Effect of acetylation on quality attributes of potato protein preparations heated to various temperatures

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Summary
The objective of this study was to determine the effect of acetylation of a potato protein concentrate (PPC) previously heat-treated to four different temperatures on the chemical composition and functional properties of the modified preparations. Results showed that the composition and properties of the modified preparations depended on the temperature of heat treatment before acetylation. An increase of temperature ranging from 50 °C to 80 °C of the native PPC prior to acetylation contributed to an increase in levels of amino acids such as leucine, valine, and phenylalanine with tyrosine and threonine in the potato protein preparation after acetylation. Solubility as well as emulsifying and foaming properties of the modified preparations decreased successively along with the temperature increase ranging from 50 °C to 80 °C. The highest water-holding capacity values were reported for the preparation heated to 60 °C and then acetylated (5.88 $g \cdot g^{-1}$). Native PPC was characterized by a high oil-binding capacity value (6.18 $ml \cdot g^{-1}$), similarly to the acetylated preparation heated previously to 60 °C with 1.0 ml of acetic anhydride (6.37 $ml \cdot g^{-1}$), which temperature was the most favourable.

Keywords
potato protein concentrate; heating; acetylation; amino acid composition; functional properties

The knowledge of consumers and food manufacturers concerning the nutritional value of potato protein is on the rise. In the starch industry, potato protein, in the form of potato juice, constitutes a waste material, with coagulable protein representing up to about 20 % of its dry matter. Potato protein discharge with potato juice to wastewater has recently become a common practice. However, it may be recovered using advanced technological methods. The dry feed protein preparation currently produced by the starch industry contains approximately 80 % of protein [1, 2].

The global starch industry processes more than 15 million tons of potato tubers annually, from which about 200 000 t of preparations containing healthy and nutritious protein can be isolated from potato juice with the use of heat coagulation. It is known that potato proteins unfold between 55 °C and 75 °C. This was documented using differential scanning calorimetry (DSC) and combination of far-ultraviolet (far-UV) circular dichroism (CD) and near-ultraviolet (near-UV) CD spectroscopy [3]. The application of higher temperatures during coagulation (over 90 °C) increases the denaturation of protein, which triggers changes in the functional properties of preparations. According to several research studies, potato protein concentrates obtained by acid and thermal-acid coagulation methods using denaturing substances like HCl, H_2SO_4, $Al_2(SO_4)_3$ or citric acid [4] as well as $(NH_4)_2SO_4$ [3, 5] and temperatures above 90 °C are characterized by non-beneficial functional properties. Those authors assert that the drastic coagulation process contributes to deterioration of solubility, water-holding capacity (WHC) and oil-binding capacity (OBC) of protein preparations.

Several authors [5, 6] claimed that it is possible to produce a potato protein concentrate (PPC) of light colour and weak potato aroma and taste, as well as beneficial functional properties. However, this requires costly separation of glycoalkaloids and phenolic compounds together with removal

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or inactivation of antinutrients. The antinutrients include protease inhibitors, which inhibit the proteolysis process of proteins, worsening their digestibility and thus decreasing their biological value. The proteolytic enzyme inhibitors present in potato tubers in relatively high amounts include specific trypsin inhibitors, inhibitors acting on trypsin and chymotrypsin, and inhibitors of wide spectrum of activity that inhibit also serine protease of microorganisms [7].

Favourable functional properties are reported for proteins isolated from potato juice in a non-denatured form with the use of membrane processes such as ultrafiltration and reverse osmosis with polymeric and ceramic membranes, ion exchange chromatography, and complexation with carboxymethylcellulose or bentonite [5, 6, 8, 9]. Such preparations display good functional properties, like solubility, foaming and emulsifying abilities, but on the other hand, they are characterized by a poor nutritional value, due to the presence of protease inhibitors. It also has to be taken into account that a protein preparation intended for consumption should be subjected to heat treatment, which leads to deterioration of its functional properties [7].

Ample research is reported in the literature regarding the effects of chemical modification, e. g. with acetic, succinic or maleic anhydrides, on the functional properties of different plant proteins [10–12]. The authors emphasize that the modified proteins have some improved functional properties, e.g. better emulsifying and foaming ability, solubility and WHC, which result from changes in protein structure, including protein dissociation, partial denaturation, change in isoelectric point and increased hydrophobicity. As a result of chemical modifications, some fractions containing the amino acids in N-terminal α- and ε-amino groups can be released. These could act as substrates in Maillard reactions.

The problem of chemical modification of proteins points to the need of analysing the effects of different types of modification on physical and functional properties and of determining the dependency between modification degree and properties of modified products. Some information is available concerning the process and effects of chemical modification, whereas the effect of these modifications on proteins is closely dependent on protein source and temperature of the isolation process [10, 13, 14]. Therefore, it is justifiable to conduct analyses aimed at application of different methods of protein recovery from potato juice, which do not deteriorate the product properties.

The aim of the study was to determine the effect of acetylation of PPC previously heat-treated at four different temperatures (50 °C, 60 °C, 70 °C and 80 °C) on the chemical composition and functional properties of the modified preparations.

MATERIALS AND METHODS

Materials

Fresh potatoes used in the experiment were delivered from an experimental station in Pawłowice, Poland. Acetic anhydride, 2,4,6-trinitrobenzenesulfonic acid (TNBS), cellulose membranes and Amberlite XAD-4 resin were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). All chemicals used in the experiments were of analytical grade. Distilled water used (0.35 μS, pH 6.20) was obtained from HLP 10 demineralizer (Hydrolab, Straszyn, Poland).

Preparation of potato protein concentrate

PPC was prepared using the method described by MIEDZIANKA et al. [15]. Briefly, it was obtained using membrane techniques in combination with dialysis membranes retaining substances of molecular weight (MW) > 12 kDa against distilled water. Clarified potato juice, generated by the method described above, was kept in contact with Amberlite XAD-4 resin (250 $g \cdot l^{-1}$) under mechanical stirring overnight to remove a part of phenolic acids. The extract was then dialysed against distilled water and freeze-dried to produce PPC.

Coagulation and acetylation of potato protein concentrate

The 1% PPC solution was heated to 50 °C, 60 °C, 70 °C or 80 °C for 20 min in the presence of 810 $mmol \cdot l^{-1}$ $CaCl_2$, the ionic strength of which is 0.045 and allows coagulation of potato protein at a temperature not causing its denaturation [3]). After cooling, the protein solution was acetylated with 1.0 ml of acetic anhydride per gram of PPC during 90 min at pH 7.5–8.0 with 1 $mol \cdot l^{-1}$ NaOH, based on the earlier authors' studies and accepted as the most favourable condition [15], using a pH meter to maintain the appropriate reaction conditions. Afterwards, the samples that had previously been heated to 50 °C or 60 °C were heated to 70 °C for 15 min, to precipitate almost all fractions of potato proteins and to increase the amount of coagulated protein subjected subsequently to the acetylation process. Then, the solutions were again cooled to laboratory temperature and the precipitated protein was separated from the supernatant by centrifugation. Samples of coagu-

lated protein after removing the supernatant were mixed with distilled water till the conductivity of the liquid was similar to distilled water (50 mV; CC-411 conductometer, Elmetron, Zabrze, Poland). The obtained preparations of coagulated and acetylated potato protein were freeze-dried during 24 h at 400 Pa in Modulyo 4K (Edwards, Crawley, United Kingdom), sifted through a sieve with a pore size of 420 μm and stored at –20 °C until further analysis.

Chemical composition determination

The total and coagulable protein content (protein precipitated with $CuSO_4$ solution, it means with Bernstein I and II compounds) was determined using the Kjeldahl method [16]. Protein content was calculated by multiplying the nitrogen content by a conversion factor of 6.25. To determine ash content, the samples were gradually heated to 550 °C, after which the residues were weighed. Fat content was determined by the Soxhlet method [16].

Amino acid composition

The composition of amino acids was determined after a 24 h hydrolysis with the use of 6 mol·l^{-1} HCl at a temperature of 110 °C. Analysis was done by high-performance liquid chromatography, using AAA 400 instrument (Ingos, Prague, Czech Republic). As a detector, a two-wavelength photometer (440 nm and 570 nm) was employed. The column of 350 mm × 3.7 mm was packed with ion exchanger Ostion LG ANB (Ingos). The column temperature was maintained at 40–70 °C and the detector temperature was 121 °C. The prepared samples were analysed using the ninhydrin method. Amino acid composition was expressed as grams of amino acids per kilogram of protein [17]. No analysis was carried out for tryptophan.

Extent of chemical modification

The trinitrobenzenesulfonic acid (TNBS) method by Habeeb [18] was used to determine the extent of acetylation. This procedure involved the addition of 1 ml of 4% $NaHCO_3$ and 1 ml of 0.1% TNBS to protein suspensions. Afterwards, the samples were incubated in a water bath at 60 °C for 2 h. After cooling to laboratory temperature, 1 ml of 10% sodium dodecyl sulfate (SDS) and 3 ml of 0.1 mol·l^{-1} HCl were added to the protein solutions. The absorbance of solutions was read at 390 nm in a spectrophotometer UV-2601 (Rayleigh, Beijing, China) against a reagent blank. The absorbance of the control protein concentrate was set to 100% of free amino groups and the extent of acetylation of the modified samples was calculated based on the decrease in absorbance caused by a reduction in the number of amino groups able to react with the TNBS reagent.

Functional properties of protein preparations

Protein solubility index

Protein solubility index was determined by the method described by Jackman and Yada [19]. Sample protein preparations (1 g·l^{-1}) were dispersed in distilled water and the slurries were shaken for 1 h at laboratory temperature. They were then centrifuged at 5 000 ×g for 30 s. Finally, protein content in the supernatants was determined by the Kjeldahl method [16]. Protein solubility index (*PSI*) was expressed as the percent of soluble protein content to total protein content.

Water-holding capacity

Protein preparations (1 g) were diluted in 30 ml of distilled water by mixing for 30 s every 10 min using a glass rod. Once they had been mixed seven times, they were centrifuged at 5 260 ×g for 25 min (Rotofix 32A, Hettich, Tuttlingen, Germany). The supernatants were carefully decanted and the sediment inside the tubes allowed to drain at 50 °C for 25 min and then weighed. Water-holding capacity (*WHC*) was expressed in grams of water per gram of protein preparation [20].

Oil-binding capacity

The protein suspensions (1 g) were mixed with 5 ml of sunflower oil for 1 min. The samples were then allowed to stand for 30 min. The protein–oil mixtures were centrifuged at 3 000 ×g (Rotofix 32A) and the unabsorbed oil was carefully collected with a 5 ml calibrated pipette. Oil binding capacity (*OBC*) was expressed in millilitres of oil bound per gram of protein preparation [21].

Emulsifying properties

The protein suspensions (1 g) with added oil (50 ml) were emulsified for 1 min in a 302 MPW homogenizer (Praiston, Leszno, Poland) at 167 Hz. Next, the samples were centrifuged at 3 000 ×g for 5 min. Emulsion stability was determined by centrifugation after heating at 80 °C for 30 min [20]. The emulsion activity (*EA*) and emulsion stability (*ES*) were expressed in percent and calculated as:

$$EA = \frac{a}{b} \times 100 \quad (1)$$

where a is the volume of emulsified layer in a tube (in millilitres), b is the volume of the total contents in a tube (in millilitres).

$$ES = \frac{c}{d} \times 100 \quad (2)$$

where c is the volume of emulsified layer after heating (in millilitres), d is the volume of the total contents before heating (in millilitres).

Foaming properties

The protein suspensions were emulsified for 1 min in a homogenizer 302 MPW at 167 Hz. The blends were immediately transferred into a graduated cylinder. The volumes were recorded before and after stirring. Foaming capacity (*FC*) was expressed as the volume increased due to stirring. To determine foaming stability (*FS*), changes in foam volume in a cylinder were recorded after 10 min of storage [20]. *FC* and *FS* were calculated as:

$$FC = \frac{a}{b} \times 100 \quad (3)$$

where a is the volume after whipping (in millilitres), b is the volume before whipping (in millilitres).

$$FS = \frac{c}{d} \times 100 \quad (4)$$

where c is the volume after standing (in millilitres), d is the volume before whipping (in millilitres).

Statistical analysis

Analyses of the chemical composition and functional properties were performed in duplicate or triplicate. Results were calculated with the assumption that the moisture content of the preparation was 5 %. Data were subjected to a two-way (differences between temperatures of protein coagulation) Duncan's multiple range test ANOVA using Statistica 9.0 software (StatSoft, Tulsa, Oklahoma, USA). Differences were taken as significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Chemical composition

Data on the chemical composition of native PPC and of the preparations of acetylated PPC previously heated to different temperatures are presented in Tab. 1. The unmodified concentrate was characterized by the highest content of total proteins (768.3 g·kg^{-1} dry matter, DM) and the highest content of coagulable protein (719.4 g·kg^{-1} DM) in comparison to the heated and acetylated preparations. However, along with increasing the temperature of the protein solution from 50 °C to 80 °C, a tendency was observed for total and coagulable protein content to increase in the obtained preparations, which was related to considerable loss of solubility. Moreover, the acetylated samples of potato protein heated to temperatures from 60 °C to 80 °C were characterized by a higher protein content than the preparation obtained after heating to 50 °C followed by acetylation. The loss of protein content as affected by heat treatment and acetylation amounted to 90–160 g·kg^{-1} for the total protein and to 60–110 g·kg^{-1} for the coagulable fractions in comparison to the native PPC. The content of coagulable protein in total protein increased as an effect of the heating and acetylation processes from 805.2 g·kg^{-1} up to 969.4–996.2 g·kg^{-1} DM. Additionally, the study demonstrated the influence of heating the protein to temperatures higher than 60 °C on its properties prior to acetylation. Similar findings were presented by KLEPACKA et al. [22] who studied the effect of heat treatment on chemically modified proteins of legume seeds.

The degree of acetylation increased with raising the heating temperature (Tab. 1). The greatest extent of lysine modification in the concentrate was observed in the preparations heated to 70 °C and 80 °C (973.2 g·kg^{-1} and 965.3 g·kg^{-1} DM, respectively). Such temperatures can cause changes inside the spatial structure of proteins,

Tab. 1. Characteristics of potato protein concentrates heated to different temperatures and acetylated with a definite dose of acetic anhydride.

Type of preparation	PPC	APC 50	APC 60	APC 70	APC 80
Dry matter [g·kg^{-1}]	938.9 ± 2.0^{a}	921.1 ± 1.1^{b}	901.8 ± 3.4^{b}	881.3 ± 4.5^{c}	872.6 ± 1.1^{c}
Total protein [g·kg^{-1}]	768.3 ± 8.0^{a}	503.4 ± 7.0^{d}	640.0 ± 10.8^{c}	658.9 ± 8.6^{c}	698.9 ± 13.3^{b}
Coagulable protein [g·kg^{-1}]	719.4 ± 1.6^{a}	405.2 ± 5.0^{d}	637.6 ± 1.1^{c}	643.5 ± 11.9^{c}	677.5 ± 9.3^{b}
Degree of acetylation [g·kg^{-1}]	–	892.1 ± 11.2^{c}	902.6 ± 14.3^{b}	962.4 ± 8.2^{a}	957.1 ± 9.9^{a}

Values represent mean ± standard deviation. The same letters in superscript in columns related to individual preparations indicate homogenous groups.
PPC – potato protein concentrate; APC 50, APC 60, APC 70, APC 80 – acetylated protein concentrate heated to 50 °C, 60 °C, 70 °C and 80 °C, respectively.

Tab. 2. Amino acid composition of potato protein concentrates heated to different temperatures and acetylated with a definite dose of acetic anhydride.

Type of preparation	PPC	APC 50	APC 60	APC 70	APC 80	Standard protein [27]
Essential amino acids [g·kg^{-1}]						
Isoleucine	45.5 ± 2.0^{a}	31.8 ± 1.5^{c}	38.8 ± 3.5^{bc}	36.8 ± 4.5^{c}	41.1 ± 10.1^{b}	30.1
Leucine	39.9 ± 10.0^{d}	58.5 ± 4.5^{c}	75.2 ± 5.9^{b}	73.4 ± 9.3^{b}	80.2 ± 19.4^{a}	53.0
Lysine	5.20 ± 7.0^{d}	48.7 ± 3.4^{c}	60.3 ± 5.6^{ab}	58.3 ± 7.4^{b}	64.4 ± 16.0^{a}	45.0
Methionine + cysteine	55.7 ± 1.3^{a}	26.3 ± 2.0^{c}	32.4 ± 2.6^{b}	31.3 ± 3.9^{b}	35.0 ± 9.2^{b}	22.1
Valine	12.0 ± 1.2^{d}	39.0 ± 3.8^{c}	50.7 ± 3.6^{ab}	48.6 ± 6.4^{b}	53.7 ± 12.7^{a}	39.0
Phenylalanine + tyrosine	84.9 ± 1.4^{b}	70.8 ± 5.6^{c}	92.5 ± 6.9^{ab}	88.9 ± 11.5^{b}	98.9 ± 24.2^{a}	38.1
Relatively essential amino acids [g·kg^{-1}]						
Threonine	73.0 ± 6.0^{a}	32.6 ± 2.7^{c}	43.2 ± 4.2^{b}	41.7 ± 5.5^{b}	44.9 ± 10.7^{b}	23.0
Arginine	237.5 ± 8.0^{a}	34.7 ± 2.8^{b}	38.2 ± 4.0^{b}	37.3 ± 4.6^{b}	41.0± 9.8^{b}	–
Histidine	15.8 ± 1.7^{b}	15.0± 1.8^{b}	17.0± 1.8^{a}	15.4 ± 2.1^{bc}	16.9 ± 4.5^{a}	–
Non-essential amino acids [g·kg^{-1}]						
Aspartic acid	48.1 ± 1.0^{d}	69.6 ± 5.3^{c}	90.3 ± 6.9^{a}	89.3 ± 11.1^{b}	99.1 ± 24.9^{a}	–
Serine	71.2 ± 8.0^{a}	30.7 ± 3.7^{c}	39.6 ± 4.4^{b}	39.9 ± 5.5^{b}	43.3 ± 10.9^{b}	–
Glutamic acid	105.7 ± 1.4^{a}	59.3 ± 4.8^{d}	72.7 ± 6.1^{b}	68.8 ± 9.2^{c}	75.0 ± 18.8^{b}	–
Proline	75.1 ± 1.2^{a}	27.7 ± 1.1^{d}	36.7 ± 2.7^{c}	37.0 ± 6.0^{c}	40.5 ± 6.9^{b}	–
Glycine	40.9 ± 6.0^{a}	29.3 ± 2.4^{c}	35.8 ± 2.9^{b}	35.7 ± 4.8^{b}	39.3 ± 9.9^{a}	–
Alanine	41.3 ± 1.3^{a}	35.2 ± 2.5^{c}	39.5 ± 3.7^{b}	36.7 ± 4.8^{bc}	40.5 ± 10.5^{a}	–
Total	951.8 ± 14.8^{a}	609.2 ± 45.3^{d}	762.9 ± 62.3^{c}	739.1 ± 95.3^{c}	813.8 ± 198.1^{b}	–

Values represent mean ± standard deviation. The same letters in superscript in columns related to individual preparations indicate homogenous groups.
PPC – potato protein concentrate; APC 50, APC 60, APC 70, APC 80 – acetylated protein concentrate heated to 50 °C, 60 °C, 70 °C and 80 °C, respectively.

which lead to the exposure of new polypeptide chains or functional groups, among them the non-polar (hydrophobic) ones. Such exposed groups can more easily form bonds with acetic anhydride [22].

Amino acid composition

Tab. 2 presents data on the amino acid composition of PPC and of acetylated preparations obtained after its heating to different temperatures. Although the native PPC contained high amounts of essential (316.2 g·kg^{-1}) and non-essential (382.3 g·kg^{-1}) amino acids, its value was low due to the low contents of leucine (39.9 g·kg^{-1}), lysine (5.2 g·kg^{-1}) and valine (12.0 g·kg^{-1}). The amino acid composition of acetylated preparations depended on the temperature of the heating process preceding the chemical modification by acetylation. Contents of all amino acids increased as the heating temperature was increased from 50 °C to 80 °C. The increase of temperature to 60 °C of the native PPC before its acetylation contributed to the increased contents of amino acids like leucine, valine, phenylalanine with tyrosine and threonine in the modified potato protein preparations. Further increase of temperature caused no significant further increase in the content of these amino acids. However, contents of methionine together with cysteine and threonine in the modified preparations were reduced by almost half when compared to the control sample of native PPC. This is explained by the fact that acetic anhydride can react with different nucleophilic groups of amino acids that are blocked after the acetylation process. These include amino groups (N-terminal α-amino groups and ε-amino groups of lysine), phenolic groups (in phenylalanine and tyrosine) and thiol groups (in methionine and cysteine) [23]. Moreover, heating to lower temperatures (50 °C, 60 °C and 70 °C) led to a decrease in the sum of essential amino acids in acetylated preparations in comparison to the native PPC (951.8 g·kg^{-1}). This fact is in accordance with results of the study conducted by Wojnowska et al. [5], who produced potato preparations using different membrane processes (ultrafiltration, cryoconcentration and

polyelectrolysis) and heated them to 100 °C for 15 min. They noticed that the thermal treatment led to considerable losses in the content of amino acids of proteins and had an adverse effect on the physico-chemical properties of the dried preparations. Potato proteins were found to be more reactive after heating, mainly at temperatures between 40 °C and 50 °C, when an increase was observed in the number of free functional groups, which are usually blocked during the acetylation process. According to ADEYEYE [24], heat treatment at high temperatures (85–90 °C) can cause isomerization of amino acid residues. GRUENER and ISMOND [25] reported a slight reduction in essential amino acids following acetylation after they modified the canola globulin. They observed the highest decrease in the lysine content, mainly due to its susceptibility to chemical modification. The lower content of essential amino acids in an acetylated protein isolate might be caused by different mechanisms of substitution of functional groups of amino acids by acetic anhydride, and by differences in protein solubility after chemical modification. Authors who determined the amino acid composition of PPC by the dialysis method [7, 26] stated that endogenous amino acids represented more than 900 $g \cdot kg^{-1}$, including glutamic acid and aspartic acid, as well as threonine. As shown in Tab. 2, regardless of the coagulation temperature, the content of essential amino acids, except for threonine, was similar or higher in the modified preparations than that established in the FAO protein standard [27]. FRIEDMAN [28] confirmed the high nutritional value of potato proteins in comparison to other plant proteins. Some authors also underlined that the amino acid composition of protein preparations obtained from potato juice depends on the method of isolation [5], climate [29], and storage conditions [30]. The latter authors noticed that the protein content of the isolates prepared from the soybean stored under adverse conditions was not markedly lower until after seven months of storage.

Functional properties

Protein solubility index

Among all functional properties of protein preparations, the most important one is solubility of nitrogen compounds, due to its significant influence on other properties [31]. PPC was characterized by total (100%) solubility in distilled water (Fig. 1). Along with increasing the heating temperature from 50 °C to 80 °C, *PSI* of the modified preparations gradually decreased. This can be explained by a degree of denaturation [19]. The intensive particle aggregation of PPC, obtained with the use of calcium ions, caused a decrease in solubility of the modified preparations. By increasing the ionic strength after use of the $CaCl_2$ solution, denaturation temperatures of the majority of potato fractions increased and destruction of the secondary structure of proteins occurred prior to complete denaturation [7]. These facts were confirmed by HOLM and ERIKSEN [8], who observed an increase in the *PSI* value of concentrates heated to 50 °C and 60 °C before acetylation, in comparison to heating to 70 °C and 80 °C. The authors explained it by higher potato protein hydrophobicity at these lower temperatures, which caused its higher reactivity. Also, studies conducted by BARANIAK et al. [13] confirmed that factors accelerating protein agglomeration through an increase in surface hydrophobicity (thermal coagulation) caused lower solubility of the obtained preparations. Furthermore, LAWAL and ADEBOWALE [10] noticed that acetylation reduced protein solubility in the acidic pH range below the isoelectric point (4.5) of the protein concentrate, and linked this effect to the combination of intra- and intermolecular charge repulsion, promoting protein unfolding and producing fewer protein–protein interactions and more protein–water interactions.

Water-holding and oil-binding capacity

Data on the effect of the heating temperature before acetylation on *WHC* and *OBC* of the modified preparations is presented in Fig. 2. PPC was characterized by the lowest *WHC* (1.06 $ml \cdot g^{-1}$), as compared with all modified preparations, which

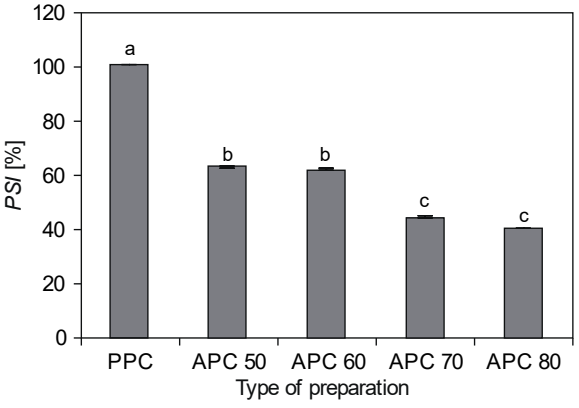

Fig. 1. Effect of temperature on protein stability index of acetylated potato protein concentrate.

The same letters above columns related to individual preparations indicate homogenous groups.
PSI – protein solubility index, PPC – potato protein concentrate; APC 50, APC 60, APC 70, APC 80 – acetylated protein concentrate heated to 50 °C, 60 °C, 70 °C and 80 °C, respectively.

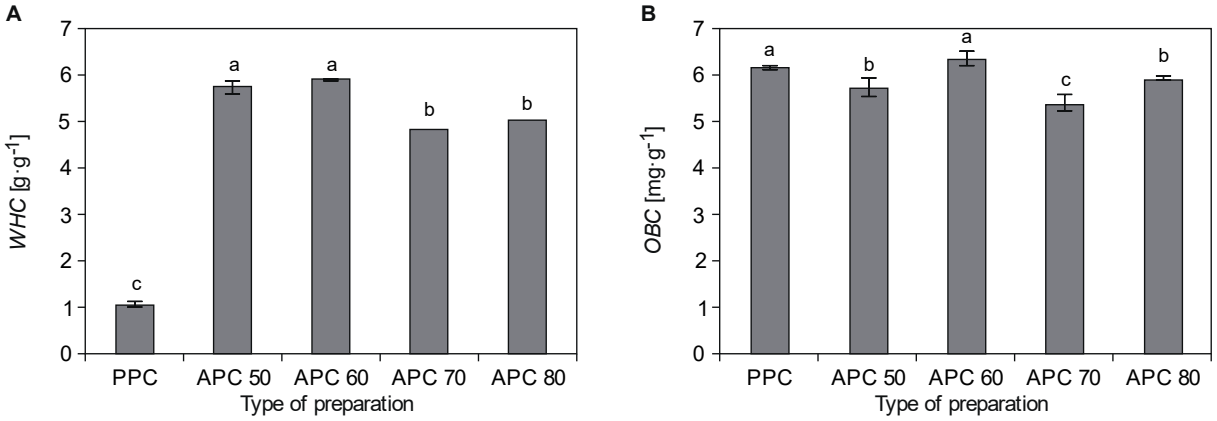

Fig. 2. Effect of temperature on water-holding capacity and oil-binding capacity of acetylated potato protein concentrate.

A – water-holding capacity, B – oil-binding capacity.
The same letters above columns related to individual preparations indicate homogenous groups.
WHC – water holding capacity (expressed as grams of water per gram of preparation), *OBC* – oil-binding capacity (expressed as millilitres of oil per gram of preparation), PPC – potato protein concentrate; APC 50, APC 60, APC 70, APC 80 – acetylated protein concentrate heated to 50 °C, 60 °C, 70 °C and 80 °C, respectively.

is related to the exposure of a smaller number of hydrophilic groups [31] by native potato protein. However, the PPC preparations heated to 50 °C and 60 °C and then acetylated were characterized by higher *WHC* as compared to the preparations heated to higher temperatures (70 °C and 80 °C) before acetylation. The highest *WHC* value (5.88 $g \cdot g^{-1}$) was reported for the preparation heated to 60 °C and then acetylated. This resulted from sufficient exposure of hydrophilic groups and higher amounts of polar amino acids present in protein particles, mainly lysine (75.2 $g \cdot kg^{-1}$) and aspartic acid (90.3 $g \cdot kg^{-1}$) (Tab. 2). A similar explanation of this phenomenon was offered by Bora [32], who acetylated lentil globulin, and by Lawal and Dawodu [11], who acylated locust bean.

Native PPC was characterized by a high *OBC* value (6.18 $ml \cdot g^{-1}$), which was similar to *OBC* of the acetylated preparation heated previously to 60 °C (6.37 $ml \cdot g^{-1}$), this temperature appearing to be the most favourable (Fig. 2). This could be explained by the presence of several non-polar side chains in the protein concentrate, which may bind the hydrocarbon chains of lipids. Bora [32] observed the improvement of *OBC* of acetylated rapeseed proteins, but simultaneously he pointed out that the deterioration of this feature could be connected to an increase in the extent of protein modification. Krause [14] stated that properties of chemically modified protein preparations depend not only on the degree of their modification, regarding accessibility of functional amine groups, but on the type of protein preparation used for modification. Acetylated preparations obtained from PPC were characterized by higher *OBC* values than a commercial soya protein (3.29 $ml \cdot g^{-1}$) [33] or pea protein (1.7 $ml \cdot g^{-1}$) [34].

Emulsifying properties

Fig. 3A presents data on the effect of heating before the acetylation process on *EA* and *ES* of the modified preparations. PPC obtained from potato juice subjected to a cleaning processes, including dialysis and adsorption on the ion-exchange resin, was characterized by good emulsifying properties in terms of both activity (44.4%) and stability (50.0%). These properties are related to its total solubility in distilled water, as suggested by Ortiz and Wagner [35]. Those authors noticed that the emulsifying properties of proteins were related to protein solubility, as soluble proteins are surface-active, which facilitates emulsion formation. Moreover, the dialysis process, by removing low-molecular substances such as phenolic acids, is known to have a beneficial effect on emulsifying properties [36]. However, when increasing the temperature of PPC in the presence of $CaCl_2$ from 60 °C to 80 °C, a decrease in *EA* and *ES* was observed. This phenomenon can be explained by reduced surface tension from adding the salt ($CaCl_2$), which causes the protein surface binding (van der Waals forces outweigh repulsion) and an increase in coalescence and in flocculation [37]. Additionally, our results indicated that increasing the treatment temperature from 50 °C to 80 °C caused a decrease in emulsion-forming ac-

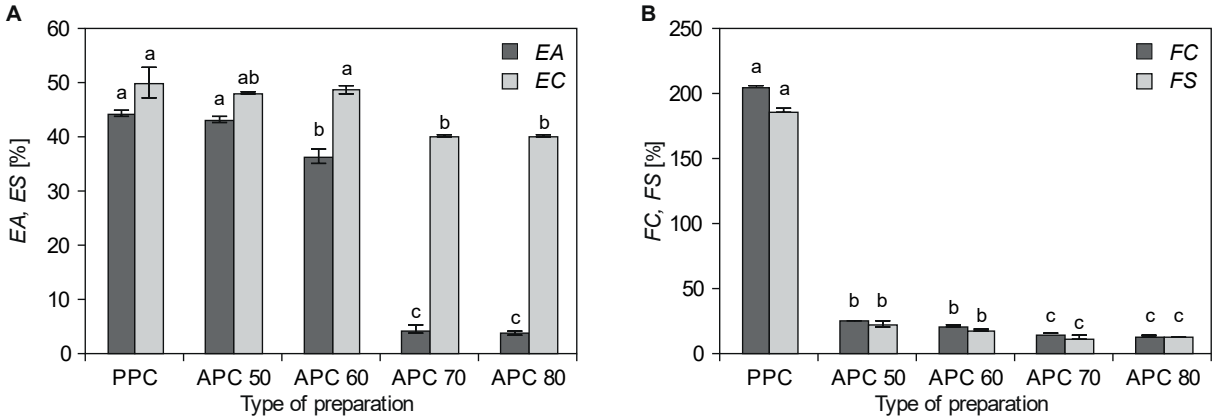

Fig. 3. Effect of temperature on emulsifying and foaming properties of acetylated potato protein concentrate.

A – emulsifying activity and emulsifying stability; B – foaming capacity and foaming stability.
The same letters above columns related to individual preparations indicate homogenous groups.
EA – emulsifying activity, *ES* – emulsifying stability, *FC* – foaming capacity, *FS* – foaming stability, PPC – potato protein concentrate; APC 50, APC 60, APC 70, APC 80 – acetylated protein concentrate heated to 50 °C, 60 °C, 70 °C and 80 °C, respectively.

tivity of acetylated protein preparations. Similarly, Lee et al. [38] observed a decrease in *EA* and *ES* of Australian lentil preparations with temperature increase during protein extraction.

Foaming properties

Fig. 3B presents data on the effect of heating temperature of PPC and acetylation process on *FC* and *FS* of the modified protein preparations. PPC revealed good foaming properties with respect to both capacity and stability (206.1 % and 186.7%, respectively), which could be explained by a low molecular mass of proteins, as lighter molecules are known to penetrate the interface more easily [39].

Due to their surface-active properties, plant proteins exhibit foaming abilities, which has been confirmed in many investigations [10, 13, 22, 24]. Increase in the temperature before the acetylation process from 50 °C to 80 °C resulted in a decrease in both the foam volume and stability. The preparations heated to higher temperatures (70 °C and 80 °C) were characterized by the lowest *FC* and *FS* values, which was related to their low solubility in water. Low *FC* values of potato preparations subjected to heating and modification processes could be caused by the closed, homogenous and globular structure of denaturated protein, which inhibited their ability to achieve effective re-orientation [10, 40]. Likewise, Lee et al. [38] noted a decrease in *FC* values with increased coagulation temperature during extraction of proteins from two Australian lentil varieties.

CONCLUSIONS

Potato juice obtained in starch factories in significant quantities can be an inexpensive and valuable raw material for producing protein preparations with a wide spectrum of applications. The present work describes the influence of heating native PPC, followed by an acetylation process, on properties of the modified preparations. Results from the presented study show that PPC heated to 60 °C and then acetylated with 1.0 ml of acetic anhydride per gram of preparation had the best chemical composition and most beneficial functional properties. Specifically, increasing the temperature of PPC prior to acetylation led to increased contents of such amino acids as leucine, valine and phenylalanine with tyrosine and threonine in the modified preparation. Generally, the acetylation process is known to improve solubility in the area above the isoelectric point due to a shift in the acid direction. Changes observed in functional properties result from modification of protein structure, including dissociation, partial denaturation, changed isoelectric point and increased hydrophobicity. Due to their high WHC, such preparations could be used in viscous products (dough, processed cheese). The obtained preparations characterized by high binding capacity may, therefore, be applied as meat product extenders. The described modification could be a viable alternative method of producing commercial protein preparations. Our results provide information on acetylated derivatives, which could

be useful in future experiments on the chemical modification of potato protein preparations appropriate for food purposes.

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