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Maltogenic amylase has a non-typical impact on the molecular and rheological properties of starch

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Abstract

The effects of different amylases on the rapid visco analysis rheological properties of starch were studied and the accompanying changes in the starch molecular properties were analyzed with high performance size exclusion chromatography. Different amylases affect the rheological properties of starch slurries and the molecular weight of the starch molecules to a degree depending on their mode of action and properties such as thermostability. Endo-amylases generally reduced the viscosity and the amylose and amylopectin molecular weight (MW) whereas an exo-amylase had little effect. In contrast, a maltogenic amylase from *Bacillus stearothermophilus* had a markedly different effect on the rheological and molecular properties of starch. Remarkably, the cold paste viscosity (CPV) exceeded that of the control slurry even at high enzyme dosages. Furthermore, this enzyme specifically affected the amylose population, which gained in monodispersity while largely maintaining its MW. In addition, an interesting relationship between CPV and amylose peak degree of polymerisation was found. These results may contribute to a better understanding of amylase functionality in bread making.

Keywords: Amylase; Starch; Partial hydrolysis; RVA; Molecular properties

1. Introduction

The semi-crystalline radially anisotropic native wheat starch consists of two glucose polymers, (the essentially linear) amylose and (the highly branched) amylopectin. When a starch granule suspension is heated above its gelatinization temperature, it gelatinizes and hence undergoes a series of changes which eventually lead to the irreversible destruction of the granular molecular order (Atwell, Hood, Lineback, Varriano-Marston, & Zobel, 1988). This phase transition is a non-equilibrium process associated with diffusion of water into the granules, hydration and swelling, uptake of heat, loss of crystallinity and leaching of (mainly) amylose from the granules (Slade & Levine, 1991). Upon cooling of starch pastes at sufficiently high starch concentrations, a gel is formed (Miles, Morris, Orford, & Ring, 1985). Starch retrogradation is the process whereby starch molecules reassociate in an ordered structure. Shortly after gelatinization (within a few hours), starch retrogradation is dominated by gelation and crystallization of amylose leached out during gelatinization. Retrogradation of amylopectin is only complete after several days of weeks (Ring, Miles, Morris, Turner, & Colonna, 1987).

In the production of starchy foods, the structure and properties of the final product are determined by the transformation of the starch fraction during heating and storage (Zobel & Kulp, 1996). In particular, the rate and extent of the retrogradation have a profound effect on the texture and, hence, acceptability of many starch-containing foods in general, and bread in particular. The starch functionality can be impacted in several ways, such as by using enzymes. A typical example is the use of amylases to affect the firming rate, particularly the firmness of bread crumb (Bowles, 1996; Kulp & Ponte, 1981). Different mechanisms by which different amylases delay crumb firming have been proposed. Either the released dextrins (Akers & Hoseney, 1994; Beleia, Miller, & Hoseney, 1996; Defloor & Delcour, 1999; Martin & Hoseney, 1991) or the modified residual starch (Duedahl-Ohlesen, Zimmermann, & Delcour, 1999; Gerrard, Every, Sottun, & Gilpin, 1997;

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Miles et al., 1985; Zobel & Kulp, 1996) can impact the rheological properties of the entire system and the subsequent starch retrogradation. The different views on the antifirming mechanism of starch degrading enzymes reflect the still incomplete understanding of the firming mechanism itself (Hug-Iten, Handschin, Conde-petit, & Escher, 1999). In addition, the comparison is often difficult because different amylases have been used.

The present study subjects starch slurries supplemented with amylase to a heating step comparable to that of the baking phase during bread making. The impact of different amylases on the rheological properties during gelatinization, pasting and gelation was evaluated with a rapid visco analyzer (RVA). In addition, the molecular structure of the materials recovered by freeze-drying starch gels after RVA analysis was studied by high performance size exclusion chromatography (HPSEC). The amylases used represent a range of enzymes with different mode of action (endo, exo and maltogenic) and thermal stability.

2. Materials and methods

2.1. Materials

Wheat starch was from Amylum (Aalst, Belgium). Moisture content (12.2%) was determined based on weight loss at 130 °C for 2 h of ca. 1.0 g accurately weighed starch. Differential scanning calorimetry (DSC) onset, peak and conclusion gelatinization temperatures determined with a Seiko DSC 120 (Kawasaki Kanagawa, Japan, starch/water ratio 1:3 w/w, heating rate 4 °C/min) were 52.3, 57.3, and 65.4 °C, respectively. The level of damaged starch (Starch Damage Assay Kit, Megazyme, Bray, Ireland, AACC Method 76-31) was 2.1%. The close packing concentration, determined as described earlier (Vandeputte, Derycke, Geeroms, & Delcour, 2003) was 5.2% starch dry matter (dm).

Amylases used were *Bacillus subtilis* alpha-amylase (BSuA), porcine pancreatic alpha-amylase (PPA) and sweet potato β-amylase (SPB), all from Sigma Aldrich Chemie (Bornem, Belgium). *Bacillus licheniformis* alpha-amylase (BLA) and *Aspergillus oryzae* α-amylase (TAKA) were from Megazyme. TAKA is widely used in bread making. *Bacillus stearothermophilus* maltogenic amylase (BStA) from Novozymes (Novamyl®, Bagsvaerd, Denmark) is an effective anti-staling enzyme. All cited enzymes are endo-alpha-amylases (E.C. 3.2.1.1), apart from SPB, an exo-enzyme (E.C. 3.2.1.2), and BStA (E.C. 3.2.1.133). Earlier work with BStA revealed properties that distinguish it from other alpha- en beta-amylases (Christophersen, Otzen, Norman, Christensen, & Schafer, 1998; Outtrup & Norman, 1984). In particular, while the main hydrolysis product is alpha-maltose, which points to an exo-action, its action on cyclodextrins and beta-limit dextrins (Christophersen et al., 1998) and the structure of its active site (Dauter et al., 1999) indicate that the enzyme does not require chain ends.

2.2. Amylase activity and thermal stability

Amylase activities were assayed by quantifying the reducing sugars released from soluble starch (Merck, Darmstadt, Germany) according to the Somogyi-Nelson method (Somogyi, 1952) with maltose as reference. One enzyme unit (1 EU) was the amount of enzyme which released 1 μmole maltose/min at 40 °C and pH 6.0 (100 mM sodium maleate + 5 mM $CaCl_2$). The thermal stability of the amylases was defined as the residual activity after 5 min incubation at several temperatures (50, 70, 80, 100 °C) and was determined with the amylase activity assay as well.

2.3. RVA

Rapid viscosity analysis was with a rapid visco analyzer (Model RVA-4D; Newport Scientific, Sydney, Australia) interfaced with a computer equipped with Thermocline software. Starch suspensions (11% dm w/v, total weight 25.0 g), with or without amylase addition at the start of the RVA-run, were subjected to a temperature increase from ambient temperature to 40 °C (0–1 min), followed by a linear temperature increase from 40 to 95 °C at 3.95 °C/min, a holding step (5 min at 95 °C), a cooling step with a linear temperature decrease from 95 to 50 °C (6.4 °C/min) and a final isothermal step at 50 °C. A BStA dosage of 9.03 EU/g starch was recommended in bread making (Spendler, Nilsson, & Fuglang, 1999). Enzyme dosages of the other amylases were chosen to have a similar effect on the peak viscosity as did the above mentioned BStA dosage. The viscograms measured by RVA were excellently reproduced. RVA parameters were onset temperature, peak viscosity (PV), peak time (PT), hot paste viscosity (HPV, i.e. the minimum viscosity value read after the peak), cold paste viscosity (CPV, i.e. viscosity after 70 min), and breakdown, i.e. the difference between the PV and the HPV. All viscosity readings were in Poise (*P*; 0.1 Ns/m^2) which is a unit of dynamic viscosity. After each RVA run, the starch gels were freeze-dried.

2.4. High performance size exclusion chromatography (HPSEC)

HPSEC of the material obtained by freeze drying the starch gels was as described by Klucinec and Thompson (1998) with modifications. Fractionation was with a Sepharose CL-2B column (74×1.6 cm^2, Amersham Pharmacia Biotech, Sweden) by ascending chromatography at a flow rate of 30 ml/h using degassed 0.1 M potassium hydroxide containing 0.02% (w/v) sodium azide. Starch samples (15 mg) were dispersed in 1 ml of concentrated mobile phase (1.0 M KOH) for 8 h under mild magnetic stirring and then diluted to 10 ml with purified water.

After filtration (0.45 μm; regenerated cellulose syringe filter), 5 ml of the solubilised samples were injected. Detection was with a differential refractometer (Shimadzu, Japan). Fractions (5 ml) were collected and their iodine binding (620 nm) was examined. A 1.0 ml aliquot of each SEC fraction was neutralized with 25 μl 1.0 M HCl and mixed with 5.0 ml of iodine solution (0.38 mg I_2 and 0.90 mg KI/ml).

The column was calibrated with Shodex pullulan standards (Showa Denko, Japan). Pullulans can replace linear amylose standards for chromatographic calibration purposes when 0.1 M potassium hydroxide is used as eluent (Roger, Axelos, & Colonna, 2000). A second order polynomial correlation was found between the logarithm (M_r standards) and the partition coefficient (Kav). The polydispersity (P) was estimated for each amylose fraction ($0.2 < \text{Kav} < 0.87$) based on the above mentioned pullulan calibration. P is defined as the ratio of the weight average DP (DP_w) to the number average DP (DP_n) (Gelders, Vanderstukken, Goesaert, & Delcour, 2004).

3. Results and discussion

3.1. Activity and thermal stability of the amylases

Table 1 presents the activities and thermal stabilities of the amylases used. The endo-amylases BSuA and BLA are of intermediate and high thermostability, respectively. Under the experimental conditions (incubation at 80 °C during 5 min), they retained ca. 55 and 99%, respectively, of their activity. Incubation at 100 °C during 5 min inactivated BSuA but not BLA which retained ca. 69% of its activity. TAKA and PPA had low thermostabilities and were inactivated after 5 min at 50 and 70 °C, respectively. The exo-amylase SPB with low thermostability was largely inactivated after 5 min incubation at 50 °C. The bacterial maltogenic amylase BStA, characterized by an intermediate thermostability, retained ca. 76% of its activity following incubation at 80 °C during 5 min, but was inactivated after 5 min at 100 °C.

3.2. Influence of amylase addition on the pasting characteristics of wheat starch

Fig. 1 presents the results of the RVA profiles. The control sample had a PV of 5000 cP, a PT of 14.3 min and a CPV of 7225 cP (Table 2).

The starch concentration used (11% dm starch w/v) clearly exceeded the close packing concentration (5.2% dm starch w/v). In the concentrated regime, starch granules cannot swell to their equilibrium volume, and the rheological features of the suspensions are mainly determined by the rigidity of the swollen granules (Steeneken, 1989), which in its turn affects the starch swelling power and the amylose leaching in solution (Morris, 1990). Under the experimental conditions, the addition of amylases slightly decreased the close packing concentration (data not shown).

3.2.1. The influence of endo-amylases on the pasting characteristics of wheat starch

The endo-amylases BSuA, BLA, TAKA and PPA changed the rheological behavior of the starch suspensions (Fig. 1(a–d)). In general, higher rates of viscosity development and an extensive viscosity reduction, in particular of PV and CPV were observed (Table 2). These effects were more pronounced with increasing enzyme dosages. In the Steeneken (1989) line of thinking, this can be interpreted, as a weakening of the granule structure by the endo-amylases. As a result of the amylolysis, the less rigid granules have an increased swelling rate and are more susceptible to disintegration, particularly under the shear conditions in the RVA. Consequently, PT generally decreased with increasing enzyme dosages. This effect was more pronounced for BSuA (PT decrease from 13.5 to 10.2 min) and BLA (PT decrease from 13.9 to 12.0 min), while PPA (PT decrease from 13.9 to 13.3 min) and TAKA (PT decrease from 13.7 to 13.3 min) had only a minor impact on the PT. The more thermostable endo-amylases (BSuA and BLA) most likely remained more active during/after gelatinization, when starch is more accessible and susceptible to hydrolysis. The less thermostable enzymes, such as PPA and TAKA, were presumably partly inactivated

Table 1
Action mechanism, thermostability, and residual activity (i.e. percentage of the activity recorded at 40 °C and pH 6.0) of amylases following 5 min incubations at 50, 70, 80 or 100 °C

Enzyme	Action mechanism	Thermostability	50 °C (%)	70 °C (%)	80 °C (%)	100 °C (%)
BSuA	Endo	Intermediary	100	94	55	0
BLA	Endo	High	100	99	99	69
TAKA	Endo	Low	48	0	0	0
PPA	Endo	Low	90	0	0	0
SPB	Exo	Low	46	0	0	0
BStA	*Maltogenic*	Intermediary	96	96	76	0

BSuA, *Bacillus subtilis* alpha-amylase; BLA, *Bacillus licheniformis* alpha-amylase; TAKA, *Aspergillus oryzae* alpha-amylase; PPA, porcine pancreatic alpha-amylase; SPB, sweet potato beta-amylase and BStA, *Bacillus stearothermophilus* maltogenic amylase.

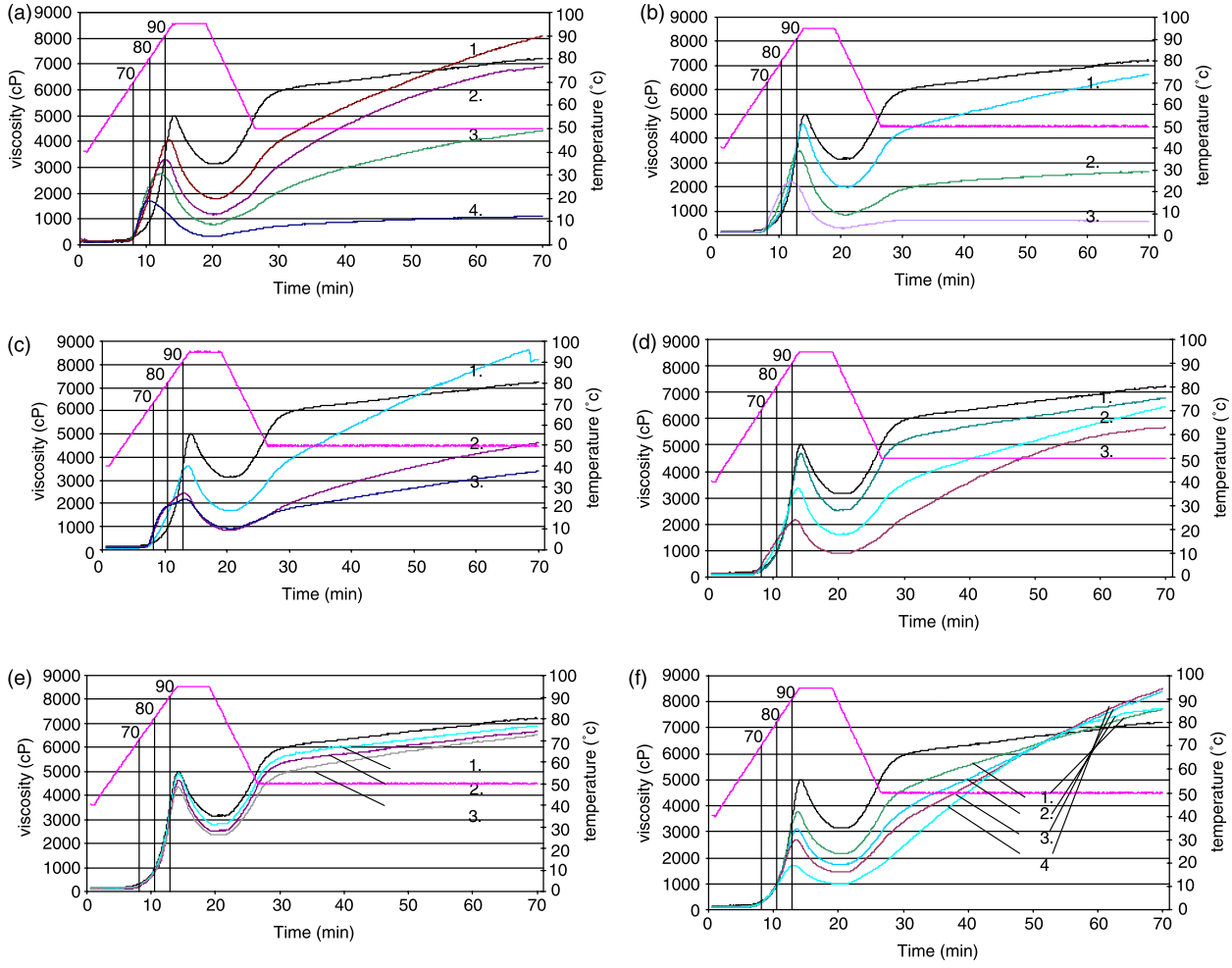

Fig. 1. Effect of amylase addition on rapid visco-analyzer (RVA) profiles of wheat starch slurries: (a) BSuA: 1, 0.0920 EU/g ST, 2, 0.139 EU/g ST, 3, 0.170 EU/g ST, 4, 0.920 EU/g ST; (b) BLA: 1, 0.0107 EU/g ST, 2, 0.0533 EU/g ST, 3, 0.107 EU/g ST; (c) TAKA: 1, 0.434 EU/g ST, 2, 1.09 EU/g ST, 3, 2.16 EU/g ST; (d) PPA: 1, 0.174 EU/g ST, 2, 0.348 EU/g ST, 3, 0.695 EU/g ST (e) SPB: 1, 13.3 EU/g ST; 2, 26.3 EU/g ST, 3, 132.5 EU/g ST and (f) BStA: 1, 4.52 EU/g ST, 2, 9.03 EU/g ST, 3, 18.1 EU/g ST, 4, 36.1 EU/g ST.

before full starch gelatinization which can explain the minor influence on the PT.

Low dosages of BLA, BSuA and TAKA resulted in a larger breakdown than in the control sample. This can be related to the decreased granular rigidity or the increased susceptibility of starch granules to disintegration/deformation. With increasing amylase dosages, a decreasing breakdown was observed.

When the mixture was subsequently cooled, the viscosity increased, leading to the CPV. This can, to a certain extent, be attributed to the re-association of starch molecules in the starch paste and in particular to the leached amylose (Olkku & Rha, 1978). In general, the CPV decreased with increasing enzyme concentration. At high enzyme concentrations (PV $<$ 2000 cP) only a small setback was observed for BSuA and BLA and no gel could be recovered after RVA-analysis. Even though TAKA had probably been largely inactivated before full starch gelatinization, at low dosage, a higher CPV was found for the control sample. In contrast, PPA had only a slight influence on the CPV.

We were not able to rationalize the influence of TAKA on the CPV based exclusively on the granule rigidity in the concentrated regime. Some other factors, which might influence starch rheological properties, such as interactions between the dispersed and continuous phase in the starch paste, may also have been altered. In addition, the shear during pasting and holding in the RVA may have affected the rheological properties as well (Jacobs, 1998).

3.2.2. The influence of exo-amylase on the pasting characteristics of wheat starch

SPB had only a small influence on starch rheology (Fig. 1(e)) and viscosity parameters (Table 2). This can be attributed to its exo-mechanism of action, its limited (if any) activity on ungelatinized starch and its limited thermostability. β-Amylase cannot hydrolyze native undamaged starch, and hydrolyses damaged starch only slowly (Colonna, Leloup, & Buléon, 1992), hence only small changes were found.

Table 2
Rapid visco analyzer parameters of starch (11% dm) in the presence or absence of varying levels different amylases

Enzyme	EU/g starch	Peak			Hot paste			Breakdown viscosity (cP)	Cold viscosity (cP)
		Viscosity (cP)	Time (min)	T (°C)	Viscosity (cP)	Time (min)	T (°C)		
Control	0	5000	14.3	94.9	3150	20.0	94.5	1850	7225
BSuA									
	0.0920	4075	13.5	92.6	1800	20.8	84.2	2275	8070
	0.139	3785	13.2	91.5	1460	20.7	84.6	2325	7690
	0.170	2740	12.2	87.3	785	20.3	87.6	1955	4430
	0.920	1710	10.2	78.7	315	20.0	89.2	1395	1110
BLA									
	0.0107	4600	13.9	94.6	1975	21.1	82.2	2625	6635
	0.0533	3480	13.3	92.0	835	20.9	85.1	2645	2635
	0.107	2205	12.0	86.4	280	20.6	85.5	1925	870
TAKA									
	0.217	4055	14.2	95.0	2365	20.1	88.1	1690	9440
	0.434	3630	13.7	93.8	1690	20.3	87.1	1940	8610
	1.09	2440	13.1	90.9	840	20.7	87.6	1600	4625
	2.16	2185	13.3	92.0	920	20.8	84.2	1265	3390
PPA									
	0.174	3785	13.9	95.5	1820	19.7	91.0	1965	6500
	0.348	3380	13.8	94.1	1620	20.4	89.1	1760	6455
	0.695	2160	13.3	92.0	885	20.1	88.2	1275	5480
SPB									
	1.33	4930	14.3	94.9	2785	19.9	90.7	2145	6915
	66.3	4645	14.3	95.0	2500	19.8	90.3	2145	6665
	132.5	4375	14.2	94.9	2360	19.8	90.3	2015	6520
BStA									
	4.52	3750	13.9	94.2	2175	19.9	89.8	1575	7725
	9.03	3105	13.7	93.4	1740	19.5	92.1	1365	8405
	18.1	2700	13.5	92.9	1440	19.8	90.1	1260	8515
	36.1	1710	13.1	91.2	990	19.5	92.3	720	7726

BSuA, *Bacillus subtilis* alpha-amylase; BLA, *Bacillus licheniformis* alpha-amylase; TAKA, *Aspergillus oryzae* alpha-amylase; PPA, porcine pancreatic alpha-amylase; SPB, sweet potato beta-amylase and BStA, *Bacillus stearothermophilus* maltogenic amylase.

3.2.3. The influence of a maltogenic amylase on the pasting characteristics of wheat starch

Much as the bacterial endo-amylases, the maltogenic amylase BStA decreased PV (Fig. 1(a)). However, in contrast to what was observed with BLA and BSuA, PT was only slightly reduced (from 13.9 to 13.1 min) (Table 2). The endo-mechanism suggested for BStA (Christophersen et al, 1998; Dauter et al., 1999) is in line with a reduction in PV and a weakening of the granule structure, although it was less pronounced than with the intermediately and highly thermostable endo-amylases. As BStA did not increase the rate of viscosity development, it was probably mainly active at higher temperatures and thus on gelatinized and more enzyme susceptible granules. Other effects of BStA addition clearly differed from those observed for the endo-amylases. Upon BStA addition, a marked decrease of the breakdown viscosity was observed, even at low concentration. Remarkably, the CPV was higher than that of the control slurry even at high enzyme dosages (PV $<$ 2000 cP). Furthermore, CPV increased with enzyme concentration and, intriguingly, under the conditions of the analysis, the different viscosity traces crossed in a single point at about 50 min. At higher starch concentrations (15% dm w/v), the effect of BStA on CPV was even more pronounced (data not shown). However, at lower starch concentration (8% dm w/v), a higher CPV than for the control was not found (data not shown). It seems that, at concentrations closer to the close packing concentration, the structural properties of the starch paste and in more particular of the amylose molecules are probably insufficient to form an effective network during cooling.

3.3. HPSEC of freeze-dried gels from amylase supplemented wheat starch slurries

In order to better understand the relation between the impact of the amylases on the starch polymers and the rheological properties of the amylase supplemented starch slurries, the starch fraction at the end of the RVA-run was analyzed by HPSEC. Fig. 2 shows the Sepharose Cl-2B elution profiles of the freeze-dried starch samples. The chromatograms can be divided into two regions with Kav either lower than or exceeding 0.2. The former region represents material eluting in the void volume. We observed a marked shift in iodine binding indicating a sharp transition from amylopectin to amylose. Amylopectin eluted in the void volume of the column, which in many cases complicated interpretation of the influence of the enzymes

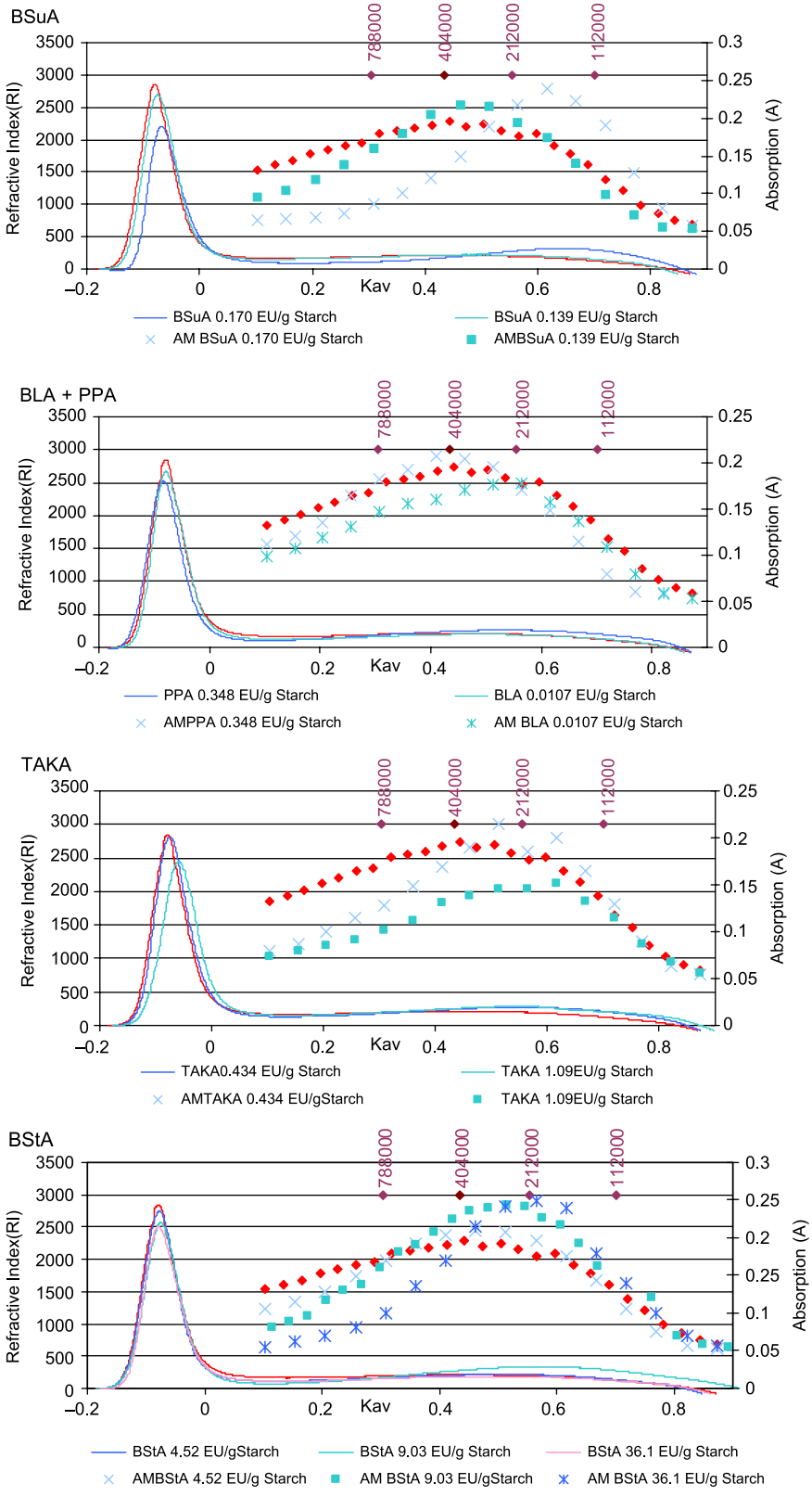

Fig. 2. High performance size-exclusion (HPSEC) elution profiles after rapid visco-analysis (RVA). HPSEC-chromatograms of amylose fractions in the starches obtained by freeze drying starch gels produced in the RVA in the absence or presence of specified levels of different amylases. Total carbohydrate (solid line) (control: highest AP-peak) and iodine binding (broken line) (control: ♦).

on amylopectin MW. The control starches obtained after RVA-analysis had a broad, polydisperse amylose fraction ($P=2.13$) with a peak DP around DP 2150.

3.3.1. The influence of endo-amylases on the molecular composition of starch

The influence of the endo-amylases BSuA, BLA, TAKA and PPA on the molecular composition of the starch molecules was visualized by HPSEC (Fig. 2). BSuA (0.182 and 0.139 EU/g starch) markedly reduced both amylopectin and amylose MW. This can be related to the significant decrease of particularly PV and HPV. In addition, a more monodisperse ($P=1.51$ and $P=1.91$) enriched amylose distribution was observed with peak DP of ca. 1800–1850 and ca. 900–950, respectively (Table 2). The partial hydrolysis by BLA (0.0107 EU/g starch) led to a lower apparent amylose content, as confirmed by iodine binding, with a peak DP 1650–1700, and a polydispersity similar to that of the control sample. With the SEC column used, only minor differences were detected between native amylopectin and that after BLA action. Much as BSuA, TAKA also reduced amylose and amylopectin MW, especially at higher concentrations. The MW distribution of the resulting amylose population depended on the amylase concentration added. At low dosage (TAKA 0.434 EU/g starch), two maxima could be observed in the amylose profile with peak DPs of ca. 1650–1700 and ca. 1000, whereas, at higher dosages the maximum around DP 1650–1700 disappeared. PPA mainly affected the longer amylose molecules, resulting in a more monodisperse ($P=1.91$) amylose fraction with peak DP of ca. DP 2370. The SEC column used detected only minor differences between the native amylopectin fraction and that obtained after PPA action (Table 3).

Table 3
Peak DP, weight (DPw), number (DPn) average degree of polymerization and polydispersity (P) of amylose fractions in the starches obtained by freeze drying starch gels produced in the rapid visco-analyzer in the absence or presence of specified levels of different amylases

Enzyme added EU/g starch	Peak DP	DPw	DPn	P
None	2147	2786	1307	2.13
BSuA 0.139	1812	2009	1333	1.51
BSuA 0.17	925	1937	1013	1.91
BStA 4.52	1955	2009	1341	1.50
BStA 9.03	1604	2493	1764	1.41
BStA 18.1	1345	2404	1284	1.87
BStA 36.1	1277	2097	1135	1.85
TAKA 0. 434	1610	2411	1201	2.01
TAKA 1.09	988	2969	1551	1.91
PPA 0.348	2372	2969	1551	1.91
BLA 0.0107	1679	2655	1263	2.10

BSuA, *Bacillus subtilis* alpha-amylase; BLA, *Bacillus licheniformis* alpha-amylase; TAKA, *Aspergillus oryzae* alpha-amylase; PPA, porcine pancreatic alpha-amylase; SPB, sweet potato beta-amylase and BStA, *Bacillus stearothermophilus* maltogenic amylase.

The above described effect of BSuA and TAKA, and to a slightly lesser extent also of BLA and PPA, on the pasting properties and molecular characteristics of starch is in agreement with the generally accepted mode of action of endo-amylases, i.e. a more or less random degradation of starch to low MW dextrins and oligosaccharides resulting in a decrease in viscosity.

3.3.2. The influence of the exo-amylase on the molecular composition of starch

SPB had only a small influence on amylose and amylopectin MW (data not shown), in agreement with the limited, if any, influence on the RVA properties.

3.3.3. The influence of the maltogenic amylase on the molecular composition of starch

BStA reduced amylose MW, but no reduction in the amylopectin MW could be detected as a result of BStA action (Fig. 2). Starch hydrolysis by BStA (4.52 EU/g starch) yielded an amylose population with peak DP of 1925–1975. Even with an overdosage of BStA (36.1 EU/g starch; PV < 2000 cP), the amylose population still retained a relatively high MW with a peak DP of ca. 1250–1300. Furthermore, the amylose molecules were narrowly distributed around the peak DP, indicative of a highly monodisperse amylose population ($P=1.41$ and $P=1.85$ for 9.03 and 36.1 EU/g starch, respectively). The suggested endo-mechanism of BStA (Christophersen et al., 1997; Dauter et al., 1999) is in line with the reduced MW of the amylose molecules and a reduction of the PV.

3.4. Relation between molecular and pasting properties (CPV) of starch slurries

Remarkably, under the conditions of the analysis, the influence of the amylases on CPV was not uniform. While most amylases reduced CPV, some amylases had little effect or even increased the CPV, particularly at low concentrations. These observations could not be rationalized exclusively in terms of the Steeneken (1989) view involving the close packing concentration and rigidity properties of the granules.

It is generally accepted that leached amylose forms a three dimensional network during cooling, involving the conversion of soluble amylose chains into a gel in which starch granule remnants are present (Miles et al., 1985), and hence increases the viscosity. Fig. 3 The correlation has been evaluated between the peak DP of the amylose fraction after RVA-analysis on the one hand and the CPV in the corresponding RVA-run on the other hand (Fig. 3). The results obtained with BLA were not included in this graph. Because of its high thermostability this enzyme was not inactivated during the holding step at 95 °C. Under the conditions of the RVA-analysis, a maximal CPV correlated with starch slurries containing an amylose population with peak DP between ca. 1400 and 1700 (region II in Fig. 3).

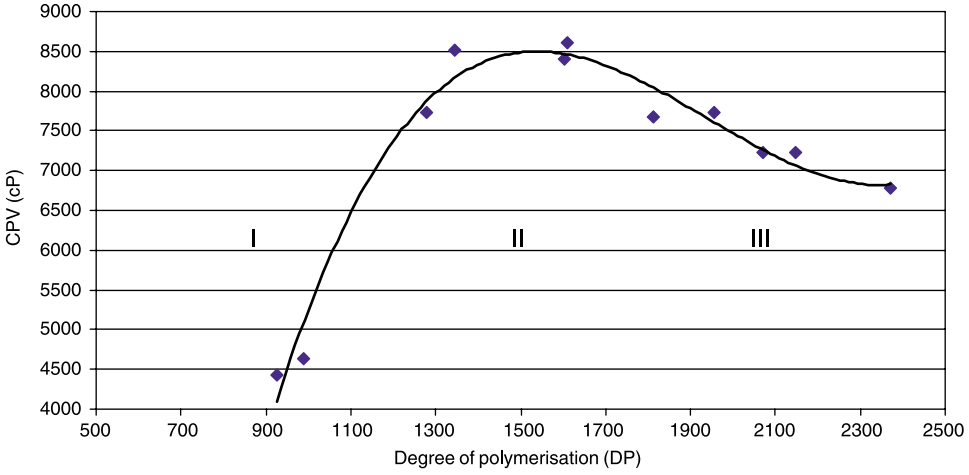

Fig. 3. Relationship of RVA cold past viscosity to amylose chain length of wheat starch slurries.

This indicates that a limited reduction of the amylose peak DP, preferably combined with a higher monodispersity than for the amylose fraction of the native starch, enhances structure formation during cooling. Presumably, the aggregation rate of the amylose molecules was increased and/or a better three-dimensional network was formed leading to a higher CPV. However, this is in contrast with the observations of Clark, Gidley, Richardson, and Ross-Murphy (1989), who assumed that aggregation of amylose is slower for DP > 200 with increasing chain lengths in aqueous amylose solutions. For amylose molecules with DP < ca. 1400 (region I in Fig. 3), the CPV decreased proportional with the chain length and with DP > ca. 1700 (region III in Fig. 3) the CPV decreased with increasing chain length. A possible explanation is that the aggregation of the longer amylose chains would be retarded or hindered after a relatively small number of cross-links, thereby resulting in a decreased CPV. The correlation between amylose chain length and CPV does not exclude that the properties of the starch paste may also be determined by interactions between the dispersed and continuous phases.

Furthermore, it is of note that addition of BStA, even when overdosing (PV < 2000), only to a limited extent reduced amylose MW and at the same time markedly increased its monodispersity. This probably promotes its ability to build an intergranular network, resulting in a smaller breakdown and higher CPV. This is in agreement with Hug-Iten et al. (1999), who in the case of bread making, assumed an enhanced amylose aggregation as a result of BStA action. Moreover, this may explain initial bread firmness increases upon BStA addition (Hug-Iten, Escher, & Conde-Petit, 2001). It can be hypothesized that retrogradation of starch in bread and bread model systems does not only involve changes in the amylopectin fraction (Zobel & Kulp, 1996), but also in the amylose polymers. More in particular, it is conceivable that an enhanced amylose aggregation also influences the firming of bread and gels by forming a network which rearranges less on aging and/or by limiting the interactions between the starch components (Hug-Iten et al., 2003). This indicates that, besides the concentration of the amylose fraction (Miles et al., 1985), also the amylose chain length and its polydispersity determine the texture of starch containing food products.

4. Conclusions

RVA can differentiate the effects of various amylases on starch pasting and gelation characteristics. Pasting and gelation of starch slurries is significantly affected by and greatly depended on the mode of action and properties of the amylase added.

In general, addition of endo-amylases to a starch slurry reduced viscosity, in particular PV, HPV and CPV, and PT, which goes hand in hand with a drastic decrease in the amylose and amylopectin MW. The more thermostable endo-amylases remained active during gelatinization/gelation and hence had a more pronounced influence on the rheological behavior of the slurries during heating and cooling. Furthermore, an exo-amylase had only minor impact on the molecular and rheological properties of the starch slurries. Like the endo-amylases, BStA decreased PV but with only little reduction in PT. It decreased breakdown and increased CPV, even at high dosages (PV < 2000). In addition, the amylose population was specifically affected and became more monodisperse, while the amylose MW was slightly reduced.

The influence of different amylases on the CPV could not be rationalized, based on the granule rigidity in the concentrated regime (Steeneken, 1989). Under the conditions of the analysis, a correlation was found between amylose chain length and CPV. This indicates that the properties of the starch paste are also determined by interactions between the dispersed and continuous phase.

A maximal CPV was observed with a (monodisperse) amylose population with peak DP 1400–1700. Due to its limited influence on the amylase molecular chain length, BStA probably promotes the aggregation of amylose. Further experiments are required to evaluate the impact of amylases on starch structure and properties which may be important for product and process development, in particular the firming properties of bread and starchy products.

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