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Influence of High Pressure or Autoclaving-Cooling Cycles and Pullulanase Treatment on Buckwheat Starch Properties and Resistant Starch Formation

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Buckwheat starch was subjected to cycles of high pressure-cooling (P-CC) or autoclaving-cooling (A-CC) combined with pullulanase debranching to determine changes in resistant starch (RS) content, digestibility, rheological properties and microstructure. Native buckwheat starch had 11.9 g/kg of RS, while the highest RS content (58.7 g/kg) was reached after A-CC and 6 h of pullulanase treatment. Among the P-CC samples, the highest RS content (43.3 g/kg) was obtained after treatment with 600 MPa/9 min and 6 h pullulanase debranching. The digestibility of the starch samples was negatively correlated with RS content and its highest values were noted for native and P-CC 200 MPa preparations subjected to 2 and 16 h of pullulanase treatment (95.18–95.35%). Buckwheat starch A-CC preparations after 6 h of pullulanase treatment exhibited the lowest digestibility (85.87%). Rheological analysis of 6% starch pastes showed that all investigated samples demonstrated a non-Newtonian flow, pseudoplastic properties and thixotropy. The Ostwald de Waele rheological model was very well fitted to the flow curves of the investigated pastes (R^2>0.98). Both P-CC and A-CC reduced the consistency coefficient (K) and thixotropy values, while the flow behavior index (n) was increased only after P-CC treatment. The P-CC and A-CC treatment resulted in starch granule breakdown and porous gel structure formation, differing in surface properties.

INTRODUCTION

Starch occurs in the plant world as a storage material in the form of granules. It is a natural biopolymer composed of linear amylose and branched amylopectin, which has multiple applications both in the food and non-food industry. However, it is mainly used as an energy source in the human diet. Several reports indicate that starch present in food products is not completely digested by human digestive enzymes, therefore being a substrate for the intestinal microbiota, providing health benefits and reducing caloric value of food [Le Thanh-Blicharz *et al.*, 2012; Wasserman *et al.,* 2007; Rahman *et al.*, 2007].

Based on the action of digestive enzymes, starch may be classified into three groups: as rapidly digestible starch, slowly digestible starch, and resistant starch (RS). According to Englyst *et al.* [1992], resistant starch is a fraction of starch that is resistant to *in vitro* hydrolysis by α-amylase and pullulanase. Nowadays, RS is classified as a fraction of starch which is not digested in the small intestine and is measured as a difference between total starch and the sum of rapidly and slowly digestible starch. Resistant starch is subdivided into 4 fractions from RS_1 to RS_4. RS_1 is the fraction of starch which is physically inaccessible due to its entrapment in a non-digestible matrix, *e.g.* coarsely ground grains or seeds. RS_2 is described as native, ungelatinized granular starch. RS_3 is a fraction of retrograded starch, mainly amylose, formed during cooling of thermally-processed starch. RS_4 is a fraction where bonds other than α-(1–4) or α-(1–6) are formed during various types of chemical treatment, *e.g.* cross-linking [Sajilata *et al.,* 2006; Zhao & Lin, 2009]. Some authors also report a fraction of starch described as RS_5, of which resistance to amylolysis is a consequence of amylose and lipid complex formation [Le Thanh-Blicharz *et al.,* 2016].

Delivering adequate amounts of energy to a starch-water slurry leads to destabilization of the granular structure and starch gelatinization. The energy may be supplied not only in the form of heat (during thermal processing), but also in the form of high hydrostatic pressure (HHP). The gelatinization phenomenon varies depending on the form of energy delivered to the system – heat treatment is related to an increase of kinetic energy of the molecules, while the HHP processing involves compression and decompression [Buckow *et al*., 2007]. The HHP processing can evoke gelatinization of starch granules in an excess of water already at room tem-

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perature. Intensive HHP treatment is expected to affect not only the granule morphology but also its physicochemical properties, and therefore offers a new possibility for the application of starch to food products [Błaszczak *et al.,* 2005]. As it was shown, processing of buckwheat starch with the pressure of 600 MPa for 20 min at ambient temperature significantly increased its pasting temperature and thermostability. Moreover, the pressurized buckwheat starch manifested lower *in vitro* hydrolysis as compared to its native form, reduced amount of rapidly digestible starch, and had a higher level of slowly digestible starch (505 g/kg) and RS (67 g/kg) [Liu *et al.,* 2016].

It is generally believed that slowly digested starches (SDS) and/ or RS are desirable in the food products since they reduce risk factors for the diet-related diseases. Methods leading to RS formation found in literature vary in the type and number of processes applied, treatment temperature and time or the activity of enzymes used [Lertwanawatana *et al.,* 2015]. Pullulanase (EC 3.2.1.41), a specific kind of glucanase, is used as a debranching enzyme in polysaccharide processing due to its ability to hydrolyze α-1,6 linkages. As reported by various authors, debranching of amylopectin prior to thermal treatment increased the yield of RS formation [Berry, 1986; Ozturk *et al.*, 2009]. The study of Lertwanawatana *et al*. [2015] demonstrated that the RS yields can be distinctly enhanced (from 24 g/kg up to 174 g/kg) already after a single autoclaving (121°C/15 min) and pullulanase (50° C/24 h) treatment of cassava starch-water suspension (starch-water ratio of 1:5). Moreover, further autoclaving and cooling (60°C 4 h) of the mentioned preparation led to the highest RS content of 365 g/kg. Zhao & Lin [2009] found that maize starch subjected to three autoclaving-cooling cycles (at 121°C/20 min and 4°C/ 24 h, respectively) had 85 g/kg of RS. As a result of further processing. *i.e.* autoclaving-cooling cycles and pullulanase treatment, the RS content increased, reaching a maximum of 324 g/kg.

As above reported, the potential of RS formation in the structure of starch resulting from autoclaving-cooling cycles and pullulanase treatment has been widely documented in the literature. However, very little is known about RS formation particularly in the buckwheat starch structure following combination of HHP processing and pullulanase debranching.

Therefore, this study explores the potential of forming RS in the structure of buckwheat starch gelatinized under HHP conditions (at different levels of pressure and time of treatment), debranched by pullulanase and subjected to high pressure-cooling cycles (P-CC). Moreover, the obtained preparations were characterized regarding their digestibility, rheological properties, and microstructure. All the aforementioned evaluations were also performed in relation to buckwheat starch preparation obtained after autoclaving-cooling cycles (A-CC) coupled with pullulanase debranching.

This study aimed to evaluate the use of P-CC *vs.* A-CC coupled with pullulanase debranching to maximize the production of RS from buckwheat starch.

MATERIALS AND METHODS

Material and chemicals

Raw buckwheat flour, obtained after grinding of dehulled buckwheat ahene, was a gift from Melvit S.A., Szczytno, Poland.

All chemicals used were of analytical grade. α-Amylase from porcine pancreas Type VI-B (≥10 U/mg), maleic acid, calcium chloride dihydrate, dimethyl sulfoxide, iodine, and potassium iodide were purchased from Sigma-Aldrich (Poznań, Poland). Pullulanase M2 from *Bacillus licheniformis* (900 U/mL), resistant starch assay kit (K-RSTAR), and amyloglucosidase (3300 U/mL) were purchased from Megazyme (Wicklow, Ireland). Sodium hydroxide, sodium azide, potassium hydroxide, ethanol, urea, propan-1-ol, and acetic acid anhydride were obtained from POCH (Gliwice, Poland). Licquick Cor-Glucose kit was purchased from PZ Cormay S. A. (Łomianki, Poland). Water was purified using the Milli-Q system (Millipore, Bedford, USA).

Native starch isolation and analysis

Starch was isolated from the obtained material according to the method of Christa *et al.* [2009], which involved washing with a solution of 1 g/L NaOH in order to separate proteins and non-starch compounds, followed by centrifugation (1800×*g*, 10 min) and washing with distilled water until pH=7 was reached. Basic chemical analyses of the isolated native buckwheat starch were carried out. Amylose content was determined spectrophotometrically [Morrison & Laignelet, 1983]. Official methods [AOAC, 2006] were used in the analysis of ash (method 942.05), moisture (method 934.01), and protein content (method 984.13A), while surface and total lipid content was determined following Kaukovirta-Norja *et al.* [1997] assay procedure.

Preparation of samples treated with pressure-cooling cycles (P-CC)

Starch-water (30 g dry matter (d.m.)/100 g) suspensions were subjected to high pressure treatment in a high pressure unit (U-33, Unipress, Warsaw, Poland) at pressure levels of 200, 400, and 600 MPa for 3 min or 9 min at room temperature. After decompression, pullulanase at a concentration of 1 U per 1 g dry basis (d.b.) of starch was added and the samples were incubated for 2, 6, 10, and 16 h in a water bath with shaking (200 rpm) at the temperature of 60°C. The samples were treated at 650 MPa/10 min in order to inactivate the added enzyme (as it was checked experimentally) and then stored under cooling conditions (4°C) for 24 h before the next two pressure-cooling cycles at 200, 400 or 600 MPa/3 min or 9 min. The obtained P-CC preparations were rapidly frozen, freeze-dried (Labcono 195, Kansas City, USA), grinded using a laboratory grinder and passed through a sieve to obtain a fraction below 120 μm.

Preparation of samples treated with autoclaving-cooling cycles (A-CC)

The A-CC samples were prepared alike the P-CC samples, with some modifications. Starch-water (30 g d.m./100 g) suspensions were autoclaved (121°C/20 min/0.2 MPa) and cooled down to 60°C prior to pullulanase (1 U per 1 g d.b. of starch) addition. After incubation with the added enzyme (2–16 h), the samples were again autoclaved in order to inactivate the enzyme, cooled down to room temperature and kept at 4°C for 24 h before the next autoclaving-cooling cycle. A total of three autoclaving-cooling cycles were per-

formed. The obtained samples were rapidly frozen, freeze--dried (Labcono 195, Kansas City, USA), grinded and sieved to a fraction below 120 μm.

Determination of resistant starch content

Resistant starch (RS) content analysis was carried out using a Megazyme K-RSTAR kit. The starch preparation (100 mg) was hydrolyzed with a solution (4 mL) of pancreatic α-amylase (10 mg/mL) and amyloglucosidase (3 U/mL) at 37°C in a water bath with shaking for 16 h. The reaction was stopped by adding 4 mL of absolute ethanol and the RS was recovered as a pellet on centrifugation (1500×*g*/10 min). The pellet was again twice washed with 8 mL of an ethanol:water mixture (50:50, v/v) and centrifuged. Free liquid was removed by decantation. RS in the pellet was dissolved in 2 mL of 2 mol/L KOH by vigorous stirring in an ice-water bath over a magnetic stirrer. The solution was neutralized with 8 mL of 1.2 mol/L sodium acetate buffer (pH=3.8) and starch was quantitatively hydrolyzed to glucose with 0.1 mL of amyloglucosidase (3300 U/mL) in a water bath at 50° C for 30 min. After centrifugation (1500×*g*/10 min), 0.1 mL aliquots of supernatants were transferred into glass test tubes, 3 mL of GOD-POD reagent were added and the samples were incubated at 50°C for 20 min. The amount of released D-glucose was determined spectrophotometrically (Beckman DU 7500 spectrophotometer, California, USA) at 510 nm against the reagent blank. The measured absorbance was used in calculation of Resistant Starch content (g/kg d.m.) using an equation from the kit. Three measurements were performed for each sample.

Determination of starch digestibility

The rate of digestion of starch was determined according to the method of Le Thanh *et al*. [2007] using a Liquick Cor-Glucose diagnostic kit Starch (50 mg) was suspended in 5.8 mL of 0.1 mol/L tris-maleate buffer (pH=5.25) and 4 mL of a solution of pancreatic α-amylase (10 mg/mL) and 0.2 mL of amyloglucosidase (6 U/mL). The suspension was incubated in a water bath with continuous shaking at 37°C for 16 h, then cooled down to room temperature, neutralized with 0.6 mL of 4 mol/L KOH and centrifuged at 4000×*g* for 10 min. The pellet was twice washed with distilled water and centrifuged. The collected supernatants were transferred into a volumetric flask and distilled water was added to a final volume of 50 mL. The supernatant (0.1 mL) was added to 1.5 mL of GOD-POD reagent and after 30 min glucose content was determined spectrophotometrically (Beckman DU 7500 Spectrophotometer, California, USA) at λ=650 nm against reagent blank. Starch digestibility was expressed as percentage of the released glucose in dry matter of the sample analyzed. Three measurements were performed for each sample.

Determination of rheological properties

Starch preparations were suspended in distilled water and heated to 100°C for 30 min, so that the final paste concentration was 6 g of starch/100 g. The obtained pastes were cooled to room temperature prior to the analysis. The rheological measurements were carried out on a RheoStress 1 rheometer (Haake, Vreden, Germany) equipped with a Z20 DIN Ti rotor and C25P refrigerated bath with a Phoenix II controller. The samples were relaxed and thermostated in the measurement cylinder for 5 min. The flow curves were determined with increasing and decreasing shear rate in the range of 1–600 s^{-1} for 4 min at 20°C. RheoWin 3.40 software was used to record and calculate the results. The obtained flow curves were described using Ostwald de Waele ($\tau = K \times \dot{\gamma}^n$) rheological model. The thixotropy values were calculated as a difference between the areas under the upward and downward flow curves. Two measurements were performed for each sample.

Microstructure analysis

The starch preparations obtained after P-CC and A-CC treatment were stuck on a specimen holder using copper tape for samples in a form of powder and silver paste for gel particles. The samples were coated with gold in a vacuum evaporator (JEE 400, Jeol, Tokyo, Japan) and viewed in a scanning electron microscope (Jeol JSM 5200, Tokyo, Japan) at an accelerating voltage of 10 kV.

Statistical analysis

Significant differences between the samples were determined using ANOVA post-hoc Duncan's test (95% confidence limit) using IBM SPSS Statistics v.22 software.

RESULTS AND DISCUSSION

Chemical composition of the raw material

The investigated native buckwheat starch had 249.3 g/kg of amylose. The protein content reached 14.1 g/kg, while surface and total lipids accounted for 2.1 and 18.8 g/kg, respectively. Moisture and ash content was 114.4 and 2.6 g/kg, respectively.

Liu *et al.* [2016] studied the properties of starch isolated from common buckwheat seeds and reported that native starch had a higher amylose (281 g/kg) and ash (11 g/kg) content and a lower content of both protein (3.5 g/kg) and lipid (5 g/kg), while its moisture content was at a similar level (112.0 g/kg). The obtained differences may be attributed to the botanical origin of the starches or isolation and analytical methods applied.

Resistant starch content and starch digestibility

Results of resistant starch content determination are shown in Table 1. Native buckwheat starch had 11.9 g/kg of RS. The RS content of A-CC and P-CC preparations ranged from 53.8 to 58.7 g/kg and from 12.3 to 43.3 g/kg, respectively. Both in A-CC and P-CC preparations, the observed differences were related to pullulanase treatment time, where prolonged enzymatic incubation led to irregular changes of RS level. The most efficient pullulanase incubation time was 6 h, while shorter or extended enzyme action time resulted in a lower RS content. The highest resistant starch content among all investigated preparations was observed in buckwheat starch preparations subjected to A-CC. Compared to native buckwheat starch, more than a threefold increase in RS content was noted in all A-CC preparations. In the case

TABLE 1. Resistant starch content (g/kg) in native buckwheat starch and pressure-cooling cycles (P-CC) or autoclaving-cooling cycles (A-CC) starch preparations treated with pullulanase.

Processing conditions	Pullulanase treatment time			
	2 h	6 h	10 h	16 h
P-CC: 200 MPa/ 3 min	12.3 ± 1.7^{DEb}	18.5 ± 0.7^{Da}	17.6 ± 0.8^{Da}	12.4 ± 0.7^{Eb}
P-CC: 200 MPa/ 9 min	12.4 ± 0.3^{DEb}	19.5 ± 0.7^{Da}	18.5 ± 0.7^{Da}	12.2 ± 0.1^{Eb}
P-CC: 400 MPa/ 3 min	13.7 ± 0.2^{CDd}	25.3 ± 1.0^{Ca}	20.2 ± 1.0^{Cb}	16.4 ± 0.8^{Dc}
P-CC: 400 MPa/ 9 min	14.1 ± 0.4^{Cd}	26.4 ± 0.9^{Ca}	21.1 ± 0.9^{Cb}	17.9 ± 0.8^{Cc}
P-CC: 600 MPa/ 3 min	29.7 ± 0.9^{Bc}	42.1 ± 1.0^{Ba}	36.6 ± 1.0^{Bb}	30.2 ± 0.5^{Bc}
P-CC: 600 MPa/ 9 min	29.4 ± 0.9^{Bc}	43.3 ± 1.2^{Ba}	35.9 ± 1.2^{Bb}	30.4 ± 0.5^{Bc}
A-CC	53.8 ± 0.8^{Ac}	58.7 ± 0.7^{Aa}	57.1 ± 0.4^{Ab}	54.8 ± 0.4^{Ac}
Native*	11.9 ± 1.2^{E}			

*Value for a native starch was included in statistical analysis carried out for each of the columns. Different letters in superscript show significantly different means ($p<0.05$): capital letters (A-E) within the same column represent the influence of starch preparation; small letters (a-d) within the same row represent the influence of pullulanase treatment time.

TABLE 2. Digestibility (%) of native buckwheat starch and pressure-cooling cycles (P-CC) or autoclaving-cooling cycles (A-CC) starch preparations treated with pullulanase.

Processing conditions	Pullulanase treatment time			
	2 h	6 h	10 h	16 h
P-CC: 200 MPa/ 3 min	95.18 ± 0.08^{Aa}	94.04 ± 0.12^{Bb}	94.20 ± 0.03^{Bc}	95.25 ± 0.11^{Aa}
P-CC: 200 MPa/ 9 min	95.22 ± 0.18^{Aa}	93.81 ± 0.11^{Bb}	94.01 ± 0.06^{BCc}	95.28 ± 0.14^{Aa}
P-CC: 400 MPa/ 3 min	94.92 ± 0.25^{Ba}	92.85 ± 0.23^{Cb}	93.82 ± 0.15^{Cc}	94.63 ± 0.09^{Ba}
P-CC: 400 MPa/ 9 min	94.93 ± 0.07^{Ba}	92.40 ± 0.21^{Db}	93.45 ± 0.22^{Dc}	94.18 ± 0.14^{Cd}
P-CC: 600 MPa/ 3 min	91.84 ± 0.07^{Ca}	89.39 ± 0.10^{Eb}	90.43 ± 0.12^{Ec}	91.63 ± 0.19^{Da}
P-CC: 600 MPa/ 9 min	91.85 ± 0.18^{Ca}	89.18 ± 0.17^{Eb}	90.54 ± 0.11^{Ec}	91.68 ± 0.16^{Da}
A-CC	86.96 ± 0.21^{Da}	85.87 ± 0.20^{Fb}	86.34 ± 0.11^{Fc}	86.68 ± 0.29^{DEa}
Native*	95.35 ± 0.06^{A}			

*Value for a native starch was included in statistical analysis carried out for each of the columns. Different letters in superscript show significantly different means (at $p<0.05$): capital letters (A-F) within the same column represent the influence of processing conditions; small letters (a-d) within the same row represent the influence of pullulanase treatment time.

of preparations obtained *via* P-CC, the intensification of pressure was followed by RS formation. The starch pressurized at a level of 600 MPa offered the highest RS content among all the P-CC samples, resulting in an increase in the range of 147 to 264% (depending on the pullulanase treatment time) in comparison to RS noted for native buckwheat starch. Statistical analysis revealed that the duration of pressure treatment (3 or 9 min) had no significant effect on the RS content ($p>0.05$). Moreover, no significant differences were observed between RS contents in native buckwheat starch and P-CC samples that were treated with high hydrostatic pressure at a level of 200 MPa and subjected to 16-h pullulanase debranching ($p>0.05$).

The recrystallization of amorphous polymers is a three--step process, involving nucleation, propagation, and maturation [Slade & Levine, 1987]. The intensity of the mentioned processes is related to amylose content, orientational mobility of the starch molecules and their chain length, autoclaving temperature, storage time and temperature [Silverio *et al.*, 2000; Eerlingen *et al.*, 1993]. RS formation involves starch hydrolysis during autoclaving at high temperatures and recrystallization upon cooling. Pullulanase-induced debranching of amylopectin increases the molecule alignment or aggregation, thus leading to the formation of crystalline structures and resistant starch [Zhao & Lin, 2009]. As it was reported, hydrolysis of α-1–6 linkages in amylopectin structure upon pullulanase treatment triggered the formation of more linear structures similar to the amylose chains and/ or may form amylopectin A-chains in a form of a double helix or crystalline segments. Therefore, these debranched structures closely pack into the crystal formation as retrograded starch (RS3) during retrogradation process [Lertwanawatana *et al.,* 2015]. It can be stated that the RS3 could be strongly enhanced when the starch was debranched to increase the number of linear

molecules prior to autoclaving or HPP process (600 MPa/ 3 or 9 min).

Digestibility of native buckwheat starch, A-CC and P-CC preparations is presented in Table 2. Native buckwheat starch, as well as P-CC preparations obtained after 200 MPa of pressure and 2 or 16 h of pullulanase treatment, exhibited the highest digestibility, exceeding 95%. Moreover, no significant differences of digestibility rates were observed between the mentioned samples ($p>0.05$). The A-CC preparations after 6 h of pullulanase treatment were the least susceptible to digestion among all preparations studied and demonstrated a 10% lower digestibility compared to native starch. Among all the P-CC preparations an increase of pressure values was followed by reduction of digestibility, whereas the duration of pressure treatment (3 or 9 min) did not change the digestibility in a significant way ($p>0.05$). All preparations studied demonstrated lower values of digestibility after 6 h of pullulanase treatment, compared to 2, 10 and 16 h. A very strong, negative linear correlation ($r=-0.997$; $p<0.01$) was found between RS content and digestibility (Figure 1), which may indicate that the level of RS formed is a key factor influencing the digestibility of the obtained buckwheat starch preparations.

Liu *et al.* [2016] studied the influence of high hydrostatic pressure, in the range of 120–600 MPa and 20 min at room temperature, on common buckwheat starch. They observed that pressure increase up to 600 MPa was followed by an increase in RS level (up to 82 g/kg) and a 27.5% decrease of digestibility, expressed as rapidly digestible starch. The influence of heat-moisture treatment (20–35% moisture, 110ºC/ 16h) on common buckwheat starch also resulted in a 6.3% decrease of rapidly digestible starch and an increase of slowly digestible starch (3.1%) and resistant starch content (1.4%), compared to native buckwheat starch [Liu *et al.,* 2015]. The mentioned authors reported that the applied heat-moisture treatment resulted in strong amylose-amylose or amylose-amylopectin interactions which could limit the accessibility of the digestive enzymes to starch chains. Starch susceptibility to enzymatic digestion is mainly related to the botanical origin of starch, amylose to amylopectin ratio, crystalline structure, and granule or particle size [Zavareze & Dias, 2011].

Summarizing, the present study proved that the buckwheat starch subjected to A-CC or P-CC (600 MPa/ 3 or 9 min) coupled with pullulanase treatment has potential to be used in the design of foods with a modified course of starch digestion. Moreover, taking into consideration that starch digestibility is relevant to the glycemic index and to the prevention of non-insulin-dependent diabetes [Liu *et al.,* 2016], it can be stated that the starch preparation obtained may also demonstrate a potential in the prevention of chronic diseases as compared to the native form of starch.

Rheological properties and microstructure of starch preparations

Taking into consideration the obtained results concerning the high resistant starch content and low digestibility (Table 1–2), starch preparations subjected to 6 h pullulanase treatment and P-CC (400, 600 MPa/ 9 min) or A-CC treatment, as well as native starch (as a reference), were selected for rheological and microstructural characteristics.

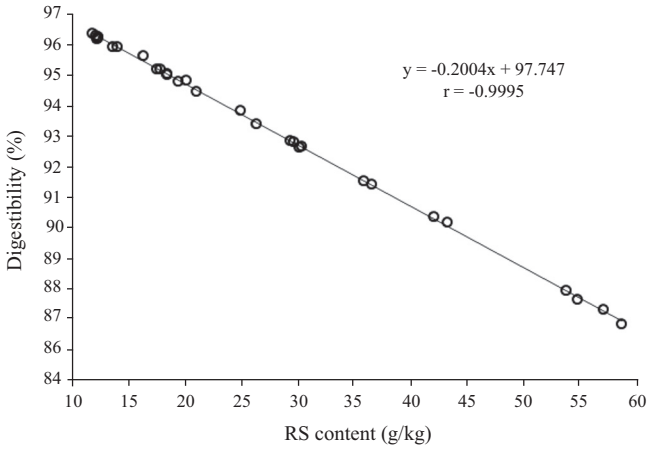


FIGURE 1. Correlation between RS content and digestibility of buckwheat starch preparations.

TABLE 3. Rheological parameters of the Ostwald de Waele model (K, n, R^2) and thixotropy values of native buckwheat starch and pressure-cooling cycle (P-CC) or autoclaving-cooling cycle (A-CC) starch preparations obtained after 6 h of pullulanase debranching.

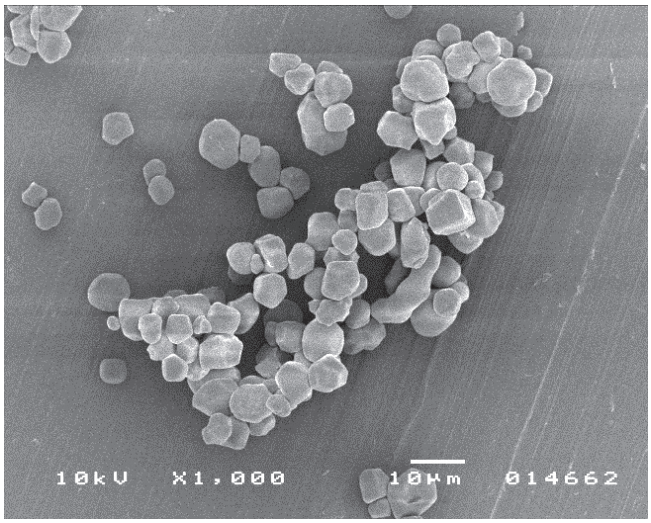
Processing conditions	K (Pa s^n)	n (-)	R^2	Thixotropy (Pa/s)
Native	89.31	0.19	0.98	38160
P-CC 400 MPa/ 9 min	17.27	0.30	0.99	11160
P-CC 600 MPa/ 9 min	11.41	0.39	1.00	5488
A-CC	74.33	0.20	0.99	31450

The rheological properties of pastes obtained from native buckwheat starch and its preparations are presented in Table 3. All investigated samples demonstrated a non-Newtonian flow, pseudoplastic properties and thixotropy. The applied Ostwald de Waele rheological model was characterized by a very good fit to the flow curves of the examined pastes, as evidenced by the high coefficients of determination (R^2).

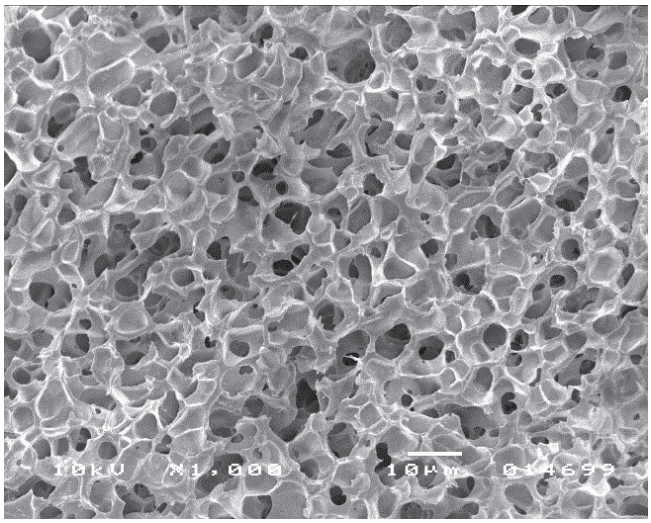
The highest consistency coefficient (K), which is a measure of paste viscosity, was noted for native starch pastes. The A-CC treatment resulted in a 17% decrease of the K value, while starch pastes after pressure treatment were characterized by a considerable decrease of the consistency coefficient, which amounted to 81% for 400 MPa and 87% for 600 MPa P-CC starch pastes, compared to the native starch paste.

The pastes obtained from P-CC samples demonstrated a higher flow behavior index (n), compared to the pastes prepared from native starch or A-CC samples. An increase of pressure from 400 to 600 MPa during the preparation of P-CC samples was followed by an 30% increase of the flow behavior index (n), which indicates that the 600 MPa-treated pastes were less susceptible to shear thinning. The flow indices observed for native and A-CC starch pastes were at a comparable level.

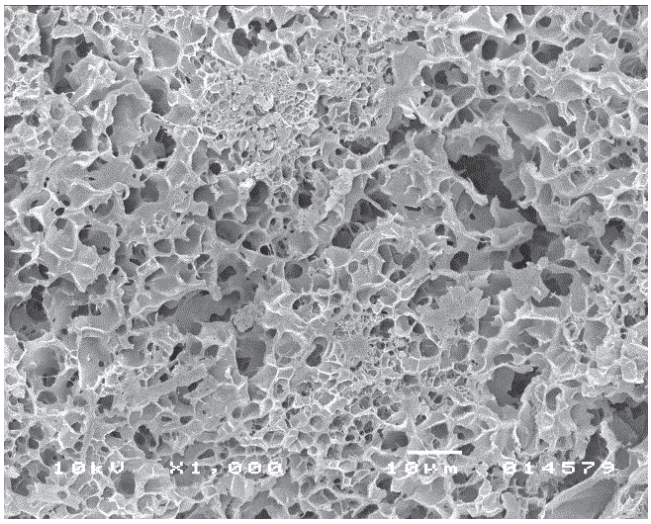
Thixotropy is an isothermal, reversible, time-dependent transformation taking place under the influence of mechanical stress, which destroys structures present in the solution. The area between the upward and downward flow curves



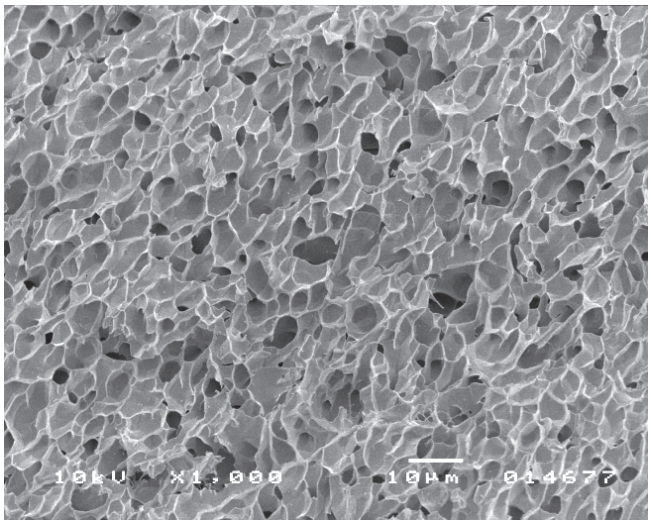
native



P-CC 400 MPa/ 9 min



P-CC 600 MPa/ 9 min



A-CC

FIGURE 2. Microstructure of native buckwheat starch, pressure-cooling cycle (P-CC) and autoclaving-cooling cycle (A-CC) starch preparations.

is the value of thixotropy, representing the energy supplied to the shear volume of the solution. It is therefore a continuous process of reduction of the apparent viscosity under shear conditions, with the subsequent recovery of the viscosity at rest [Achayuthakan & Suphantharika, 2008]. The investigated native starch paste demonstrated the highest thixotropy value, meaning that it formed the most unstable paste structure. Compared to native starch paste, a minor decrease (18%) of thixotropy value was observed in the pastes obtained from A-CC, while major reductions were noted in the case of P-CC starch preparations: 71% for 400 MPa and 86% for 600 MPa treated samples. The applied processing led to an increase in the paste stability with emphasis on HHP treatment. As demonstrated, the P-CC starch paste treated with pressure volume of 600 MPa was characterized by the lowest susceptibility to the breakdown of the thixotropic structure. The results distinctly proved that P-CC treatment enhanced the formation of shear-induced network structure with reduced breakdown of the network by the shear. These phenomenon probably occurred since some rigid rod-like conformation was formed upon P-CC treatment of buckwheat starch [Achayuthakan & Suphantharika, 2008].

Changes in the microstructure of the investigated starch preparations are presented in Figure 2. Native buckwheat starch granules were polygonal and their size distribution ranged from 5 to 12 μm. The surface of granules was smooth, with pores and channels observed on some of them (Figure 2a). The observed small indentations were probably related to amylase activity in the seeds [Gregori & Kreft, 2012]. Both applied thermal and pressure-cooling cycles combined with 6 h pullulanase treatment resulted in a complete loss of integrity of the granules. Starch breakdown was followed by the formation of a continuous gel network structure. Due to the applied freeze-drying process and free/bound water removal, a porous starch matrix was obtained. As evidenced by SEM, the microstructure of starch matrices obtained *via* P-CC and A-CC varied in porosity and gel network structure. The P-CC 400 MPa treated starch matrix was characterized by a homogeneous, coarse structure with rounded edges and oval pores with a diameter up to 10 μm (Figure 2b).

Increasing the pressure value to 600 MPa during the P-CC treatment led to the formation of a biphase network structure, consisting of regions of rugged, lamellar starch matrix with 3–10 μm pores and areas characterized by a highly perforated, spongy-like structure with pore diameter smaller than 1 μm (Figure 2c). The autoclaving-cooling cycles of buckwheat starch led to the formation of a homogeneous, thin-walled gel structure with pore diameter not exceeding 8 μm (Figure 2d).

Vallons & Arendt [2009] observed changes in the shape and surface of buckwheat starch granules subjected to 400–600 MPa treatment for 10 min at room temperature. Although the granules were swollen, deformed and (as evidenced by DSC) lost their crystallinity, most of them retained some degree of integrity after the pressure treatment, showing a significantly different structure compared to the investigated in this study P-CC starch preparations. The applied pullulanase treatment and the number of pressure cycles may be the key factors influencing the structural differences of the high pressure-treated starch preparations.

CONCLUSIONS

Buckwheat starch subjected to autoclaving-cooling or high pressure-cooling cycles, in combination with pullulanase debranching, demonstrated physico-chemical and structural changes. The applied treatment resulted in resistant starch content increase and decrease of digestibility. Although the resistant starch content was higher after autoclaving-cooling cycles, high pressure-cooling cycles may be of interest due to the possibility of resistant starch formation already at room temperature. This provides a possibility to replace high energy and time-consuming thermal methods with mild processing, such as high pressure treatment. Moreover, high pressure--cooling cycle treatment alters the rheological and structural properties of starch in a different way compared to thermal treatment, thus providing the possibility of producing preparations with novel functional properties.

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CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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