

# Modification of Starches with Different Amylose/ Amylopectin Ratios Using the Dual Approach with Hydroxypropylation and Subsequent Acid-Thinning: II. Impacts on Gelatinization and Solution Properties

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Starches with different amylose (AM)/amylopectin (AP) ratios (waxy potato: WxPS; regular potato: PS; high AM corn: HACS) are hydroxypropylated (HP, two levels) and subsequently acid-thinned (AT) to produce dual-modified samples. The physicochemical characteristics and techno-functional properties of the solutions are investigated, and the data are evaluated statistically (analysis of variance). Differential scanning calorimetry gelatinization experiments reveal a reduction of the transition temperatures particularly due to HP. The subsequent AT decreases the specific enthalpy significantly. HACS samples have lower solubility (*S*) compared to their potato-based counterparts (WxPS and PS), but HP enhances the starch's *S*. The *S* of the AP of the HACS samples is comparatively high, and HP particularly facilitates the additional solubilization of AM. Dual-modified samples show characteristics of *S* similar to the corresponding HP samples. Or particular note, the HP-modified WxPS and PS, respectively, tend to cause molecular degradation of the polymers dissolved. The hot paste viscosity is impacted significantly by all varied factors.


Since the utilization of native starch is limited for most industrial applications, physically, enzymatically, converted, or chemically modified products are used. Moreover, combinations of different approaches, so-called dual-modified starch products, are common in practice. HP is one chemical modification method changing the properties comprehensively by reaction with propylene oxide.<sup>[4,5]</sup> A comprehensive review was recently given by Fu et al.<sup>[6]</sup> The granular state of the starch and the semicrystalline structure and its type are generally preserved, and the molecular size being basically not reduced during HP is commonly accepted.<sup>[6]</sup> However, the reliable determination of the molecular composition of HP starches is very challenging and sophisticated, and the impact of the HP on the molecular starch structure strongly depending on the starch type was evidently

## 1. Introduction

Starch is used in several food-applications. The AM/AP ratio of common starches is usually/roughly about 25/75, whereupon pea starches are an exception having higher relative AM portions between about 33% and 70% (w/w) depending on the specific type.<sup>[1,2]</sup> Generally, commercial starches with differing AM/AP ratios are available, so-called genotypes. Waxy varieties consist actually only of AP, whereas high AM types have relative AM contents of 50% (w/w) or higher (e.g., amylo maize V and VII, respectively).<sup>[3]</sup>

shown recently.<sup>[7]</sup> The preparation of a molecularly dispersed starch sample is the crucial factor and an essential requirement for examinations based on SEC techniques with high validity. Influences by the HP process conditions (e.g., paste and slurry, drying method) on the molecular properties were reported by Vorwerk et al.<sup>[5]</sup> Thermal properties of the HP starch like gelatinization (DSC), granular swelling and *S* are impacted by the modification. The transition temperatures (onset temperature ( $T_o$ ), peak temperature ( $T_p$ ), conclusion temperature ( $T_c$ ), the specific enthalpy ( $\Delta H_{gel}$ ) as well as the glass transition temperature ( $T_g$ ) are normally decreased, most probably due to internal plasticization caused by the introduction of the substituents. Since the HP group is actually less hydrophilic than the (original) OH group, the explanation for increased granule swelling and accompanied *S* by leaching should not be merely limited to the simple introduction of a hydrophilic group to the starch structure.<sup>[6]</sup> The increase of both attributes is presumably related to a weakening of the granule structure 1) and to internal plasticization 2) by the HP groups acting as a spacer. However, there is strictly speaking, a lack of information regarding the specific properties (molecular structure of the specific starch polymer fraction) of the respective phases, i.e., swollen particles (including granule remnants) and dissolved molecules, respectively. Moreover, most investigations vary in terms of the analysis conditions including, e.g., starch concentration 1), temperature range examined 2), swelling and leaching time 3), and other factors such as stirring, separation

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DOI: 10.1002/star.202000145

**Table 1.** Denotations and molecular data of the starch samples.

		$M_w$ starch <sup>a)</sup> [10 <sup>6</sup> g mol <sup>-1</sup> ]	Recovery rate [%]	$M_w$ AM fraction <sup>b)</sup> [10 <sup>5</sup> g mol <sup>-1</sup> ]	MS <sup>c)</sup>
Native	WxPS	40.50	64.8	2.44	
	PS	33.20	60.7	5.73	
	HACS	3.67	74.4	1.95	
Single modification (HP <sup>d)</sup> and AT <sup>e)</sup> )	WxPS-HP-1	39.50	73.4		0.08
	WxPS-HP-2	40.70	66.3		0.19
	PS-HP-1	21.20	71.9		0.10
	PS-HP-2	14.70	66.1		0.17
	HACS-HP-1	5.21	79.8		0.04
	HACS-HP-2	4.82	77.1		0.13
	WxPS-AT	8.02	78.8	3.09	
	PS-AT	6.50	82.3	2.34	
	HACS-AT	0.87	78.9	1.08	
	Dual modification (HP-AT) <sup>f)</sup>	WxPS-HP-1-AT	8.72	75.8	
WxPS-HP-2-AT		7.02	73.8		0.16
PS-HP-1-AT		7.93	79.9		0.08
PS-HP-2-AT		4.05	73.1		0.13
HACS-HP-1-AT		1.53	82.2		0.04
HACS-HP-2-AT		1.41	78.4		0.09

<sup>a)</sup> Determined by means of SEC-MALS-DRI; <sup>b)</sup> Determined by means of SEC-MALS-DRI after mathematical peak separation<sup>7)</sup>; <sup>c)</sup> Molar substitution; <sup>d)</sup> Hydroxypropylated starch samples and single modification; <sup>e)</sup> Acid-thinned starch samples and single modification; <sup>f)</sup> Acid-thinned hydroxypropylated starch samples and dual modification.

of the phases and the methodical approach of the determination altogether. The importance of the “classic” properties swelling and *S* is actually questionable, since the paste preparation under industrial circumstances is different from that (e.g., pressure cooking). In any case, the flow behavior of the starch dispersion or the hot paste viscosity (HPV) of the starch solution, respectively, is one of the most important techno-functional characteristics, which controls the processability and thus impacts the industrial application. Most investigations on pasting and paste viscosity are based on RVA measurements. Since the actual experimental conditions are often similar or in a manner of speaking standardized, the data obtained have good reproducibility. However, since the heating is limited to 95 °C in most cases, no good solution state of the starch polymers is obtained by the method. The literature data regarding the “final viscosity” after heating the suspension to 95 °C can be termed to be inconsistent, since the findings differ widely depending on the starch source and the impact of the respective degree of modification, i.e., molar substitution (MS). Decreased values (Chuenkamol et al.,<sup>8)</sup> 6% w/w, canna starch, MS 0.01–0.11; Pal et al.,<sup>9)</sup> 5% w/v, corn starch with MS 0.05–0.10, amaranth starch with MS 0.05–0.10; Shi and BeMiller,<sup>10)</sup> 8.9 w/w, common corn starch, MS 0.096–0.186; Karim et al.,<sup>11)</sup> mung bean starch), almost unchanged values (Han,<sup>12)</sup> 7% w/w, waxy corn and PS), slightly enhanced val-

**Table 2.** ANOVA for the impacts AM (starch type), HP, AT, and C on the DSC gelatinization characteristics ( $T_o$ ,  $T_c$ ,  $\Delta T_{gel}$ , and  $\Delta H_{gel}$ ), *S*, and HPV (at 40 s<sup>-1</sup>); boldface type: statistically significant effect with a 95.0% level of significance.

Impact		<i>p</i> -Value					
		gelatinization <sup>a)</sup>				<i>S</i> <sup>b)</sup>	HPV <sup>c)</sup>
		$T_o$	$T_c$	$\Delta T_{gel}$	$\Delta H_{gel}$		
Single	AM <sup>d)</sup>	0.0748	<b>0.0000</b>	<b>0.0000</b>	<b>0.0355</b>	<b>0.0014</b>	<b>0.0289</b>
	HP <sup>e)</sup>	<b>0.0160</b>	<b>0.0128</b>	0.0633	0.0550	<b>0.0000</b>	<b>0.0002</b>
	AT <sup>f)</sup>	0.7325	0.3846	0.1326	<b>0.0202</b>	0.9122	<b>0.0297</b>
	C <sup>g)</sup>					0.2012	<b>0.0001</b>
Interaction	AM-HP	0.7047	0.3366	0.0643	0.3419	<b>0.0021</b>	0.1483
	AM-AT	0.7115	0.1638	<b>0.0259</b>	0.2131	0.1439	0.8259
	AM-C					0.0586	<b>0.0263</b>
	HP-AT	0.3291	0.7543	0.1687	0.2379	0.1068	<b>0.0470</b>
	HP-C					0.0817	<b>0.0013</b>
	AT-C					0.4570	0.1578

<sup>a)</sup> Without investigation of impact starch concentration (C), *n* = 18; <sup>b)</sup> Solubility, *n* = 36; <sup>c)</sup> Hot paste shear viscosity at 40 s<sup>-1</sup>, *n* = 36; <sup>d)</sup> Starch types with different AM contents (WxPS, PS, and HACS); <sup>e)</sup> Hydroxypropylation to different degrees (HP-1 and HP-2); <sup>f)</sup> Acid-thinning; <sup>g)</sup> Starch concentration of pastes (6% and 9% w/w).

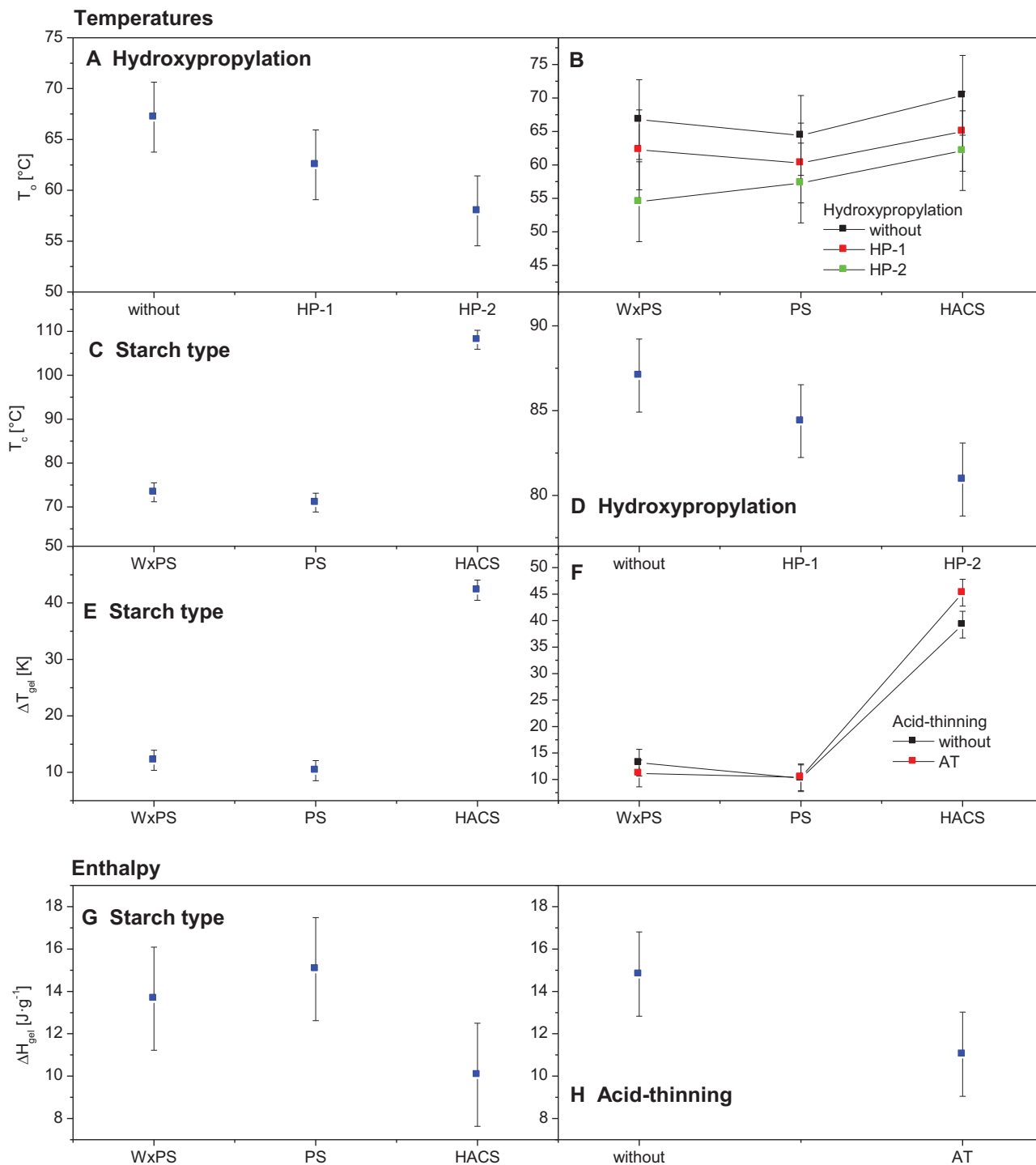
ues (Kim,<sup>13)</sup> 3% w/w, potato starch, MS 0.045–0.125; Hung and Morita,<sup>14)</sup> 8% w/w, A- and B-type granules of wheat starch) and enhanced values (Han,<sup>12)</sup> 7% w/w, normal corn starch; Karim et al.,<sup>11)</sup> corn starch) were reported with increasing MS. Lawal<sup>15)</sup> found increasing values with increasing MS, but lower compared to the native counterpart (5.66% w/v, pigeon pea starch, MS 0.06–1.17). Based on rotational experiments, Oladebeye et al.<sup>16)</sup> clearly showed increasing HPV with an increasing level of HP (lima bean and jack bean starch, MS 0.26–0.37 and 0.24–0.30, respectively).

To combine the properties of both HP and partially molecular degraded starches, starches of different origins and AM contents (0–74% w/w; WxPS, PS and HACS) were initially HP to different degrees (–HP-1 and –HP-2) and subsequently acid-modified (–AT).<sup>7)</sup> Effects on the microscopic and overall molecular properties of the systematically produced samples were investigated in a previous study and published recently.<sup>7)</sup> The present study summarizes the characteristics in terms of physicochemical (differential scanning calorimetry (DSC) gelatinization) as well as state (*S* and molecular data of the phases) and flow behavior (HPV) of the starch dispersions prepared by means of pressure cooking, which is meaningful within the scope of the technical application in the food industry. Statistical evaluation of the data facilitates the identification of significant impacts by the starch type itself (varying molecular composition of the initial material), the modification and the polymer concentration of the paste investigated as well.

## 2. Material and Methods

### 2.1. Starch and Preparation of Modified Starch Samples

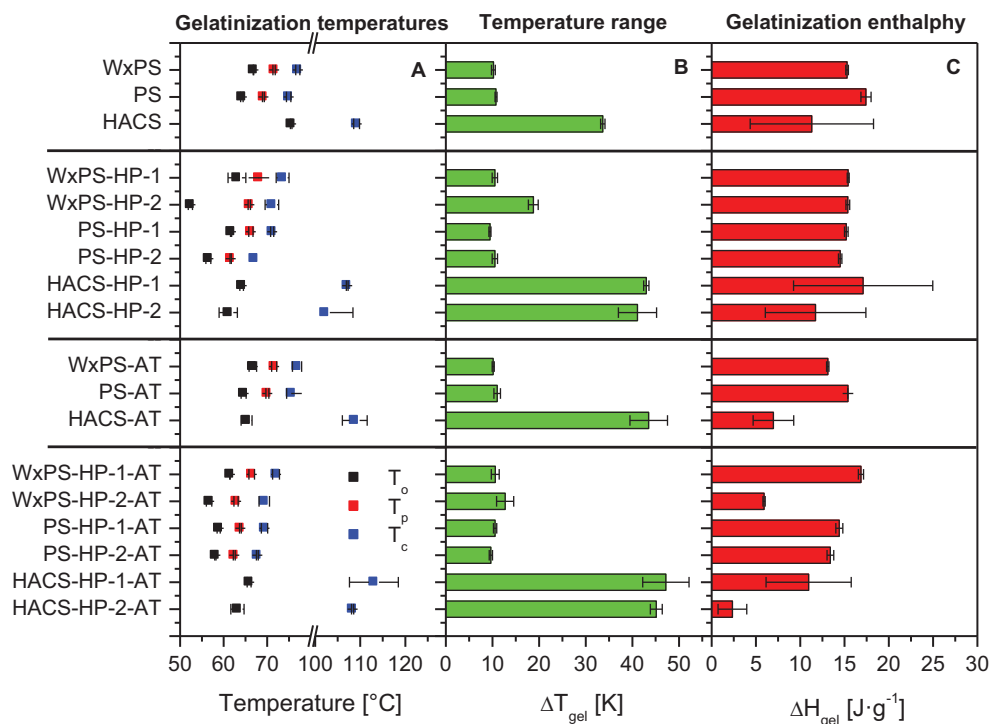
A commercial native WxPS (Empure AKS 100), a commercial native regular PS (Superior) and a commercial native HACS



**Figure 1.** Mean A,C–E,G,H) and selected interaction diagrams B,F) with a 95% confidence interval of the impacts starch type (WxPS, PS, and HACS), degree of HP (without, HP-1, and HP-2) and AT (without and with acid treatment) on the DSC gelatinization properties ( $T_o$ ,  $T_c$ ,  $\Delta T_{gel}$ , and  $\Delta H_{gel}$ ).

(Hylon VII), respectively, were used as initial starch material for the experiments (WxPS and PS: Emsland-Stärke GmbH, Emlchheim, Germany; HACS: Ingredion Germany GmbH, Hamburg, Germany). The modification was executed in two steps. Initially, the starches underwent an HP to two different levels of MS (–HP-1 and –HP-2 referring to low and high MS,

respectively) by grading the amount of propylene oxide added, and the AT samples were prepared by a partial acid hydrolysis, respectively (single modification). The HP samples were subsequently AT (dual modification). The details of the initial starch samples, the modification procedures as well as the comprehensive molecular characteristics are detailed in a previous study.<sup>[7]</sup>



**Figure 2.** DSC gelatinization properties ( $T_o$ ,  $T_p$ ,  $T_c$ ,  $\Delta T_{gel}$ , and  $\Delta H_{gel}$ ) of the starch samples ( $T_p$  was not determined from the thermograms of the HACS samples).

**Table 1** summarizes the data. Deionized water was used for all experiments.

## 2.2. DSC Gelatinization Experiments

The thermal gelatinization properties of the native and modified starch samples were examined using DSC (DSC 204 F1 Phoenix equipped with an intercooler, Netzsch, Selb, Germany) according to the description elsewhere with minor modification.<sup>[17]</sup> The starch powder was weighted in an aluminum pan, water was added (about 5 mg starch and 20  $\mu$ L water, ratio about 1:5) and the crucible finally hermetically sealed before measurement. The scanning conditions were as follows: the initial temperature was kept at 20 °C for 2 min (isothermal segment), ramped to 120 °C at a heating rate of 10 K  $\text{min}^{-1}$  (nonisothermal segment, heating), maintained at 120 °C for 2 min (isothermal segment), and then cooled to 20 °C with a rate of 40 K  $\text{min}^{-1}$  (nonisothermal segment, cooling). The obtained thermograms were evaluated (Netzsch Proteus thermal analysis-Version 7.0.1 software) in terms of gelatinization temperatures ( $T_o$ ,  $T_p$ , and  $T_c$ ), gelatinization range ( $\Delta T_{gel}$ ;  $T_c - T_o$ ), and  $\Delta H_{gel}$ . The experiments were performed in repeated determination, and the arithmetic mean as well as the corresponding standard deviation were calculated.

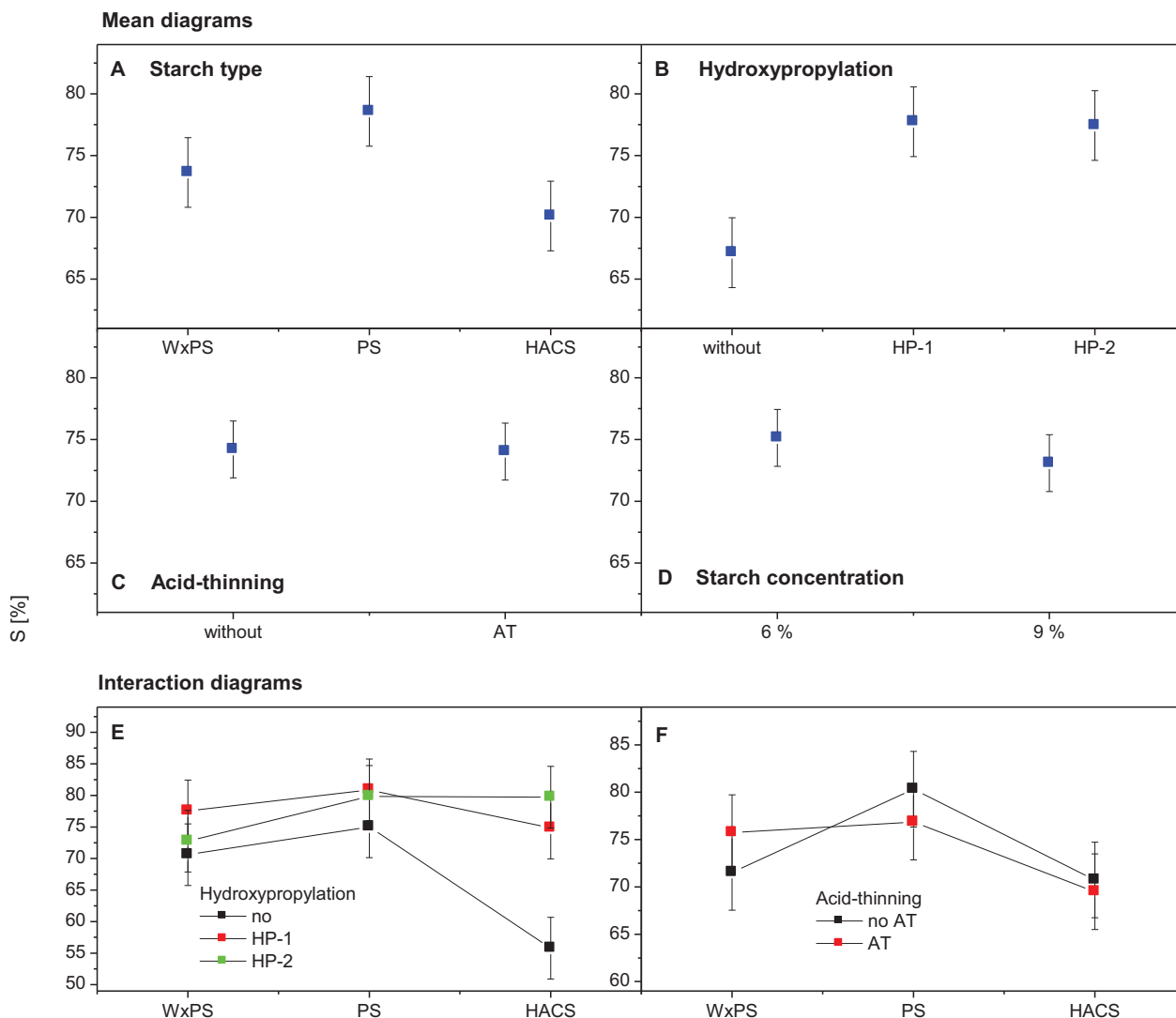
## 2.3. Paste Preparation

Concentrated pastes of the starch samples with different polymer concentrations (C) were prepared based on aqueous dispersions

of 6% and 9% (w/w), respectively, by means of pressure cooking at 145 °C using an autoclave (Model I, Carl Roth GmbH & Co. KG, Karlsruhe, Germany) under continuous stirring (300  $\text{min}^{-1}$ ) for 20 min. The dispersion obtained subsequently underwent a high-shear treatment using an Ultra-Turrax T25 (IKA-Werke GmbH & Co. KG, Staufen, Germany) at 24 000  $\text{min}^{-1}$  for 2 min at about  $85 \pm 5$  °C. The freshly prepared aqueous starch solutions were immediately analyzed (S and HPV).

## 2.4. Solubility and Molecular Characterization of the Dispersion Phases

The comprehensive determination of S and the molecular analysis of the dispersion phases was the same as described elsewhere with modifications.<sup>[18]</sup> An aliquot of the respective starch dispersion was diluted with water to a concentration of 3.0% (w/w). A mass of 12.0 g was subsequently centrifuged at 10 000  $\text{min}^{-1}$  (11 180 g) for 15 min (Biofuge 28RS, Heraeus, Hanau, Germany). After the centrifugation step, the amount of supernatant (SUP) was determined by decanting and weighing, and an aliquot of 0.5 mL was diluted 1:10 (v/v) in DMSO. Additionally, a part of the sediment (SED) was dispersed in DMSO and finally dissolved by stirring (400  $\text{min}^{-1}$ ) at 90 °C (oil bath) for 24 h. Both solutions (SUP and SED) were characterized by means of SEC-MALS-DRI. The chromatograms were obtained and the weight average molar mass ( $M_w$ ) calculated, respectively. Additionally, the polysaccharide concentration of the SUP was determined from the SEC-chromatograms and, based on the amount of the SUP phase, the total starch content solubilized within the SUP phase was



**Figure 3.** Mean A–D) and selected interaction diagrams E,F) with a 95% confidence interval of the impacts of starch type (WxPS, PS, and HACS), degree of HP (without, HP-1, and HP-2), AT (without and with acid treatment), and C (6% and 9%) on *S*.

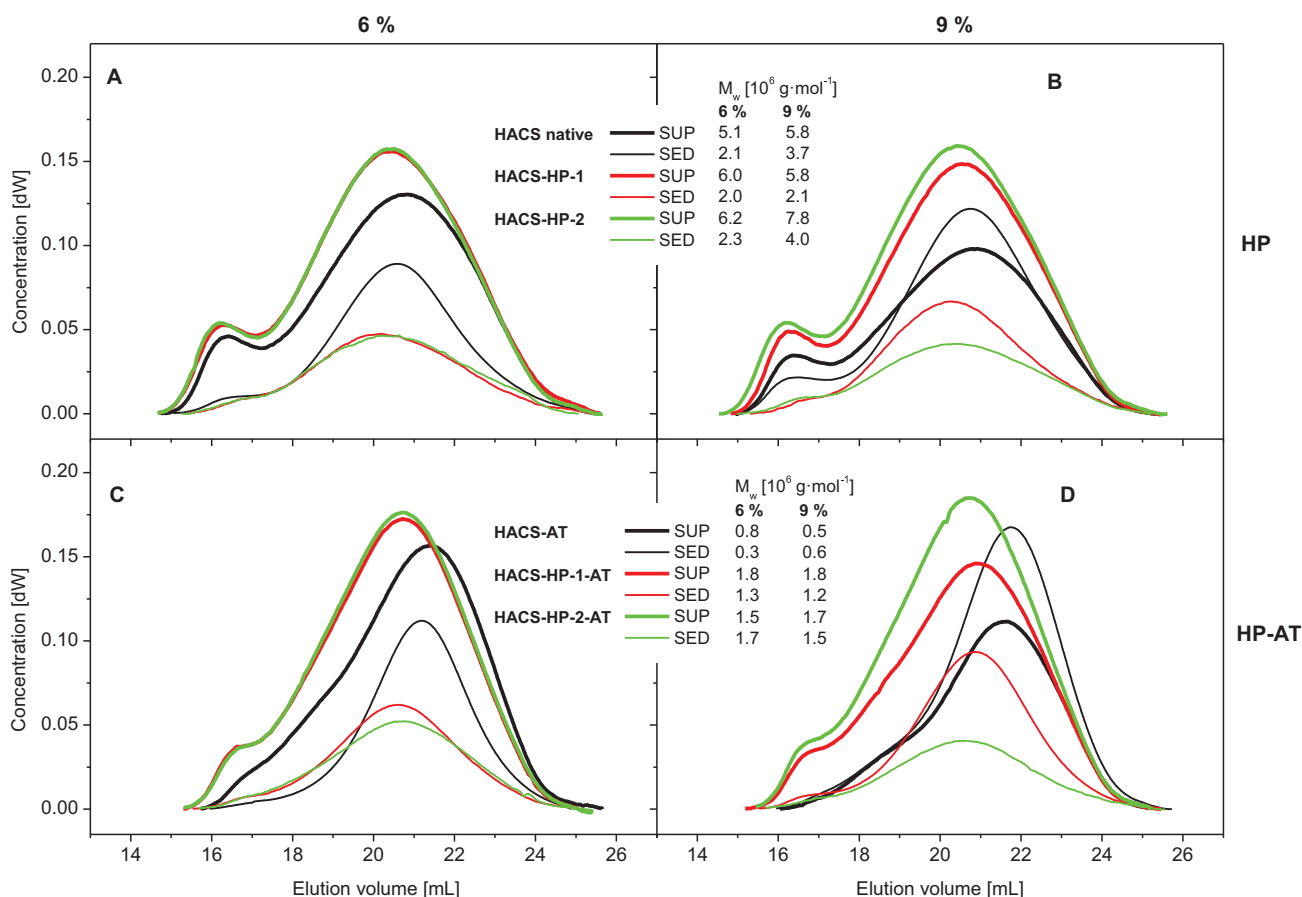
calculated. The portion of the latter fraction related to the starch content of the initial dispersion (12.0 g of a 3.0% w/w solution) was multiplied by 100 and expressed as % *S*. Based on the *S*, the SEC-chromatograms corresponding to the dissolved (SUP) and swollen (SED) dispersion phase, respectively, were weighted according to the relative portion of the starch polymers.

The molecular characterization of the polydisperse solutions (SUP and SED) was carried out by means of SEC-MALS-DRI. The separation was executed with an SEC-3010 module (WGE Dr. Bures GmbH & Co. KG, Dallgow-Doeritz, Germany) including degasser, pump and auto sampler connected to a MALS detector and a differential refractive index detector (DRI). The MALS detector was a Bi-MwA (Brookhaven Instruments Corporation, Holtsville, NY, USA) fitted with a diode laser operating at  $\lambda_0 = 635$  nm and equipped with seven detectors at angles ranging from 35° to 145°. The DRI was a SEC-3010 RI detector operating at  $\lambda = 620$  nm. Three columns in a row were used: AppliChrom ABOA DMSO-Phil-P-100 (100–2500 Da), P-350 (5–1500 kDa)

and P-600 (20 to >20 000 kDa) (Applichrom, Oranienburg, Germany). The samples were eluted with degassed DMSO (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) containing 0.1 M NaNO<sub>3</sub> at a flow rate of 0.5 mL min<sup>-1</sup> and a temperature of 70 °C. During the sample run on the SEC-MALS-DRI system (single determination), the data from the MALS and DRI detectors were collected and processed using ParSEC Enhanced V5.6 1 chromatography software to give the concentration of the eluted solution and MM at each retention volume (*M<sub>i</sub>*). The basis for the molecular characterization by means of SEC-MALS-DRI has been described elsewhere.<sup>[19,20]</sup>

## 2.5. Hot Paste Shear Viscosity

The rheological measurements were carried out using a rotational rheometer (MCR 302, Anton Paar GmbH, Graz, Austria) equipped with a cone-plate geometry (CP50-1/TG; 50 mm and



**Figure 4.** Weighted SEC chromatograms of the dissolved (SUP) and corresponding undissolved (SED) polymer fraction of the single (HP) A,B) and dual-modified HACS (HP-AT) C,D) samples after disintegration at different C (6% and 9%, respectively).

1°, True Gap). The starch solutions (6 and 9% w/w, respectively; preparation section 2.3) were filled in the measuring system, and the HPV curves were determined in the shear rate range 0.1–125 (up) and 125–0.1 s<sup>-1</sup> (down) at 60 °C.

## 2.6. Statistical Evaluation

The impact of the different modification parameters (starch type with different AM content, degree of HP, AT treatment and C) on the DSC gelatinization properties (except impact of C;  $T_o$ ,  $T_c$ ,  $\Delta T_{gel}$ , and  $\Delta H_{gel}$ ;  $n = 18$ ),  $S$  ( $n = 36$ ) and HPV ( $n = 36$ ) was investigated based on respective experimental designs using Statgraphics Plus 5.0 software. The values for the probability of error ( $p$ -value) were listed in the ANOVA table (analysis of variance; **Table 2**). With a  $p$ -value less than 0.05, the factor investigated had a statistically significant effect with a 95.0% level of significance (boldface type in the ANOVA table). Additionally, selected results from the statistical evaluation were summarized in mean and interaction diagrams showing the calculated means of each category and the confidence interval with a 95.0% confidence level. With no overlapping of the confidence intervals in the diagrams, there was a statistically significant difference with a 95.0% level of confidence.

## 3. Results

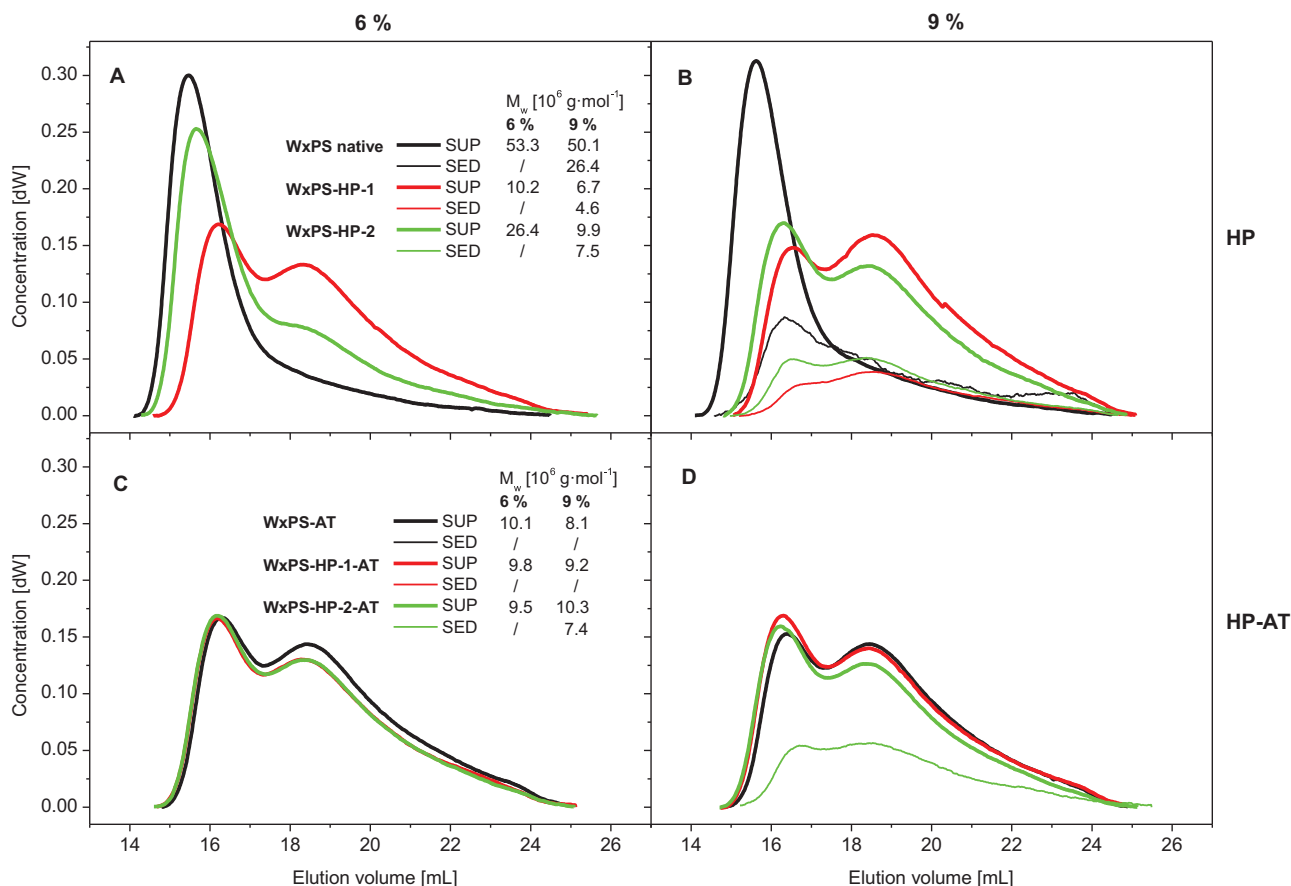
### 3.1. Statistical Analysis

Table 2 shows the ANOVA resulting from the statistical analysis of selected experimental data. The impacts of the varied parameters AM (starch type), HP, AT, and C on the gelatinization characteristics ( $T_o$ ,  $T_c$ ,  $\Delta T_{gel}$ , and  $\Delta H_{gel}$ ),  $S$  and HPV were investigated. The investigation of the DSC gelatinization of the starch samples was performed without variation of C analogous to  $S$  and HPV. Hence, no values are determined for the impact of C (Table 2; gelatinization). Moreover,  $T_p$  is left from the ANOVA since the DSC thermograms of all HACS samples did not provide the possibility to determine  $T_p$  due to the exceptional shape. The impacts on the specific properties and their significance are discussed in the respective sections below.

### 3.2. Gelatinization Properties

The gelatinization properties of the starch samples were determined based on DSC experiments. Since the thermograms of the HACS samples did not allow the reliable calculation of  $T_p$ , this specific value was excluded from the statistical evaluation. It is clear from the ANOVA table (Table 2) that  $T_o$  is significantly



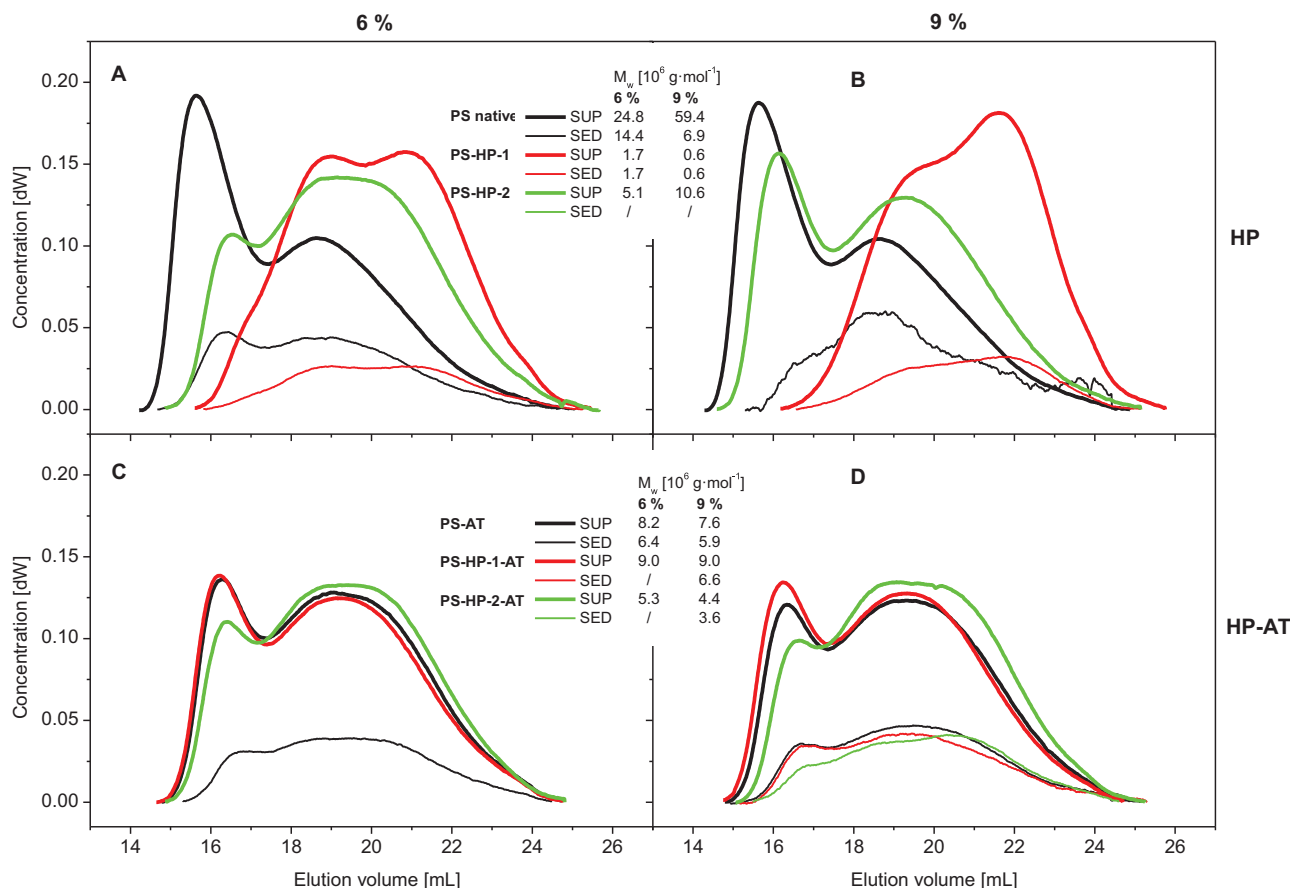


**Figure 5.** Weighted SEC chromatograms of the dissolved (SUP) and corresponding undissolved (SED) polymer fraction of the single (HP) A,B) and dual-modified WxPS (HP-AT) C,D) samples after disintegration at different C (6% and 9%, respectively).

impacted by the HP. An increasing level of HP decreased  $T_o$  (Figure 1 A), independent of the starch type (Figure 1B). This is also obvious from the data summarized in Figure 2A and confirmed by the results of others. The  $T_c$  was impacted in a statistically significant manner by both the starch type (AM) and the level of HP (Table 2).  $T_c$  of the HACS samples was on a considerable higher level (about 105 °C; Figures 1C and 2A) compared to the WxPS and the PS (about 70–75 °C; Figures 1C and 2A), which is basically in accordance with literature data. Jane et al.<sup>[3]</sup> reported the  $T_c$  for a HACS even at about 130 °C, resulting in a  $\Delta T_{gel}$  of about 60 K (Amylomaize VII), which is also similar with the findings of Liu et al.<sup>[21]</sup> However, they reported a successive narrowing of the gelatinization peak with an increasing MS. In the present study, the HP decreased  $T_c$  successively with an increasing level of modification (Figure 1D). The  $\Delta T_{gel}$  was also found to be significantly impacted by the starch type (AM; Table 2), in particular due to the enormous higher  $T_c$  and accordingly higher  $\Delta T_{gel}$  of the HACS compared to the potato based starches (WxPS and PS; Figure 1E). This is underlined by the data summarized in Figure 2B showing the temperature range of the HACS samples being between about 35 and 45 K, which is different from the other starches ( $\Delta T_{gel}$  about 10 K). The combined impact AM-AT was also found to be a statistically significant effect at the 95.0% confidence level, since the  $p$ -value was below 0.05 (Table 2). From Figure 1F it becomes obvious, that this resulted

mostly from the HACS samples. The AT enhanced  $\Delta T_{gel}$  in that group (Figure 1F). This is also evident from the data in Figure 2B showing that  $\Delta T_{gel}$  increased particularly when the native HACS was modified. Chuenkamol et al.<sup>[8]</sup> reported a systematic shift of  $T_o$ ,  $T_p$ , and  $T_c$  to a lower temperature of about 10 K due to the successively enhanced MS of canna starch (MS 0.01–0.11). Moreover,  $\Delta T_{gel}$  increased slightly. Similar results were presented by Yeh and Yeh,<sup>[22]</sup> Van Hung and Morita<sup>[14]</sup> and Hoover et al.<sup>[23]</sup> Kim et al.<sup>[13]</sup> found systematically reduced pasting temperatures with increasing MS of HP samples based on RVA measurements (PS, MS 0.045–0.17). In contrast to the present study,  $\Delta T_{gel}$  was reported to increase remarkably with increasing MS (PS, AM content about 25% w/w, MS 0.11–0.25).<sup>[24]</sup> Changed gelatinization temperatures owing to HP can be ascribed to partial disruption of the H-bonds between the starch polymers within the amorphous regions due to introduction of the HP groups (spacer).<sup>[25]</sup> The weakened granular integrity probably increases the mobility of the polymer chains and the accessibility for water and thus, decreases the transition temperatures ( $T_o$ ,  $T_p$ , and  $T_c$ ) of starch crystallites.<sup>[8]</sup>

The  $\Delta H_{gel}$  was significantly impacted by both the starch type (AM) as well as the AT (Table 2). The  $\Delta H_{gel}$  of the HACS samples was found to be lower compared to the other starch samples<sup>[26,27]</sup> (Figure 1G), and the AT treatment reduced the  $\Delta H_{gel}$  likewise. Conspicuously, the evaluation of the thermograms of the HACS



**Figure 6.** Weighted SEC chromatograms of the dissolved (SUP) and corresponding undissolved (SED) polymer fraction of the single (HP) A,B) and dual-modified PS (HP-AT) C,D) samples after disintegration at different C (6% and 9%, respectively).

samples regarding the area was all in all difficult, consequently leading to comparatively high standard deviations of the calculated arithmetic means (Figure 2C). The reasons for this are primarily the very high  $\Delta T_{\text{gel}}$  and the irregular shape of the thermograms impeding the analysis. A (systematic) reduction of  $\Delta H_{\text{gel}}$  caused by the HP, which was found in the case of PS, was actually expected for all starch types investigated (Figure 2C). Kaur et al.<sup>[28]</sup> reported a systematic decrease of  $\Delta H_{\text{gel}}$  with an increasing MS of the starch, which is attributed to loss of crystallinity and primarily loss of molecular (double-helical) order.<sup>[29]</sup> Similarly, Perera et al.<sup>[24]</sup> revealed a systematic decrease of  $\Delta H_{\text{gel}}$  with an increasing level of HP (MS 0.11–0.25).

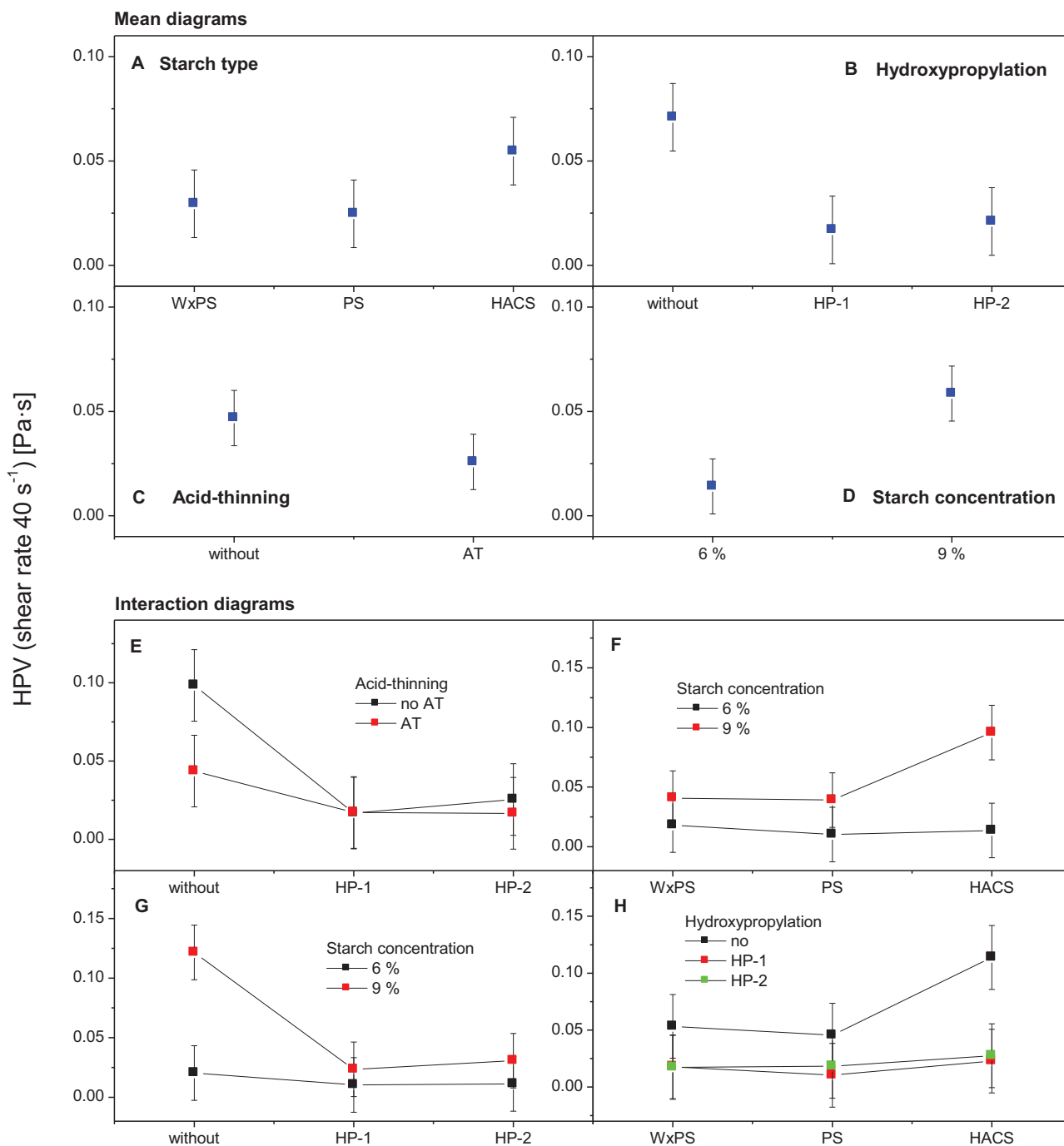
### 3.3. Solubility

The starch's *S* was impacted statistically significant by the single factors starch type (AM) and HP as well as the combination of both (Table 2). The statistical evaluation of the molecular composition ( $M_w$ ) of both the dissolved (SUP) and the not-dissolved (SED) phase of the paste was not possible since the data obtained were incomplete. In some cases, the recovery of the SED and a sufficient amount, respectively, was not a certainty. Hence, the molecular characterization of the not-dissolved phase by means of SEC-MALS-DRI was impossible in some cases (WxPS and PS).

Figure 3 shows the mean (A–D) and interaction diagrams (E and F) of the impacts starch type (AM), HP, AT, and C. As shown by the analysis of the *p*-values (ANOVA, Table 2), the impacts starch type and the modification by means of HP were found to be statistically significant factors on *S* (Figure 3A,B). The *S* of the PS-based samples was found to be higher compared to the WxPS samples (not significant), and even significantly higher compared to the HACS samples, which are commonly known to have a restricted tendency to swell and dissolve (Figure 3A). The HP enhanced the starch's *S* significantly, but the specificity of the HP did not impact the *S* in general (Figure 3B). The AT modification did not change the *S* (Figure 3C), but the increase of the C from 6% to 9% decreased it by trend (Figure 3D; not significantly). The combined impacts shown in Figure 3E,F did not provide evidence of a remarkable influence of the AT on the one hand and the C on the other hand. In contrast, the specific impact of the HP depending on the starch type (AM) was distinct in particular for the HACS (Figure 3E). The HP of the HACS enhanced the *S* gradually, and that specific characteristic was also not impacted by the additional AT of the HACS samples (Figure 3F; dual modification).

Figure 4A shows that the chromatograms of the SUP and the SED of the single-modified HACS samples disintegrated at 6% polymer concentration in the paste. The chromatogram area corresponds to the relative amount of starch. In comparison to the

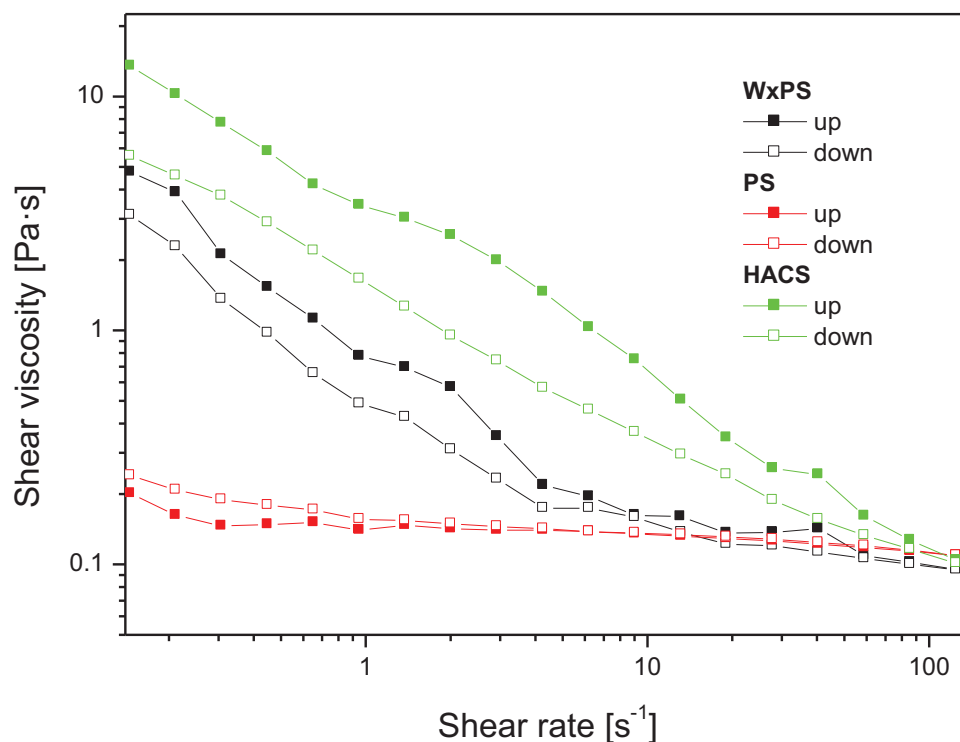




**Figure 7.** Mean A–D) and selected interaction diagrams E–H) with a 95% confidence interval of the impacts of starch type (WxPS, PS, and HACS), degree of HP (without, HP-1, and HP-2), AT (without and with acid treatment), and C (6% and 9%) on the HPV (at a shear rate of  $40 \text{ s}^{-1}$ ).

native HACS, the HP enhanced the *S*, which is evident by the higher chromatogram area of the SUP of the HP samples and corresponding decreased area of the SED. The  $M_w$  of the dissolved starch fraction was generally higher than the undissolved fraction, i.e., related to their relative portion within the starch product, the higher MM polymers (AP) are dissolved basically to a greater extent compared to the lower MM fraction (AM). Based on

the chromatograms in Figure 4A it is reasoned, that the HP provoked in particular, a reduction of the relative AM content within the SED. Altogether, the enhancement of the *C* from 6% to 9% (w/w) did not change that characteristic and correlation, respectively (Figure 4B). However, at a higher *C*, the supporting effect of the HP on the *S* becomes obvious. The higher the MS of the HACS, the higher the dissolved starch content and concurrently



**Figure 8.** HPV curves (up: 0.1–125 s<sup>-1</sup>; down: 125–0.1 s<sup>-1</sup>) of 9% (w/w) pastes of the native starches (WxPS, PS, and HACS).

lowered the undissolved portion. The respective molecular composition of the fractions is comparable to the paste prepared with 6% (w/w) starch.

Since the AT induced a partial molecular degradation of the starch samples, the chromatograms of the -AT samples (dual modification; Figure 4C,D) were shifted to higher elution volume compared to their counterparts (Figure 4A,B). The difference regarding the dissolved starch fraction between the HP-AT samples and the AT starch was remarkable (Figure 4C,D), however, the overall tendency or systematic of the -AT samples was in accordance with the HP samples (Figure 4A,B). The  $M_w$  of the SUP was higher in most cases compared to the SED.

Figure 5A–D shows the chromatograms of the SUP and the SED phase of the single- and dual-modified WxPS samples disintegrated at 6% and 9%, respectively, polymer concentration in the paste. Some of the SED are left, since they could not recover during the experimental steps. Although the HP actually did not cause a noticeable molecular degradation of the modified WxPS per se, the dissolved starch polymers were remarkably degraded after disintegration at 6% and 9% (w/w), respectively (Figure 5A,B). It was hypothesized that this phenomenon is due to (strongly) enhanced sensitivity against chain cleavage of the branched high MM polymers at comparatively high starch concentration in the dispersion combined with the high temperature during autoclave cooking and subsequent high-shear treatment. However, the suggested sensitivity ceased after additional AT (dual-modified samples), since the relative portion and molecular composition of the dissolved starch fraction (SUP) were comparable/similar (Figure 5C,D).

The chromatograms of the dissolved and the undissolved phase of the single- and dual-modified PS samples disintegrated

at 6% and 9%, respectively, polymer concentration in the paste, are shown in Figure 6A–D. According to the WxPS, some undissolved fractions (SED) were not recovered during the examinations. Compared to the molecular composition of the native PS ( $M_w$  about  $33 \times 10^6$  g mol<sup>-1</sup>), the SUP fraction contained an enhanced amount of dissolved higher MM polymers (AP), which is especially distinctive for the 9% (w/w) solution with a very high average MM ( $M_w$  about  $59 \times 10^6$  g mol<sup>-1</sup>; Figure 6A,B). Astonishingly, the dissolved fraction of the HP samples was remarkably degraded, which is on the other hand similar to the systematic found for the respective WxPS samples (Figure 6A,B). The degradation to that high extent cannot be comprehensively and conclusively explained. In particular the remarkably higher degree of molecular cleavage with lower degree of chemical modification (SUP, -HP-1) of the potato-based starches (WxPS and PS) is surprising. In the case of the dual-modified PS (Figure 6C,D), the differences in terms of the molecular composition of the dissolved fraction were comparatively small. However, in particular, the HP-2-AT sample was visibly degraded for both investigated starch concentrations (Figure 6C,D). For all samples considered, the soluble fraction (SUP) had a higher MM compared to the highly swollen fraction (SED).

The partially remarkable molecular degradation of the starch polymers of the HP samples based on potato (WxPS and PS) after disintegrating independently on the C (6% and 9% w/w) raises a question. The phenomenon was not found for the corn based high AM variety (AP content about 30%) but just for both potato-based starches having different AM-AP-ratios (AP content about 75% (PS) and 100% (WxPS), respectively). Moreover, when applied to a 2.5% starch dispersion (for molecular characterization), the same disintegration procedure using the autoclave at the

same temperature (145 °C) including the high-shear-treatment of the hot solution also did not result in the described degradation. A strong degradation basically did not happen when there was additional and subsequent, respective, modification by means of acid hydrolysis (AT) including a step when the product was washed. It is assumed, that the thermal disintegration technique as described, including a shear aftertreatment, can induce a molecular degradation of the starch, mainly of the branched polysaccharide fraction. The distinct cleavage could be probably supported by remnants of chemicals (from HP), the high AP content and simultaneously high starch polymer concentration in combination with the high-shear impact. In the case of the additional AT modification (dual-modified samples), the AP was partially degraded and the starch sample repeatedly washed removing solubilized carbohydrates, solved NaCl as well as the remaining chemicals from the previous chemical modification.

An increase in swelling power and *S* caused by HP or with increasing MS, respectively, was reported by, e.g., Kaur et al.<sup>[28]</sup> (PS, AM content 20–27% w/w, MS 0.098–0.118, dispersion: 2% heated to 90 °C), Oladebeye et al.<sup>[16]</sup> (lima bean and jack bean starch; MS 0.26–0.37 and 0.24–0.30, respectively), Liu et al.<sup>[30]</sup> (corn starches with different AM contents (3.3–66% w/w), dispersion: 1.25% w/w, heated incremental between 60 and 90 °C) and Karim et al.<sup>[11]</sup> (corn and mung bean starch; AM contents 28% and 56% w/w, respectively; MS about 0.02 and 0.002, respectively; 80 °C). The latter speculated that the soluble material consisting mostly of leached AM and low MM polysaccharides. Lawal<sup>[15]</sup> also found increased *S* with increasing MS (pigeon pea starch; MS 0.06–0.17; dispersion: 0.133% w/w, heated incremental between 30 and 90 °C, 1 h). The reason for this was suggested to be enhanced leaching of AM mainly from the amorphous regions due to enhanced accessibility of the granular structure for water after HP. In addition, the disruption of inter- and intramolecular H-bonds weakens the supramolecular structure, and the motional freedom of starch chains increases as the temperature increased, facilitating an increased *S* of the starch.<sup>[15]</sup> Moreover, Shi and BeMiller<sup>[31]</sup> revealed that the greater the extent of the modification, the easier AM leaches out (1), but the preference for leaching derivatized AM decreases with an increasing MS of the whole starch (corn starch; 4% w/v; MS 0.043–0.093). Ulbrich and Flöter<sup>[32]</sup> showed a higher relative *S* of the AM for a native PS, but elevated disintegration temperatures (95–155 °C) facilitated the solubilization of the AP fraction successively.

### 3.4. Hot Paste Shear Viscosity

The HPV of the dispersions was determined by means of rotational rheometer measurements. The viscosity at 40 s<sup>-1</sup> was used for the statistical evaluation (ANOVA, mean and interaction plots). From the ANOVA, all single factors (AM, HP, AT, and C) as well as some interactions (AM-C, HP-AT, and HP-C) were estimated to have a statistically significant effect on the viscosity (Table 2). The mean and selected interaction diagrams are displayed in Figure 7A–H.

The viscosity of the HACS samples was higher compared to the potato based samples (Figure 7A), which is mainly caused by the significantly enhanced viscosity owing to increased starch concentration (Figure 7F) and the not-modified HACS

(Figure 7H). Probably, limited gelation of the starch polymers of the HACS samples occurred during the measurement period or even before under prevailing methodical conditions, in particular, presumably due to the high AM content (about 74%). However, this is suggested for the most part, for the starches without HP, but is basically supported by Ulbrich et al.<sup>[33]</sup> who revealed significantly higher viscosity level with higher AM content (corn starch genotypes). Shear viscosity curves are exemplarily shown for the 9% (w/w) pastes of the native starch samples (Figure 8). At a low shear rate, the maximum difference in HPV was about one order of magnitude. The significantly higher level of the WxPS compared to the PS is probably caused by differences in terms of the solution state including the presence of granule remnants or simply the molecular size. For almost all starch samples investigated in the present study, a shear rate dependency of the HPV was confirmed. HP decreased the HPV significantly (Figure 7B), but the enhancement of the level of HP from HP-1 to HP-2 actually did not change the viscosity further. Decreasing viscosity with an increasing MS of low-substituted canna starch was reported by others based on RVA experiments (6% w/w, MS 0.01–0.11).<sup>[8]</sup> However, Oladebeye et al.<sup>[16]</sup> clearly showed increasing HPV with an increasing level of HP (lima bean and jack bean starch; MS 0.26–0.37 and 0.24–0.30, respectively), which contradicts the findings in the present study. Reasons are probably different paste preparation conditions as well as the determination at 25 °C. The higher HPV of the samples without modification by means of HP was seen in the case without AT (Figure 7E) and with an increased C (Figure 7G), respectively. Furthermore, the HPV decreased due to AT as a result of reduced molecule size by partial hydrolysis (Figure 7C) on the one hand, and increased due to the increased polymer concentration of the paste (C) on the other hand (Figure 7D,F). Reduced HPV caused by partial molecular degradation (enzymatic hydrolysis:  $\alpha$ -amylase and glucoamylase, RVA experiments) of HP starches was likewise revealed by Karim et al.<sup>[11]</sup>

## 4. Conclusions

Starches with different AM/AP ratios were systematically modified by means of HP and subsequently AT to produce dual-modified samples. The investigation of the DSC swelling properties was successful, and the evaluation of the data statistically provided valuable correlations. All varied factors impacted the gelatinization characteristics. In particular, the HP caused a decrease in the transition temperatures. The remarkably broad gelatinization range of the HACS samples remained despite HP, possibly limiting the industrial application of corresponding dual-modified samples. In contrast to HACS, the potato-based HP starches tended to degrade molecularly while preparing concentrated pastes using a laboratory technique including pressure cooking (autoclaving) and subsequent high-shear treatment (Ultra-Turrax), especially the branched polysaccharide fraction within the dissolved phase. Obviously, primarily the HP modified AP fraction is sensible to heat and shear-induced degradation, which presumably impacts the techno-/functional properties of such products. Conspicuously, both native and HP modified HACS samples contained a higher relative portion of AP within the dissolved phase of a paste compared to the corresponding undissolved one. Hence, there is evidence for a limited *S*

particularly of the AM fraction of the high AM starch samples, which actually contradicts the commonly accepted behavior on the one hand. On the other hand, it underlines the exceptional position of such high AM genotypes in terms of the disintegration and processing. The detailed examination of the HPV at the comparatively high shear rate of  $40 \text{ s}^{-1}$  is most probably an appropriate level, since it feasibly represents the strain during, e.g., pump and other steps during industrial processing. Altogether, the techno-functionality of the starch (processing characteristics) is assessed at an importance equivalent to functional properties (development of desired product characteristics) achieved in the end product.

## Acknowledgements

This research was financially supported by the German Federal Ministry for Economic Affairs and Energy (BMWi) within the scope of the Industrial Collective Research (IGF) of the German Federation of Industrial Research Associations (AIF; research project 20248 N). The authors would like to thank Emsland-Stärke GmbH for preparing the HP starches. The authors also thank Eugenia Kuhl for her assistance in performing numerous experiments and Donna Hastings for checking the manuscript linguistically.

## Conflict of Interest

The authors have declared no conflict of interest.

## Data Availability Statement

Research data are not shared.

## Keywords

DSC gelatinization properties, dual modification approach, hot paste viscosity, starch solubility

Received: July 10, 2020  
Revised: September 3, 2020  
Published online:

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