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PHYSICO-CHEMICAL CHANGES OCCURRING IN GAMMA IRRADIATED FLOURS STUDIED BY SMALL-ANGLE X-RAY SCATTERING

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Abstract

Commercial samples of wheat, rye and potato flour and flaked oats, as well as ground flaked oats and fresh potatoes were gamma-irradiated (20 or 30 kGy dose). The products and appropriate control samples were investigated by small-angle X-ray scattering (SAXS) in the range of 2θ from 0.357 to 6.46 degree (CuK_{α} radiation). The results were compared with those obtained for pure potato starch. Effects of grinding and heating at 100° C on SAXS results were also examined.

Different scattering curves were obtained for various unirradiated products. Reflections derived from starch were detected for some unirradiated as well as irradiated products. A reflection connected with long-range ordering in starch (corresponding to the distance d ≅ 10 nm) was observed for potato, wheat, rye flour and potato powder. A reflection corresponding to the distance d ≅ 1.6 nm (due to short-range ordering) was observed in the cases of potato flour and potato powder. Comparison of diffractograms of gamma-irradiated and unirradiated products, have revealed changes of the reflection corresponding to d ≅ 10 nm intensity and elevation of scattering curves, connected with changes of long-range ordering in starch granules. The effect of gamma-irradiation on long-range ordering in starch was also observed after heating of starch at 100°C. Heating at 100°C as well as grinding, cause diminution of long-range ordering in starch granules.

KEY WORDS: Small-angle X-ray scattering, flour, cereal products, starch; gamma-irradiation, heating, grinding; structural changes in gamma-irradiated cereal products.

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Introduction

The present development of gamma-irradiation techniques as a promising method of food preservation requires elaboration of appropriate trade control for detection of irradiated foodstuffs. Currently instrumental methods are required for such control. The necessity of recognition of the physico-chemical changes occurring after gamma-irradiation and estimation of the applicability of the particular instrumental methods to check irradiated foodstuffs are closely related to these requirements.

Our previous papers [2, 4] presented results of investigations of gamma-ray irradiated potato starch using small-angle X-ray scattering (SAXS). Reflections have been observed corresponding to an interspacial distance d ≅ 10 nm connected with an occurrence of long-range ordering in starch granules (ordered distribution of polysaccharide macromolecules). It has been discovered that gamma-irradiation causes destruction of such ordering, as a result of starch degradation. The efficiency of the process is different in dried preparations than in their water suspensions [4].

It is therefore advisable to carry out similar experiments for different kinds of cereal products and for potato flour. It has been pointed out that the diffractograms of these products are likely to differ from diffractograms of pure starch due to the presence of other components (i.e., proteins, lipids, fatty acids, saccharides, water) and due to the grinding process being carried out. The additional components are capable of affecting the course of starch degradation process occurring under gamma-irradiation [6, 7] and thereby to change the experimental results obtained using SAXS. Gamma-irradiation is able to modify these components, (e.g., proteins [5, 7]).

It has been observed that water suspensions of starch grow dark during irradiation and that yellow water suspensions result when prepared from irradiated starch samples. This phenomenon seems to be caused by the rearrangement of the residual proteins. Similarily, starch samples, gamma-irradiated and then heated at the temperatures as high as 100°C turn yellow, while unirradiated samples remain white. Therefore, we have investigated the ability of SAXS method to detect changes caused by gamma-irradiation in heat treated foodstuffs containing starch.

In this work results are presented of the SAXS method applied to various commercial flours (wheat flour, rye flour, potato flour) and flaked oats - untreated and gamma-irradiated - as well as for potato powder prepared under similar conditions from unirradiated and irradiated potatoes. Irra-

diation doses of 20 kGy or 30 kGy were applied. The effects of grinding and heating at 100°C on the results obtained by the SAXS method have also been investigated.

Experimental

Preparations of the samples

Commercial potato flour (PF), wheat flour (WF), rye flour (RF), and flaked oats /commercial (FO) as well as powdered oats (GFO; ground in a mortar for 0.5 h.)/ were gamma-irradiated applying a 30 kGy dose.

Two samples of potato powder were prepared from potato of Fauna type, harvested in 1988 and kept at 4°C. Sample P2 was prepared from potatoes (10 tubers) irradiated in April 1989 using 20 kGy dose. After irradiation the potatoes were grated, dried at 30°C for 5 days and powdered in a mortar for 0.5 h. Simultanously a reference sample (P1) was prepared from unirradiated potatoes.

A sample of starch (S1) was extracted from potatoes of Fauna type in April 1989 (from potatoes harvested in 1988 and kept at 4°C) and dried at 60°C for 18 h.. Part of sample S1 was ground in a mortar for 15 min. The other part of sample S1 was irradiated applying a 20 kGy dose. Afterwards, a part of the initial sample S1 and irradiated product were heated at 100°C for 70 h.

Irradiation

Irradiations were carried out at ambient temperature in a gamma cell "Issledovatiel" having an activity of 3 kCi ^{60}Co. Commercial flour (potato, wheat, rye flour), as well as commercial and ground flaked oats (FO and GFO samples) were irradiated at a dose rate of 10 kGy per h.. For irradiation of potatoes and starch a dose rate of 1.58 kGy per h. was applied. All the samples were placed in closed polyethylene capsules.

Small-angle X-ray Scattering

Dense water suspensions of the samples were investigated using a Siemens D-500 Diffractometer with a small-angle camera of Kompakt Kratky type (production of A. Paar, Austria), K 800 generator and scintillation counter with single channel analyzer.The $Cu_{K\alpha}$ (Ni filtered radiation $\lambda = 0.154178$ nm) was employed with a tube voltage 50 kV and a tube current 25 mA. The widths of input and output slits were 150 μm and 300 μm, respectively. The suspensions were put into cuvettes under Mylar film. The measurements were carried out in the range 2θ from 0.357 to 6.46 degree (d values from 24.7 to 1.368 nm) with a step $\Delta 2\theta = 0.057$ and counting time of 40 s.

Results

The measuring range consisted of the small-angle region followed by begining of the wide-angle region.

The scattering curves obtained for commercial wheat flour, rye flour and potato flour are presented in Fig. 1 in comparison with the scattering curve of the sample of pure potato starch S1. Fig. 2 shows the scattering curves of flaked oats, ground flaked oats and potato powder P1.

The diffractogram of commercial potato flour was similar to the diffractogram of pure starch (Fig. 1; PF). Two reflections, derived from starch, corresponding to the interplanar distances d ≅ 10 nm (in the small-angle range) and d ≅ 1.6 nm (the first starch reflection in the wide-angle range), were recorded in the scattering curve. The intensities of these reflections were similar to those recorded in the case

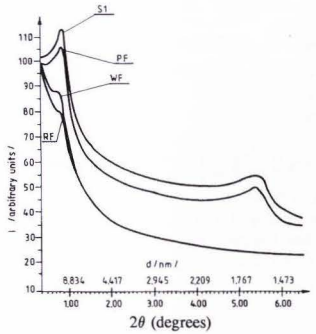

Fig. 1. Scattering curves recorded in the full measuring range for: potato starch S1), potato flour (PF), white flour (WF), and rye flour (RF). The background levels of the curves recorded for samples S1 and PF are shifted relatively to the background level of the curves recorded for samples WF and RF.

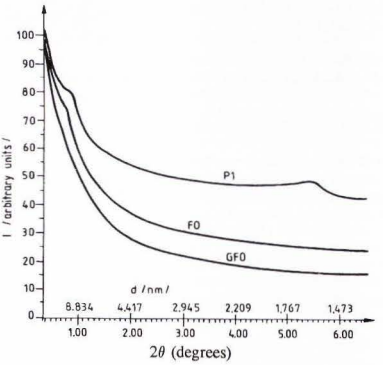

Fig. 2. Scattering curves recorded in the full measuring range for: flaked oats (FO), ground flaked oats (GFO), and potato powder (P1). The background levels of the curves recorded for samples FO and P1 are shifted relative to the background level of the curve recorded for sample GFO.

of starch sample S1. Both these reflections were also recorded in the case of potato powder (Fig. 2; P1) but at very weak intensities. In the cases of wheat flour and rye flour (Fig. 1; WF, RF) weak intensity reflections were observed in the small-angle range, particularly in the range of the first reflection derived from starch (corresponding to the interplanar distance d ≅ 10 nm in the case of potato starch).

No significant reflections were observed in the scattering curves of commercial and ground flaked oats (Fig 2; FO, GFO). However in the case of commercial flaked oats a

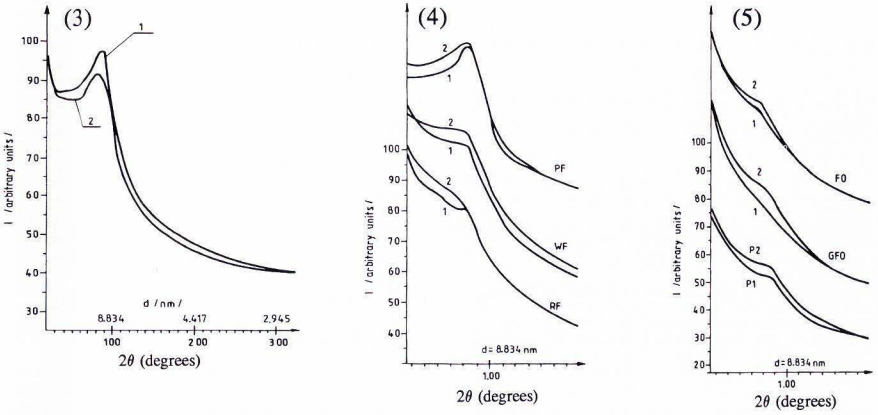

Fig. 3. Comparison of the scattering curves recorded in the range 2θ to 3.3 degree (d value to 2.68 nm) for the initial sample S1 (curve 1) and the product obtained after grinding of sample S1 in a mortar for 15 minutes (curve 2).

Fig. 4. Comparison of the scattering curves recorded in the range 2θ to 1.7 degree (d value to 5.2 nm) for unirradiated and irradiated with 30 kGy dose: potato flour (PF), white flour (WF), and rye flour (RF). The curves are assigned as 1 for the cases of unirradiated samples and as 2 for the cases of irradiated products. The curves drawn for potato and wheat flour are shifted relative to the curves of rye flour.

Fig. 5. Comparison of the scattering curves recorded in the range of 2θ to 1.7 degree (d value to 5.2 nm) for unirradiated to those recorded for irradiated with 30 kGy dose, respectively: flaked oats, ground flaked oats (FO, GFO; the curves are assigned as 1 for the cases of unirradiated samples and as 2 for the cases of irradiated products) as well as of the scattering curves recorded for potato powder, prepared from unirradiated potatoes (P1) and from potatoes irradiated with 20 kGy dose (P2). The curves drawn for flaked oats and ground flaked oats are shifted relative to the curves of potato powder.

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small inflection on the scattering curve in the region of the first reflection of starch was visible when compared with the scattering curve of ground flaked oats.

No other reflections besides those corresponding to the reflections of starch could be identified in the scattering curves in any case.

To determine how grinding influences the course of the scattering curves of starch - the main component of each cereal product - in the small-angle range, the product obtained after grinding of sample S1 in a mortar for 15 min was examined by SAXS method. For the ground sample the intensity of the reflection corresponding to the distance d $\cong$10 nm is lower than in the case of initial sample S1 (Fig. 3). Differences between the intensities of the reflection corresponding to distance d $\cong$ 1.6 nm were not observed.

Scattering curves were recorded of gamma-irradiated (30 kGy dose) potato flour, wheat flour, rye flour, flaked oats, ground flaked oats as well as potato powder P2 prepared from potatoes gamma irradiated applying a 20 kGy dose. In all cases the scattering curves of irradiated products were elevated in the small-angle range (the region of occurrence of the reflection corrresponding to d $\cong$ 10 nm), as compared with the scattering curves of unirradiated samples. When the reflection corresponding to the distance 10 nm was clearly observed on the scattering curves of the unirradiated flour (potato, rye, wheat flour), the intensity of this reflection was lower in the case of irradiated product. In the cases of flaked oats (commercial and ground) weak inflections in this region were seen in the scattering curves of irradiated products, when compared with those of appropriate unirradiated samples. The reflection corresponding to the interplanar distance d $\cong$ 1.6 nm was - as in the diffractograms of initial samples - weak or not visible (except for potato flour). As in the case of the unirradiated samples, no other reflections besides those derived from starch were detected in the scattering curves.

Figs. 4 and 5 show comparison of the scattering curves for irradiated products and appropriate reference (unirradiated) samples in the range of the first starch reflection.

The products obtained by heating at 100^{0}C (without melting) for 70 h. of initial and irradiated (20 kGy dose) starch sample S1 were investigated applying SAXS. The results were compared with those obtained for unheated samples.

Reflections, corresponding to distances d $\cong$ 10 nm and d $\cong$ 1.6 nm were still observed in diffractogramms of heated samples. However, compared with diffractograms of appropriate unheated samples, both reflections were less intensive and the scattering curves were elevated in the range of

the first reflection.

The scattering curves of the products obtained after heating at 100°C of unirradiated and irradiated samples revealed similar differences as noted in the scattering curves recorded for corresponding unheated samples (lower intensity of the reflection corresponding to the distance d ≅ 10 nm and elevation of scattering curve in this range, in the case of irradiated sample).

Fig. 6 presents the scattering curves for starch sample S1 before and after irradiation (20 kGy dose) after heating at 100°C, in comparison with the scattering curves of initial sample S1 and irradiated (20 kGy dose) product.

Discussion

Only reflections derived from starch, corresponding to d ≅ 10 nm (small-angle reflection, connected with long-range ordering in starch granules) and d ≅ 1.6 nm (the first reflection in the wide-angle region, connected with short range ordering) were distinquished in diffractograms of control and irradiated cereal and potato products.

Different scattering curves were obtained for particular unirradiated products. The intensities of the reflections were smaller (except for commercial potato flour) than recorded for a reference sample of potato starch. In the case of flaked oats the reflection corresponding to d ≅ 10 nm) was not visible at all. This reflection is suggested to be related to the ordered distribution of amylose and amylopectyn macromolecules in starch granules [1]. The reflection related to short-range ordering of starch (d ≅ 1.6 nm) was recorded only for potato flour and - though with very small intensity - potato powder. This is in accordance with literature data [8]. Four types of starch are distinquished on the basis of wide-angle diffractograms. The appearence of the first reflection in this range is considered as specific for starch of the B-type (distance of 1.6 nm corresponds to the a parameter of orthorombic unit cell [9]). Only potato starch among those investigated - belongs to this type, while wheat, rye and oat starch belong to the A type.

The weak intensity of small angle reflection (d ≅ 10 nm) of starch in the case of cereal products, in comparison to the reference sample of potato starch, arises because starch is only one - though predominant - of their components. However a variability in intensity, shape and the angle position of these reflections depends on the variability in the starch structure (macromolecular ordering in starch granules). It can vary for different kinds of starch derived from different varietes of cereals and depends on their ripeness. In fact, such ordering is practically not visible in the case of our commercial flaked oats. Moreover, our investigations carried out on control and ground samples, have revealed, that grinding (into flour) diminished the long range ordering in starch grains, probably because of cracking on the microfibrils' interfaces. Therefore, in the case of pure potato starch, the smaller intensity of the reflection corresponding to d ≅ 10 nm and elevated scattering curve were recorded for ground rather than the initial sample. Even in the case of flaked oats, for which the small-angle reflection was not detected, the scattering curve appears flatter in the case of the ground than in the case of initial sample in the range of this reflection.

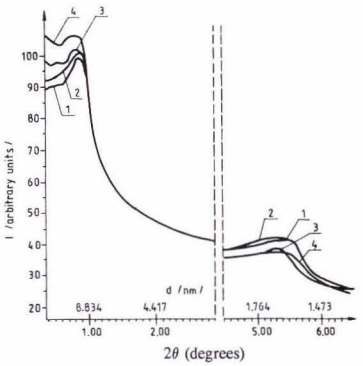

Fig. 6. Comparison of the scattering curves in the ranges of occurrence of both starch reflections recorded for the initial starch sample S1 (curve 1) and for the products obtained after irradiation of the sample S1 with 20 kGy dose (curve 2) and after heating of the sample S1 at 100°C for 70 hours (curve 3) as well as after irradiation with 20 kGy dose and afterwards heating at 100°C for 70 hours (curve 4). In the range of the second reflection, the curves 1 and 2 are shifted relative to the curves 2 and 3.

Similarily, grinding causes the diminution of short-range ordering (amorphization of crystalline starch [3]).

Weak intensities of both starch reflections in the case of the sample P1 of potato powder can additionally be explained by the long period of sample drying (5 days); it arises from our previous examinations [2] that the long-range as well as short-range ordering in starch granules are influenced by prolonged water treatment.

In the case of irradiated products, in comparison with unirradiated ones, changes were observed in diffractograms, suggesting an alteration of macromolecular ordering in starch granules occurring under gamma-irradiation. In the cases of potato, wheat and rye flour the diminution of this ordering can be concluded. Similarily, the smaller intensity of the reflection corresponding to d ≅ 10 nm and elevated scattering curve in this range were recorded previously in the case of irradiated potato starch sample, when compared with scattering curve of unirradiated sample [2, 4]. It was concluded [2] that starch degradation during gamma-irradiation results in destruction of ordered distribution of amylopectyn and amylose macromolecules. In the case of flaked oats, both commercial and ground, a little different phenomenon was observed. Simultaneously as in the cases of pure potato starch, the scattering curves of irradiated samples

were elevated compared with those of appropriate unirradiated samples. But, in contrast with starch, weak reflections appeared in the range of the first starch reflection only in the cases of irradiated products. This suggests the appearence of polysaccharide ordering catalyzed by gamma-irradiation though the rearrangement of other molecules is not unlikely. In fact, it can be expected that the rearrangement of other molecules (i.e. proteins, lipids), occurring under gamma-irradiation in cereal and potato products, cannot be detected under the present conditions by SAXS. In the cases of diffractograms of unirradiated as well as of irradiated potato, rye, wheat flour and potato powder no additional reflections, connected with ordered distribution of these molecules, were distinquished, though the course of scattering curves depends on their presence in the samples.

No correlation between gamma-irradiation being applied and intensity of the reflection of potato starch corresponding to the interplanar distance $d \cong 1.6$ nm was found previously in the cases of pure starch samples [4], nor was it found here for potato flour and potato powder.

The starch granules still preserved an ordered structure at temperatures as high as 100^0C (below melting point) even after 70 h. However, a diminution of long-range ordering, as compared to the unheated samples, is revealed on the basis of SAXS results (diminution of intensity of the reflection corresponding to $d \cong 10$ nm and elevation of the scattering curve in the range of this reflection). After heating up to 100^0C similar differences between long range ordering in irradiated and unirradiated starch could be seen as in the case of unheated samples.

Therefore, it can be predicted - on the basis of comparison of the results obtaind for heated irradiated and unirradiated starch with those obtained for irradiated and unirradiated cereal and potato products - that the scattering curves in the small-angle range will be elevated and the reflection corresponding to $d \cong 10$ nm will be less intense also in the cases of cereal and potato products, irradiated and then heated at temperatures to 100^0C than in the case of unirradiated sample heated under the same conditions.

Conclusions

Differences between flours, obtained from different varieties of cereals and of potatoes, as well as between unirradiated flour and irradiated product can be detected applying small-angle X-ray scattering. Reflections related to ordered distribution of starch macromolecules can be distinquished in diffractograms, corresponding to $d \cong 10$ nm (connected with long-range ordering; in the cases of potato, wheat, rye flour) and $d \cong 1.6$ nm (connected with short range ordering; in the cases of potato flour and potato powder). Starch rearrangement occurring under gamma-irradiation in flour can be detected under conditions applied in this work. Differences between long range ordering in initial and in irradiated starch can be found even after heating the samples at temperature as high as 100^0C.

Heating (without melting), as well as grinding, leading to flour production, caused diminution of the long-range ordering in starch granules. However, even after heating for 70 h. at 100^0C the long range ordering in starch granules is partially preserved. Grinding causes visible destruction of the short-range ordering in a very short period of time.

Detection of gamma-irradiation of flour by the SAXS method requires a reference sample of unirradiated flour, as was stated before for pure starch [4].

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Discussion with Reviewers

P. Colonna: Is SAXS the most pertinent method for detecting changes?

Authors: Development of the methods of irradiated foodstuffs detection is a continuing current problem. The most elaborate present methods are based on free radical detection (electron spin resonance, thermoluminescence, chemiluminescence), chemical changes (gas chromatography) and electrical measurements [10]. Other physico-chemical methods (i.e., liquid chromatography, viscosimetry, thermal analysis, infrared spectroscopy) as well as biological methods are also being developed. The aim of our paper is to detect structural changes brought about under gamma-irradiation and to recognize the possibility to adopt the SAXS method to study them.

P. Thomas: What was the purpose of irradiating potato tubers to a dose of 20 kGy? For sprout inhibition, the dose needed and permitted is in the range of 0.075 to 0.15 kGy. Under no circumstances can fresh potato tubers be exposed to the very high dose of 20 kGy used in this study. The tubers will show severe radiation injury at this dose level.

Authors: High doses of gamma-irradiation of flours and potato tubers were applied for the purpose of enhancing the

irradiation effects. Similarly, to enhance the heating effects, a long period of heating (70 hours) at relatively high temperature (100°C) was carried out.

P. Thomas: What was the objective of irradiating flour to a dose of 30 kGy? In these products, irradiation is normally used for insect disinfestation for which purpose the maximum recommended is 1 kGy. However, if the purpose is radiation sterilization and improvement of quality for use as food or modification of starch for industrial materials, then a dose of 30 kGy is justified. Please clarify.
Authors: This study was performed with regard to elucidating phenomena occurring during radiation sterilization. Indeed, doses permitted for flour and potato products are lower than applied in this work. However, our results are also interesting from the point of view of irradiation processes which can be applied for modification of cereal products. Feedstuff modification by gamma-irradiation have been recently performed. We expect gamma irradiation to be a possible step in production of amylose or high amylose starch (additives for some kinds of instant products, i.e., legumes, puddings, creams) as well as during production of high quality foodstuffs with assimilable starch, ready for consumption without or after very short boiling (i.e., instant flours, instant porridge, breakfast cereals, chips, snacks, etc.).

P. Colonna: Two orderings at 10 and 1.6 nm are observed. What is the minimal starch concentration necessary to observe these two reflections and what is the influence of gamma irradiation intensity on changes?
Authors: Answers to questions, such as minimal starch concentration necessary to observe long-range ordering and short range ordering and the influence of gamma-irradiation conditions on the destruction of this ordering, must await future studies.

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