High pressure impact on changes in potato starch granules

Lucyna Słomińska[8*], Roman Zielonka[1], Leszek Jarosławski[2], Aldona Krupska[4], Andrzej Szlaferek[5], Wojciech Kowalski[6], Jolanta Tomaszewska-Gras[3], Marek Nowicki[7]

[1]*Institute of Agricultural and Food Biotechnology, Department of Food Concentrates and Starch Products, Poznań, Poland*
[2]*Institute of Agricultural and Food Biotechnology, Department of Food Concentrates and Starch Products, Poznań, Poland*
[3]*Poznań University of Life Sciences, Faculty of Food Sciences and Nutrition, Poznań, Poland*
[4]*Polish Academy of Sciences, Institute of Molecular Physics, Poznań, Poland*
[5]*Polish Academy of Sciences, Institute of Molecular Physics, Poznań, Poland*
[6]*Polish Academy of Sciences, Institute of Molecular Physics, Poznań, Poland*
[7]*Poznań University of Technology, Faculty of Technical Physics, Poznań, Poland*
[8]*University of Zielona Góra, Faculty of Biological Sciences, Zielona Góra, Poland*
Corresponding author: e-mail: ls@man.poznan.pl

Air dry potato starch (84.9% d.s.) was subjected to pressurizing under the pressure of 50, 100, 250, 500, 750, 1000 and 2000 MPa for 1 h. The physical properties of pressurized starch, such as morphology, surface and crystalline structure, gelatinization parameters, were studied by means of scanning and atomic force microscopy (SEM/AFM), X-ray diffraction (X-ray), differential scanning calorimetry (DSC). The susceptibility to the amylolytic enzyme (α-amylase) was also measured. Application of pressure in the range of 50–2000 MPa results in an increase in the compressed potato starch bulk density, change in the contours of the granules from oval to polyhedral, increase in the roughness of the granule surface, vanishing of the X-ray reflexes generated by the orthogonal structure and weakening of the reflexes generated by the hexagonal structure, lowering of the enthalpy of starch gelatinization, and the enhancement of hydrolytic susceptibility of starch granules to the amylolytic enzyme.

Keywords: potato starch, high pressure, shape and structure of starch, gelatinization-enthalpy.

INTRODUCTION

Starch granules can be modified by high hydrostatic pressure (HHP) treatments. First papers on the effect of HHP on starch were published in 1981[1] (250 MPa) and 1982[2] (up to 400 MPa).

Under high pressure, starch undergoes morphological and structural changes. It also exhibits different gelatinization parameters (starch granules swell easier and have a lower initial gelatinization temperature) as well as different rheological properties[3–15]. Depending on the crystalline structure of starch (type A, B, or C), its humidity and pressurizing conditions (pressure, duration of pressurization, temperature) – the structural changes can be so deep that the semi-crystalline in form starch granule, insoluble in cold water, can transform to the amorphous form, partially soluble in cold water and characterized by a higher susceptibility to α-amylase[16, 17].

An advantage of the HHP treatment of the starch, that could find a practical application in the future, is the possibility of reduction of the content of thermally generated free radicals[18].

Among all kinds of starches, the potato starch – classified on the basis of X-ray diffraction as a type-B starch – is distinguished by the highest resistance to the action of HHP[17, 19].

It may be supposed that the high resistance of the potato starch to pressure, in particular that of air-dry starch, explains why most investigations into the applications of HHP pertained to the type-A starch (corn, waxy corn, wheat, rice, oat, barley) and type-C starch (tapioca, smooth pea, water chestnut) in high water excess conditions, and hence dealt with conditions and substrates that relatively easily showed distinct changes in their characteristics at pressures up to 1000 MPa. For example, with high water excess (mostly, starch 5÷25% suspension), the pressure of 400 MPa suffices for gelatinization of the wheat starch, and the pressure of 800÷900 MPa – for gelatinization of potato starch[4]. Only few investigators[3] tried to apply HHP (800÷1200 MPa) to air-dry potato starch.

Hence, a gradual tendency can be noted to apply higher and higher pressures in the investigations of the native and gelatinized starch. For example, the pressure of 1500 MPa was applied to potato, maize and wheat starch granules[9] and pressures from 1500 to 5900 MPa to maize starch gels[20].

In the past investigations, HHP pressurization was performed for various intervals, ranging from several minutes[21] up to several dozen of hours[22]. The results indicate that prolongation of the duration of the pressurizing to over one hour is not very effective[17, 23, 24].

Accordingly, studies were undertaken with the aim of defining the effect of high pressure up to 2000 MPa, acting for one hour at ambient temperature (20°C), on air dry potato starch, and a further characterization of its physical properties as well as determination of its susceptibility to enzymatic depolymerization at a relatively low temperature (45°C), lower than the gelatinization temperature of native starch.

MATERIAL AND METHODS

Material

Starch

Potato starch (air dry – pneumatic drying) with 15.1% of water and 0.4% of the aggregate content of non-starch substance (ash – 0.29%, protein – 0.06%, fat – 0.05%), was produced by Zakłady Przemysłu Ziemniaczanego at Niechlów (Poland). The spread in size of starch granules ranged between 8 and 85 μm, the sizes of most grains

falling between 30 and 50 μm (Fritsch Particle Sizer Autosieb/a20 apparatus).

Enzyme

α-amylase – the enzyme produced by submerged fermentation of a genetically modified microorganism *Bacillus licheniformis* – Liquozyme® Supra (Novozymes Co.) with activity of 135 KNU/g (1 KNU – the amount of enzyme which breaks down 5.26 g starch per hour using the Novozymes standard method).

Methods

Preparation of physically modified starch

Potato starch was subjected to the HHP of 50, 100, 250, 500, 750, 1000 MPa for 1 h at room temperature in the hydraulic press, the construction of which was described in the paper[18]. Starch powder was inserted into the appropriate pressure chamber. Then it was put into the hydraulic press. Pressure was gradually raised up to 1000 MPa (about 2 min). The sample was pressurized for 1 h, with subsequent reduction down to the atmospheric pressure (about 1–2 s). After the set time of the pressurizing (1 h), there was no increase in the sample temperature in relation to ambient temperature (about 20ºC). Potato starch was also pressurized under 2000 MPa (about 3 min.) in a home-made device developed for attaining super-high pressures up to 3000 MPa[25]. In the standard hydraulic press theoretically we can obtain higher pressure than 1000 MPa. However, in practice it is difficult to obtain this value of pressure. The standard pressure chamber is made of hardened steel, which can not withstand pressures higher than 1000 MPa. It is necessary to use the materials harder than hardened steel, such as tungsten carbide (WC). This device was constructed for measurements the ferroelectric effects in TGS[25].

Density measurement

The density measurements were performed according to the requirements of the Polish Standard (PN-A-74702--3:1998 – Potato products. Test methods. Determination of bulk density).

Scanning electron microscopy (SEM)

Morphological characteristics of granules of potato starch were viewed using scanning electron microscopy ZEISS EVO 40 XVP voltage of 16 kV. The sample of starch was placed on a bilateral carbon sticker fixed to the SEM table. Subsequently, gold was sputtered on the sample in an ion sputter (voltage 250 V, sputter time 240 s). All experiments were carried out at room temperature and ambient humidity.

Atomic force microscopy (AFM)

AFM imaging was performed with Bruker Icon atomic force microscope (AFM). Images were recorded in tapping mode using PPP-NCLR silicon probes (NanoAnd--More GmbH) and Bruker SNL cantilevers. AFM images were collected and analyzed using WS×M computer program[26]. All experiments were carried out at room temperature and ambient humidity.

The roughness (RMS) was analyzed by WSxM software. Used formula:

$$R_q = \sqrt{\frac{1}{n}\sum_{i=1}^{n} y_i^2}$$

were: R_q is the RMS roughness,
y_i is the vertical distance from the mean line to the ith data point, n is number.

X-ray diffraction

The X-ray patterns of starches were obtained using a goniometer HZG 4 made by Freiberger Präcisionsmechanik. Cobalt Kα radiation with crystalline filter type Fe was used. The radiations were collimated by a system of diaphragms and Soller slits. The operation setting for the diffractometer was 20 mA and 30 kV. The samples of diffraction scanned were from 5° to 30° with 0.05° steps and 6 s count time. The powder samples were filled in disk-like containers. The angles and intensities of diffraction reflexes were recorded using a detector and specialized software.

Differential scanning calorimetry (DSC)

Differential scanning calorimeter (Perkin Elmer, DSC-7. Norwalk, CT, USA), equipped with an intercooler, was used to determine the endothermic transitions of native and high-pressure treated starch. Prior to analysis, the DSC calorimeter was calibrated using indium (m.p. 156.6°C, ΔH_f = 28.45 J/g) and n-dodecane (m.p. –9.65°C, ΔH_f = 216.73 J/g). Potato starch samples of 3 ±0.2 mg were precisely weighted. Subsequently, 6 mg of deionized water was added, sample pans of 20 µl (Perkin Elmer, No. 0219-0062) were hermetically sealed and finally stored in a refrigerator (4°C) overnight to attain a uniform moisture distribution. Samples were analyzed by heating from 10 to 100°C at a scanning rate of 5°C/min[27]. An empty pan served as a reference. The onset (T_o), peak (T_p) and completion (T_c) temperatures and the enthalpy (ΔH) in J/g of dry starch were recorded using DSC 7 software (PYRIS). The enthalpy (ΔH) in J/g per 1 g d.s. of starch was calculated.

Enzymatic hydrolysis of starch

The preparation of α-amylase Liquozyme® Supra with the ratio of 0.54 KNU/g of dry substance (d.s.) of starch was added to the 10% starch-water suspension of native and physically modified (0, 50, 100, 250, 500, 750, 1000 and 2000 MPa for 60 min) potato starch of 15.1% humidity, after bringing it to pH 5.2÷5.4. Hydrolysis was performed at 45°C for 24 h. In the hydrolysates the yield ratio was determined[28], that is, the ratio of the amount of dry substance of soluble components obtained from starch hydrolysis to the amount of dry substance of starch introduced to the reaction, expressed in weight percent. At the same time, another reaction without the addition of the enzyme was performed.

Carbohydrate composition of hydrolysates

The qualitative and quantitative carbohydrate composition was analyzed by chromatography (HPLC Hewlett--Packard Model 1050) with use of Aminex HPX-42A column (4% cross-linking of the cation exchange resin

silver form) – 300 × 7.8 mm (Biorad Laboratories), equipped with a refractive index detector. Samples of 20 μl were filtered through a 0.45 μm membrane (Millipore Laboratories) and eluted with distilled and degassed water at a rate of 1.5 cm³ min⁻¹ at 85°C.

Statistical analysis

The Statistica 10 software (StatSoft, Tulusa, USA) was used for statistical data DSC analysis. All the analyses were performed in triplicates. The first stage in the statistical analysis and density measurement consisted in the verification of variance homogeneity using the Hartley-Cochorn-Bartlett test. One-way ANOVA and the Tukey test were used to test the significance of differences at the level α = 0.05.

RESULTS AND DISCUSSION

Density of granules

Native starch in a powder form was pressurized in a hydraulic press. The draw-pieces consisted of cylinders of a solid hard rock form. Due to the action of HHP the compressed starch granules became adjusted to the environment. The bulk density of the compressed starch in the form of draw-pieces is the measure of that adjustment. The influence of HHP on bulk density of compressed potato starch is shown in Figure 1. At atmospheric pressure, bulk density of the potato starch amounts to 770 kg/m³. Bulk density of compressed starch increases from 770 to 1310 kg/m³ with pressure increasing from the atmospheric pressure up to 100 MPa. In the range from 100 to 1000 MPa, compressed starch bulk density is stable and climbs from 1310 to 1390 kg/m³. Under the pressure of 2000 MPa, the compressed starch bulk density attains the value of 1700 kg/m³. It is a value higher than that of the specific density of the granules of native potato starch, which amounts to 1650 kg/m³ [29, 30]. It may be considered an evidence of disintegration of the original structure of starch granules.
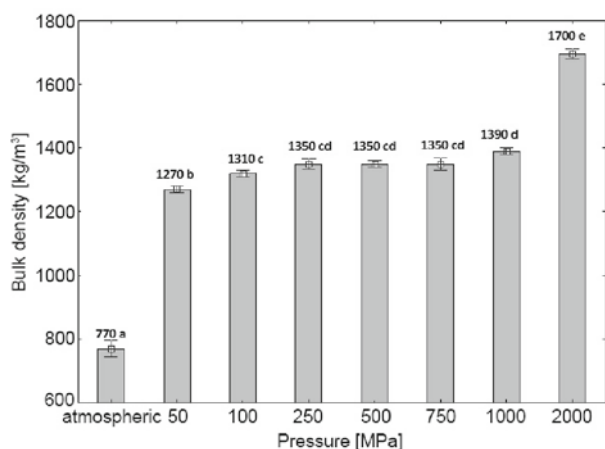
Figure 1. Influence of high pressure on bulk density of potato starch

Morphology of granules

The comparison of shapes of granules of native starch and those pressurized at 50, 100, 250, 500, 750, 1000 and 2000 MPa for 1 h with the use of SEM is presented in Figure 2. It was established that starch granules subjected to high pressure have a different appearance as compared
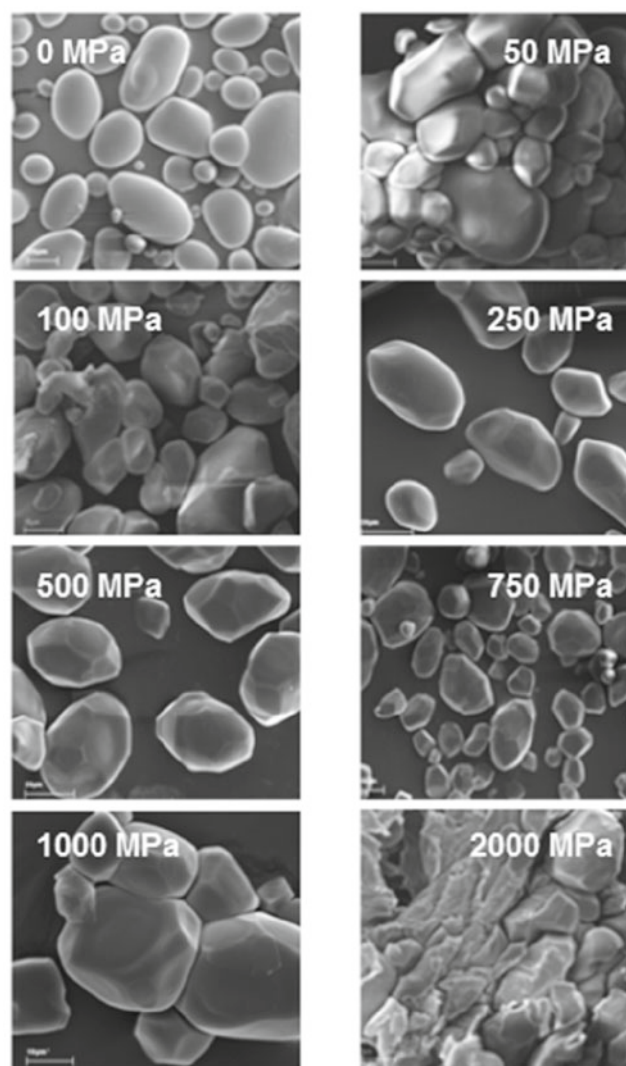
Figure 2. SEM microstructure of potato starch: native and pressurized starches under pressures in the range of 50–2000 MPa

to the native starch granules. Application of pressure of 50 MPa leads to noticeable changes in the shape of granules from oval to the polyhedral. As pressure increased, especially from 500 MPa and above, these changes are increasingly pronounced; flattened walls appear which meet along lines of intersection forming certain angles. These changes result from a denser packing of individual granules, which is a consequence of the ever increasing pressure. At the pressure of 2000 MPa, an overwhelming transformation of the grain takes place. The appearance of amorphous form takes place, even though deformed granules having the form of polyhedra are still visible.

Other researchers also observed changes in form of the native starch granules under high hydrostatic pressure, but changes as significant as the current ones were not detected, because distinctly lower pressures were applied by them. Liu, Selomulyo & Zhou[9] have shown changes in the shape and surface of starch granules of various botanical origins (maize, wheat, potato) under high pressure (740÷880 MPa). These results have been confirmed by Błaszczak, Valverde & Fornal[8], who also found deformations of granules under high pressure (600 MPa).

Surface structure of granules

A scanning atomic force microscope (AFM) has been applied to the study of surface structure of potato starch granules, making possible the studies on micrometric as well as nanometric scales. The surface of native starch (Fig. 3Aa) is characterized by the presence of ordered blocklets with diameters in the range of 20÷30 nm. The structure of those blocklets is similar to that described earlier in the samples observed with TEM investigations[31] and those investigated with AFM[32–35]. The surfaces of the starch granules pressurized at 750 MPa (Fig. 3Ab) are coarser than those of native granules. The measure of coarseness of the surface is the roughness (RMS), which for native starch granules amounts to 1.7 nm and for the granules pressurized at 750 MPa amounts to 2.5 nm. The surfaces of starch granules pressurized at 2000 MPa (Fig. 3Ac) lose the typical lamellar structure. The latter structure is characterized by a still higher coarseness: its roughness (RMS) amounts to 9.19 nm. In Figure 3B, images of surfaces of the aforementioned granules in a larger blowup than in Figure 3A along with the material contrast (phase) images are shown. Analysis of contrast images indicates differences in the mechanical properties of pressurized granules as contrasted to the native starch ones. It is noticeable in particular for granules pressurized at 2000 MPa, where
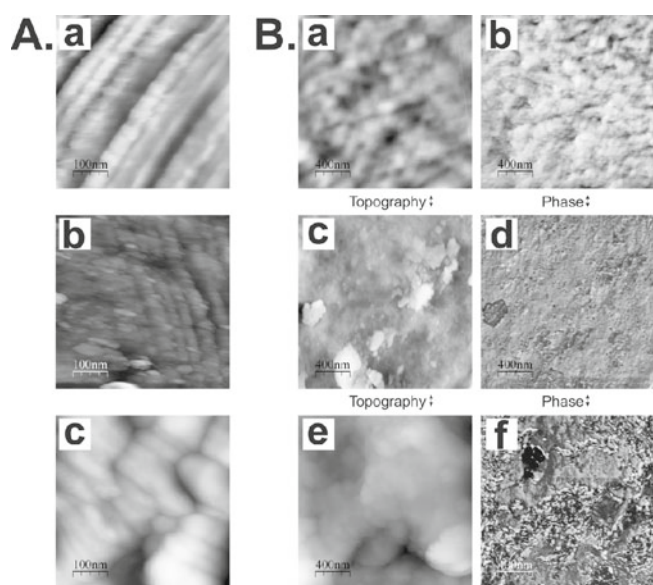

Figure 3. AFM topographic image. A) granule surface of native potato starch (a) and starch pressurized at 750 MPa (b) and 2000 MPa (c) obtained for scan size 500 nm x 500 nm. Color scale (sample high) is 12, 58 nm and 39 nm, respectively; B) granule surface and material contrast images (b, d, f) of native starch (a, b) and starch granules pressurized at 750 MPa (c, d) and at 2000 MPa (e, f)

the contrast images testify for large differences in local mechanical properties.

Research carried out by atomic force microscopy (AFM) and electron microscopy (SEM) shows significant differences between the effects on the starch granules of pressures up to 1000 MPa inclusive and the pressure of 2000 MPa. The use of pressures up to 1000 MPa is followed by a more or less deformed shape of the starch grains. On the other hand, pressurizing under

2000 MPa is followed by the destruction of the existing structure of granules. An amorphous form appears, next to which granules in the form of polyhedra are present (as shown by SEM images). Analysis of AFM contrast images of the granules pressurized under 2000 MPa can indicate that there occurs a non-uniform transformation of starch grain.

The surface of starch plays an essential role in the process of its transformation and storage – it is the main factor affecting penetration speed of water and other components into the interior of starch granules. The state of the surface also affects the process of gelatinization of starch as well as its susceptibility to the acidic or enzymatic hydrolysis. Modification of the surface of starch can essentially change the functionality and possibilities of technological applications of starch.

Thomson et al.[36] and Fannon et al.[37] observed that the presence of fractures, cracks and pores at the surfaces of starch granules enhances the susceptibility of starch to enzymes. Gallant et al.[31] noted that the susceptibility of starch to enzymes increases with the number of pores.

Crystallographic structure of granules

The influence of HHP on crystallographic structure of potato starch was investigated also by means of X-ray analysis. In the native starch, the amylose and amylopectin polymers occur alternately, forming semi-crystalline and amorphous layers. In the semi-crystalline layers, crystalline and amorphous layers occur. The former have the shape of double helices. Double helices can be arranged in an orthogonal pattern forming the A-type crystal, or in a hexagonal pattern forming the B-type crystal. Helices form fibrous structures with mutually parallel fibers. The voids between helices can be filled with molecules of absorbed water[16, 38].

Crystallographic structures of native starch pressurized under HHP of 50, 100, 250, 500, 750, 1000 and 2000 MPa were investigated on the basis of X-ray diffraction patterns (Fig. 4A).

A diffraction pattern of native starch is characterized by a diffuse background amorphous halo coming from amorphous structures, with the peaks stemming from the crystalline structures visible upon it. Peaks at the scattering angles 2θ (two theta) = $6.4°$ and $20°$ come from the hexagonal structure of type-B starch, but the maxima at the angles $2\theta = 17.2°$ and $26.8°$ originate from the orthogonal structure of type-A starch[16, 38].

With the increase in pressure the reflexes related to the orthogonal structure gradually vanish and those related to the hexagonal structure also decrease. The increase in pressure causes a significant broadening of the peaks related to the reflexes.

In the diffraction pattern of the native starch reflexes recognized as related to crystalline phases are observed. With the increase in pressure, the intensities of peaks related to crystalline phases of both types of starch decrease. The half-height width of those peaks increases. As pressure increases, one observes vanishing of reflexes from starch of orthogonal structure for the scattering angles $2\theta = 17.2°$ and $26.8°$ as well as that of B-type starch for $2\theta = 6.8°$. However, the peak at the scattering angle $2\theta = 20°$ (Fig. 4B) is considerably broadened. The possible origin of that phenomenon might lie in a
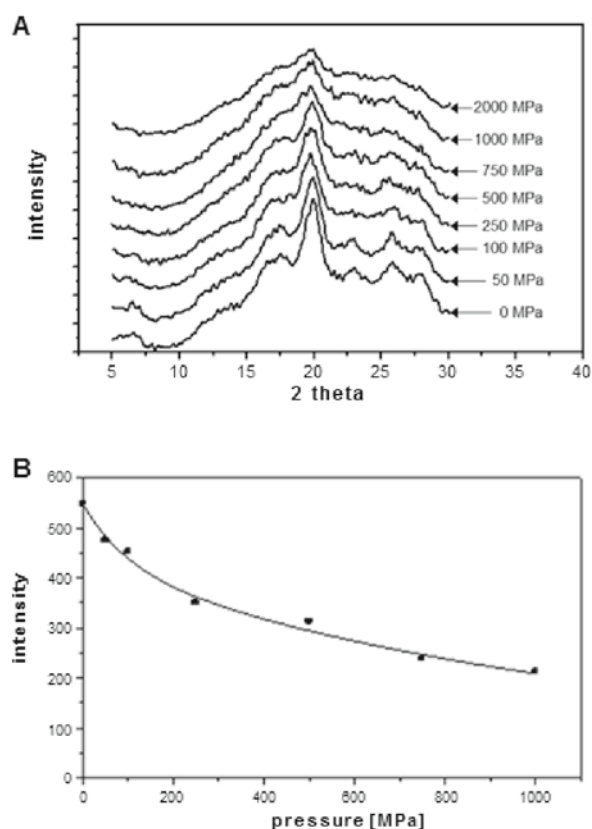
Figure 4. X-ray diffraction. A) X-ray diffraction patterns of native (0) and high-pressurized at 50–2000 MPa starches; B) Changes in X-ray diffraction intensity for scattering angle two-theta = 20° as a function of applied pressure

probable partial breaking of hydrogen bond in starch inducing a transformation from the crystalline structure to the amorphous one.

X-ray analysis leads to the conclusion that starch with B-type hexagonal structure becomes amorphous to a large extent and in the A-type there occurs a complete amorphization of the crystalline structure.

Our investigations complement those performed by Hibi et al.[19] under HHP up to only 500 MPa (suspension of starch and water 1:1, w/v). They found no possibility

of disintegration of the crystalline structure of starch granules. Kudła & Tomasik[3, 39] and Liu et al.[9] stated that the degree of disintegration of the crystalline structure of starch increases proportionally to the pressure applied and to the duration of its action. The influence of water content in the starch pressurized at 690 MPa on its crystalline structure was investigated by Katopo et al.[40] They concluded that a higher water content in the starch favors the disintegration of its crystalline structure (at the water to starch ratio 1:1) or causes a complete change of the crystalline structure into the amorphous one (water to starch ratio 2:1). Investigations by Błaszczak et al.[8] confirm the effect of HHP on the structure of starch granules. In the latter research the 10% starch-water suspension was pressurized under 600 MPa for 2–3 min. It was found that starch pressurized for 2 min retains its typical granule form but prolonging the HHP treatment to 3 min leads to the formation of a fibrillar structure.

Gelatinization parameters

The results of research on gelatinization of the native potato starch and the pressurized one in the range from 50 to 2000 MPa, done with the differential scanning calorimeter (DSC), were collected in Table 1, and the DSC thermograms were shown in Figure 5.

The DSC thermograms and the data in Table 1 show that with increasing pressure applied to potato starch the values of all gelatinization parameters decrease. For the peak gelatinization temperature (T_p), the decrease ranges from 62.5°C (native starch) to 56.7°C (starch pressurized at 2000 MPa), which is as much as 5.8°C, or 9.3% reduction. Decrease in gelatinization enthalpy is even more profound. Gelatinization enthalpy of native starch (ΔH) amounts to 16.5 J/g, and its lowest value was found for starch pressurized at 2000 MPa, namely 7.2 J/g; it means lowering by a factor greater than two of that parameter. The height of the thermal peak decreases most markedly for native starch, amounting to 0.1733 W/g, and only 0.0550 W/g for starch pressurized at 2000 MPa; it means lowering by a factor of 3.1.

Comparative studies of native and pressurized (200––430 MPa) potato starch were conducted also by Molina-
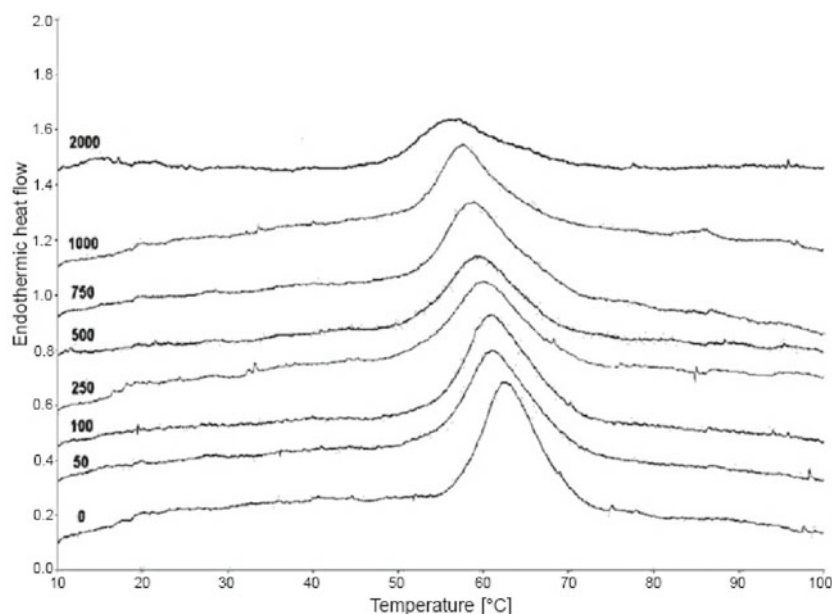

Figure 5. DSC thermograms of native (0) and pressurized starches (50 to 2000 MPa)

Table 1. DSC thermodynamic characteristics of native and high-pressure treated starches

| Pressure [MPa] | Gelatinization temperature | | | ΔH [J/g] | Peak height [W/g] |
	T_o [°C]	T_p [°C]	T_c [°C]		
0	56.9 [a] ±0.18	62.5 [a] ±0.01	69.7 [a] ±0.08	16.5 [a] ±0.42	0.1733 [a] ±0.00
50	54.1 [b] ±0.07	61.2 [b] ±0.1	69.8 [a] ±0.53	15.0 [a] ±1.26	0.1250 [b] ±0.00
100	54.7 [b] ±0.36	61.1 [b] ±0.17	70.6 [a] ±0.43	13.8 [b] ±0.78	0.1190 [b] ±0.01
250	52.9 [c] ±0.13	60.6 [b] ±0.66	69.7 [a] ±0.61	12.6 [bc] ±0.5	0.1035 [bc] ±0.00
500	51.7 [c] ±0.5	59.2 [c] ±0.39	68.7 [a] ±0.57	11.4 [c] ±0.69	0.0972 [bc] ±0.01
750	52.3 [c] ±0.12	58.7 [c] ±0.49	69.0 [a] ±1.28	10.8 [c] ±0.72	0.0988 [bc] ±0.01
1000	52.1 [c] ±0.42	57.5 [d] ±0.33	68.5 [a] ±1.22	8.8 [d] ±0.40	0.0850 [c] ±0.01

±SD – group averages ±standard deviations.
a,b,c,d – group averages with different superscripts differ significantly within columns (p≤0.05).

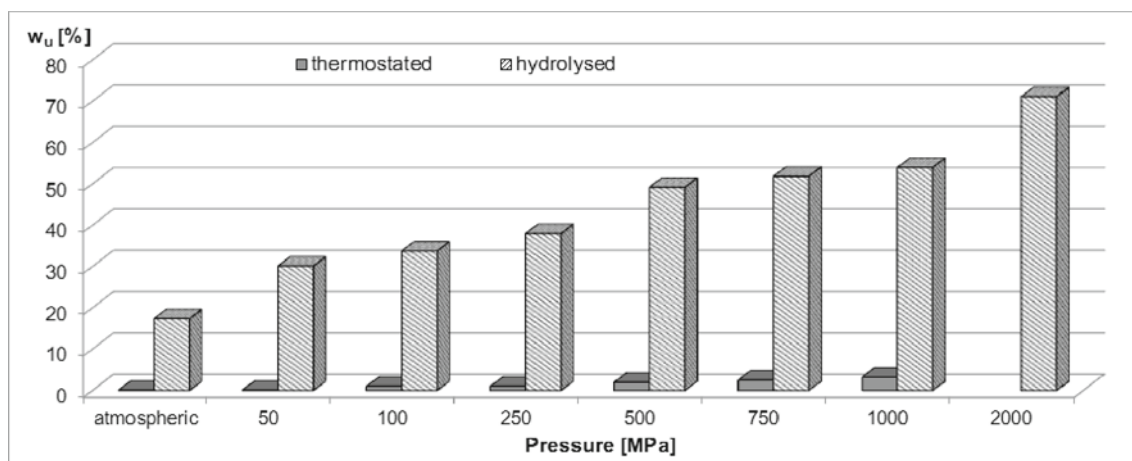-Garcia et al.[6] They revealed a decrease in the values of gelatinization enthalpy of about 30% due to the pressurization. Kawai et al.[22] investigated a 10–70% potato starch-water suspension pressurized at 600–1000 MPa at 40°C for 18 to 66 h. They found that the gelatinization enthalpy value decreased with the decreased starch content and increased treatment pressure. The increase in the duration of pressurizing had only a marginal influence on the change in enthalpy. Liu et al.[41] also found a lowering of the gelatinization enthalpy of maize starch during pressurization in the range from 0.1 to 10 MPa. Liu et al.[42] obtained similar results during their study of structure and properties of tapioca and waxy maize starch obtained by pressurization (300–600 MPa for 30 min); they found that gelatinization temperature and viscosity of the gels decreased.

The results obtained in the current research agree with those by Katopo et al.[40] Both indicate that dry starch subjected to HHP exhibits lower gelatinization temperature and enthalpy than the native starch. Pressurization induces damage and disruption of the molecular order and crystallinity of the starch granules. Reduced temperature and enthalpy of gelatinization under HHP (120–600 MPa) in starch-water suspension of bean starch was observed also by Li et al.[43]

Effect of α-amylase on the yield of starch hydrolysis

Susceptibility of potato starch to the action of amylolytic enzymes, including α-amylase, is important in particular for the production of starch hydrolysates, the largest domain of potato starch manufacturing industry. The research of other authors, i.a., Molina-Garcia et al.[6] and the current investigations show, that the application of high pressure to starch leads to a lowering of the temperature and enthalpy of gelatinization.

The employed methods of the enzymatic hydrolysis of starch entail the stage of its liquefaction, which means the hydrolysis of gelatinized starch with the use of α-amylase in the temperature range 80÷105°C, by applying the bacterial α-amylase with suitable thermal resistance[44, 45]. The pressurizing of starch, which lowers the temperature and enthalpy of gelatinization, creates a potential for starch hydrolysis at a lower temperature. Accordingly, the investigations covered the susceptibility of HHP pressurized air dry potato starch to α-amylase at a relatively low temperature of 45°C, distinctly lower than that of potato starch gelatinization of 56÷66°C[44], and even lower than its swelling temperature of 46°C[28]. The yield of starch hydrolysis expressed by the yield factor (y_u) is the measure of susceptibility of starch to α-amylase. The yield factor values for native and pressurized and thermostated (with no enzyme applied) potato starch as well as the hydrolyzed one (with enzyme application) were determined at 45°C – Figure 6. The

Figure 6. Yield ratio yr in thermostatic conditions and enzymatic hydrolysis of native and pressurized starch under pressures in the range of 50–2000 MPa

collected data shows that potato starch pressurized under HHP and subsequently hydrolyzed with the application of α-amylase – as well as the thermostated one – show higher values of the yield ratio than native potato starch. In case of thermostated starch, the increase in the value of the yield ratio rises from 0.1% (native starch) to 3.2% (starch pressurized at 1000 MPa). The yield ratio for starch pressurized at 2000 MPa, in controlled temperature conditions, could not be determined due to the change in the starch structure – transition to the amorphous form. In the case of hydrolysis with application of α-amylase, the increase in the yield ratio ranges from 17.4% (native starch) to 53.9% (starch pressurized at 1000 MPa) and 71.1% (starch pressurized at 2000 MPa). The application of α-amylase to potato starch pressurized at 2000 MPa increases the real yield of soluble components by 53.7 percentage points.

The data presented hereinbefore show that as the pressure acting on potato starch increases during hydrolysis below gelatinization temperature (45°C) with application of the liquefying enzyme, the susceptibility of potato starch to the liquefying enzyme significantly increases and the increase of the pressure leads to an enhancement of the hydrolysis yield, as measured by the value of the yield ratio.

On the basis of the analysis of the determined yield factors it can be assumed that the application of pressures higher than 2000 MPa (the highest in current research) to air-dry potato starch could help reach an even higher yield factor and, in this way, the yield of hydrolysis at 45°C would come closer to the yield of starch hydrolysis at 85°C.

In order to determine the influence of HHP on the carbohydrate composition, using starch pressurized at 1000 MPa as an example, we compared the corresponding carbohydrate compositions of hydrolysates of pressurized and native starch. The following results were obtained for carbohydrate relative contents (%) – for pressurized starch (native starch): glucose – 2.7 (2.5), maltose – 11.7 (10.9), maltotriose – 16.2 (13.2), maltotetraose – 4.6 (7.3), higher sugars – 64.8 (66.1), respectively. It can be observed that the carbohydrate compositions of hydrolysates obtained from native and pressurized starch are close to one another and typical for the applied enzyme – α-amylase. It means that the pressurizing of the potato starch does not affect the carbohydrate characteristics of the obtained hydrolysates.

Investigations of the susceptibility of previously pressurized starch to amylolytic enzymes were carried out also by Selmi et al.[46] They used starch of a different botanic origin (maize and wheat) and found that application of HHP (400 and 600 MPa) significantly enhances the yield of wheat starch hydrolysis. However, the yield of hydrolysis of maize starch is comparable to that of thermally gelatinized starch.

Recapitulation

High hydrostatic pressure acting on the granules of air-dried potato starch causes their higher mutual adhesion and compaction. The starch granules that undergo compression adapt to the environment, which on one hand results in an increase in bulk density and on the other hand – in the change of the granule shape from the oval to the polyhedral. Already a pressure of 50 MPa leads to a change in the shape of the starch granules, however, they become easily identifiable from the pressure of 500 MPa (SEM and AFM micrometric scale). The bulk density of the compressed starch granules increases distinctly already at a pressure of 50 MPa (1270 kg/m³, compared to a value of 770 kg/m³ for native starch). Pressure of 2000 MPa produces an increase in the bulk density up to 1700 kg/m³. This value exceeds the density of the native potato starch, which amounts to 1650 kg/m³. It reflects the significant transformation of the existing structure of the grain. These transformations of the grain structure are confirmed by the criteria for evaluating the changes in the physical properties of starch in terms of quality, such as crystallographic studies (X-ray) and those using the atomic force microscope (AFM). X-ray analysis indicates the coexistence of amorphous and crystalline structure of starch. As the pressure gets higher and higher the granules show a tendency to gradual amorphization. The AFM analysis shows that at the surface of the grain pressurized at 2000 MPa the typical lamellar structure characteristic of native starch disappears. As a result, the coarseness of the grain increases. A measure of the coarseness is the roughness (RMS). With increasing pressure acting on starch, roughness increases by about 47% under pressure of 750 MPa, and more than four times (440%) under pressure of 2000 MPa. The non-homogeneous transformation of potato starch granules under pressure of 2000 MPa, which means the presence of the amorphous form, next to which one still can observe deformed polyhedral granules, , is confirmed via the determination of the parameters of starch gelatinization by DSC. The DSC technique quantifies the gelatinization parameters on the basis of the measured enthalpy. Although the gelatinization curve for starch pressurized at 2000 MPa still indicates an endothermic transformation, the value of the enthalpy of that transformation amounts to only 7.2 J/g and is more than two times lower than that observed in the case of native starch (16.5 J/g). Such a low value of enthalpy can be explained by the occurrence of the amorphous and granular forms of starch next to one another, which has been observed in SEM and AFM. Gelatinization temperature (T_P) is also reduced; it is full 5.8°C lower than the gelatinization temperature of the native starch.

In the native starch grain, amylose and amylopectin molecules are intertwined and are loosely connected by means of hydrogen bonds with participation of hydroxyl groups. We can assume that – as a result of high pressure – the structure of the starch granules is destroyed due to weakening and eventual breakage of the hydrogen bonds, which stabilize the conformational structure of macromolecules. This leads to changes in the physical properties of starch, which are described in the current research. The observed changes in the physical properties of the potato starch are confirmed by their practical result, which is a significant increase in its susceptibility to α-amylase, as evidenced by the high yield ratio value (71.1% for a pressure of 2000 MPa).

CONCLUSION

Air-dry potato starch subjected to the high pressure exhibits modified physical properties as compared to the native starch.

As the pressure acting on the starch granules increases, the following changes occur:
– change in the form of the granules from the oval to the polyhedral,
– increase in the bulk density of compressed starch,
– change in the crystal structure; x-ray reflexes from the orthogonal structure disappear; the intensity of reflexes from a hexagonal structure decreases,
– increase in roughness of the granules,
– marked reduction in the gelation parameters – temperature and enthalpy of gelatinization.

The action of the high pressure on the air-dry potato starch granules leads also to a gradual increase (coupled with increasing pressure) in susceptibility of starch (hydrolysis yield) to the liquefaction enzyme (α-amylase), which creates the possibility of conducting the enzymatic depolymerization at reduced temperatures below the gelatinization temperature However, the pressure treatment of potato starch does not change the carbohydrate characteristics of the resulting hydrolysates.

The action of extremely high pressure – 2000 MPa – on potato starch causes fundamental changes in its properties. Starch obtained in this manner shows a dual structure: the amorphous (cauliflower-like) one and the granular one in the form of polyhedra. The starch that underwent such a treatment shows significant changes in physical parameters (morphology, bulk density, surface and crystallographic structure, gelatinization parameters – temperature and enthalpy), which affect its technological usefulness, in particular its very high susceptibility to the hydrolytic action of amylolytic enzyme. Probably, at a sufficiently high pressure (higher than 2000 MPa), potato starch can be obtained with properties similar to the gelatinized starch; hence, in our opinion, it would be advisable to continue this type of research.

Laboratory investigations of the effect of high hydrostatic pressure on potato starch in the air dry state indicate that the high pressure techniques can prove to be effective in changing its nature and physicochemical properties. However, it should be clearly stated that any attempts at the practical application of that technique will become possible only after a practical solution to the problem of the construction of the industrial apparatus destined for economically viable high pressure processing of starch will be found.

LITERATURE CITED

1. Thevelein, J.M. & Assche, J.A.V. (1981). Gelatinization temperature of starch as influenced by high pressure. *Carbohydr. Res.* 93, 304–307. DOI: 10.1016/S0008-6215(00)80862-9.

2. Muhr, A.H. & Blanshard, J.M.V. (1982). Effect of hydrostatic pressure on starch gelatinization. *Carbohydr. Polym.* 2, 61–74. DOI: 10.1016/0144-8617(82)90055-8.

3. Kudła, E. & Tomasik, P. (1992). The modification of starch by high pressure Part I: air – and oven-dried potato starch. *Starch/Staerke.* 44, 167–173. DOI: 10.1002/star.19920440704.

4. Stute, R., Heilbronn, R.W., Klingler, R.W., Boguslawski, S., Eshtiaghi, M.N. & Knorr, D. (1996). Effect of high pressures treatment on starches. *Starch/Staerke.* 48, 399–408. DOI: 10.1002/star.19960481104.

5. Douzals, J.P., Perrier-Cornet, J.M., Gervais, P. & Coquille, J.C. (1998). High-pressure gelatinization of wheat starch and properties of pressure-induced gels. *J. Agric. Food Chem.* 46, 4824–4829. DOI: 10.1021/jf971106p.

6. Molina-Garcia, A.D., Horridge, E., Sanz, P.D. & Martino M.N. (2007). Nanostructure of starch high-pressure treated granules discovered by low temperature scanning electron microscopy. In Méndez-Vilas, A. & Diaz, J. (Eds.), *Modern Res. Educ. Top. Micros.* (719–725). Formatex.

7. Buckow, R., Heinz, V. & Knorr, D. (2007). High pressure phase transition kinetics of maize starch. *J. Food Eng.* 81, 469–475. DOI: 10.1016/j.jfoodeng.2006.11.027.

8. Błaszczak, W., Valverde, S. & Fornal, J. (2005). Effect of high pressure on the structure of potato starch. *Carbohydr. Polym.* 59, 377–383. DOI: 10.1016/j.carbpol.2004.10.008.

9. Liu, Y., Selomulyo, V.O. & Zhou, W. (2008). Effect of high pressure on some physicochemical properties of several native starches. *J. Food Eng.* 88, 126–136. DOI: 10.1016/j.jfoodeng.2008.02.001.

10. Oh, H.E., Hemar, Y., Anema, S.G., Wong, M. & Pinder, D.N. (2008). Effect of high pressure treatment on normal rice and waxy rice starch-in-water suspensions. *Carbohydr. Polym.* 73, 332–343. DOI: 10.1016/j.foodhyd.2007.01.0208.

11. Baks, T., Bruins, M.E., Janssen A.E.M. & Boom, R. M. (2008). Effect of pressure and temperature on the gelatinization of starch at various starch concentrations. *Biomacromolecules* 9, 296–304. DOI: 10.1021/bm700814a.

12. Vallons, K.J.R. & Arendt, E.K. (2009). Effect of high pressure and temperature on structural and rheological properties on sorghum starch. *Innov. Food Sci. Emerg. Technol.* 10, 449–456. DOI: 10.1016/j.ifset.2009.06.008.

13. Nasehi, B. & Javaheri, S. (2012). Application of high hydrostatic pressure in modifying functional properties of starches: a review. *Middle East J. Sci. Res.* 11, 856–861.

14. Schenck, F.W. (2012). Starch hydrolysates: an overview. *Int. Sugar J.* 1238, 82–89.

15. Le Bail, P., Chauvet, B., Simonin, H., Rondeau-Mouro, C., Pontoire, B., Carvalho, M. & Le Bail, A. (2013). Formation and stability of amylase ligand complexes formed by high pressure treatment. *Innov. Food Sci. Emerg. Technol.* 18, 1–6. DOI: 10.1016/j.ifset.2012.10.006.

16. Tester, R.F., Karkalas, J. & Qi, X. (2004). Starch-composition, fine structure and architecture. *J. Cereal Sci.* 39, 151–165. DOI: 10.1016/j.jcs.2003.12.001.

17. Liu, P.L., Hu, X.S. & Shen, Q. (2010). Effect of high hydrostatic pressure on starches. A review. *Starch/Staerke.* 62, 615–628. DOI 10.1002/star.201000001.

18. Krupska, A., Więckowski, A.B., Słomińska, L., Jarosławski, L. & Zelonka, R. (2012). Influence of heating time and pressure treatment of potato starch on the generation of radicals: EPR studies. *Carbohydr. Polym.* 89, 54–60. DOI: 10.1016/j.carbpol.2012.02.037.

19. Hibi, Y., Matsumoto, T. & Hagiwara, S. (1993). Effect of high pressure on the crystalline structure of various starch granules. *Cereal Chem.* 70, 671–676.

20. Yang, Z., Gu, Q. & Hemar, Y. (2013). *In situ* study of maize starch gelatinization under ultra-high hydrostatic pressure using X-ray diffraction. *Carbohydr. Polym.* 97, 235–238. DOI: 10.1016/j.carbpol.2013.04.075.

21. Kweon, M., Slade, L. & Levine, H. (2008). Role of glassy and crystalline transitions in the responses of corn starches to heat and high pressure treatments: Prediction of solute-induced barostability from solute-induced thermostability. *Carbohydr. Polym.* 72, 293–299. DOI: 10.1016/j.carbpol.2007.08.013.

22. Kawai, K., Fukami, K. & Yamamoto, K. (2007). Effect of treatment pressure, holding time, and starch content on gelatinization and retrogradation properties of potato starch-

-water mixtures treated with high hydrostatic pressure. *Carbohydr. Polym.* 69, 590–596. DOI: 10.1016/j.carbpol.2007.01.015.

23. Stolt, M., Oinonen, S. & Autio, K. (2001). Effect of high pressure on the physical properties of barley starch. *Innov. Food Sci. Emerg. Technol.* 1, 167–175. DOI: 10.1016/S1466-8564(00)00017-5.

24. Bauer, B.A. & Knorr, D. (2004). Electrical conductivity: A new tool for the determination of high hydrostatic pressure-induced starch gelatinization. *Innov. Food Sci. Emerg. Technol.* 5, 437–442. DOI: 10.1016/j.ifset.2004.02.005.

25. Stankowski, J., Waplak, S., Jurga, W. & Krupski, M. (2010). Size-driven ferroelectric effects in TGS induced by high hydrostatic pressure. *J. Non-Crystal. Solids.* 356, 1305–1309. DOI: 10.1016/j.jnoncrysol.2010.04013.

26. Horcas, I., Fernandez, R., Gomez-Rodriguez, J.M., Colchero, J., Gomez-Herrero, J. & Baro, A.M. (2007). WSXM: A software for scanning probe microscopy and a tool for nanotechnology. *Rev. Sci. Instrum.* 78, 013705. DOI: org/10.1063/1.2432410.

27. Chena, L., Renb, F., Zhanga, Z., Tonga, Q. & Rashedb, M.M.A. (2015). Effect of pullulan on the short-term and long-term retrogradation of rice starch. *Carbohydr. Polym.* 115, 415–421. DOI: 10.1016/j.carbpol.2014.09.006.

28. Zielonka, R., Jarosławski, L. & Słomińska, L. (2010). Elaboration and comparison of methods for efficient determination of starch hydrolysis. *Zesz. Probl. Postęp. Nauk Rol.* 557, 423–433. DOI: 10.2478/pjct-2013-0037.

29. Nowotny, F. (1969). Ogólne właściwości skrobi. In Nowotny, F. (Eds.), *Skrobia* (pp. 18–32). Warszawa, Poland: WNT.

30. Lisińska, G. & Leszczyński, W. (1989). *Potato Science and Technology*. London & New York: Elsevier Applied Science.

31. Gallant, D.J., Bouchet, B. & Baldwin, P.M. (1997). Microscopy of starch: evidence of a new level of granule organization. *Carbohydr. Polym.* 32, 177–191. DOI:10.1016/S0144-8617(97)00008-8.

32. Krok, F., Szymońska, J., Tomasik, P. & Szymoński, M. (2000). Non-contact AFM investigation of influence of freezing process on the surface structure of potato starch granule. *Appl. Surf. Sci.* 157, 4, 382–386. DOI: 10.1016/S0169-4332(99)00554-1.

33. Baker, A.A., Miles, M.J. & Helbert, W. (2001). Internal structure of the starch granule revealed by AFM. *Carbohyd. Res.* 330, 249–256. DOI: 10.1016/S0008-6215(00)00275-5.

34. Szymońska, J. & Krok, F. (2003). Potato starch granule nanostructure studied by high resolution non-contact AFM. *Int. J. Biol. Macromol.* 33(1–3), 1–7. DOI: 10.1016/S0141-8130(03)00056-4.

35. Juszczak, L. (2003). Surface of triticale starch granules – NC-AFM observations. *Electron. J. Pol. Agric. Univ.* 6, 1–10.

36. Thomson, N.H., Miles, M.J., Ring, S.G., Shewry, P.R. & Tatham, A.S. (1994). Real-time imaging of enzymatic degradation of starch granules by atomic force microscopy. *J. Vac. Sci. Technol. (N. Y., NY, U. S.)* 12, 1565–1568. DOI: org/10.1116/1.587287.

37. Fannon, J.E., Hauber, R.J. & BeMiller J.N. (1992). Surface pores of starch granules. *Cereal Chem.* 69, 284–288.

38. Stevenson, D.G., Doorenbos, R.K., Jane, J. & Inglett, G.E. (2006). Structures and functional properties of starch from seeds of three soybean (*Glycine max* (L.) Merr.) varieties. *Starch/Staerke.* 58, 509–519. DOI: 10.1002/star.200600534.

39. Kudła, E. & Tomasik, P. (1992). The modification of starch by high pressure Part II: Compression of starch with additives. *Starch/Staerke.* 44, 253–259. DOI: 10.1002/star.19920440704.

40. Katopo, H., Song, Y. & Jane, J. (2002). Effect and mechanism of ultrahigh hydrostatic pressure on the structure and properties of starches. *Carbohydr. Polym.* 47, 233–244. DOI: 10.1016/S0144-8617(01)00168-0.

41. Liu, H., Yu, L., Dean, K., Simon, G., Petinakis, E. & Chen, L. (2009). Starch gelatinization under pressure studied by high pressure DSC. *Carbohydr. Polym.* 75, 395–400. DOI: 10.1016/j.carbpol.2008.07.034.

42. Liu, P.L., Zhang, Q., Shen, Q., Hu, X.S. & Wu, J.H. (2012). Effect of high hydrostatic pressure on modified non-crystalline granular starch of starches with different granular type and amylase content. *LWT Food Sci. Technol.* 47, 450–458. DOI: 10.1016/j.lwt.2012.02.005.

43. Li, W., Zhang, F., Lin, P., Bai, Y., Gao, L. & Shen, Q. (2011). Effect of high hydrostatic pressure on physicochemical, thermal and morphological properties of mung bean (*Vigna radiata* L.) starch. *J. Food Eng.* 103, 388–393. DOI: 10.1016/j.jfoodeng.2010.11.008.

44. Tegge, G. (2004). Physikalische Eigenschaften. In G. Tegge, (Eds.), *Stärke und Stärkederivate* (pp. 37–49). Hamburg: Behr's Verlag GmbH &Co.

45. Tomasik, P. & Horton, D. (2012). Enzymatic conversions of starch. In D. Horton, (Eds.) *Adv. Carbohyd. Chem. Biochem.* (pp. 59–436). Oxford. DOI: 10.1016/B978-0-12-396523-3.00001-4.

46. Selmi, B., Marion, D., Perrier Cornet, J.M., Douzals, J.P. & Gervais, P. (2000). Amyloglucosidase hydrolysis of high-pressure and thermally gelatinized corn and wheat starches. *J. Agric. Food Chem.* 48, 2629–2633. DOI: 10.1021/jf991332u.