

## Article

# The Effect of Chemical Modification on the Rheological Properties and Structure of Food Grade Modified Starches

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**Abstract:** Starch in its pure form can be used as a functional component of numerous food products; however, much better results both in terms of technological and economical aspects are obtained with the use of modified starches. The aim of the work was to establish how chemical modification affects the molecular structure of potato starch and, as a consequence, its rheological properties as well as texture forming ability. Commercial food-grade potato starch preparations oxidized starch, acetylated starch, distarch phosphate, acetylated distarch phosphate and acetylated distarch adipate were the investigated material. The experimental methods included: viscographic analysis of pasting properties, flow rheometry, texture profile analysis, size exclusion chromatography with triple detection. The obtained data were further analyzed employing principal component and hierarchical cluster analysis. It was found that chemical modification leads to substantial changes in the molecular and functional properties of starch products. Oxidation process leads to depolymerization, which causes a substantial decrease in viscosity. Acetylation results mostly in stabilization of rheological properties during thermal processing. Crosslinking of starch leads to an improvement in thickening capabilities, while the type of crosslinking agent used for modification has a secondary effect. Sterilization of all types of modified potato starch pastes leads only to minor changes in their texture and rheological properties.

**Keywords:** modified starch; potato starch; rheological properties; TPA; molecular structure; food quality; cluster analysis



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## 1. Introduction

Starch in its pure form, like other polysaccharides, can be used as a functional component of numerous food products thus influencing their quality [1,2]. However, much better results, both in terms of technological and economical aspects, are obtained with the use of modified starches. This is because starch can be processed in numerous ways, and the properties of modified starches can be precisely tailored for specific applications [3–7]. The industry is still witnessing growth in the production of chemically modified starches and their application in food manufacturing [8–10]. This is despite consumer preferences for foods with clean labels, which in the case of starch restricts the use of E-labeled additives [11]. While clean-label starch products are expected to substitute 10% of food-grade chemically modified preparations by 2026 [12], the starch market of chemically modified products is also expected to grow from USD 10.4 billion in 2018 to USD 12.7 billion in 2023 globally [13]. Such a great popularity of modified starches would not be possible if their consumption was associated with any risks to the health of consumers. It is so as modified starches are among the safest food additives and can be used according to the *quantum*

*satis* rule. This means that their amount that can be used in food production is limited only by technological considerations. This has been confirmed by international bodies grouping authorities in food, nutrition and health. Moreover, these data are systematically updated and published on the websites of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA) [14,15].

Technological processes involved in food production include different forms of thermal treatment. As a result, starch in food products exists in gelatinized form, thus revealing its functional properties. Therefore, the evaluation of the suitability of modified starch is assessed mainly employing rheological methods [5,16–18]. However, the multitude of applications and the complexity of food matrices implies different measurement standards proposed for analyzing various modified starches. In starch studies, it should be taken into consideration that both starch pastes and most food products in which it is used as a thickener or stabilizer are pseudoplastic fluids often also thixotropic. For analyses of that type of materials, rheometers should be used, i.e., apparatuses of defined shear rate. Unfortunately in industrial laboratories, other cheaper devices are often used, which means that comparison of the results obtained by different analysts is often impossible [16–19]. Another type of rheological studies, analyses with the use of viscosgraphs, is of great importance in the assessment of the functional properties of starches. These devices perfectly illustrate the phenomena occurring during starch pasting, which is extremely important from the technological point of view [20]. Moreover, pasting characteristics recorded with viscosgraphs perfectly correlate with some structural features of starch [21].

The structure of starch can be studied using several methods, but part of them, such as X-ray diffractometry [22] or scanning electron microscopy [21], apply to starch in its non-gelatinized, granular form. Chemical methods that enable the determination of the degree of substitution or chromatographic analyses of the composition of hydrolysis products using starch debranching enzymes bring us closer to knowing the molecular structure of starch [23], but none of these methods directly correlates with the rheological properties of this biopolymer. The method for analyzing the molecular structure of starch that perfectly reflects its rheological properties is size exclusion chromatography, as the correlation between the viscosity of diluted solutions of polymers and their molecular mass, described by the Mark–Houwink equation, has been well known in polymer physics for years. However, it is not very popular as SEC apparatuses are expensive. Moreover achieving molecular dispersion of starch in solution is also a difficult task as it can involve degradation of macromolecules; this is particularly a problem in native and crosslinked starch preparations [24–27]. As a consequence, results may be understated if harsh solubilization conditions are used or understated due to cut-off at the filtration stage.

Although starches of all plant species undergo chemical modification, mostly waxy corn and potato starch are used as raw materials for the production of food-grade modified preparations [28,29]. Cassava starch is used to a much lesser extent. Moreover, starches approved by nutrition authorities for food applications have different popularity [10,14]. Taking into consideration the above, the aim of the work was to establish how chemical modification affects the molecular structure of potato starch and, as a consequence, their rheological properties as well as texture-forming ability. The most popular, commercial, food-grade chemically modified starches were studied in terms of molecular mass distribution, degree of branching and hydrodynamic parameters of macromolecules in solution. Moreover, comprehensive rheological and textural characteristics of their pastes were performed.

## 2. Materials and Methods

### 2.1. Starch

Commercial food-grade modified starches kindly provided by Potato Processing Company “Zetpezet” Ltd. (Piła, Poland) were the basic working material:

- Oxidized starch E 1404;
- Distarch phosphate E 1412;

- Acetylated distarch phosphate E 1414;
- Acetylated starch E 1420;
- Acetylated distarch adipate E 1422.

Native potato starch was used as a reference material. All samples were manufactured in year 2018 and were produced from starch obtained during one production campaign; applicable product data sheets are included in form of Supplementary Material (S1).

## 2.2. Determination of Pasting Characteristics

Pasting characteristics of 4% starch suspensions (except E 1404, where due to low viscosity 8% suspension was used) were recorded with a Brabender Viscograph (Duisburg, Germany). Measurement parameters used: measuring cartridge 0.07 Nm, a heating and cooling rate of 1.5 °C/min (within the 25–92.5–25 °C range), a holding period of 20 min at 92.5 °C. The following pasting parameters were analyzed: pasting temperature °C, peak viscosity BU (Brabender units), breakdown BU, setback BU and final viscosity BU. All analyses were performed in duplicate.

Such prepared starch pastes were divided into two even parts and sealed in a glass container. One part was additionally subjected to sterilization process at 121 °C for 15 min (SA232X, Sturdy Industrial, New Taipei, Taiwan). All further analyses performed on starch pastes were based on samples prepared in that manner, minimum after 24 h, but not later than 30 h from preparation.

## 2.3. Determination of Rheological Properties

Rheological properties were determined using a RotoVisco1 rheometer (Haake Technik GmbH, Vreden, Germany) equipped with DC30-K10 refrigerated bath with immersion circulator. Before the measurement, samples were thermostated at 20 °C and relaxed in measuring cylinder for 5 min. Data collection and calculations were made using RheoWin 3.61 software. Measurements were performed under the following conditions: Z20 DIN coaxial measurement system, 0.1–600–0.1 s<sup>-1</sup> shear rate range and time of 2 min. Obtained flow curves were described with the Ostwald de Waele equation:

$$\tau = K \cdot \dot{\gamma}^n \quad (1)$$

where  $\tau$  is shear stress Pa,  $K$  is the consistency index Pa·s<sup>n</sup>,  $\dot{\gamma}$  is the shear rate s<sup>-1</sup>, and  $n$  is the flow behavior index (a dimensionless number that indicates the closeness to Newtonian flow).

Moreover, thixotropy, understood as area of hysteresis loop formed between upward and downward flow curve, was calculated.

## 2.4. Determination of Universal Texture Profile

Universal texture profiles were determined with assistance of TA-XT2 texturometer (Stable Micro Systems, Godalming, UK). A standard “double-bite test” was performed with an aluminum cylindrical head (35 mm diameter) in a 68 mm diameter vessel at a depth of 20 mm with a test speed of 0.5 mm/s and pre-test speed of 1.0 mm/s. Hardness (N), adhesiveness (N·s), cohesiveness, resilience, springiness and gumminess (N) were determined.

## 2.5. Determination of Molecular Mass Distribution and Hydrodynamic Parameters

Molecular mass distribution and hydrodynamic parameters of starch were determined using high-performance size exclusion chromatography (SEC) with triple detection. OM-NISEC system (Malvern Instruments, Malvern, Great Britain) equipped with Viscotec TDA 305 multi-detector was used for that purpose.

Starch samples were dissolved in dimethyl sulfoxide (DMSO):water(H<sub>2</sub>O) binary mixture in ratio 9:1, at temperature of 80 °C for 24 h, accompanied by gentle stirring at 125 rpm using Reacti-Therm™ (Thermo Fisher Scientific, Waltham, MA, USA). The concentration of

sample was 1.5 mg/mL. Prior to that, the analysis sample was filtered through a 5 µm filter (Millipore, Burlington, MA, USA). The separation was on GRAM series column (10 µm, 3000 Å, 8 × 300 mm) with a guard column (10 µm, 8 × 50 mm) supplied by PSS Polymer Standards Service GmbH (Mainz, Germany), using DMSO:H<sub>2</sub>O in ratio 9:1 as an eluent at a flow rate of 0.3 mL/min. Columns and detectors were thermostated at 65 °C. The calibration was performed with pullulan standard (113,000 g/mol). The refractive index (RI) was 1.449 and 0.074 for the solvent and investigated sample, respectively. The calculations were performed using the OmiSEC 4.7. software (Malvern, TX, USA). Calculation principles of the average molecular masses ( $M_n$ ,  $M_w$ ,  $M_z$ ), intrinsic viscosity (IV), hydrodynamic radius ( $R_h$ ), radius of gyration ( $R_g$ ) and number of branches per molecule ( $B_n$ ) were described previously by Szwengiel and co-authors [26].

### 2.6. Statistical Analysis

All analyses were performed in triplicate (unless otherwise stated), and the results are presented as mean value ± standard deviation. Experimental data were studied using a one-way analysis of variance and Tukey's post hoc test. Moreover, two-way analysis of variance that took into consideration type of chemical modification (Factor 1) and thermal treatment–sterilization (Factor 2) was performed where applicable. Principal component analysis (PCA) was performed based on the correlation matrix. Cluster analysis (CA) was conducted based on single-linkage method, and Euclidean distance was used as a measure of similarity. The statistical analyses were performed using Statistica 13.3 (TIBCO Software Inc., Palo Alto, CA, USA).

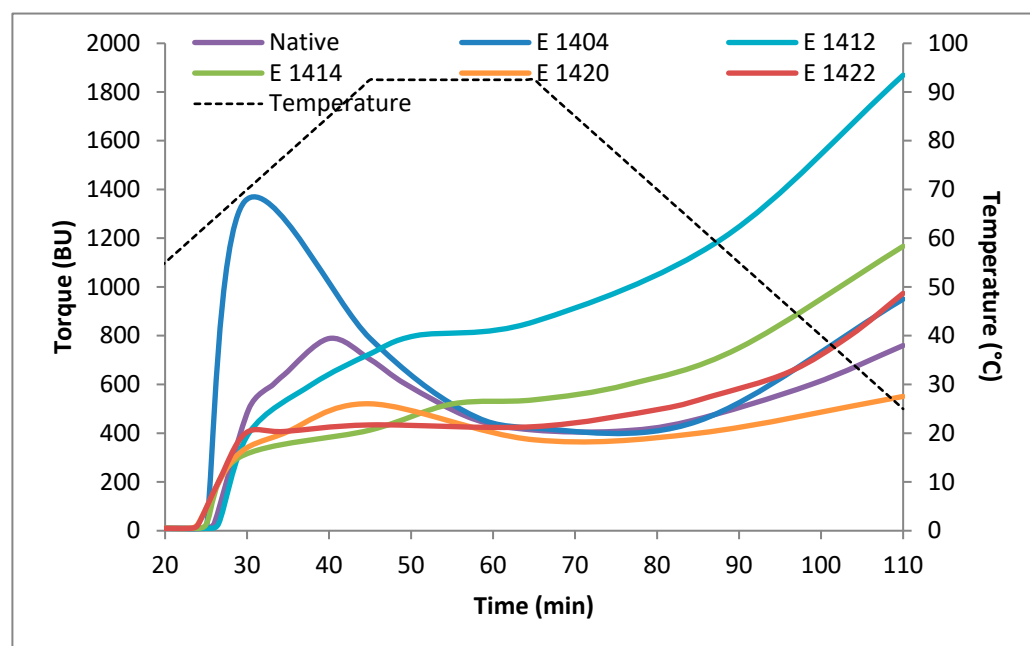
## 3. Results and Discussion

### 3.1. Pasting Characteristics

Pasting characteristics of starch can be divided into three major patterns according to their swelling characteristics, namely [30]:

- High type—characterized by a sharp viscosity peak and significant breakdown as well as a setback;
- Medium type—characterized by a moderate increase in viscosity in the heating phase, stabilization in the holding period and a further setback in the cooling phase;
- Restricted type—characterized by a moderate and constant increase in viscosity through all phases of the pasting process.

Native potato starch is believed to exhibit the high type of swelling characteristics [18], which is especially obvious at higher concentrations [30]. The investigated sample of native potato starch did not deviate from that pattern (Figure 1). A more pronounced pattern of the high type of swelling characteristics could be observed for oxidized starch (E 1404), which had to be analyzed at double the concentration as a result of low viscosity caused by depolymerization resulting from the modification process. All other types of chemical modification of starch led to a significant change in pasting behavior. Crosslinking with sodium trimetaphosphate (E 1412) resulted in a product with the most restricted type of swelling characteristics, whereas acetylation with acetic anhydrite (E 1420) influenced the swelling characteristics to a much lesser extent. Combination of both of these methods for starch modification (E 1414) provided a preparation with intermediate (medium) characteristics. The same conclusion can be drawn from the analysis of the course of gelatinization of other dually modified starch—acetylated and further crosslinked with adipic anhydrite (E 1422)—which exhibited the typical medium type of swelling characteristics. The above conclusions are supported by the analysis of pasting parameters presented in Table 1.



**Figure 1.** Pasting curves of native and modified potato starches recorded by Brabender viscosograph.

**Table 1.** Pasting parameters of modified potato starches recorded by Brabender viscosograph.

Starch	Gelatinization Temperature (°C)	Peak Viscosity (BU)	Breakdown (BU)	Setback (BU)	Final Viscosity (BU)
Native	62.6 ± 0.4 <sup>cd</sup>	789 ± 16	348 ± 5	348 ± 7	760 ± 11
E 1404	61.4 ± 0.1 <sup>bc</sup>	1351 ± 38	929 ± 17	455 ± 9 <sup>a</sup>	950 ± 9 <sup>a</sup>
E 1412	63.5 ± 0.3 <sup>d</sup>	-	-	1009 ± 19	1869 ± 18
E 1414	61.2 ± 0.4 <sup>b</sup>	-	-	631 ± 12	1167 ± 9
E 1420	59.6 ± 0.3 <sup>a</sup>	527 ± 6	154 ± 2	177 ± 6	551 ± 6
E 1422	59.8 ± 0.2 <sup>a</sup>	-	-	442 ± 11 <sup>a</sup>	974 ± 7 <sup>a</sup>

Explanatory notes: mean value ± standard deviation. Values marked with the same letter do not differ significantly  $p > 0.05$ .

The type of performed chemical modification significantly influences the pasting temperature of starch suspension, which was especially evident in the case of preparations subjected to the acetylation process. A minor decrease in pasting temperature was also observed for oxidized starch, but due to the fact that it had to be analyzed at a higher concentration, drawing any unambiguous conclusions is not possible. Nevertheless, the range of the whole data set was below 5 °C, which indicates that those changes were irrelevant when considering applications in food technology. Changes in the values of peak viscosity, breakdown and setback confirmed previous observations regarding swelling characteristics of the investigated starch products. Crosslinked preparations were characterized by the lack of a viscosity peak and breakdown followed by a significantly higher value of setback. The most important parameter relating to pasting analysis is final viscosity, which reflects the sample performance in industrial applications. Taking into consideration the concentration of suspension subjected to analysis, the viscosity of the starch preparations increased in the following order: E 1404 < E 1420 < Native < E 1422 < E 1414 < E 1412. This indicates that crosslinking enhances the thickening capability of starch preparation, while oxidization as well as acetylating significantly lowers it.

### 3.2. Rheological Properties

Starch pastes are considered as non-Newtonian, pseudoplastic and thixotropic fluids, and their flow can be quite accurately described by Ostwald de Waele Equation (1) [18,31].

Values of consistency and flow index are presented in Table 2. The employed model was very well fitted to experimental data with values of the coefficient of determination exceeding 0.97. The performed multivariate analysis of variance indicated that although investigated starch pastes differ in rheological properties as a result of preformed modification (Factor 1), further thermal processing (sterilization) does not have a significant effect on their flow behavior (Factor 2). Consistency index values followed a similar pattern as in the case of pasting analysis and their final viscosity. Crosslinking resulted in an increase in the consistency index, which was especially evident in the case of distarch phosphate. Acetylation caused insignificant changes when compared to native starch but substantial when considering acetylated distarch phosphate. Lastly, a relatively high value of the consistency index recorded for oxidized starch paste is a result of higher starch concentration and presumably gelling phenomena, which is quite commonly observed for preparations modified by means of oxidation. The hypothesis regarding the substantial effect of sol–gel transition is also supported by a very low value of the flow index and high area of the hysteresis loop of thixotropy recorded for this sample. Those values indicate that this paste formed a structure that can be easily destroyed when shear forces are applied. The conclusions regarding the thickening performance of starch preparations that can be drawn based on the analysis of the flow curves are similar as in the case of viscographic analysis, i.e., the viscosity of the starch preparations increased in the following order: E 1404 < E 1420 < Native < E 1422 < E 1414 < E 1412. However, it should be noted that as a result of all types of modification, the flow index of starch pastes decreased, thus indicating more non-Newtonian flow behavior. This phenomenon relates to the lower “stability” of viscosity when higher shear forces are applied; nevertheless, extremely high shear rate values are rarely observed during preparation or consumption of semi-solid food products.

**Table 2.** Parameters describing flow behavior of modified starch pastes.

Sample *		Consistency Index (Pa·s <sup>n</sup> )	Flow Index (-)	Thixotropy (Pa·s <sup>-1</sup> )
Native		5.4 ± 0.1 <sup>ab</sup>	0.646 ± 0.003	8 600 ± 499 <sup>bc</sup>
Native	S	1.9 ± 0.1 <sup>a</sup>	0.740 ± 0.011	276 ± 192 <sup>a</sup>
E 1404		24.6 ± 6.4 <sup>d</sup>	0.445 ± 0.031 <sup>b</sup>	17,080 ± 4639 <sup>d</sup>
E 1404	S	30.7 ± 1.5 <sup>de</sup>	0.392 ± 0.004 <sup>a</sup>	14,615 ± 1747 <sup>cd</sup>
E 1412		38.8 ± 1.0 <sup>f</sup>	0.405 ± 0.005 <sup>ab</sup>	39,695 ± 2737 <sup>e</sup>
E 1412	S	34.1 ± 1.7 <sup>ef</sup>	0.411 ± 0.010 <sup>ab</sup>	32,950 ± 2220 <sup>e</sup>
E 1414		11.9 ± 0.8 <sup>c</sup>	0.508 ± 0.013 <sup>cd</sup>	7617 ± 476 <sup>b</sup>
E 1414	S	8.7 ± 2.0 <sup>abc</sup>	0.542 ± 0.023 <sup>de</sup>	5988 ± 4609 <sup>ab</sup>
E 1420		5.9 ± 0.4 <sup>abc</sup>	0.544 ± 0.006 <sup>de</sup>	4863 ± 837 <sup>ab</sup>
E 1420	S	3.5 ± 0.8 <sup>a</sup>	0.570 ± 0.021 <sup>e</sup>	3828 ± 1107 <sup>ab</sup>
E 1422		10.5 ± 1.0 <sup>bc</sup>	0.500 ± 0.012 <sup>c</sup>	9165 ± 1450 <sup>bc</sup>
E 1422	S	6.5 ± 0.2 <sup>abc</sup>	0.562 ± 0.002 <sup>e</sup>	4755 ± 908 <sup>ab</sup>
Two-way ANOVA <i>p</i> -value				
Factor 1		0.0004	0.0037	0.0001
Factor 2		0.2846	0.2328	0.0188

Explanatory notes: mean value ± standard deviation. Values marked with the same letter do not differ significantly  $p > 0.05$ . \* Samples denoted as S were additionally subjected to sterilization process.

### 3.3. Texture Profile Analysis

Texture is a multi-parameter sensory attribute of a food product that derives from its macro-, micro- and molecular structure. Texture is a sensory property, and thus it can be only perceived and described by a human being. Nevertheless, several approaches toward instrumental determination of texture can be used. The most common is the profiling method of texture description (TPA) which is applicable both for sensory and instrumental measurements [32]. Table 3 compares four primary texture parameters as well as one secondary property (gumminess) of the original TPA method. Moreover,

resilience, being a closer approach to the description of elastic recovery of the sample, was calculated. Similarly to rheological measurements, the results of multivariate analysis of variance indicated that the sterilization process did not influence significantly the texture profile of the analyzed starch pastes. However, the lowest p-values were observed for both parameters relating to the elasticity of the sample, i.e., resilience and stringiness—indicating a possible outcome of sterilization in different conditions/food matrixes. The effect of chemical modification on the texture profile was mainly observed for: hardness, cohesiveness, resilience and gumminess (product of the two first values). Oxidation and crosslinking led to the formation of starch paste with a “stiffy” gel-like texture—relatively hard, less cohesive and elastic as well as gummier—when compared to native starch. On the other hand, all types of preparations that were subjected to the acetylation process formed pastes with a “softer” sol-like texture, indicated by low hardness accompanied by minor changes in cohesiveness that resulted in low gumminess. The only difference that can be seen between acetylated preparations is related to resilience. Pure acetylation gave starch paste with the highest values of resilience, which is related to the low viscosity of the sample—it easily restores its original height which is more liquid-like. At the same time, crosslinked and acetylated starch pastes were more solid-like resulting in lower values or resilience.

**Table 3.** Universal texture profile of potato starch pastes.

Sample *		Hardness (N)	Adhesiveness (N·s)	Cohesiveness (-)	Resilience (-)	Springiness (-)	Gumminess (-)
Native		1.26 ± 0.14 <sup>b</sup>	1.35 ± 0.29 <sup>a</sup>	0.54 ± 0.02 <sup>b</sup>	0.43 ± 0.02 <sup>d</sup>	0.90 ± 0.03 <sup>abc</sup>	0.68 ± 0.06
Native	S	0.64 ± 0.06 <sup>ab</sup>	0.19 ± 0.04 <sup>a</sup>	0.61 ± 0.03 <sup>c</sup>	0.56 ± 0.03	0.93 ± 0.02 <sup>bc</sup>	0.39 ± 0.03 <sup>a</sup>
E 1404		5.22 ± 0.65 <sup>c</sup>	7.97 ± 0.59	0.41 ± 0.02 <sup>a</sup>	0.11 ± 0.02	0.95 ± 0.03 <sup>c</sup>	2.14 ± 0.17
E 1404	S	4.90 ± 0.56 <sup>c</sup>	21.15 ± 2.08	0.37 ± 0.02 <sup>a</sup>	0.04 ± 0.01	0.91 ± 0.03 <sup>abc</sup>	1.83 ± 0.12 <sup>b</sup>
E 1412		3.40 ± 0.24	5.32 ± 0.37	0.49 ± 0.01 <sup>b</sup>	0.23 ± 0.01 <sup>a</sup>	0.97 ± 0.03 <sup>c</sup>	1.67 ± 0.09 <sup>b</sup>
E 1412	S	4.75 ± 0.28 <sup>c</sup>	0.68 ± 0.07 <sup>a</sup>	0.60 ± 0.02 <sup>c</sup>	0.39 ± 0.01 <sup>cd</sup>	0.93 ± 0.03 <sup>bc</sup>	2.84 ± 0.12
E 1414		0.52 ± 0.04 <sup>ab</sup>	1.31 ± 0.14 <sup>a</sup>	0.64 ± 0.02 <sup>dc</sup>	0.25 ± 0.01 <sup>a</sup>	0.88 ± 0.03 <sup>ab</sup>	0.33 ± 0.02 <sup>a</sup>
E 1414	S	0.51 ± 0.05 <sup>ab</sup>	1.28 ± 0.09 <sup>a</sup>	0.60 ± 0.02 <sup>c</sup>	0.31 ± 0.01 <sup>b</sup>	0.85 ± 0.02 <sup>ab</sup>	0.31 ± 0.02 <sup>a</sup>
E 1420		0.42 ± 0.03 <sup>a</sup>	0.11 ± 0.02 <sup>a</sup>	0.70 ± 0.01 <sup>e</sup>	0.62 ± 0.03 <sup>e</sup>	0.95 ± 0.02 <sup>c</sup>	0.29 ± 0.02 <sup>a</sup>
E 1420	S	0.40 ± 0.03 <sup>a</sup>	0.29 ± 0.06 <sup>a</sup>	0.70 ± 0.00 <sup>e</sup>	0.67 ± 0.02 <sup>e</sup>	0.91 ± 0.01 <sup>abc</sup>	0.28 ± 0.02 <sup>a</sup>
E 1422		0.48 ± 0.04 <sup>ab</sup>	0.60 ± 0.13 <sup>a</sup>	0.66 ± 0.02 <sup>de</sup>	0.36 ± 0.01 <sup>bc</sup>	0.91 ± 0.04 <sup>abc</sup>	0.32 ± 0.03 <sup>a</sup>
E 1422	S	0.50 ± 0.02 <sup>ab</sup>	1.45 ± 0.32 <sup>a</sup>	0.60 ± 0.01 <sup>c</sup>	0.34 ± 0.02 <sup>bc</sup>	0.83 ± 0.03 <sup>a</sup>	0.30 ± 0.01 <sup>a</sup>
Two-way ANOVA <i>p</i> -value							
Factor 1		0.0006	0.1095	0.0151	0.0026	0.0870	0.0096
Factor 2		0.8187	0.5987	0.8334	0.2166	0.0677	0.7192

Explanatory notes: mean value ± standard deviation. Values marked with the same letter do not differ significantly  $p > 0.05$ . \* Samples denoted as S were additionally subjected to sterilization process.

### 3.4. Molecular Structure

Technological properties of starch and its chemically modified products are largely dependent on the hydrodynamic parameters of its molecules in solution. These parameters can be assessed by means of size exclusion chromatography (SEC) with the triple detection technique, which is based on the refractive index, viscometer and light scattering detectors [33,34]. Molecular weights of starch preparations are presented in Figure 2, while hydrodynamic parameters of starch molecules in the solution are listed in Table 4. All methods for chemical modification resulted in significant changes in the molecular weight and values of hydrodynamic parameters of starch molecules. As one could expect, oxidation led to a decrease in the number ( $M_n$ ), weight ( $M_w$ ) and Z average ( $M_z$ ) molecular weights, while the decrease in  $M_w$  was the smallest. This consequently led to a significant increase in the polydispersity index ( $M_w/M_n$ ). Depolymerization of oxidized starch was also manifested by a significantly lower degree of branching, followed by a decrease in the radius of gyration ( $R_g$ ), which eventually led to the adoption of a very compact shape

by oxidized starch macromolecules in the solution (as indicated by  $R_g/R_h$ ). In the case of acetylated starch, only minor changes in SEC results could be observed, while changes in  $M_w$ ,  $R_g/R_h$  and branching were statistically insignificant. These observations are expected taking into consideration previous results of pasting, rheological and texture analysis, in which acetylating was the method of modification that influenced starch properties to a much lesser extent. Crosslinking with both types of modifying agents (sodium trimetaphosphate or adipic anhydrite) resulted in an increase in  $M_n$  and  $M_w$ , while  $M_z$  decreased. This phenomenon can be partially explained by the hypothesis that the crosslinking process of extremely large molecules ( $M_z$ ) made them insoluble or they were cut off during filtration of the sample. The decrease in the polydispersity index caused by definitely higher  $M_n$  values after the crosslinking process suggests that this process mainly involves molecules with lower molecular weight. Moreover, crosslinked starch preparations were more branched, and its molecules adopted looser conformation in the solution. This further proves the above conclusions, as values of  $R_g/R_h$  above 2.0 can be only observed for elongated and branched molecules [35]. The increase in the molecular weight of crosslinked preparations was also manifested in preceding studies where increased values of viscosity parameters were observed for this type of starch pastes.

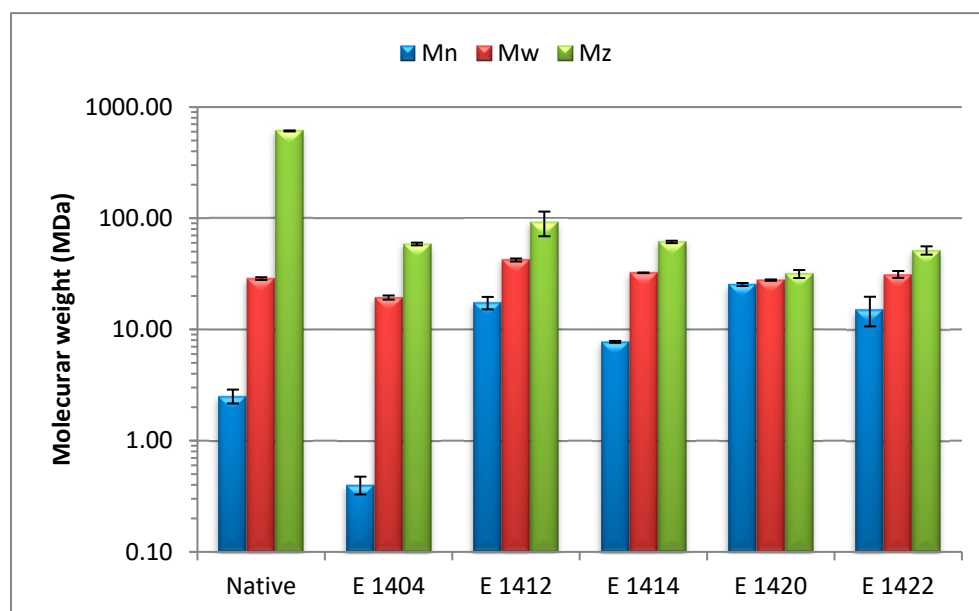


Figure 2. Molecular weight distribution of chemically modified potato starches.

Table 4. Hydrodynamic parameters of modified starch preparations.

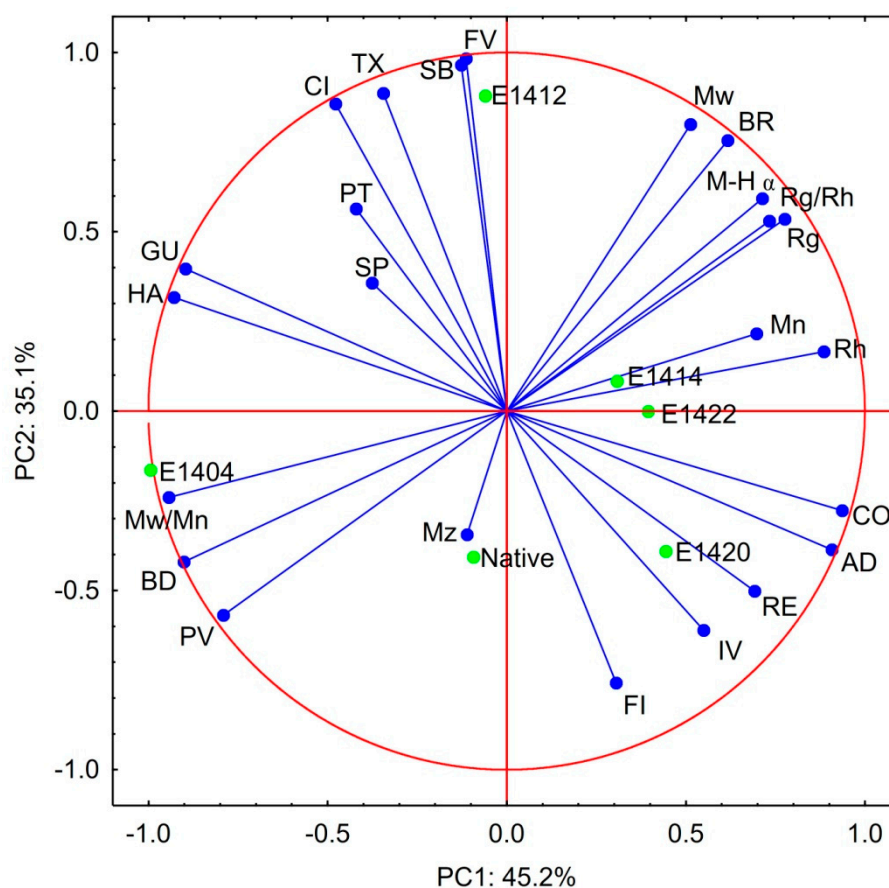
Starch	$M_w/M_n$ (-)	IV (dl/g)	$R_h$ (nm)	$R_g$ (nm)	$R_g/R_h$ (-)	M-H $\alpha$ (-)	Branches (-)
Native	$11.52 \pm 1.92^d$	$1.41 \pm 0.05^b$	$79 \pm 2^b$	$96 \pm 6^b$	$1.22 \pm 0.10^b$	$0.279 \pm 0.073^a$	$1010 \pm 56^b$
E 1404	$48.98 \pm 6.83^e$	$1.20 \pm 0.03^{ab}$	$66 \pm 3^a$	$39 \pm 3^a$	$0.60 \pm 0.02^a$	$0.262 \pm 0.014^a$	$131 \pm 12^a$
E 1412	$2.46 \pm 0.40^b$	$1.13 \pm 0.22^a$	$85 \pm 2^c$	$187 \pm 7^a$	$2.19 \pm 0.12^c$	$0.459 \pm 0.010^c$	$2481 \pm 347^d$
E 1414	$4.19 \pm 0.07^c$	$1.35 \pm 0.02^{ab}$	$80 \pm 3^b$	$197 \pm 9^{cd}$	$2.46 \pm 0.11^b$	$0.446 \pm 0.022^b$	$1488 \pm 68^c$
E 1420	$1.09 \pm 0.05^a$	$1.79 \pm 0.03^c$	$90 \pm 2^d$	$126 \pm 10^c$	$1.40 \pm 0.07^b$	$0.420 \pm 0.017^b$	$1250 \pm 92^{bc}$
E 1422	$2.14 \pm 0.49^b$	$1.34 \pm 0.06^{ab}$	$83 \pm 4^{cd}$	$212 \pm 15^d$	$2.56 \pm 0.15^c$	$0.389 \pm 0.025^b$	$1629 \pm 88^c$

Explanatory notes: mean value  $\pm$  standard deviation. Values marked with the same letter do not differ significantly  $p > 0.05$ .  $M_w/M_n$ —polydispersity index, IV—intrinsic viscosity,  $R_h$ —hydrodynamic radius,  $R_g$ —radius of gyration, M-H  $\alpha$ —Mark–Houwink equation  $\alpha$  parameter.



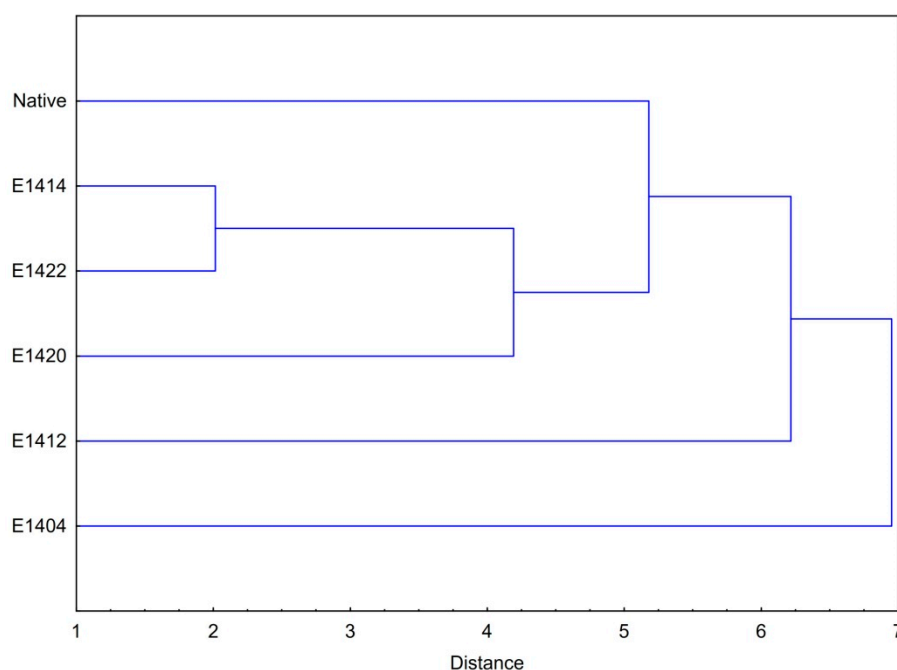
### 3.5. Principal Component and Hierarchical Cluster Analysis

In order to systematize the relation between different parameters and properties of the analyzed starch preparations, principal component analysis was performed. The presented PCA biplot (Figure 3) represents over 80% of total variation between samples. All investigated preparations were scattered throughout the whole area of the biplot; the only exception was noted for crosslinked and further acetylated preparations. Native starch stood out from other samples as a result of high  $M_z$  values while oxidized starch due to its high polydispersity. Lastly, distarch phosphate which was the sample with the highest viscosity was separated by values of Brabender's final viscosity and breakdown. Among noticeable correlations between the study parameters of starch preparations, those between the consistency index, thixotropy and flow index should be mentioned. Moreover, TPA parameters including hardness, adhesiveness, cohesiveness and gumminess should be noted as well. Similar correlations were also reported for native waxy as well as normal starches of potato and corn origin [18]. Surprisingly, no indisputable correlation was found between parameters originating from SEC with triple detection with rheological or texture measurements. This indicates that a single parameter of molecular structure cannot be used as an indicator of functional properties of starch products and has to be analyzed as a whole data set.



**Figure 3.** PCA of chemically modified potato starches. Explanatory notes: PT—pasting temperature, PV—peak viscosity, BD—breakdown, SB—setback, HA—hardness, AD—adhesiveness, CO—cohesiveness, RE—resilience, SP—springiness, GU—gumminess, CI—consistency index, FI—flow behavior index, TX—thixotropy,  $M_n$ —number average molecular weight,  $M_w$  weight average molecular weight,  $M_z$ —Z-number average molecular weight,  $M_w/M_n$ —polydispersity index, IV—intrinsic viscosity,  $R_h$ —hydrodynamic radius,  $R_g$ —radius of gyration, M-H  $\alpha$ —Mark-Houwink equation  $\alpha$  parameter, BR—number of branches per molecule.

Hierarchical cluster analysis (Figure 4) proved very similar properties of crosslinked and acetylated starch preparations indicated by PCA, as E 1414 and E 1422 formed one cluster. The next closest linkage was calculated to acetylated starch, which in various aspects of technological properties reassembled the dually modified starch products. Nevertheless, the process of acetylation influenced starch properties to a much smaller extent, which was also proven by formation of the next closest linkage to native starch. Both oxidized starch and distarch phosphate stood out for their unique properties but for various different reasons. While they produced starch pastes with stiff texture, they significantly differed in thickening performance due to changes in molecular structure. As a result of depolymerization, oxidized starch (which had to be analyzed at double the concentration) was less effective in thickening capabilities when compared to distarch phosphate. Meanwhile, the thickening performance of E 1412 was further increased due to an increase in molecular mass as a result of the crosslinking process.



**Figure 4.** HCA of chemically modified potato starches.

#### 4. Conclusions

Chemical modification leads to substantial changes in the molecular and functional properties of starch products. Oxidation process leads to depolymerization, which is accompanied by a significant increase in polydispersity and decrease in viscosity. Oxidized starch forms highly thixotropic pastes with stiff texture, which is related to its gelling capability. Acetylation when used as a standalone modification process causes only minor changes to the starch macromolecule resulting mainly in the stabilization of rheological properties during processing. Crosslinking of starch leads to an increase in molecular weight resulting in improvement of thickening capabilities of starch preparation, while the type of crosslinking agent used for modification has a negligible effect.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pr10050938/s1>, Document S1: Product data information.

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