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THE APPLICATION OF THE REPLICA AND ELECTRON DIFFRACTION METHODS FOR THE INVESTIGATION OF SOME COMPONENTS OF BREAD

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Key words: bread, starch, electron diffraction, electron microscope.

With the replica and electron diffraction methods such bread components as starch gel, bread crumb and starch cristals were investigated.

The chemical structure of starch is well known and is not the subject of our present study. The literature data [1-6], however, prove that the structure of bread and starch gel is not well known. There is no method which would allow the investigation of the structure of starch gel at the level of colloid dispension, and there is no method, which would allow the investigation of the structure of a bread crumb on this level of matter organization by means of electron transmission microscope, either. In order to bridge this gap, we had proposed and experimentally checked the methods of preparation of bread samples for the investigation in electron transmission microscope. The results are presented below.

METHODS AND MATERIALS

To investigate the structure of starch gel, both wheat and rye starch were investigated. Starch gel was placed on the microscopic screen covered with triofol film. In order to strengthen the film and increase the contrast of the microscopic picture, the screen with the gel was vaporized in carbon and platinum. Samples of starch gels prepared this way were then examined and photographed in an electron microscope JEM-100C applying the accelerating voltage of 60-80 KV. The magnification 5000-100 000 was used.

To investigate the structure of a bread crumb by means of electron microscope of following procedure of the preparation of the sample were proposed: Triofal was immersed briefly in methyl acetate and then placed on the bread crumb. The structure of bread is reflected on the film of triofal. After the removal from the bread sample, the triofal film was vaporized with carbon and platinum. From the replica prepared this way, triofal was removed by immersion in methyl acetate, where it dissolves. The replica was then washed in acetone and distilled water. The replica obtained this way, reflecting accurately the structure of bread, was examined and photographed in the electron microscope. A great advantage of the replica method is that the bread sample is not damaged and the replica may by taken from the same place again, thus giving the possibility to investigate the changes in the bread crumb during staling.

RESULTS

Fig. 1 shows the picture of wheat starch gel as seen in the electron microscope. As it may be seen from the picture, the starch gel consists of small granules of the size of 10-500 nm i.e. in the colloid dispersions. It is our supposition that the "big" granules are formed from amylopectin, while the "small" are from amylose. The structure of rye starch gel is shown in Fig. 2, and seems to be very similar to wheat starch.

It was interesting to examine the structure of bread crumb on the same level of structure organization. Fig. 3 shows the picture of the rep-

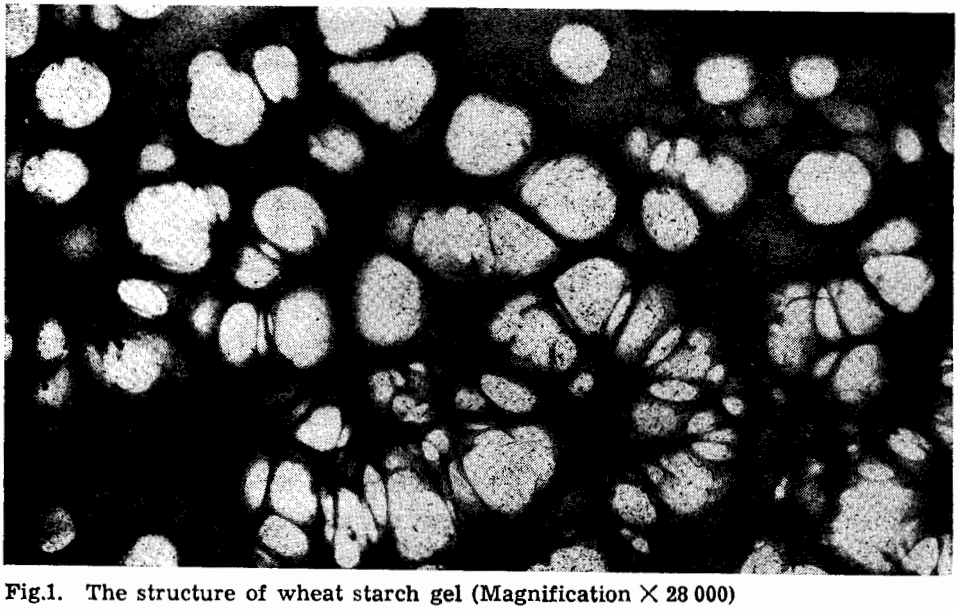

Fig.1. The structure of wheat starch gel (Magnification × 28 000)

lica taken from rye bread and photographed in the electron microscope. It seems obvious that the structure of starch gel is also present in the bread crumb. Fig. 4 shows the picture of the replica taken from wheat bread. This replica in not typical, however. Part of starch from the bread stuck to the triofal film and remained in the carbon-platinum replica, but from Fig. 4 and 1 it may be seen that both structures are very similar. The replica method confirms the existence of the structures presented in Fig. 1 and 2.

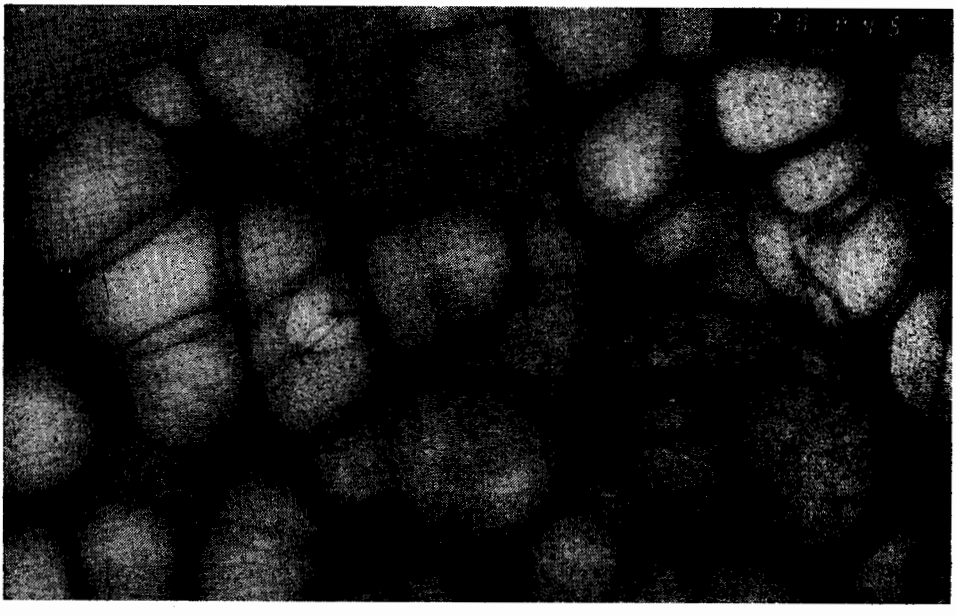

Fig.2. The structure of rye starch gel (Magnification × 28 000)

The replica method has one more advantage. With the stream of electrons penetrating immediately the investigated starch preparation some artefacts may occur. This is not the case with the replica, where the stram of electrons penetrates the platinum replica, in which the bread or starch structure is accurately reflected.

In our opinion, the replica method may prove useful in investigating the structures of food products in the electron transmission microscope, and may be very important both for technological and biochemical investigations reaching beyond the science of cereals.

We have also made a strong effort to investigate the crystalline structure of starch, which is the subject of many papers in world literature. In no paper, however, have we found either the picture of starch crystals, or the electron difraction being one of the basic criteria of its identity. In starch gel no crystalline form of starch was found. We have found,

however, the crystalline forms of starch in bread. The "older" the bread the more crystalline forms of starch could be found. This problem, however, will be further investigated.

In Fig. 5, the picture from the electron transmission microscope showing starch crystals is presented, and in Fig. 6, the electronic diffractions

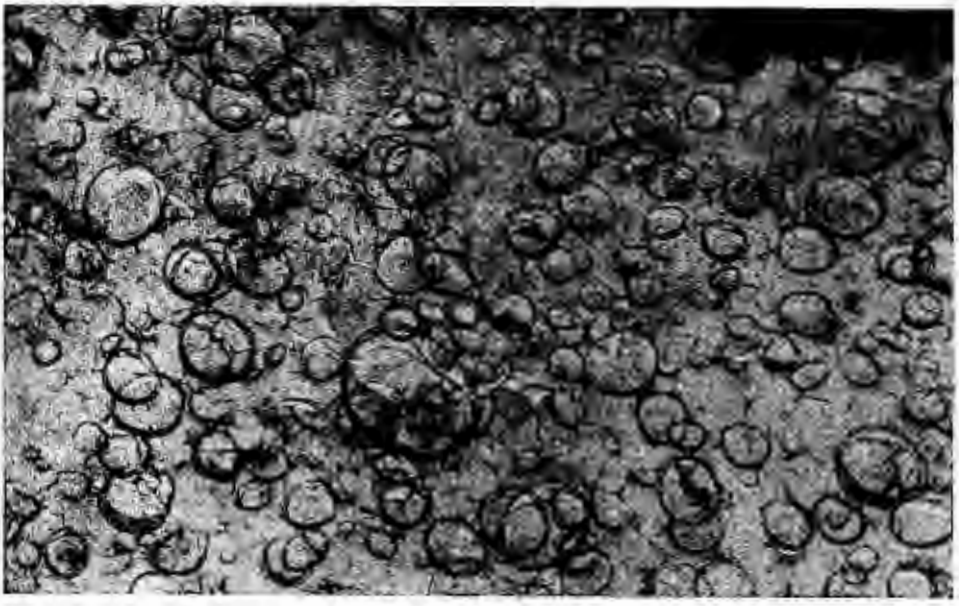

Fig.3. Replica of rye bread structure (Magnification × 14 000)

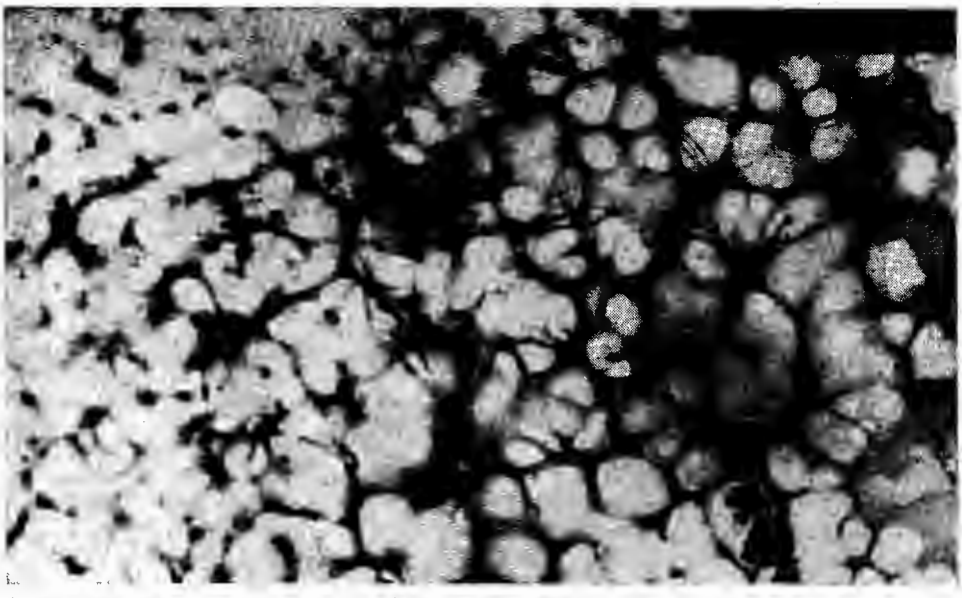

Fig.4. Replica of wheat bread structure (Magnification × 28 000)

of this crystal. The location of the points of electronic diffractions on the picture do confirm the crystalline structure. The above starch was found in the rye bread crumb, 12 hours after baking. Fig. 7 shows the picture of crystalline starch from wheat bread.

Fig.5. The starch crystal from rye bread (Magnification × 70 000)

Fig.6. The electronic diffraction on starch crystal shown in Fig.5

For comparison, the crystals of salt (Fig. 8) and sugar (Fig. 10) are shown. Fig. 9 and 11 show respectively electronic diffractions on these crystals.

Fig.7. The starch crystal from wheat bread (Magnification × 70 000)

Fig.8. The salt crystal (NaCl)

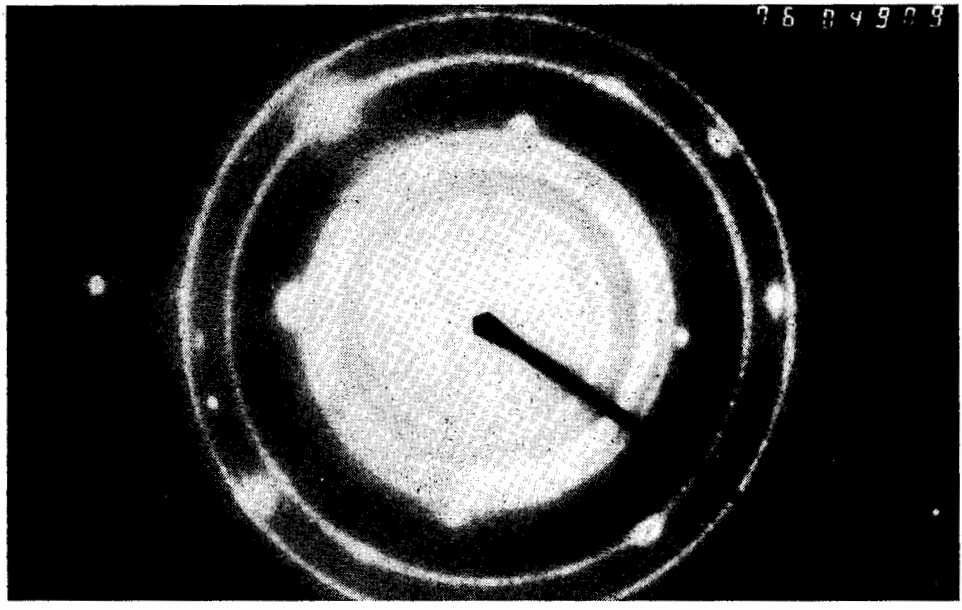

Fig.9. The electronic diffraction on salt crystal

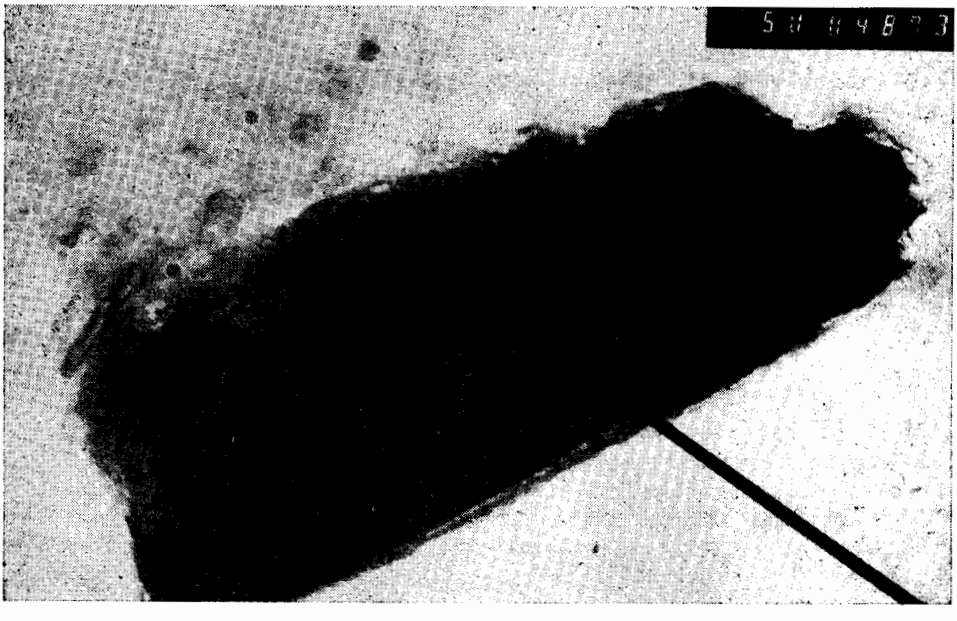

Fig.10. The crystal of sugar

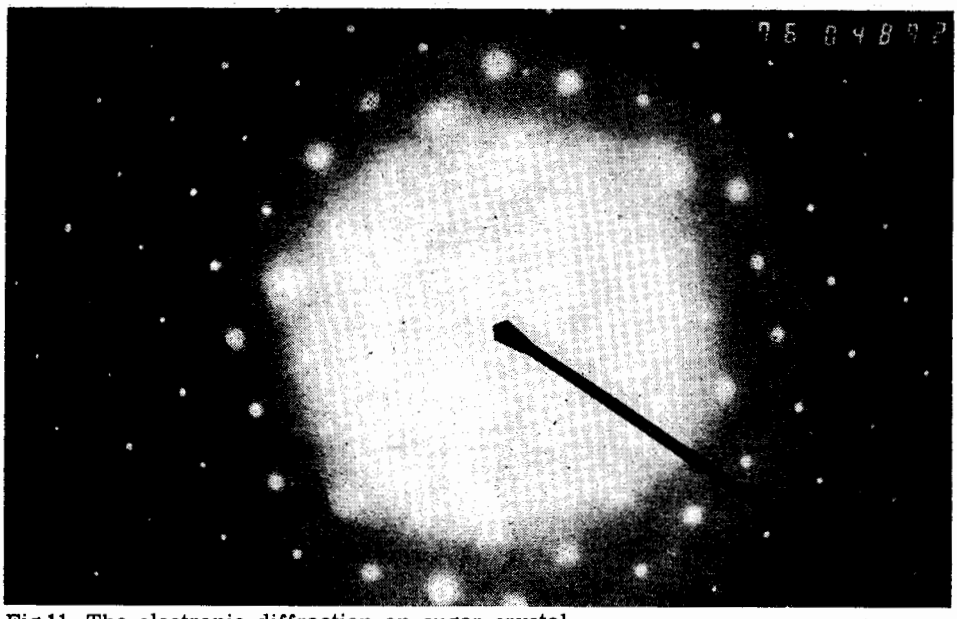

Fig.11. The electronic diffraction on sugar crystal

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ZASTOSOWANIE MIKROSKOPU ELEKTRONOWEGO DO BADANIA NIEKTÓRYCH SKŁADNIKÓW CHLEBA

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Streszczenie

Przeprowadzono studia nad wykorzystaniem metody repliki i dyfrakcji elektronowej do badania struktury skrobi w chlebie. Autorzy opracowali własne modyfikacje tych metod do badania kleików skrobiowych oraz miękiszu chleba. Stosując powiększenia od 14 do 26 tys. razy obserwowano i opisano struktury kleiku skrobiowego i miękiszu. Ponadto stosując powiększenie 70 tys. razy stwierdzono obecność kryształów skrobi zarówno w miękiszu chleba pszennego, jak i żytniego. Ponadto przedstawiono w celu porównania zdjęcia mikroskopowe kryształów cukru i soli.