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STRUCTURAL PROPERTIES OF STARCH IN FOOD SYSTEMS

Abstract

In the present work, structural changes of starch granules as seen by LM, SEM, TEM, CLSEM under different processing were shown, in relation to a function they were playing in the ready products. Special emphasis was also paid to starch change during different modification processes of the isolated starches for food and non-food uses. The structure of irradiated starches, resistant starch obtained by different methods, starches as encapsulating materials, high pressure treated starches as well as packaging materials were presented.

Starch, the most important storage component in many of the plant materials, is not only a source of energy for developing seed but also an important component of human diet. Its properties, depending on botanical source as well as processing are crucial for many functional properties of food.

Starch granules formed in amyloplasts differed in shape, size, localization within the cells as well as in proportions of granules fraction. Their appearance in cells is closely related to other cell components, mainly protein, being different in cereals (Phot. 1a), where protein matrix surrounds starch granules and in legume seeds (Phot. 1b), where additionally protein bodies envelope the granules [7]. Cereal starches are characterized by the presence of at least two fractions: large (10–45 μm) and small (1–10 μm). The first ones lenticular in shape are 70–90% by weight but only 30–10% by number, of the whole granule population, while the latest are spherical and more resistant to technological parameters. This is one of the reasons why in the starch industry fractionation of potato or wheat starch granules is being increasingly popular, or why the sources of starch with uniform and smallest size are searched for. Such starchy materials are oat (Phot. 1c) and amaranth (Phot. 1d) or quinoa [1, 8, 12, 28, 36]. Internal, lamellar structure and organization of starch granules is shown in Phot.1e where concentric rings and crystalline and amorphous parts of the granules are clearly dem-

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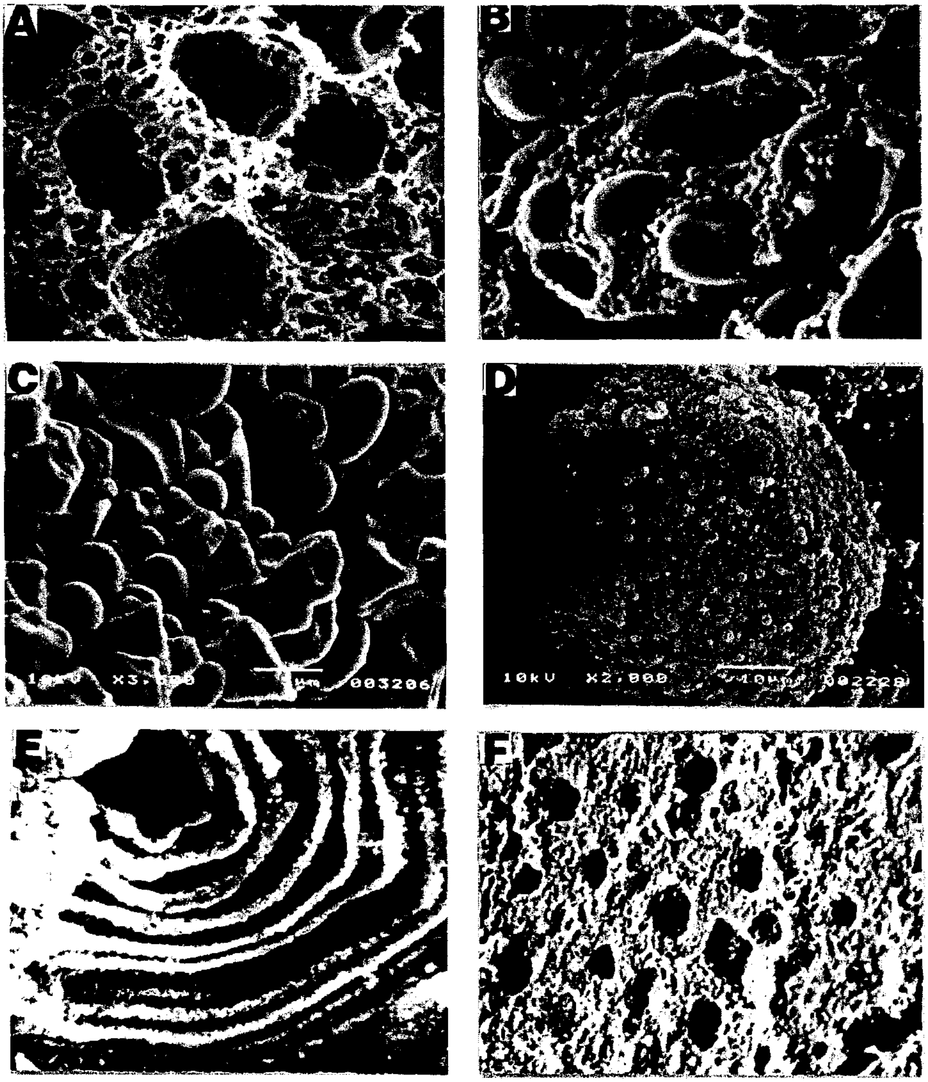
onstrated. Such picture can be obtained only after an enzymatic attack in controlled conditions (α-amylase treated starch in laboratory conditions germinated -as in the case of barley, or sprouted in non-controlled conditions).



Phot. 1. Microscope pictures of different starches;
A/ protein matrix of wheat grain in which starch granules are entrapped (amylase treated); B/ starch and protein in legume cotyledon cell; C/ oat starch granules of different shape and size; D/ amaranth starch agglomerate; E/ lammellar structure of barley starch; F/ cross-sectioned starch granules in corn grits.

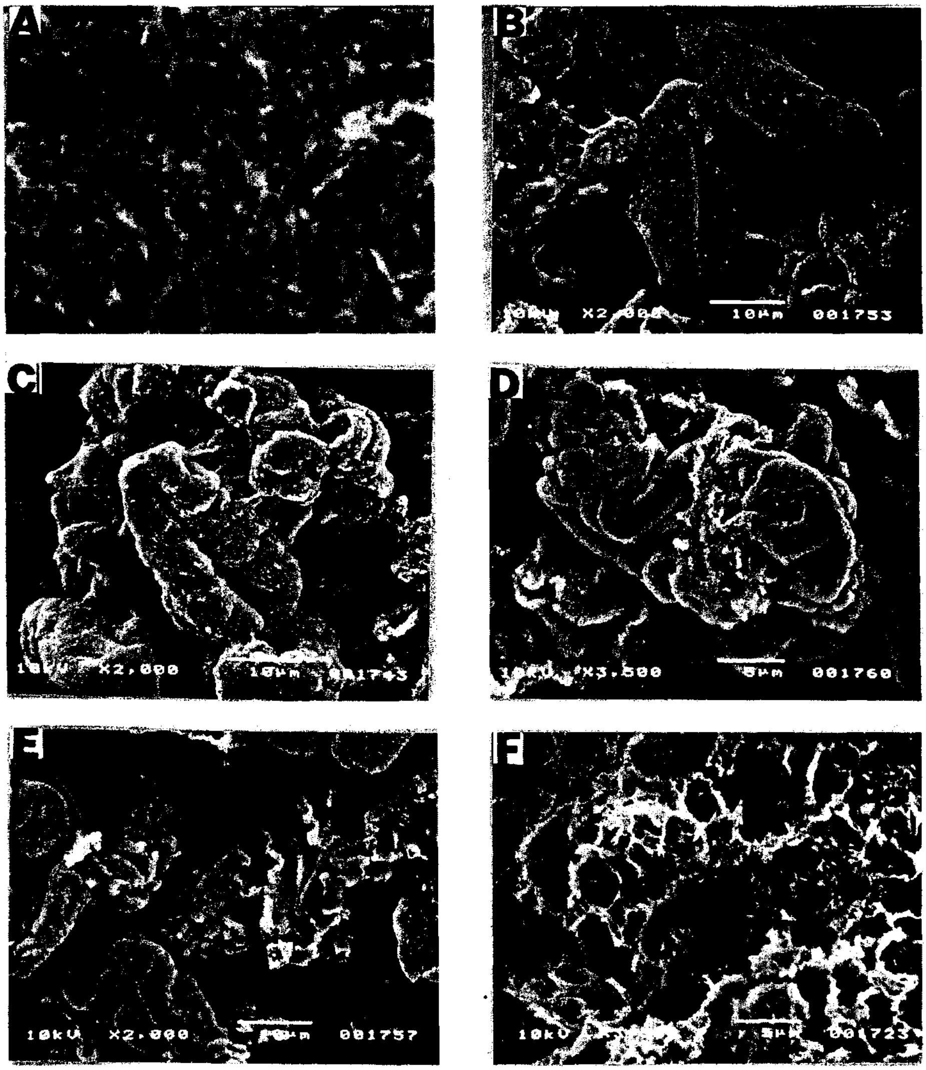
The changes in starch (e.g. among others: swelling, solubilization, gelatinization and granule breakdown) occurring during technological processing, their interactions with other food components result in the physico-chemical properties of raw materials and finally creation of the new texture of the final products. The processes dominating in food technology are of thermal or hydrothermal nature [7, 8, 10, 11, 13-17, 20, 27, 31]. The example of moisture-heat or heat tratment is production of corn flakes and popcorn [7, 11, 15]. The technological process of the former is based on corn grit cooking in sugar-salt solution (2 hr at direct steaming at $1.47 \cdot 10^5$Pa), followed by drying (two stage -90 and 80°C for 40 min) and next flaking and roasting (390°C for 5 min). The structure of corn grit is composed of many polygonal cells in which starch granules stick closely to each other being surronuded by tiny residues of protein matrix. Starch granules of the control, untreated grit reveal "intra grain cavities" with an average diameter of about 2.5 µm. Pictures taken from grits subjected to longer cooking in liquor e.g. 35, 65 and 120 min ilustrated an increase in the dimension of pits observed to 5.5, 9.5 and 16 µm, respectively [7, 12]. It seems to confirm the presence of amylose concentrated in the central part of the granules and the amylopectin in the external one. Phot.1f shows additionally the elements of starch lamellar structure indicating its hydrolysis and considerable amount of minute globular structures, most probably dextrin-like ones. While hydrolysis takes place after 1 hr of the process and probably then shortening of starch granules chain length, division of their componental parts and a high increase in branching could be dominating. An increase in gelatinization degree (from 0 to 100%), drop in molecular weght (58.000–20.000) and viscosity of pastes of isolated starches (910 to 30 BU) confirmed these statements [12]. Drying and flaking of the hydrothermally treated grit and consequent roasting of flakes, due to a very high process temperature and crushing forces, are responsible for the completely different structure of final flakes. Instead ordered cellular structure, porous, spongy-like one, with air bubbles and characteristic air cell walls composed mainly of completely gelatinized starch, is formed. Microscope structure of flakes is to a great extent similar to that of buckwheat extrudate. In the latter, additional structural elements "pipe in pipe" of air bubles surface are visible. They are created during expansion of starch granules in the flower "bud mode" [12].

An interesting structure influencing specific texture of the product is developed during popcorn popping (heating of grains at 180°C / 6 min) where pressure inside the kernel reached 135 psi. The quality of the product strongly depends on the structure of material, mainly endosperm and pericarp. Translucent endosperm is regarded as closely associated with popping expansion being responsible for forming delicate network whereas the opaque one does not expand to such extent [7, 15].

Starch granules are also of great importance in the dough and bread structure formation [19, 24, 26]. A continuous, bimodal starch-protein structure of the dough is based on the phenomenon of free water on starch granule surface which is responsible for creation of continuous starch phase with spaces between granules filled by gluten gel. Such a model of dough proposed by Eliasson and Larsson was confirmed also by Hug-Item et al. [17]. The LM picture of dough stained with iodine and Light Green is presented in Phot 2a. It shows small – round and large – lenticular starch granules embedded in protein part unequally distributed within preparation. The protein fraction is predominant in the dough structure, what was counted for about 60% of bread volume. Baking process resulting in creation of porous crumb structure of bread where swollen elongated and highly ordered at the pore surface granules are clearly visible. These granules are characterized by concentration of amylose zone along the length axis whereas outside the granules and arround protein matrix free leached amylose is seen (Phot. 2a). The bread crumb structure is highly porous, thus the pores diameter and volume as well as the pores wall structure (starch gelatinization degree) are responsible for mechanical / sensory (elasticity, hardness, cohesiveness, gumminess) properties.

Other cereal product where starch is the most important functional factor is pasta produced mainly from durum wheat [29]. Freeze fracture preparation and TEM examination illustrates unmodified starch granules and very minute globular components of gluten. After cooking starch, granules became swollen and gelatinized. Disintegration of starch granules is also clearly visible. Apearing starch subunits are separated or packed together into small clusters whereas protein matrix creates fibrillar network. Although the protein and in particular its subunits network is most responsible for the quality of pasta, such a product can be obtained without protein. Basing on the fact that starches of different origin have diverse gelatinization temperature and as such during technological pasta processing undergo different changes, pasta from rice, maize and potato starch was obtained. Crucial for good quality of such pasta is the phenomenon of starch repolymerization after cooking and creation of repolymerized starch network [29].

Except starches being produced on the large scale as maize (3.6 mln t in EU), wheat (2 mln t), potato (1.8 mln t) and tapioca in Asia, also other sources of starch are taken into consideration [6]. Among them legume starch [9, 10, 16, 18, 32] and starch from so called pseudo cereals (buckwheat, and amaranth) are worth noticing. Among legume seeds most popular are pea starches of different properties, which can be used as food ingredients. Some of them posesses the gelatinization capacity and viscosity profile comparable to these of cross-linked starches showing very good stability at high temperatures, shearing and pH levels, giving covering films or sliceable gels. When products are dried they markedly improve their crispness. Pregelatinized pea starch can be used in cold processes having high gelling capacity what makes possible, at a proper



Phot. 2. Starch granules deformation after heat and moisture treatment;
A/ starch granules in bread crumb structure (LM); B-F/ different stages deformation of pea starch granules during heating in water.

concentration and strong agitation, a rapid development of a firm gel. Therefore legume starches can be used in:

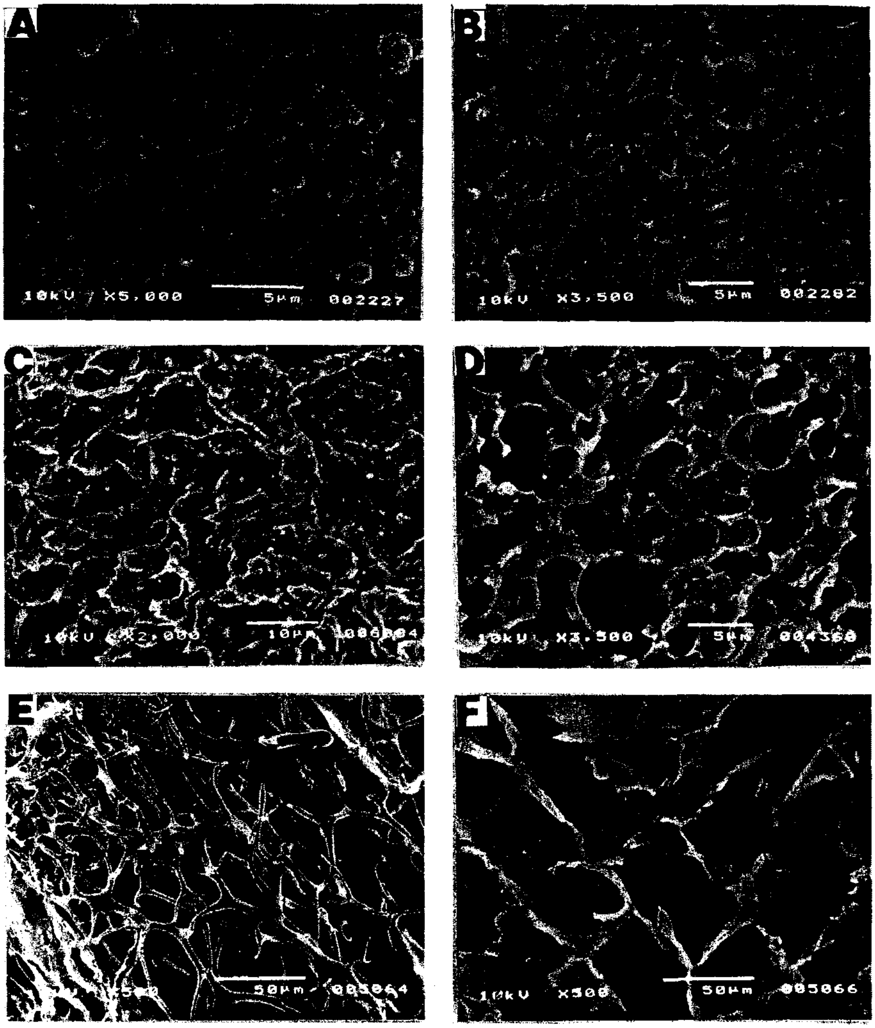
- preparation of gels (puddings) where it is possible to use up to 50% starch less than in comparable products obtained with corn starch,
- production of fruit and vegetable flakes with high cooking stability as well as pulpy texture after rehydration,

- production of instant desserts with desired texture.

The structure of legume (pea, faba bean) starch granules and their changes during heating in water suspensions are widely discussed in literature [7, 16, 20, 32]. Using the smear method, or embedding techniques for light microscope it is possible to illustrate leakage of amylose phase from the granule structure at 75°C and total disruption of granule structure at 98°C with the granule remnants of amylopectin nature embedded in the amylose phase [20]. To improve pasting properties of legume starches as well as their freeze-thaw stability, physical or/and chemical modification is used. Hindering retrogradation is visible by comparison of LM pictures taken from pea starch acetate with those of the native ones. Swelling and subsequent deformations leading at the end of heating to granule disintegration is clearly visible. The results of an additional study on extend of structural deformations that occur during heating of pea starch granules are shown in the Photos 2b-2f. The course of deformatiom and granule disintegration is in high accordance with the Bowler's theory which was developed only for lenticular wheat granules [4]. The initial swelling, which has a radial chracter, leads to flattenning of the granule shape. The amylose concentration took place in the middle part along the lenght axis of the granule folding at this particulary plane (Phot. 2b). It is a reason of folding the granule at this plane. At the next stages of deformation clear visible halves of the granule becomes closer to each other with visible preliminary division into smaller subunits. It is also evident that surface of the granules become intensively pitted and covered by amylose phase (Phot. 2c, 2d). At elevated temperatures, it is possible to observe that tendency to pucker is even higher (Phot. 2d, 2e). It means that some differences at the molecular level between granule axes are present. Probably, as in wheat lenticular starch granules, pea starch granule in xy (lenght-width) plane is composed mainly of molecules bound by covalent bonds in radial direction and in tangential direction by much weaker non-covalent ones. Higher temperatures disrupted non-covalent bonds resulting in the preferential swelling in tangential direction. It is the reason of visible deformation – puckerig of the granule out of xy plane. The last step is the disruption of granular structure of starch (Phot. 2f).

In the last decade, due to consumers demand, substantial reduction of caloric value of food is of great interest in the food manufacture. Lowering of such food ingredients as sugar, salt, cholesterol and fat in the human diet was achieved by special substitutes. Among them, fat replacers or fat substitutes are of starch origin. Native starch granules or modified ones are often used in the production of salat dressings, mayonnaises or processed cheese [21, 22, 34, 35, 36]. Due to special properties, some of native starches isolated from pseudocereals like amaranth are very useful in their manufacturing. The amaranth starch granules are extremely small and uniform in diameter 0.75–1.2 μm being much smaller than the smallest granules of the industrially produced rice starch. The content of amylose can vary, depending on species, from 4.8

to 22%, although, waxy species without amylose are also known. The shape and size of amaranth seed cells resemble that of pseudocereal buckwheat rather than of cereal grain. Starch granules are dominating structural elements whereas adherent to starch protein as well as cell walls were weakly marked. When starch is isolated from the seeds, unusually uniformed starch granules are assembled in greater agglomerates consisted of several hundreds of single granules (Phot. 3a). When amaranth starch is



Phot. 3. Pictures of different starch granules and their products;
A/ native amaranth starch garnules; B/ amaranth starch granules heated 15 min at 85°C; C/ yoghurt with starch as structurizing agent; D/ processed cheese with starch addition; E, F/ different kinds of starch gels.

heated in water suspensions at 55°C, the swelling of individual granules is visible. At elevated temperatures of 70 and 85°C respectively, the significant breaking of individual granules and their agglomeration initiating the network structure followed to form very delicate fibrous network structure after complete gelatinization (Phot. 3b) [36].

The properties of amaranth starch (low pasting temperature associated with a high rheological stability) were the base of its use in low-fat (less than 50%) mayonnaises [36]. If thickening power of amaranth starch was compared to that of potato and, it was found a close relationship between the mayonnaise viscosity and thickening agent addition, and despite of a slightly lower thickening power of amaranth starch as compared to the potato one, the concentration of 1.75% ensured thickening effect acceptable in the traditional product. It is worth stressing that low-fat mayonnaise produced with amaranth starch showed excellent sensory properties, better than these of potato starch. Most probably it resulted from the fine granularity of starch used. The only problem during long storage was the lack of rheological stability for product made from amaranth and potato starch as compared to corn starch. This can be improved by starch modification, using for example standard cross-linking and some stabilizing agents [36].

Another example of calorie-reduced products can be yoghurt and processed cheese (Phot. 3c, 3d). Fat substitutes used in both products can be based, among others, on microparticulate whey protein or starch. Modified starch preparations used under processing conditions are fused with the protein particles giving uniform matrix in which fat droplets or fat agglomerates are placed. Such a structure is responsible for desired texture properties (graininess, stickiness, mouth coating, and greasiness) as well as rheological properties (spreadability, stickiness or cohesiveness) not differing too much from the product without any fat substitute [5, 35].

Another important role that starch can play as a food component or as a pure preparation obtained by starch modification is so called resistant starch (RS) [23, 30, 34]. Resistant starch is a component of raw potatoes and green bananas or can be generated in food due to the action of heat and water. Its final amount to be present in foods is dependent on such parameters as starch concentrations, amylose/amylopectin content, starch/water ratio and energy supplied to the system. Retrograded starch is the most common RS starch in the diet and from the technological point of view, it is the most important type of resistant starch because it is formed as a result of food processing.

Resistant starch can be also produced from isolated starches by retrogradation, spray drying or by enzymatic modification. Depending on the process applied, RS (never being pure resistant starch but the mixture of its different forms) is characterized by different microscope structure and properties (water holding capacity -2.0-3.5 in comparison to 668–732 g/g dmb wheat and potato native respectively, and fat absorption 1.2–1.6 g oil) [22, 23, 34].

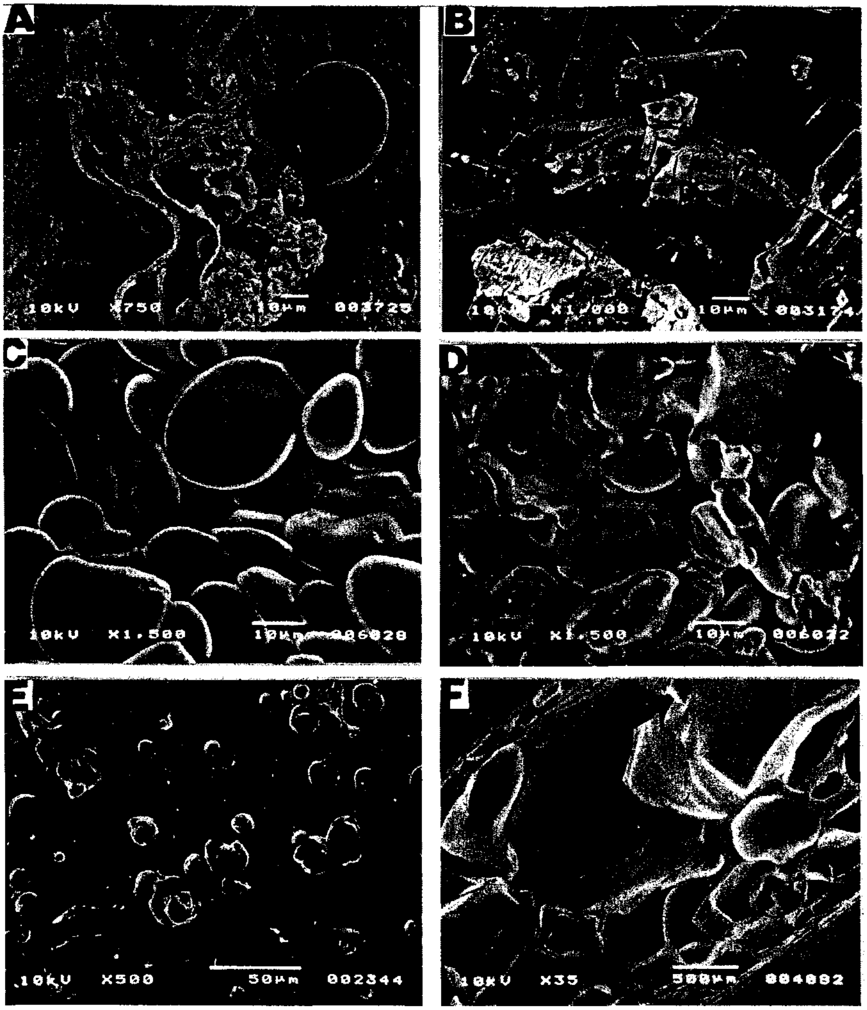
The special properties of starch in food systems – gelling properties, are shown in Phot. 3e and 3f. Structure of such gels is strongly dependent on the proportion between both polymer of starch: amylose and amylopectin, as well as processing parameters [2, 13]. For microscope determinations of the structure of such gels, the factors of great importance are also methods of specimens preparation for analysis. The most important is the step of freezing of water-containing samples which can introduce ice crystal artefacts. The extent of structure damages is dependent mostly on the size of crystals formed. Therefore, to avoid this undesired phenomenon, the special intermediate velocity of freezing (1000°C/sec) is recommended [3]. The newly introduced methods of sample preparation for microscopy as well as new microscope methods will be mentioned latter.

High water binding properties of modified starches as well as their enhanced gelling properties are very usefull for keeping quality of meat products to be portioned and long term stored in the supermarkets. The kind of modification strongly influences mentioned properties of different starches used for this purpose. This is especially visible after heating to the temperature which is reached inside the product during pasteurization [Phot. 4a].

Other popular starch derivatives used as food ingredients are such preparations as: cyclodextrins, porous starches and starch coacervates used as a vehicles for aromas, vitamins and food pigments/colourants or other substances (Phot. 4b) [38].

Except the mentioned above processing and products, very promissing ones in respect to starch properties, among others, are microvawe treatment and high pressure tratment. The changes induced in the granule structure are clearly visible for the former in LM, showing different pattern of breakdown in comparison to the structure of native granules. Spherical single structures or their agglomerates separated or perhaps tight together with brown amylopectin material are dominating in the smeared preparation. Thus, they can result in a rise of gelatinization temperature, drop in solubility of starch granules or viscosity. The extent of those changes was dependent on initial moisture of starch [21, 22]. Also high pressure can markedly influence structure and properties of starch. Depeding on processing parameters, we can obtain the products with different susceptibility to alpha-amylase and different rheological properties (Phot. 4c, 4d).

Starch granules are also used alone or in composition with polymers such as polystyrene to form structure and properties in packaging materials (foils, foams, alkogels) (Phot. 4e, 4f). The biodegradability of foils with starch addition is depending on the properties of starch in concentrates for foil obtaining, their percentage in the mixture and susceptibility to amylase attack. Being degraded first, starch is making place to other hydrolytical enzmyes slowly degrading another part of the foil. Different structure is formed in aerated products as for example plates, which become porous, and are due to many air cells of very low density [37].



Phot. 4. Examples of different use of starches;
A/ meat product; B/ cyclodextrins; C, D/ high pressure treated starch gels (3500 atm at 15 and 60 min); E/ starch in the structure of biodegradable foil; F/ structure forming properties of starch in foams.

Structural analysis of all foods, also these containing starch, in transmission and especially in giving three-dimensional structure impression, scanning electron microscopes are often difficult to interpret because of the artefacts appearing in thespecimen preparation procedure. As it was mentioned above the freezing velocity is one of the most important factors influencing the extent of damaging by ice crystals formation in foods containing water [3]. To avoid this new method of freezing the sample – High pressure freezing (HPF) was developed, which can replace such methods like, for example, jet freezing or mirror slaming. In this technique, the sample is exposed to a very high pressure (2000 atm) and immediately frozen by jet of nitrogen. In 100 μm thick sample of the gel gelatine/water -5/5vv after HPF internal structure even on the depth of 50 μm is very clearly marked, whereas in the sample prepared by the traditional method structural changes caused by ice crystals formation are present on the depth of 5 μm and are even more pronounced on the depth of 15 μm. Also in milk gels such method of preparation, in comparison with the traditional one, reveals much more details of the structure. Preservation of protein structure in the casein network and fat globules is clearly visible. Also after use of immunization and gold labelling the localisation of β-lactoglobulin is much better visualised [3].

The development of new microscope techniques also creates new possibilities for more detailed structural analysis.

Among these methods some are very promissing. Field Emission Scanning Electrone Microscopy combined with cryo preparation allows observations of, for example, different materials with lamellar structure, which until now were possible only by the replication method in TEM. High resolution and no damages occurring at 2 kV is a great advantage of this method [3, 33].

Scanning Tunneling Microscopy is able to ilustrate in real time the surface relief with resolution of 2 A. The very small structures like for example the globular protein –vicyline with molecule length of about 100 A can be investigated. This microscopy can work also in water and low electrone energy (few V) what does not distroy the sample [3, 33].

The special potential for starch structure investigations represents Atomic Force Microscopy where the action of amylolytic enzymes on individual granule as well as the geometry of resulting pits and lamelles can be calculated [33].

An interesting and modest tool for starch containig foods can be Raman Microspectroscopy. This particularly method can give not only the structural images but it is a potential tool for measurements of forces acting between food components for example starch and protein [25].

Concluding the presented paper it can be stated that undoubtely an important role of starch granules in the formation of food structure and properties can be also visualized by microscope methods. The development in microscope technology can even better support the knowledge about starch itselfs and in food systems and also on the base of the new findings expected to create new desired properties of food.

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WŁAŚCIWOŚCI STRUKTURALNE SKROBI W ŻYWNOŚCI

Streszczenie

Przedstawiono możliwości obserwowania roli skrobi w kształtowaniu struktury żywności i jej przemian w trakcie przygotowywania żywności posługując się różymi technikami mikroskopowymi, a to: mikroskopem optycznym, transmisyjnym mikroskopem elektronowym (TEM), scanningowym mikroskopem elektronowym emisji polowej, scanningowym mikroskopem tunelowym, mikroskopem sił atomowych i mikrospektroskopią ramanowską.