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Small-Angle X-Ray Scattering (SAXS) Studies of the Structure of Starch and Starch Products

Abstract

In this short review, the possibilities of using the small-angle X-ray scattering (SAXS) method in studies of native starch and starch products are presented. The paper presents two approaches, which can be used to analyse the SAXS data for native starch as well as for example interpretations of SAXS results. We describe two models of changes in starch structure, which take place during deep freezing and gelatinisation. The possibilities of using the SAXS method for studying starch products structure are also presented. A more detailed analysis of the SAXS method possibilities for examining bread and extrudates was made.

Key words: *starch, SAXS, starch products, review.*

■ Introduction

Many cereal products have been developed empirically over very many years. The structure of these new products (especially of the starch-rich domains) over a range of length scales from the macroscopic to the molecular has a potential impact on its properties.

Small-angle X-ray scattering is always observed when electron density heterogeneities whose size is between 10 and 10,000 Å exist in a sample. The scattered intensity is proportional to the square difference between the electronic density of the scattered heterogeneities and its surrounding, and also depends on the size, shape, and amount of these heterogeneities, as well as on some additional features as multiple scattering, orientation or even the degree of ordering [1].

Starch consists of two main polysaccharides, amylose and amylopectin. Amylose is essentially linear, whereas amylopectin is highly branched. The structure of native starch is now thought to be hierarchically organised on four length scales: the molecular scale (~Å), the lamellar structure (80–90Å), growth rings (~0.1 μm), and the whole granule morphology (~μm).

Thus, the SAXS method can provide information about the lamellar structure of starch. The structure of amylopectin (see Figure 1) consists of heavily branched regions connected by straight-chain, unbranched regions. In straight-chain regions, the chains form double helices with each other. It means that the amylopectin can be treated as a semicrystalline lamellar structure. The semi-crystalline lamellar structure of amylopectin is placed in quite an amorphous phase, which is built mainly by amylose (see Figure 1) [2–5].

So, when hydrated starch is measured with SAXS or SANS [6–10], a peak is seen at a value of q (q=(4πsinθ)/λ) of 0.062–0.066Å^{-1}. This peak is thought to arise from the alternating crystalline and amorphous regions of amylopectin (see Figure 1). The location of the peak depends on the size of lamella, and may differ [9] for starch originated from different plants (see Figure 2), while the peak area depends mainly on the degree of ordering in semicrystalline regions. In Table I, the comparison of the peak areas for different kinds of wheat flour is presented [11].

■ Two ways to interpret the SAXS data

At present, two approaches for envisaging starch structure from the point of view of the SAXS method have been delineated in literature [12, 13]. The first is based on the structure scheme presented in Figure 1; it assumes that in the starch structure three regions exist which differ in their values of electron density: the crystalline region of amylopectin, the amor-

phous region of amylopectin and the amorphous region in which the amylopectin is placed. For such models of starch structures, Cameron and Donald derive an equation for SAX scattering [10] from which the following parameters can be fitted: d – lamellar repeat distance, φ – fractional lamellar crystallinity, β – width of the distribution of lamellar sizes, Δρ$_I$ – electron density differences between the three regions of starch granule, N – number of semicrystalline repeats within each growth ring. Knowledge about the value of fitted parameters allows us to construct models which can explain many changes in starch structure which take place after different processes. As an example of using the above approach, the influence of low temperatures on starch structure is presented below in Figure 3 [12].

In Figure 3, the SAXS curves, both experimental (points) and calculated (line), for wet maize before and after freezing are presented, as well as the schematic model of changes which take place in starch structure under deep freezing. By analysing the values for fitted parameters

Table 1. The comparison of SAXS peak area for different kind of wheat flour.

Wheat Flour	Peak area [impulse]
T-450	1.00
T-500	0.97
T-550	0.32
T-550 high gluten	0.57
T-650 low gluten	0.38
T-750	0.18
T-850 high gluten	0.32

obtained (as described above), the following model for changes in starch structure have been proposed. In the first step, the expansion of water external to the lamellae within starch granules upon freezing and ice formation places the semicrystalline lamellar structure under compression. The amorphous part of the lamellae is preferentially compressed upon freezing, with the crystalline region remaining largely unaffected. This cause the lamellar distance to shorten, which in turn causes the peak to shift to a higher value of the scattering vector q (see Figure 3). In the next step, the compression causes the loss of correlation in the lamellar region, which causes the peak

to be broader and smaller than for the sample which is not frozen.

Figure 4 shows the example of using the **liquid crystalline approach** for understanding the changes which take place in amylopectin upon gelatinisation in intermediate stages [13].

In a liquid crystalline approach, the following values can be estimated from the SAXS data:
ϕ - mean orientational order, ψ - lamellar order, **h** – helical order . In Figure 4, the changes in the first stage of gelatinisation of starch types A and B are presented. B-type starch has a much longer
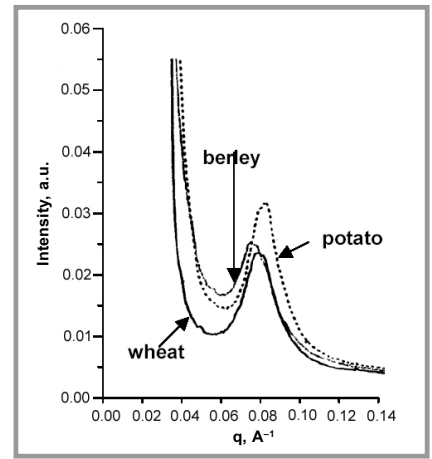
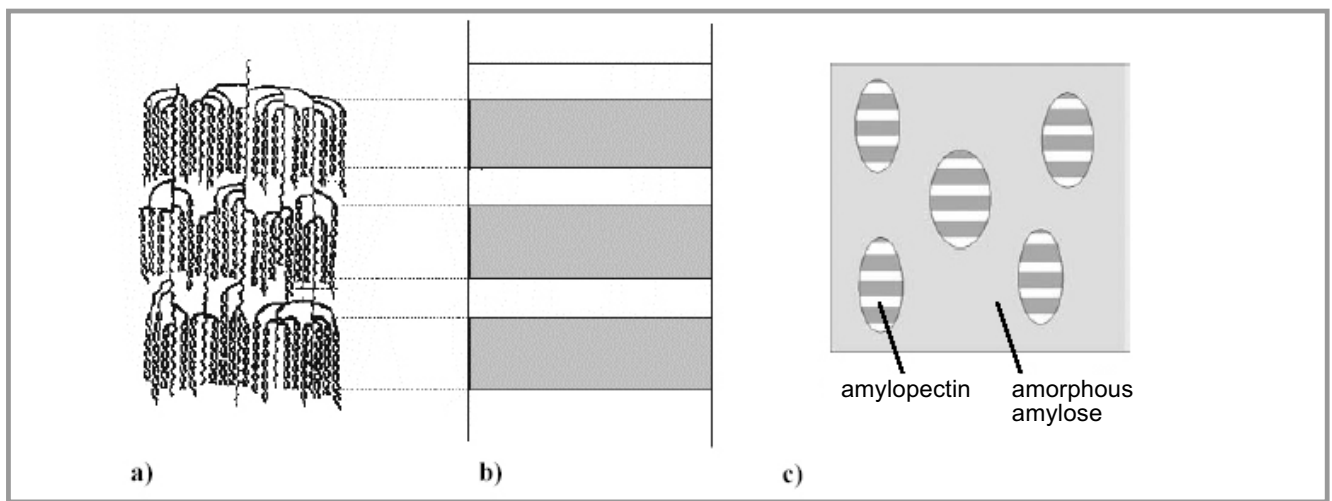**Figure 2.** *The SAXS curves for native starches originated from different sources.*

Figure 1. *Schematic diagram of starch structure: a) structure of amylopectin, b) schematic diagram of lamellar structure of amylopectin in which repeat phases – amorphous and crystalline – are shown, c) three-phase schematic system of the starch structure – the two-phase amylopectin structure is placed in the third amorphous phase, which is built mainly by amylose.*
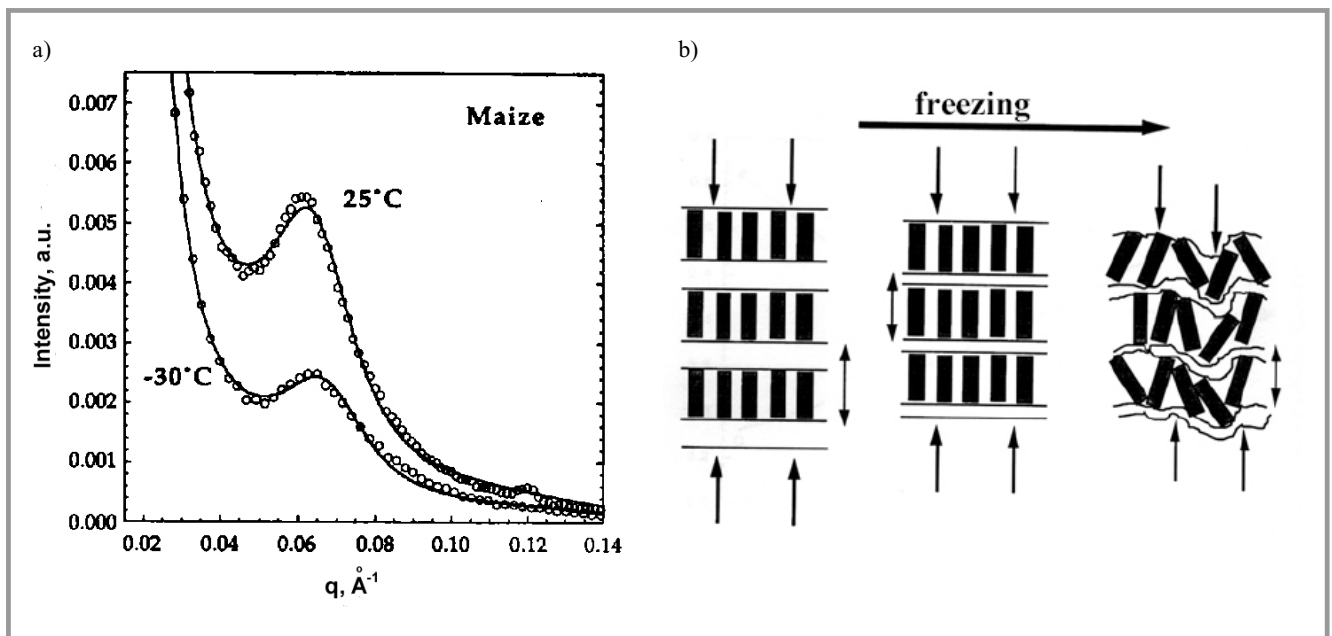
Figure 3. *The interpretation of SAXS data for wet maize starch occurring upon deep freezing: a) the SAXS curves for wet maize starch, before and after deep freezing, b) the schematic diagram of changes which take place in the amylopectin structure upon freezing.*
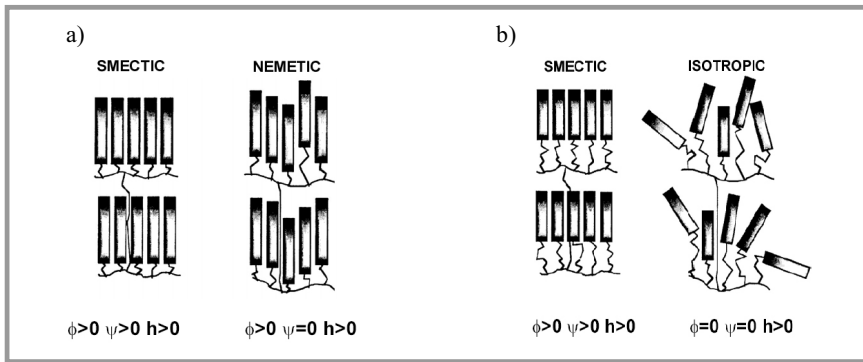
Figure 4. *Schematic diagram of changes which occur in intermediate stages, during gelatinisation by using the liquid crystal approach: a) schematic diagram for A-type starch, b) schematic diagram for B-type starch.*

amorphous backbone than starch A, and because of this, the isotropic structure is formed over gelatinisation in the intermediate stage, while for A-type starch this takes place during the nematic phase.

The SAXS investigations of starch products

In almost all starch products, the semicrystalline structure of amylopectin is quite collapsed or seriously destroyed. This causes the SAXS curves not to have any visible peak. In Figure 5, the SAXS curves for native potato starch and potato extrudate are presented [14].

In contradistinction to the SAXS curve for native potato starch, the SAXS curve for potato extrudate has no peaks. This means that the extrusion process has completely destroyed the semicrystalline structure of amylopectin. The theory of SAXS method also allows quite smooth curves to be analysed because, as mentioned above, the only condition for existing the SAX scattering is the presence of heterogeneities in electron density distribution, whose size is on a nanoscale. Thus, even a qualitative comparison of the level of SAXS intensity can yield much highly interesting information about the system under investigation. In Figure 6, the comparison of SAXS intensity for potato and wheat extrudates is presented [15].

Figure 6 clearly demonstrates that the SAXS intensity for wheat extrudates is much greater than for those from potato extrudates. It means that in wheat extrudates there are more heterogeneities than in the potato extrudates, or that existing heterogeneities in wheat extrudates have an electron density which differs from the surroundings much more than in potato extrudates. Taking into account that the wheat starch consists of a small amount of protein and lipids while potato starch is near-pure starch, the differences obtained in SAXS intensity probably indicate the participation of proteins and lipids in the formation of nanoscale heterogeneities in wheat extrudates. The comparison of SAXS level intensities can give only qualitative results, but when the Porod law [1] is met by the SAXS curves, a more detailed analysis can be realised, and for example the specific surface area can be calculated, as well as the size distribution function.

A comparison of changes in the SAXS intensity level has also been used in analysing the bread staling process [16]. In Figure 7, the SAXS curves for bread samples aged at different times are presented. Freshly baked bread can be referred to as an unstable material and bread staling as a multifaceted process involving physical, chemical and sensory changes that are all interrelated [17]. Starch is significantly involved in the bread staling process. The main transformation of the starch during aging is retrogradation; this is a process which consists in the aggregation of polysaccharide chains which may form a crystalline phase within or outside the starch granules. Also, many other compounds such as gluten, water-soluble proteins, oligosaccharides and lipids play a very important role in bread staling [17].

The curves presented in Figure 7 clearly show that bread staling significantly influences the intensity of the SAXS; the greatest intensity for 1-week bread is observed, whereas further aging of bread caused a decrease in scattering. It means that the 1-week sample mostly consists
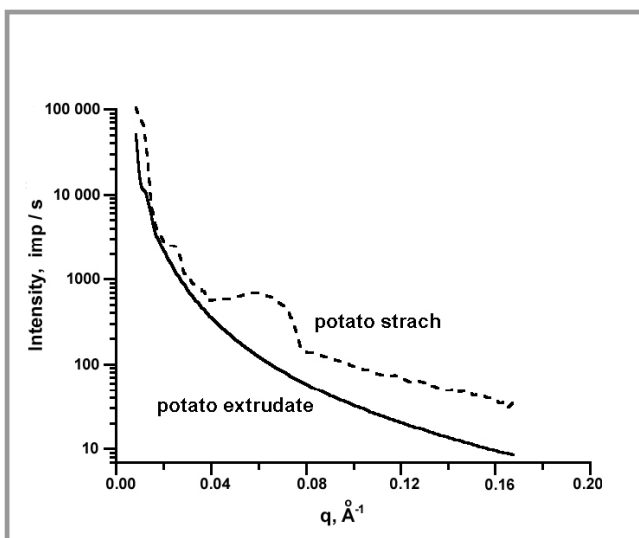

Figure 5. *The SAXS curves for potato starch and extrudate produced by using potato starch.*
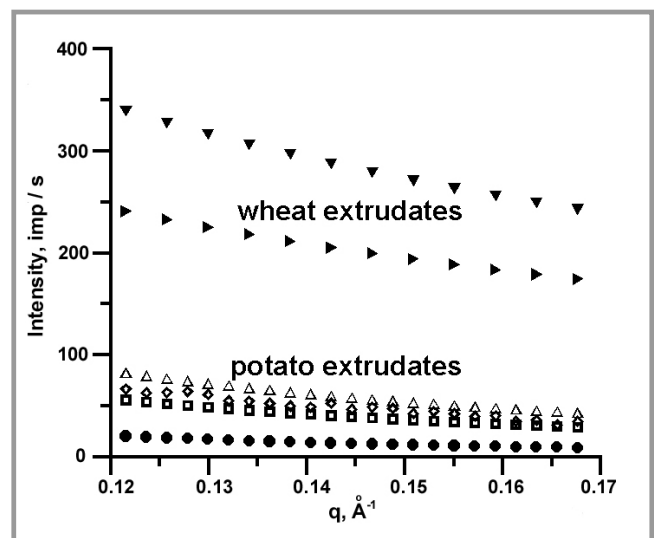

Figure 6. *The end part of the SAX scattering curves for samples of wheat and potato starch extrudates.*

of electronic density heterogeneities, which number decreased after further aging. The curves presented in Figure 7 demonstrate different runs (shapes); this means that the size of the scattered heterogeneities was changed during bread staling. Taking into account that starch is a main compound of bread, as well as results obtained by other methods [17], it can be stated that the changes in SAXS intensity should be linked to the rearrangement of starch matrix structure.

The small-angle X-ray scattering measurements can determine whether the scatterers are fractal, and allows estimating the fractal dimension D to be estimated by analysing power law scattering; $I(q)=I_o \cdot q^{-\alpha}$ where I_o and α are constant, and for $1< \alpha < 3$, $D_m = \alpha$ (D_m – mass fractal dimension), $3 < \alpha < 4$, $D_s = 6 - \alpha$ (D_s – surface fractal dimension) [18]. The α–values obtained for the curves in Figure 7 are in the range 1.75 to 2.5, which indicates a mass fractal structure. The greatest value of α–value ($\alpha=2.5$) for a 1-week aging time was observed. The decrease in the α-value during further aging suggests that after one week of aging, the species which form the scattered heterogeneities are more unfolded, and are straighter than in the fresh bread.

Sometimes, the analysis of power law scattering of SAXS curves for starch products can bring especially interesting results. In Figure 8 the log-log plots of SAXS curves for extrudates produced by using oat raw materials are presented [19]. The SAXS curve presented in Figure 8a originated from the extrudate which was made from milled oat and its bran, while the curve in Figure 8b is from the extrudate produced by near-pure milled oat. In the first figure the curve forms a near-straight line with an inclination of about 1.83, while in the second figure the SAXS curve forms two straight lines with different inclinations (0.98, 1.72). For both the curves, the values of α coefficient obtained indicate mass fractal structures. The results presented in Figure 8 are rather unexpected because they suggest that the extrudates produced by using a mixture of milled oat and its bran have a more uniform structure than the extrudates produced from near-pure milled oat. It is worth noting that both kinds of oat extrudates have different tastes.

Conclusions

Starch is a most important biological material, and a very important component of many food products or materials often used by man. Thus, knowledge of the properties and structures of native starch and of the changes, which take place in starch structure during many different processes as hydrolysis, gelatinisation, dissolving, melting, extrusion etc., is very desirable. The studies of starch structure are very important not only from the theoretical but also from the practical point of view.
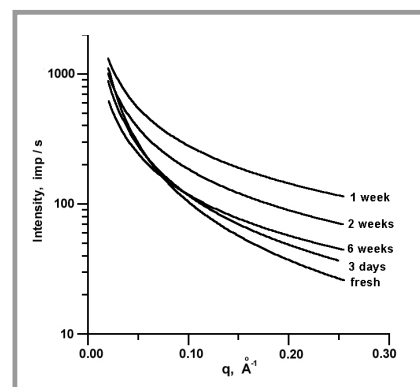

Figure 7. The SAXS curve for dry samples of bread aging by increasing time.

The small-angle X-ray method (SAXS) is very useful in investigating both native starch and starch products. For native starch, the SAXS method allows the study of structure and changes in the structure of amylopectin. The lamellar structure of amylopectin with 80-90 Å thickness of lamellae causes the SAXS curves to show a distinct, visible peak. The analysis of location and peak areas of these peaks allows several values to be estimated, namely lamellar repeat distance, fractional lamellar crystallinity, width of the distribution of lamellar sizes, electron density differences between the three regions of starch granule, the number of semicrystalline repeats within each growth ring whose details characterise the starch structure, and the changes which occur in the starch matrix. The two approaches for envisaging starch structure from the point of view of the SAXS method are familiar in literature.
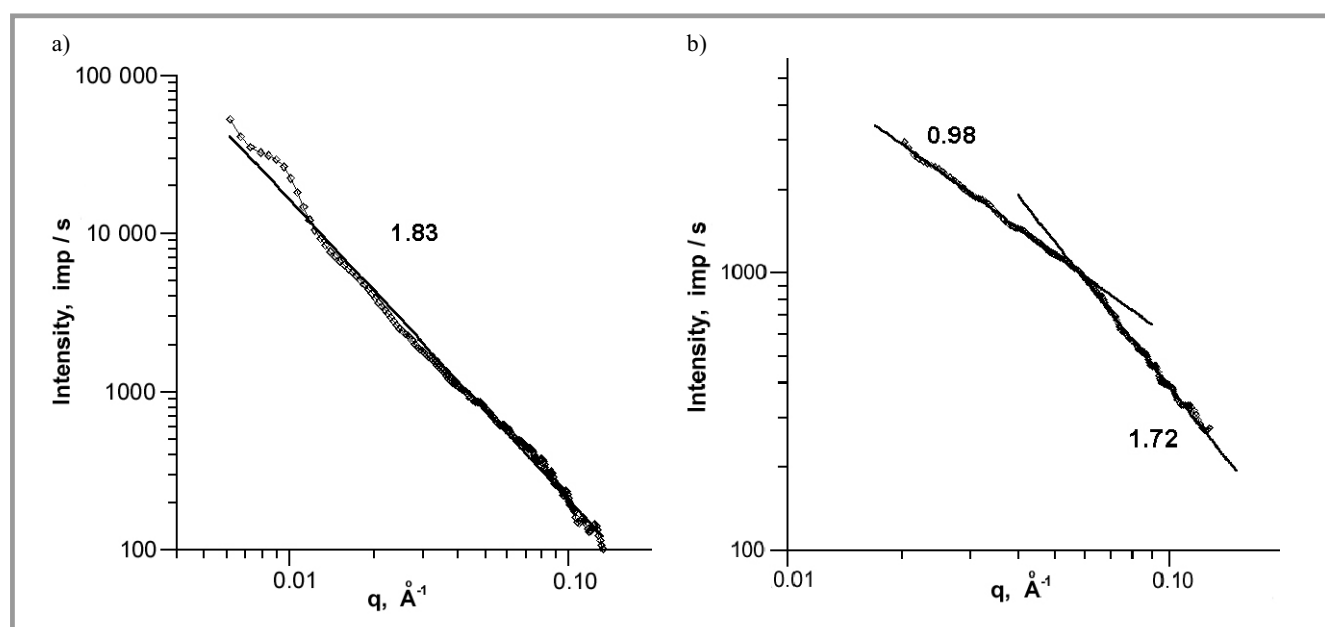

Figure 8. The log-log plots of SAXS intensity of oat extrudates: a) extrudate which was produced from milled oat and its bran, b) extrudate which was produced from the only milled oat.

The first one describes starch as a lamellar system of two phases (amorphous and crystalline) placed in a third, amorphous phase. It is worth noting that SAX scattering arises only when heterogeneities in electron density exist in the sample, and for this reason each phase means a separate region which differs in electron density from other regions. The second approach describes starch as a liquid crystal with different degrees of ordering (smectic, nemetic, isotropic).

The SAXS method has been more rarely used for studying starch products. This results from the fact that in most of these materials the lamellar structure of amylopectin is quite collapsed or seriously destroyed. Starch is not the only component in these materials, which makes the analysis of SAXS data more complicated than in native pure starch. However, for this kind of starch material (food products) the SAXS method also allows many values to be obtained which can undoubtedly help in classifying the properties of these materials. For starch products, the SAXS method allows the number and size of electron density heterogeneities to be estimated, although it is not in most cases

possible to identify these heterogeneities only by analysing SAXS data. Moreover, by analysing the power law scattering for SAXS curves, it is possible to describe the uniformity of the scattered heterogeneity sizes, as well as to determine the class of these heterogeneities.

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