, 1991; Jenkins, Cameron, & Donald, 1993). Starch has also been described as a side-chain liquid-crystalline polymer (SCLP), where amylopectin side-chains form double helices, and they are still twisted into a superhelix forming lamellae (Waigh, Gidley, Komanshek,

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& Donald, 2000; Waigh, Perry, Riekel, Gidley, & Donald, 1998).

The crystalline properties of isolated starch have been widely investigated using wide-angle and small-angle X-ray scattering (WAXS, SAXS) methods. The lattice constants for crystalline B-starch have been reported in several references. The crystal structure having the hexagonal unit cell with $a = b = 18.3$ and $c = 10.5$ Å, or $a = b = 18.5$ and $c = 10.4$ Å (Imberty & Perez, 1988 and the references therein) are generally accepted. The distance in the direction [100] is equivalent to the interhelical distance of the starch molecules. The studies have shown that the water content of a starch sample has a great effect on the scattering intensities of the reflection 100 and on the intensities arising from the lamellar structure. However, the changes in the structure become weak with the hydration level greater than 30% (w/w) (Imberty & Perez, 1988; Nara, Mori, & Komiya, 1978; Suzuki, Chiba, & Yano, 1997). The crystallinity as well as the water content of starch affects the glass transition point (and the gelatinization temperature) (Mizuno, Mitsuiki, & Motoki, 1998; Zobel, Young, & Rocca, 1988). According to Jenkins and Donald (1997) the gelatinization occurs primarily in the amorphous growth rings. As Hoover (2001) mentioned in the review, the influence of the crystallinity on the gelatinization and rheological properties of starch have not been thoroughly investigated. The physico-chemical properties of starch as well as the lipid and the phosphate contents of potato affect the texture of potato (whether potato is mealy, firm etc.) (Baianu, Yakubu, & Ozu, 1999; Jane et al., 1999; Jarvis & Duncan, 1992; Van Marle et al., 1997; Van Soest, Tournois, deWit, & Vliegenthart, 1995).

In this study the nanometer range structure of potato (*S. tuberosum*) tubers was examined using WAXS, SAXS and ultra small-angle X-ray scattering (USAXS) methods. The crystallinity of the starch and the lattice constants of the hexagonal lattice of amylopectin were estimated from WAXS data. The average crystallite size, the lamellar distance and the thickness of lamella stacks were estimated from SAXS and USAXS data. The objective was to carry out experiments on slices and mashes of raw potato tubers, without isolating the starch from tuber matrix. The changes in the structure of three different cultivars Satu, Saturna and Lady Rosetta grown in Finland were studied monthly from August to May. The cultivars Saturna and Lady Rosetta are mealy and have been used in chips production, whereas Satu is used in French fries production. Generally, the cultivars grown in Finland differ in their processing properties, especially in French fries production, from the same cultivars grown in other countries. For comparison, two cultivars grown in the Netherlands were studied in December. Correlation of the structural parameters with the physiological ageing of potato tubers, as well as the effects of sample preparation on the parameters is discussed.

2. Experimental

2.1. Materials and sample preparation

Commercial potato starch and modified starch (Paselli WA-4) were obtained from Avebe (The Netherlands). Paselli WA-4 was used as an amorphous reference sample for crystallinity determinations. Distilled water was measured in a bag made from X-ray Mylar films (6.0 μm, Chemplex ind. Inc., NY, USA). Potato tuber flesh was studied as 1 mm thick slices or mashes. The samples were covered with the Mylar films immediately after cutting or mashing to avoid drying. The samples were weighed before and after the measurement, and as air-dried.

The effect of sample preparation on the structure was studied separately. Fresh and air-dried potato samples, isolated starch, and re-hydrated samples were used. Half of a potato tuber and 100 ml of water were homogenized with Ultra Turrax (13,000 rpm, IKA, GMBH and CO, Germany). The starch fraction was separated, air-dried and pressed as a pellet with the thickness of 1 mm. A slice cut from the same tuber was covered with the films and measured immediately. The slice was air-dried after the measurement, ground to fine powder and pressed as a pellet. The dried samples and commercial starch were suspended with 80% of water (w/w) to study the effect of water on the structural parameters. The effect of water content on the structure was also studied with USAXS by measuring a fresh potato slice (cv. Satu) at room temperature during 2.5 h.

Changes in the structure of cultivars Satu, Saturna and Lady Rosetta during the winter storage were studied monthly from August to May using WAXS and SAXS. The potatoes were grown in Jokioinen, Finland, and were harvested on 9th of September. They were stored at 4 °C in 90% of relative humidity. The samples were taken from noninjured, middle-sized tubers. The slices were cut (roughly at a distance of 0.5 cm from the surface) in randomly chosen directions of the tubers. In order to obtain an average structure of the whole tuber by one experiment, the potato tuber was mashed and an aliquot was taken for the measurement. The samples were weighed before and after the measurement and as air-dried. Potatoes (cv. Satu and Lady Rosetta) grown in Rilland (Northwest of the Netherlands) were harvested on 10th of October. They were studied in December by the same manner as the Finnish cultivars.

2.2. X-ray scattering

WAXS experiments were done with symmetrical transmission geometry with Cu Kα radiation obtained from a sealed X-ray tube operated at 45 kV and 28 mA, and monochromatized with a quartz monochromator in the incident beam. The scattered intensities were measured with a scintillation counter. An angular step of 0.1 degrees and a measuring time of 60 s per point were used.

The transmission mode was preferred for the moist samples, since the films to cover the samples caused extra background when using the reflection mode. Dry samples were also measured with the reflection mode for investigating the orientation of the crystallites in the sample.

SAXS experiments were done with Cu Kα (1.54 Å) radiation, monochromatized by a Ni-filter and a total reflection from a quartz glass monochromator. The intensity curves were measured using a linear position sensitive detector (Mbraun OED50M). The distance between the sample and the detector was 160 mm and the k-range was from 0.02 to 0.8 $Å^{-1}$. The magnitude of the scattering vector k is obtained as $k = (4\pi/\lambda)\sin\theta$ where λ is the wavelength and 2θ is the scattering angle. The beam had a narrow profile: the vertical instrument function had full width at half maximum (FWHM) 0.04 $Å^{-1}$, while in the horizontal direction (the direction of the anode wire) the instrumental function had FWHM 0.008 $Å^{-1}$. The background scattering was measured separately and subtracted from the intensity curves.

USAXS experiments were carried out at the beam line BW4 at Hamburger Synchrotronstrahlungslabor (HASYLAB, Germany) (Gehrke, 1992). DORIS III was operated at 4.445 GeV and 60–150 mA. The light was monochromatized by a double-crystal Si(111) monochromator. The energy of the X-rays was 8.979 keV ($\lambda = 1.38$ Å), which was calibrated via the measurement of an X-ray absorption spectrum from a copper foil. A two-dimensional proportional counter was used in all experiments. Two different distances, 4.42 and 10.42 m, between the sample and the detector were used. These measurements covered the magnitude of the scattering vector k from 0.002 to 0.08 $Å^{-1}$.

2.3. Data analysis

The WAXS intensity curves obtained from the potato or the starch samples displayed the B-type starch structure. The measured intensity curves were corrected for absorption and other instrument effects. For determination of the crystallinity of starch, defined as the mass fraction of crystalline starch and the total mass of starch in the sample, and the lattice constants the WAXS intensity was modeled by a linear combination of experimental intensities of amorphous starch and water and a calculated diffraction pattern of crystalline starch (Fig. 1). The model diffraction pattern of crystalline starch was calculated as a sum of reflections of amylopectin. The structure factors and multiplicities corresponding to each reflection of amylopectin were calculated from the atomic coordinates of Imberty and Perez (1988) using the program PowderCell (Kraus & Nolze, 1996). The position of a reflection was calculated from its miller indices (hkl) and from the hexagonal lattice constants. The shapes of the diffraction peaks were assumed Gaussian and the half-width was assumed to be equal for every reflection. Thus the overall model for crystalline starch was governed by three nonlinear

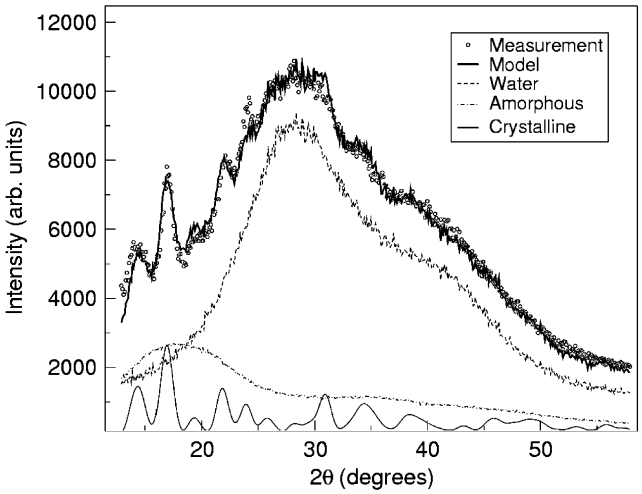

Fig. 1. WAXS intensity curve obtained from a sample of potato flesh. The data were modeled by a linear combination of experimentally determined intensity curves of amorphous starch and water and a calculated diffraction curve of crystalline starch.

parameters, the lattice constants a and c, and the width of a Gaussian lineshape. The calculated intensity was also multiplied with the Debye–Waller temperature factor with the parameter $B = 3.0$.

The experimental intensity curve was described by a model with a total of six parameters, the three nonlinear parameters needed for calculation of the diffraction pattern of amylopectin and the proportions of the intensities of water, amorphous starch and crystalline starch. The best fitting values of the parameters were obtained by minimizing the difference between the experimental and the model intensities. The scattering angle range used in the minimization was from 13 to 58 (2θ). The upper limit was chosen according to Stubicar et al. (1998). Scattering angles below 13° (2θ) were not used for fitting since small angle scattering, originating from large-scale inhomogenities, which were not included in the model, gives a large contribution to the intensity curves. An estimate of the weight fractions of the scattering components in the sample was obtained from integrals of the intensities weighed by k^2 (Balta-Calleja & Vonk, 1989). Gernat, Radosta, Damaschun, and Schierbaum (1990) also applied a similar method. The crystallinity of starch was determined as a ratio of the k^2-weighed integral of intensities of crystalline starch and the sum of crystalline and amorphous starch.

SAXS intensity curves of potato samples or moist starch included the peak corresponding to the 9 nm lamellar structure and the reflection *100* of amylopectin. Furthermore, the intensity increased towards zero obeying a power law. The electron density of water and crystalline amylopectin are about 0.33 and 0.32 electrons/$Å^3$, respectively. The unit cell of amylopectin was assumed to contain five water molecules. Thus the electron density difference between starch and water is close to that between crystalline

and amorphous starch. Electron density difference between starch and voids is roughly ten times as large as that between starch and water and that between amorphous and crystalline starch.

The lamellar distance, the lattice constant a of the hexagonal lattice of amylopectin (Imberty & Perez, 1988), and the average size of the crystallites were determined by fitting a model in the SAXS intensity curve. The lamellar peak and the reflection *100* were presented as Gaussian functions and the model intensity was a sum of these and a power law term βk^{α}. The positions, widths, and intensities of the peaks, the power law exponent α, and the scaling factor β were the fitting parameters. The lamellar distance was determined from the position of the lamellar peak, k, as $2\pi/k$. The lattice constant a was determined from the d-value of the reflection 100 by $d = \sqrt{3}/2a$. A lower bound of the average crystallite size, *Bhkl*, was determined from the width of the reflection 100 by using the well-known Scherrer formula (Klug & Alexander, 1974).

The effect of the instrumental broadening on the width of the reflections was included by smearing the model intensity with the beam height profile (Glatter & Kratky, 1982). The reflection 100 of starch samples has been modeled using Gaussian function successfully previously by, e.g. Perry and Donald (2002).

The USAXS intensity curves from two different measurement distances corresponding to k-ranges of 0.005–0.08 and 0.002–0.027 $Å^{-1}$ showed the 9 nm-lamella peak and two power-law like regimes in the smaller angles (Fig. 2). The lamellar distance and the thickness of lamella stacks were determined from the diffraction peak with a procedure similar to the SAXS data analysis. The power law exponents were obtained from a linear regression fit to the double logarithm curve of intensity versus the magnitude of the scattering vector.

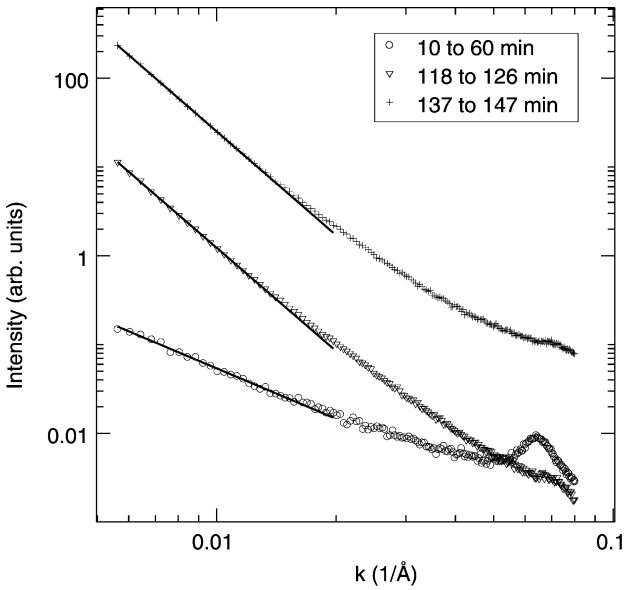

Fig. 2. USAXS intensity from a potato slice during drying and the fitted power law term. The intensity curves are normalized to the amount of incoming flux during the measurement. At the beginning of the measurement, the potato sample has a lamellar diffraction maximum and an approximate power law exponent of −1.6. After 2 h of drying the lamellar peak collapses fairly rapidly and the intensity of scattering in the low angles increases, giving an increase in the apparent power law to −3.0. Further drying increases the overall intensity of scattering dramatically, indicating that structures containing large scattering contrast (presumably voids) emerge in the sample. The apparent power law also increases to −3.8.

We also tried to fit the SAXS and USAXS data with the paracrystalline model suggested by Cameron and Donald (1992) for starch samples. They assumed that small-angle scattering arises from a finite number of infinite lamellar planes of crystalline and amorphous material, embedded in a medium with an electron density between those of the crystalline and amorphous material in the stacks. The parameters of this model have a straightforward interpretation in terms of the real space structure and the model corresponds well to the structure of starch presented in the literature. The Cameron and Donald model, even with its many parameters (8, if one takes into account the overall scaling and a constant background) failed to model the lamellar peak and the upturn of the intensity towards zero k of USAXS intensity curves of potato samples.

Statistical errors in all of the fitting results were determined by Monte-Carlo simulations (Press, Teukolsky, Vetterlingand, & Flannery, 1992). Statistical analyses of the structural parameters, comparison of the means with t-tests, were calculated with MedCalc (version 7.1.0.1).

3. Results

3.1. Crystal structure and crystallinity

A typical WAXS intensity curve measured from a sample of potato flesh is presented in Fig. 1. The lattice constants, the crystallinity of starch, the crystallite size and the lamellar distance of different starch and potato samples obtained from WAXS and SAXS experiments are listed in Table 1. The dry samples had the crystallinity of starch between 12 and 16% with water contents between 7 and 13% determined from the diffraction data. Higher crystallinities were measured from starch–water suspensions. The crystallinity of starch in potato slices and mashes was between 21 and 31% with water contents of 55–86% (Table 1). The fitting error of 5% for the crystallinity of starch and for the water content of the sample is estimated.

The lattice constants measured from fresh potato slices and mashes were nearly equal with the values obtained from starch–water suspensions by WAXS (Table 1). The fitting error for the lattice constants is 0.1 Å.

The values of the lattice constant a, determined using SAXS from the reflection 100, were in a good agreement with those obtained using WAXS both for suspensions and potato flesh samples. The fitting error for the SAXS result is about 0.03 Å. The crystallite size in that direction was

Table 1
Structural parameters obtained from different starch and potato samples by WAXS and SAXS

	Lattice constant		Crystallinity of starch (%)	Crystallite size [100] (Å)	Lamellar distance (Å)
	a (Å)	*c* (Å)			
Starch (Avebe)	18.0	10.8	16	–	–
Water suspension	18.3	10.4	24–32	127	97
Isolated starch	18.3	10.5	12	–	–
Water suspension	18.3	10.5	15	127	97
Potato powder	18.0	10.8	13	–	–
Water suspension	18.3	10.5	15	127	97
Fresh potato[a]	18.4, 18.1	10.4, 10.5	21–50	98–143	90–104

The dry samples were suspended with 80% of water (w/w).
[a] The boundary values obtained from slices and mashes of three different cultivars (number of samples ~60).

127 Å in suspensions and 98–143 Å in slices and mashes of potato tubers (Table 1).

3.2. Lamellar structure and upturn of SAXS intensity towards zero angle

As expected, the lamella peak was not seen in any of the dry samples. The lamella peak appeared reversibly in SAXS curves as water was added to the dry powders. The error for the lamellar distance is 1 Å, estimated from the fitting error for the position of the lamellar peak. The lamellar distance was around 97 Å for the suspensions. The structural parameters obtained from the peak were identical for potato flesh samples. The lamellar distance was 97 ± 3 Å and the thickness of lamella stacks was 513 ± 6 Å.

Potato slices and starch–water suspensions showed similar structures according to USAXS experiments. The general feature of small angle scattering below the 9 nm peak was the existence of two power law regimes. Interpretation of power law scattering in terms of fractal structures have been reviewed, e.g. by Schmidt (1991). The lower angle regime in the range $k < 0.01$ Å^{-1} had a power law exponent ranging from −3.5 to −4.0. The fitting error for the power law exponent was 0.1. This can be interpreted as scattering from rough surfaces (Schmidt, 1991; Suzuki et al., 1997), perhaps corresponding to the blocklet or superhelix structure of starch (Waigh et al., 1998). The second power law regime in the *k*-range 0.01–0.03 Å^{-1}, between the low angle power law and the 9 nm peak, had an exponent ranging from −1.6 to −2.5. These values are typical for mass fractal structures, but the short range in which this power law was observed does not make this evidence very convincing.

The USAXS intensities measured during the drying process of a potato slice show that the structural change happens in different stages, which are shown in Fig. 2. The native hydrated structure exhibits one power law with an exponent −1.6. The effect of water evaporation starts to show when another power law (exponent −3.0) emerges at the lower angles. This state presumably corresponds to a mixture of hydrated and dehydrated structures. In the subsequent stages the lower angle power law becomes steep towards −3.8 and also the higher angle power law steepens towards an exponent of around −3.0. The transition between the two power law regimes moves slightly towards higher angles but remains between 0.01 and 0.02 Å^{-1}. In the final stages of drying the lamellar structure collapses fairly rapidly. The overall intensity of scattering continues to increase as the rest of the water evaporates, indicating that the intensity arises now from voids (Fig. 3). Unfortunately the intensities were not determined in absolute scale, thus we cannot determine the specific surface and estimate the coherence length for the inhomogenities of the electron density causing the power law −4 behavior. As pointed out in Section 2.3, the size scale of the inhomogenities is in the range of hundreds of nanometers.

3.3. Structural changes during the storage

The mean values (± SD) for each month are calculated by combining all the three cultivars (Satu, Saturna and Lady

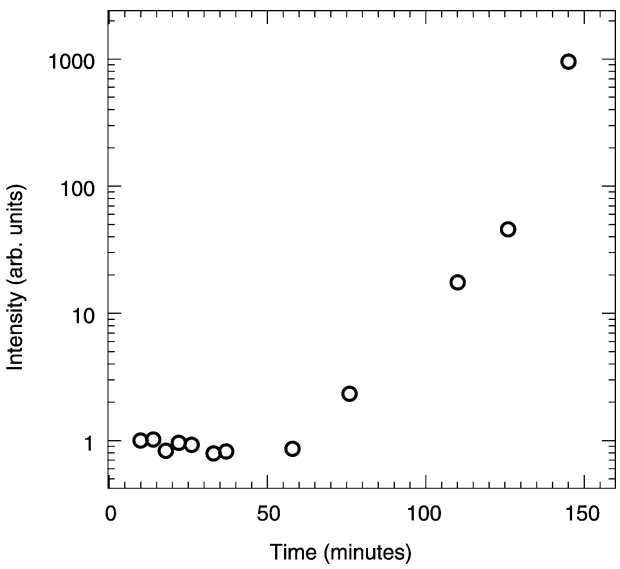

Fig. 3. Scattering intensity in the low angles ($k = 0.006$ Å^{-1}) normalized to incoming flux during the drying experiment of Fig. 2. After 80 min measuring time, an exponential increase in the intensity is observed.

Table 2
Lattice constants a and c, determined from potato slices and mashes (cv. Lady Rosetta, Satu and Saturna) during winter storage by WAXS

		Lady Rosetta	Satu	Saturna	Mean
(a) Potato slices					
August	a (Å)	18.2	18.2	18.2	18.2 ± 0.02[a,b]
	c (Å)	10.6	10.6	10.4	10.6 ± 0.11[a,b]
September–January	a (Å)	18.4 ± 0.10	18.4 ± 0.04	18.4 ± 0.06	18.4 ± 0.07[c]
	c (Å)	10.4 ± 0.06	10.4 ± 0.03	10.4 ± 0.05	10.4 ± 0.04[c]
May	a (Å)	18.2	18.2	18.2	18.2 ± 0.004[b,d]
	c (Å)	10.7	10.6	10.5	10.6 ± 0.09[b,d]
(b) Potato mashes					
August	a (Å)	18.4	18.4	–	18.4 ± 0.006[a,b]
	c (Å)	10.5	10.5	–	10.5 ± 0.006[a,b]
September–January	a (Å)	18.4 ± 0.05	18.4 ± 0.03	18.4 ± 0.04	18.4 ± 0.04[c]
	c (Å)	10.4 ± 0.03	10.4 ± 0.03	10.4 ± 0.05	10.4 ± 0.04[c]
May	a (Å)	18.3	18.1	18.1	18.2 ± 0.1[b,d]
	c (Å)	10.3	10.7	10.6	10.6 ± 0.20[b,d]

[a] $n = 2$.
[b] The means obtained in August and May were compared to the mean obtained in September to January by t-test (MedCalc Version 7.1.0.1). P-value < 0.05 indicated that the slices measured in August and May, and the mashes measured in May differ statistically significantly from the samples measured in September–January.
[c] $n = 15$.
[d] $n = 3$.

Rosetta), because the cultivars did not differ from each other markedly. The slices and the mashes differed from each other only in August. The lattice constants $a = 18.4 \pm 0.07$ and $c = 10.4 \pm 0.04$ Å for potato flesh samples were obtained from the data measured between September and January (including cv. Satu, Saturna and Lady Rosetta, number of samples = 30, mashes and slices included). The means of the lattice constants obtained from potato slices in August and in May differed statistically significantly ($P < 0.05$) from the mean value of September to January (Table 2). The changes in the lattice constants between August and May are shown in Fig. 4. The change in the lattice constants did not follow the change in the water content determined from the WAXS data (data not shown). The water content was over 55% in each sample.

The mean of 24 ± 2% for the crystallinity of starch was obtained from slices and mashes in September to January. Only the slices measured in August deviated significantly from that mean (Fig. 5). The crystallite size in the direction 100 or the lamellar distance determined by SAXS showed no significant differences between the slices and mashes, or between the months. The means for the samples measured in August to May were 118 ± 10 Å for the crystallite size and 97 ± 3 Å for the lamellar distance. The thickness of lamella stacks 513 ± 6 Å was measured only from the slices in September. Potatoes grown in the Netherlands did not differ from the Finnish potatoes in structural parameters determined by WAXS and SAXS in this study. More samples and experiments would be needed to ascertain the result.

4. Discussion

We modeled the WAXS intensity of potato samples as a linear combination of the diffraction pattern of crystalline amylopectin and experimental intensity curves of amorphous starch and water for determination of the crystallinity of starch. We have used similar method for determining the crystallinity for complex synthetic polymer materials (e.g. Jokela et al., 2002) and wood (Andersson, Serimaa, Saranpää, Pesonen, & Paakkari, 2003). As shown in Fig. 1 this model agreed well with the experimental intensity curve. The contribution of the cell walls, which contain cellulose microfibrils, has thus been ignored. The largest reflection of crystalline cellulose, 200, would be at about

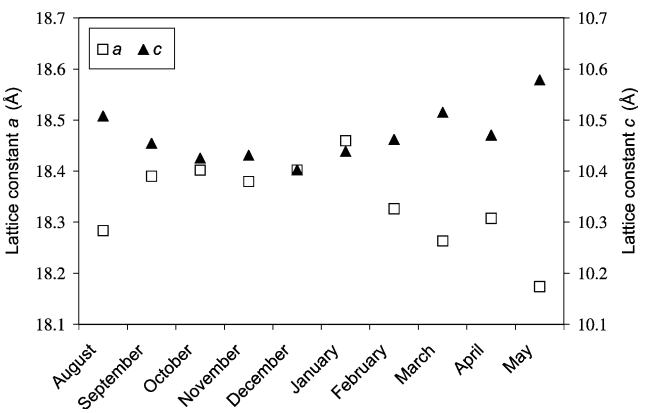

Fig. 4. Structural changes in potato samples during the winter storage determined by WAXS: the lattice constants a and c, number of samples = 6/point. The lattice constants varied during the winter storage.

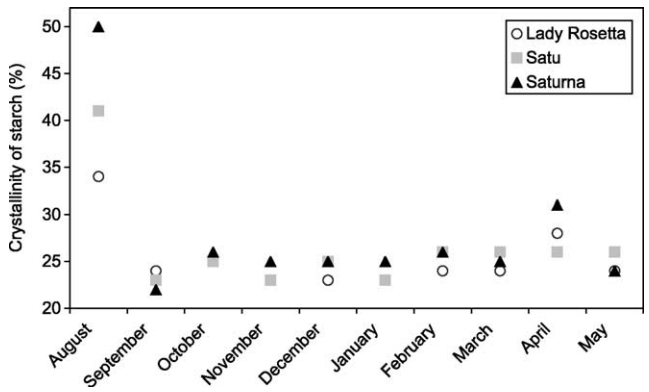

Fig. 5. Crystallinity of starch (%) in potato slices in August to May. The crystallinity is higher before harvesting time in August than in mature potato tubers.

22.6 degrees, where no discrepancy between the experimental intensity and the present simplified model is observed. The crystallinities of 12–16% obtained from dry samples are comparable with the value of 22% for native potato starch reported by Gernat et al. (1990). Furthermore, the position of the reflection 100 was determined from the SAXS intensity. This fit yielded independently the same value for the lattice constant a as WAXS data.

Since potato slices do not consist only of starch granules, it is not a surprise that the upturn of USAXS intensity towards zero angle could not be modeled only with the paracrystalline model (Cameron & Donald, 1992). We conclude that other components, e.g. blocklet surfaces and cell walls, also contribute to the scattering intensity. The model of Cameron and Donald is in a good agreement with SANS and SAXS data obtained from starch slurries (Jenkins & Donald, 1996), in which the 9 nm maximum has a width of around 0.02 $Å^{-1}$ (FWHM). However, the width of the lamellar peak is around 0.01 $Å^{-1}$ (FWHM) in the data that was obtained from potato flesh using synchtrotron radiation. The description of the lamellar structure is arguably more accurate in the Cameron and Donald model, but the emphasis on this study is on the differences observed during a time series. These points have led us to use the simpler models described above. The same value of lamellar distance, 97 Å, was obtained for humid isolated starch and potato powder. This value is in adequate agreement with the literature values, determined using the paracrystalline model (Imberty et al., 1991; Jenkins et al., 1993). The same value was obtained also for potato slices and mashes. Thus isolation of starch granules does not affect the lamellar period.

Table 1 shows that the fitting procedure yields lattice constants of water–starch and water–potato powder suspensions, which are in accordance with the published lattice constants for B-type starch (Imberty & Perez, 1988). For the dry samples different values are obtained, which may be due to the loss of water from the unit cell. The parameters obtained from the suspensions differed only slightly from the means obtained from fresh potato samples. We did not study potato powders or starch isolated from tubers during winter storage. Thus, we cannot conclude whether the drying and isolation destroys the changes observed in potato slices and mashes during the storage.

However, using potato flesh as a sample in X-ray scattering experiments takes less time without any extraction procedures. The starch in its native matrix is more interesting when complex relations between the nanometer range structure and the potato characteristics in processing or during the storage are under interest. Fine changes in the structure, which may be induced to potato with modern breeding technologies such as genetic modification, may be lost with the isolation.

The water loss from the flesh samples did not seem to cause large deviation in the parameters obtained by WAXS as the samples still contained 50–60% (w/w) of water after the measurement. Imberty and Perez (1988) have described the dependence of crystal structure of starch on water content. It has been observed that the minimum water content of 7% is needed to obtain a diffraction curve, and with water content greater than 30% the variations in the structure become weak. Furthermore, the Bragg distance of the reflection *100* and the lamellar distance did not vary with the change in water content during the SAXS measurements.

The results showed that the immature potatoes (before harvesting time) differ from mature potatoes in crystalline properties. The higher crystallinity of starch and changed lattice constants were observed only in slices in August (Fig. 5). This indicates that the structural changes are taking place nearer to the skin in maturation.

Significant structural changes in the parameters were not detected in September to January. This period is so called rest or dormant period of potato tubers. The lattice constants of both potato slices and mashes in that time period agreed with previously published values of commercial starches. However, changes in the lattice constants and in the crystallinity of starch were observed again after the sprouting process started in February and March. February could be seen as a turning point in the ageing process of the potato tubers. The changes in the crystallite size or in the lamellar distance were not statistically significant during the storage. Because the lamellae are constructed from the crystallites, it is expectable that the results for them were parallel. Jenkins and Donald (1998) have observed that even gelatinization of starch samples do not affect the lamellar distance.

Other authors have reported differences in granule size, phosphorus content, amylose contents and degree of branching of starch between varying potato cultivars, but the X-ray diffraction data have shown no specific changes in starch crystalline structure (Cottrell et al., 1995; Jane et al.,

1999; Liu et al., 2003; Svegmark et al., 2002). The differences in characteristics between the Finnish cultivars could not be explained with the structural properties studied here either. The studies on potatoes grown under varying soil compositions and day-length (f. ex. in the Netherlands) would probably give more information about the contribution of the starch crystalline properties to the behavior of potato in processing.

5. Conclusions

The crystallinity of starch, the lattice constants, the average crystallite size, the lamellar distance and the thickness of lamella stacks were obtained reliably from the WAXS, SAXS and USAXS data. A sophisticated fitting procedure for diffraction pattern of starch was applied. The method described here is suitable for screening structural changes in potato tuber flesh without isolating the starch from the matrix. The results did not explain the differences in processing characteristics between the three Finnish cultivars. The crystalline structure of starch followed ageing process of the potato tubers during the storage. This study is the basis for the further X-ray studies on interspecific hybrids of wild and cultivated potato species.

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References

Andersson, S., Serimaa, R., Saranpää, P., Pesonen, E., & Paakkari, T. (2003). The crystallinity of wood and the size of cellulose crystallites in Norway spruce. *Journal of Wood Science*, in press.

Baianu, I. C., Yakubu, P. I., & Ozu, E. (1999). Structural and hydration studies of waxy and mealy potato starch cultivars by deuterium, carbon-13 CP-MAS/MASS NMR, and electron microscopy. *Macromolecular Symposia*, *140*, 187–195.

Balta-Calleja, F. J., & Vonk, C. G. (1989). *X-ray scattering of synthetic polymers*. Amsterdam: Elsevier.

Cameron, R. E., & Donald, A. M. (1992). A small-angle X-ray scattering study of the annealing and gelatinization of starch. *Polymer*, *33*, 2628–2635.

Cottrell, J. E., Duffus, C. M., Paterson, L., & Mackay, G. R. (1995). Properties of potato starch: Effects of genotype and growing conditions. *Phytochemistry*, *40*, 1057–1064.

Gallant, D. J., Bouchet, B., & Baldwin, P. M. (1997). Microscopy of starch: Evidence of a new level of organization. *Carbohydrate Polymers*, *32*, 177–191.

Gehrke, R. (1992). An ultrasmall angle scattering instrument for the DORIS-III bypass. *Review of Scientific Instruments*, *63*, 455–458.

Gernat, Ch., Radosta, S., Damaschun, G., & Schierbaum, F. (1990). Supramolecular structure of legume starches revealed by X-ray scattering. *Starch/Stärke*, *42*, 175–178.

Glatter, O., & Kratky, O. (1982). *Small angle X-ray scattering*. New York: Academic Press.

Hoover, R. (2001). Composition, molecular structure, and physicochemical properties of tuber and root starches: A review. *Carbohydrate Polymers*, *45*, 253–267.

Imberty, A., Buleon, A., Tran, V., & Perez, S. (1991). Recent advances in knowledge of starch structure. *Starch/Stärke*, *43*, 375–384.

Imberty, A., & Perez, S. (1988). A revisit to the three-dimensional structure of B-type starch. *Biopolymers*, *27*, 1205–1221.

Jane, J., Chen, Y. Y., Lee, L. F., Mcpherson, A. E., Wong, K. S., Radosavljevic, M., & Kasemsuwan, T. (1999). Effects of amylopectin branch chain length and amylose content on the gelatinisation and pasting properties of starch. *Cereal Chemistry*, *76*, 629–637.

Jarvis, M. C., & Duncan, H. J. (1992). The textural analysis of cooked potato. 2. Swelling pressure of starch during gelatinisation. *Potato Research*, *35*, 93–102.

Jenkins, P. J., Cameron, R. E., & Donald, A. M. (1993). A universal feature in the structure of starch granules from different botanical sources. *Starch, 45*, 417–420.

Jenkins, P. J., & Donald, A. M. (1996). Application of small-angle neutron scattering to the study of the structure of starch granules. *Polymer*, *37*, 5559–5568.

Jenkins, P. J., & Donald, A. M. (1997). Breakdown of crystal structure in potato starch during gelatinisation. *Journal of Applied Polymer Science*, *66*, 225–232.

Jenkins, P. J., & Donald, A. M. (1998). Gelatinization of starch: A combined SAXS/WAXS/DSC and SANS study. *Carbohydrate Research*, *308*, 133–147.

Jokela, K., Serimaa, R., Torkkeli, M., Sundholm, F., Kallio, T., & Sundholm, G. (2002). The effect of initial matrix material on the structure of radiation grafted ion-exchange membranes: WAXS and SAXS studies. *Journal of Polymer Science: Polymer Physics*, *15*, 1539–1555.

Klug, H. P., & Alexander, L. E. (1974). *X-ray diffraction procedures*. New York: Wiley.

Kraus, W., & Nolze, G. (1996). Powder cell—A program for the representation and manipulation of crystal structures and calculation of the resulting X-ray powder patterns. *Journal of Applied Crystallography*, *29*, 301–303.

Liu, Q., Weber, E., Currie, V., & Yada, R. (2003). Physicochemical properties of starches during potato growth. *Carbohydrate Polymers*, *51*, 213–221.

Mizuno, A., Mitsuiki, M., & Motoki, M. (1998). Effect of crystallinity on the glass transition temperature of starch. *Journal of Agricultural and Food Chemistry*, *46*, 98–103.

Nara, Sh., Mori, A., & Komiya, T. (1978). Study on relative crystallinity of moist potato starch. *Starch/Stärke*, *30*, 111–114.

Perry, P. A., & Donald, A. M. (2002). The effect of sugars on the gelatinization of starch. *Carbohydrate Polymers*, *49*, 155–165.

Press, W. H., Teukolsky, S. A., Vetterlingand, W. T., & Flannery, B. P. (1992). *Numerical recipes in C, The art of scientific computing* (2nd ed.). Cambridge: Cambridge University Press.

Schmidt, P. W. (1991). Small-angle scattering studies of disordered, porous and fractal systems. *Journal of Applied Crystallography*, *24*, 414–435.

Stubicar, N., Smith, I., Stubicar, M., Tonejc, A., Janosi, A., Schurz, J., & Zipper, P. (1998). An X-ray diffraction study of the crystalline to amorphous phase change in cellulose during high-energy dry ball milling. *Holzforschung*, *52*, 455–458.

Suzuki, T., Chiba, A., & Yano, T. (1997). Interpretation of small angle X-ray scattering from starch on the basis of fractals. *Carbohydrate Polymers*, *34*, 357–363.

Svegmark, K., Helmersson, K., Nilsson, G., Nilsson, P.-O., Andersson, R., & Svensson, E. (2002). Comparison of potato amylopectin starches and

potato starches-influence of year and variety. *Carbohydrate Polymers*, *47*, 331–340.

Tamaki, S., Teranishi, K., Hisamatsu, M., & Yamada, T. (1997). Inner structure of potato starch granules. *Starch/Stärke*, *49*, 387–390.

Tester, R. F., Debon, S. J. J., Davies, H., & Gidley, M. J. (1999). Effect of temperature on the synthesis, composition and physical properties of potato starch. *Journal of the Science of Food and Agriculture*, *79*, 2045–2051.

Van Marle, J. T., Stolle-Smits, T., Donkers, J., van Dijk, C., Voragen, A. G. J., & Recourt, K. (1997). Chemical and microscopic characterization of potato (*Solanum tuberosum* L.) cell walls during cooking. *Journal of Agricultural and Food Chemistry*, *45*, 50–58.

Van Soest, J. J. G., Tournois, H., deWit, D., & Vliegenthart, J. F. G. (1995). Short-range structure in (partially) crystalline potato starch determined with attenuated total reflectance Fourier-transform IR spectroscopy. *Carbohydrate Research*, *279*, 201–214.

Waigh, T. A., Gidley, M. J., Komanshek, B. U., & Donald, A. M. (2000). Analysis of the native structure of starch granules with X-ray microfocus diffraction. *Carbohydrate Research*, *328*, 165–176.

Waigh, T. A., Perry, P., Riekel, C., Gidley, M. J., & Donald, A. M. (1998). Chiral side-chain liquid-crystalline polymeric properties of starch. *Macromolecules*, *31*, 7980–7984.

Zobel, H. F., Young, S. N., & Rocca, L. A. (1988). Starch gelatinization: An X-ray diffraction study. *Cereal Chemistry*, *65*, 443–446.