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Granule Integrity and Starch Solubility During Slow, Extended Pasting of Maize Starch – the Second Viscosity Peak

Maize starch granule integrity and starch solubilisation at specific intervals during a relatively long pasting profile–which is known to exhibit biphasic peak viscosity character–was studied by microscopy and high-performance size-exclusion chromatography, respectively. Maize starch granules, although swelling and leaching components, remained mainly intact until the second viscosity peak when they lost integrity and formed a relatively homogenous paste. The second viscosity peak coincided with a decrease in starch content in solution and amylose going out of solution was mainly responsible for this decrease. It is suggested that the loss of granule integrity during the second viscosity peak increases the potential for amylose to form complexes with lipids at this stage. It is further suggested that the second viscosity peak is due to the formation and breakdown of superstructures of amylose-lipid complexes.

Keywords: Amylose-lipid superstructures; Pasting; Starch granule; Viscosity

1 Introduction

Recently, *Nelles* et al. [1] described biphasic pasting behaviour of native maize starch, measured with a Rapid Visco Analyser (RVA) or Brabender Viscograph instrument under specific pasting parameters. In addition to the expected first viscosity peak, an unexpected relatively large second viscosity peak was observed during the holding part (constant temperature) of the pasting profile. The specific conditions were relatively long pasting times (between 32 and 45 min), holding temperatures within the range of 82 to 95 °C, maize starch concentrations from 8 to 13% with a wide range of initial heating rates (0.5-10 °C/min) and RVA shear conditions (150-500 rpm). Viscosity development of the first peak, seen during gelatinisation and pasting of starch in a viscograph-type instrument, is attributed to two main factors. The first is the swelling of the starch granule during heating in water [2] and the second is related to the leaching of starch molecules (primarily amylose) during this period [3-5]. The factors responsible for the viscosity development of the second pasting peak in maize starch are unclear. Possible factors include: granule swelling and loss of granule integrity, starch granule solubility and the formation of amylose-lipid inclusion complexes and their crystalline packing. *Nelles* et al. [1] attributed the second pasting peak to the formation of complexes between amylose and low levels of lipid present in maize starch. This was because the second pasting peak disappeared when lipid was partially removed by extraction with methanol-chloroform and re-appeared when the lipid was added back. Starch granule morphology and integrity during pasting have been studied using microscopy to establish the roles of granule swelling and component leaching during the first viscosity peak [2, 3, 5-7]. High-performance size-exclusion chromatography (HPSEC) has proven to be a useful tool to determine the solubility of starch and its molecules in various solvents and with various treatments [8]. In this study the fate of the starch granule during the second viscosity peak was followed by light microscopy, scanning electron microscopy (SEM) and HPSEC.

2 Materials and Methods

2.1 Starch pasting

A commercial native maize starch (BDH; cat. 30261 7H) was used in this investigation. Suspensions (13% dry weight basis i.e., 3.64 g of starch made up to 28 g with distilled water) of the starch were prepared fresh and loaded in a Series 3 D RVA (Newport Scientific, Warriewood, Australia). Each starch suspension was stirred rapidly at 900 rpm for 10 s before the shear input was decreased and held constant at 160 rpm for the remainder of the pasting profile. During pasting, the starch suspensions were heated from 50 to 90 °C at a rate of 4 °C/min and held at 90 °C for various periods before sampling. Samples were taken at: (a) 3.75 min pasting at 65 °C or (a') 7.5 min at 80 °C prior to the first viscosity peak; (b) 10 min pasting just after the first viscosity peak; (c)

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0038-9056/2003/0202-0072 $17.50+.50/0

22.5 min pasting in the trough between the two viscosity peaks; (d) 34 min pasting just after the second viscosity peak; (e) 50 min pasting after a decrease in viscosity (set back) had occurred after the second peak and (f) 95 min pasting.

2.2 Analyses

2.2.1 Light microscopy

A 50% glycerol-distilled water solution at ambient temperature was added to freshly pasted starch samples (a-f), and gently mixed to give a 1% starch solution/dispersion. Glycerol was added to help prevent sample drying during light microscopy. Prior to slide preparation, 15 mL 0.03 M I_2-KI solution was added to 100 mL of the starch preparation. Polarised light was used to detect birefringence in granules.

2.2.2 Scanning electron microscopy of starch suspensions/solutions

Fresh starch samples (a-f) were made up with distilled water to a 1% (w/w) starch suspension/solution. A drop (~ 5 μL) of the preparation was spread on a polished carbon planchette (5 mm diameter and 1 mm thick) and immediately plunge frozen in propane at –180 °C with the aid of a Reichert KF80 plunge freezer (Vienna, Austria). The samples were then transferred under liquid nitrogen to a freeze drier and dried at –85 °C and a vacuum of 10^{-6} Torr (7.502×10^{-3} Pa) for 48 h. The stage with samples was brought to room temperature before removing from the vacuum. After freeze drying, the planchettes were sputter coated with gold using a Polaron E5200 sputter coater (Walford, UK) and examined using a JEOL JSM-840 scanning electron microscope (Tokyo, Japan) operated at 5 kV.

2.2.3 Scanning electron microscopy of starch gels

Fresh samples (a-f) were left to cool and gel at ~25 °C for 2 h. A sliver was cut from the gel with a new, double edged razor blade and mounted onto a carbon planchette prior to rapid plunge freezing in propane as described. The samples were further prepared as for the starch suspensions/solutions.

2.2.4 HPSEC of solubilised starch

Fresh samples (a-f) were made up with a hot (65 °C) 0.02% aqueous NaN_3 solution (pH ~7.0) to give a 1% starch suspension, which was mixed thoroughly by inverting the sealed container 100 times. Starch suspensions were then centrifuged at 2000 × *g* for 5 min at 20 °C before filtering the supernatant through pre-filters (Macherey-Nagel MN 85/90; cat. 150597/0, Düren, Germany) and 1.2 μm AcetatePlus membrane filters (Osmonics Inc.; cat. A12SP02500, Minnetonka, USA). The amount of carbohydrate in the filtrate was determined by the phenol-sulfuric acid method [9]. The starch solution filtrate (200 μL) was injected into two TosoHaas HPLC columns G5000PW_{XL} and G6000PW_{XL} (TosoHaas, Tokyo, Japan) connected in series. In addition, a TSK PW_{XL} guard column was used. The columns were maintained at 60 °C. Mobile phase (0.02% aqueous NaN_3 buffer solution) was pumped with a HPLC pump (Waters model 501; Millipore Corp., Milford, USA) operating at 0.6 mL/min. The columns were connected to a Waters Associates model 401 refractive index detector set at sensitivity 8. Data were collected and peaks were integrated using Apex version 2.14 Software (Autochrom Incorporated, Milford, USA). Elution times of clearly defined peaks were used as guidelines to identify areas under broad peaks. Pullulan Molecular Weight (MW) standards (853 000 to 5300 Da) from Showa Denko K.K. (Tokyo, Japan) were dissolved in the 0.02% NaN_3 buffer and injected into the HPLC-SEC system. A standard curve was constructed using Microsoft Excel software relating retention time and $\log_{10}$ MW. The standard curve was used to calculate the apparent MW of starch molecules represented by peaks. Apparent MW was used to identify molecules on the HPSEC system according to accepted methods [8]. Ethylene glycol (~2.5%) was used as marker. Percentages of amylose, amylopectin and dextrin molecules were calculated as relative areas under peaks with boundaries defined by appropriate elution times.

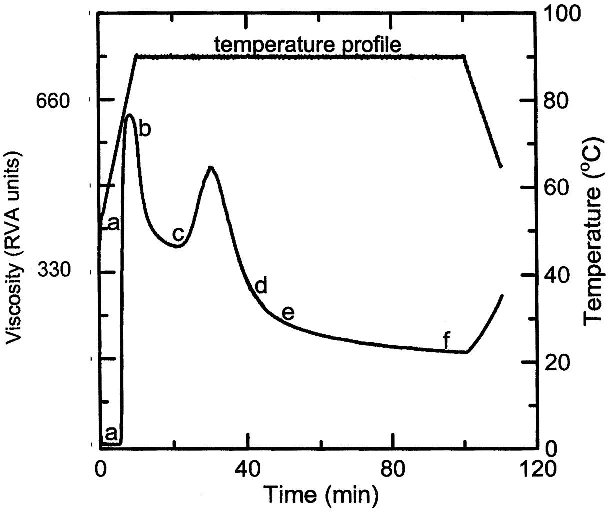

Fig. 1. Biphasic pasting curve of BDH maize starch with approximate sampling times/temperatures: a and a' – sampling prior to first viscosity peak; b – sampling just after first viscosity at 10 min; c – sampling at trough between viscosity peaks at 22.5 min; d – sampling just after second viscosity peak at 34 min; e – sampling at 50 min; f – sampling at 95 min.

2.2.5 Statistics

Analysis of variance and post-hoc comparisons of means with LSD were performed using Statistica software ’97 (StatSoft Inc., Tulsa, USA).

3 Results and Discussion

3.1 Biphasic maize starch pasting curve

Fig. 1 shows the biphasic pasting curve of the BDH maize starch, as measured with the RVA instrument. Similar curves have been obtained with a range of different maize starches, although the time and relative size of the second peak varies slightly [1]. The approximate sampling times/temperatures are indicated in Fig. 1.

3.2 Microscopy of starch granules in suspensions

Figs. 2 and 3 show SEM and light micrographs, respectively, of diluted starch suspensions taken at the various intervals during pasting. Raw maize starch granules had a mean diameter of approximately 12 µm (Fig. 2a(i)). At 3 min 45 s (8 RVA units; 65 °C) (Fig. 2a(ii)) the starch granules were slightly swollen and showed no evidence of component leaching. At 6 min 20 s (410 RVA units; 80 °C), just before the first viscosity peak, the starch granules were swollen, intact (Fig. 3a) and very few granules showed birefringence under polarised light. Their mean diameter was approximately 30 µm. This significant swelling (from 12 to 30 µm) of the granules, together with leaching of molecules from the granules is implicated as the mechanism responsible for the increase in viscosity observed during the first viscosity peak of starch gelatinisation [2-5].

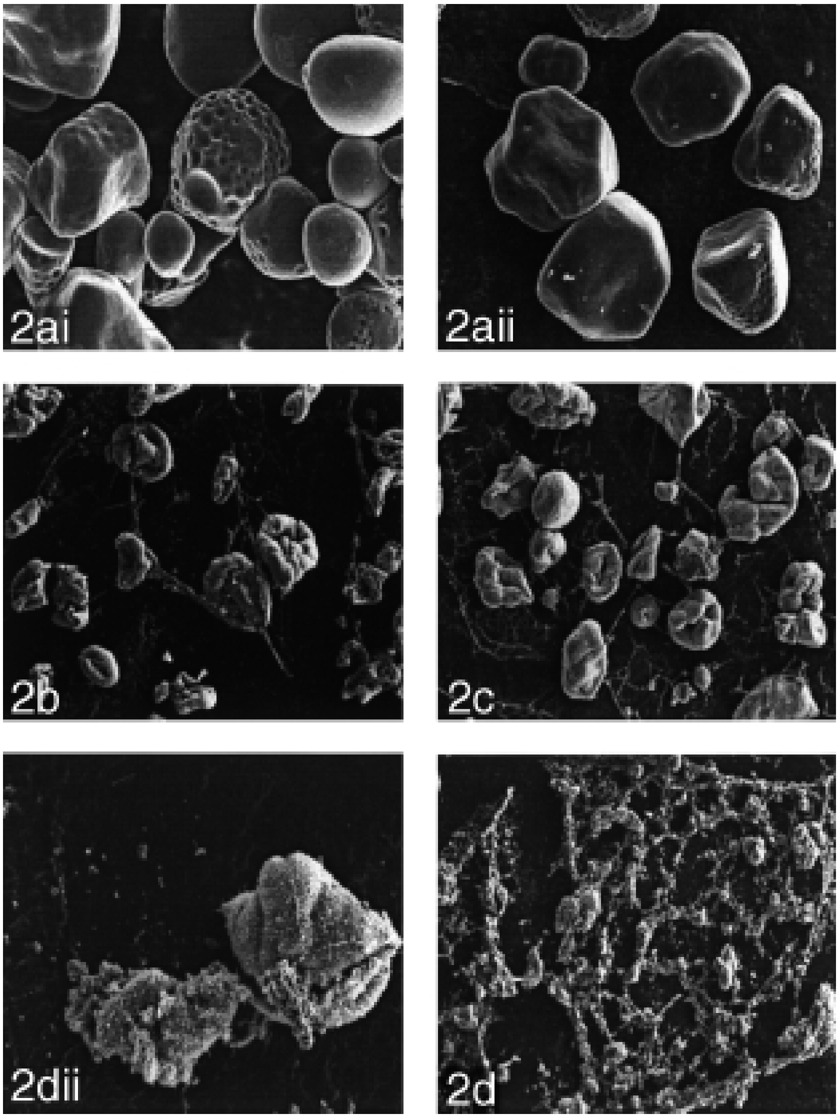

Fig. 2. Scanning electron micrographs of intact and disrupted maize starch granules prepared from starch slurries/solutions pasted for different time periods: 2a(i) – raw starch, × 2500; 2a(ii) – pasted for 3 min 45 s, × 2500; 2b – pasted for 10 min, × 500; 2c – pasted for 22.5 min, × 500; 2d(ii) – pasted for 34 min, × 2500; 2d – pasted for 95 min, × 500.

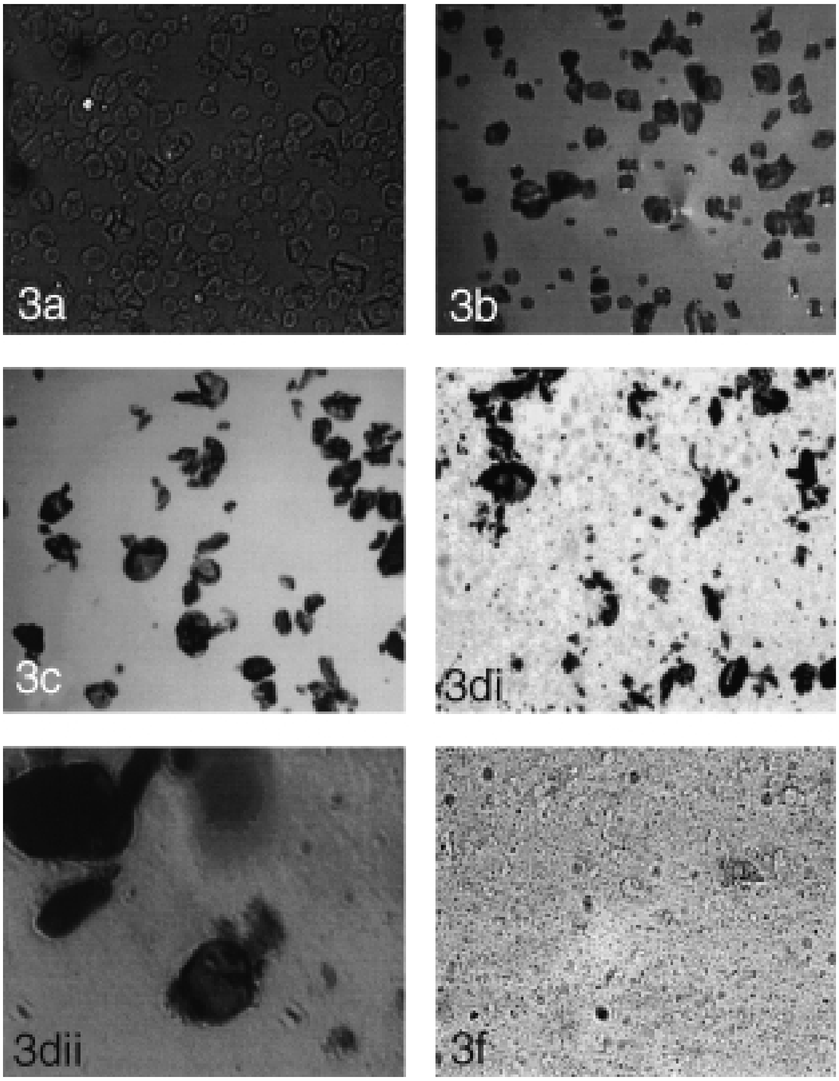

Fig. 3. Light micrographs of maize starch granule integrity prepared from starch slurries/solutions pasted for different time periods: 3a (polarised light – pasted for 6 min 2 s from 50 – 80 °C, × 100; 3b (polarised light) – pasted for 10 min, × 100; 3c (polarised light) – pasted for 22.5 min, × 100; 3d(i) – pasted for 34 min, × 100; 3d(ii) (contrast enhanced with green filter) – pasted for 34 min, × 400; 3f – pasted for 95 min, × 100.

At 10 min (520 RVA units; 90 °C) just after the first viscosity peak, the starch granules were also swollen, intact but with some evidence of leaching (Figs. 2b and 3b). Their mean diameter was approximately 29 µm. The subsequent breakdown in viscosity was attributed to disintegration of the starch granules [3, 4], and also to the alignment of amylose molecules in the direction of stirring [10]. At 22.5 min (365 RVA units; 90 °C) the trough between the two viscosity peaks, the starch granules were also still intact (Fig. 2c and 3c) but showed no birefringence (not shown). The mean diameter of these granules was 29 µm. At 38 min (455 RVA units; 90 °C) directly after the second viscosity peak, the starch granules were burst open and surrounded by granule fragments (Fig. 2d, 2d(ii) and 3d(i)). The mean diameter of the granule fragments was 10 µm. Some granules were observed to be leaching their contents into solution (Fig. 3d(ii)). It appears that the second viscosity peak coincides with the breaking down of granule structure. At 95 min (190 RVA units; 90 °C) the starch was mostly in suspension with some ghost granule structures evident (Fig. 3f). The mean diameter of the ghost granule structures was 30 µm.

3.3 Microscopy of starch granules in gels

Starch gels were prepared to obtain a more detailed picture (without possible artefacts caused by dilution) of what the pasted starch system looked like. Fig. 4 shows SEM of starch gels produced from the samples taken during pasting. It was found that starch granules in the gels from samples pasted for 6 min 20 s (410 RVA units; 80 °C) (where pasting was interrupted prior to the first viscosity peak) were intact and that leaching of a component (probably amylose) could be seen as a network surrounding the round, swollen granules (Fig. 4a). The starch granules in the gels from samples pasted for 10 min (520 RVA units; 90 °C), where pasting was interrupted just after the first viscosity peak, were also intact and swollen,

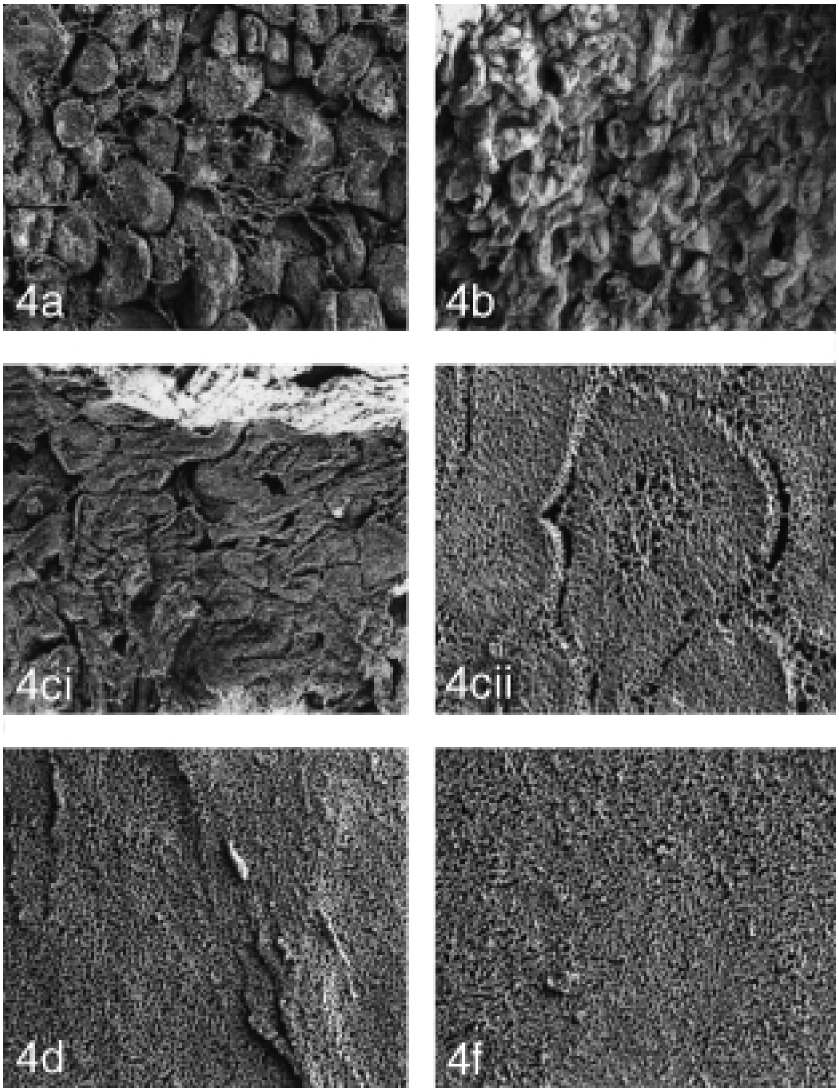

Fig. 4. Scanning electron micrographs of gels of maize starch pasted for different time periods: 4a – pasted for 6 min 2 s from 50 –80 °C, × 1200; 4b – pasted for 10 min, × 500; 4c(i) – pasted for 22.5 min, × 1600; 4c(ii) – pasted for 22.5 min, × 5000; 4d – pasted for 34 min,× 5000; 4f – pasted for 95 min, × 5000.

with evidence of component leaching (Fig. 4b). The granules in the gels from samples taken at the trough between the two viscosity peaks, at 22.5 min (365 RVA units; 90 °C) were also still intact with clearly defined perimeters but were very swollen and deformed (Fig. 4c(i) and 4c(ii)). The starch granules in the gels prepared from samples taken after the second viscosity peak, however, had lost their defined perimeters and only a homogenous mass could be seen (Fig. 4d). The observed granule integrity and loss thereof in these starch gels support the theory that the second viscosity peak coincides with the loss of granule structure. It further indicates that the forces that keep granules intact, weaken greatly during the second pasting peak. It is suggested that the loss of granule integrity allows greater interaction between starch granule molecules (in suspension) during the second viscosity peak. The homogenous mass seen in gel samples from the starch paste taken after the second pasting peak remained the same on further pasting, as can be seen from the starch gel of the sample which had been pasted for 95 min (190 RVA units; 90 °C) (Fig. 4f).

3.4 Maize starch in solution during pasting

HPSEC chromatograms of starch solubilised at different times during the pasting profiles are shown in Fig. 5. Complementary data on carbohydrate solubility after the different pasting times is given in Tab. 1. As would be expected, starch solubility after heating to 65 °C was low (Tab. 1) and the chromatogram (Fig. 5, a) shows only traces of amylose and no amylopectin in solution. This confirms that gelatinisation of the maize starch had not occurred at 65 °C. The susceptibility of raw starch granules to molecular solubility in aqueous solutions is known to be low (<5%) [11]. Gelatinisation is known to increase the solubility of starch [12]. Therefore, as expected, maize starch solubility increased significantly (10 min, 90 °C) after the first viscosity peak. After gelatinisation, as shown

Tab. 1. Total carbohydrate and carbohydrate components in solution after different pasting times.

Pasting time [min] (temp. [°C]/time at temperature [min])	Total carbohydrate in solution [%]	Amylopectin in solution [%]	Amylose in solution [%]	Dextrins and simple sugars in solution [%]
3.4 (64/0)	0.5[1] ± 0.1[2] a[3]	0.0 (0.0)[4]	0.5 (100.0)	0.0 (0.0)
10.0 (90/0)	8.4 ± 1.1 b	0.0 (0.0)	8.1 (96.4)	0.3 (3.6)
22.5 (90/12.5)	15.1 ± 1.4 d	1.5 (9.9)	13.1 (86.8)	0.5 (3.3)
34.0 (90/24.0)	12.3 ± 1.7 c	1.9 (15.4)	9.7 (78.9)	0.7 (5.7)
50.0 (90/40.0)	8.6 ± 1.5 b	2.0 (23.3)	6.1 (70.9)	0.5 (5.8)
95.0 (90/85.0)	19.8 ± 1.5 e	5.6 (28.3)	12.7 (64.1)	1.5 (7.6)

[1] Mean of replicates.

[2] Standard deviation.

[3] Mean values with the same letters do not differ significantly from each other (p>0.05).

[4] Values in brackets are the relative percentage of component in solution.

by a first peak on the RVA (Fig. 1, b), 8.4% carbohydrate was in solution (Tab. 1), of this 96% was amylose with the remainder being dextrins according to apparent MW (Fig. 5, b). These findings agree with the general knowledge that amylose is the first molecule to go into solution during the heating of starch in water [10, 13]. After a pasting period of 22.5 min at 90 °C; the time of the trough between the two pasting peaks (Fig. 1, c); a significant increase in starch solubilisation was observed (Fig. 5, c) as could be expected with additional heat and mechanical (i.e. shear) energy input. At this stage (c), 15.1% of the carbohydrate was in solution (Tab. 1). At point c, only 1% of the amylopectin and 10% of the total carbohydrate was in solution. It would seem that amylopectin only started going into solution after the first viscosity peak formed. It is known that the amount of solubilised starch increase during cooking over time (due to additional heat energy) under low-shear conditions of stirring (due to additional mechanical energy) of a viscograph-type instrument [11]. *Jackson* et al. [8] found that increasing mechanical energy, disrupted granule integrity further, resulting in more soluble amylose and amylopectin.

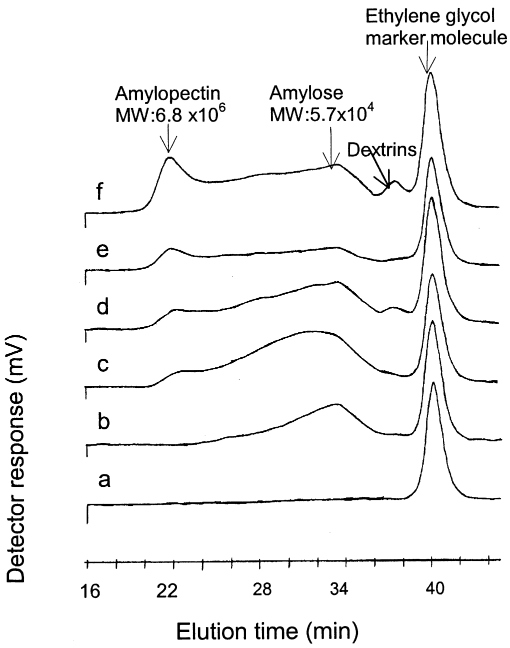

Fig. 5. High performance size-exclusion chromatography profiles of soluble carbohydrate from maize starch pasted for different time periods (a – pasted for 3 min 45 s, b – pasted for 10 min, c – pasted for 22.5 min, d – pasted for 34 min, e – pasted for 50 min, f – pasted for 95 min).

An unexpected, small, but significant reduction in carbohydrate solubility was observed after a total pasting period of 34 min at 90 °C holding temperature, the time of maximum viscosity development of the second pasting peak (Fig. 1,d). Only 9.7% of the carbohydrate in solution was amylose, whereas amylopectin represented 1.9% and dextrins represented 0.7% of the carbohydrate in solution. A further decrease in carbohydrate solubility was found after a pasting period of 50 min, the time of viscosity break down of the second viscosity peak (Fig. 1, e). Only 6.1% of the carbohydrate in solution was amylose, whereas amylopectin represented 2.0% and dextrins represented 0.5% of the carbohydrate in solution. The decrease of amylose in solution (Tab. 1) seemed to be mainly responsible for the decrease in carbohydrate in solution during the second pasting peak, as there was a steady increase in amylopectin in solution from sampling point c onwards (Tab. 1) and dextrins in solution remained relatively constant as the pasting times increased with a significant increase only at the longest pasting time. At pasting times of 34 and 50 min, the decrease of amylose in solution could be due to complexing of amylose molecules, either with another substance such as lipid or with themselves (retrogradation of amylose). The most common associations of starch in foods are: retrogradation (amylose-amylose), recrystallisation (amylopectin-amylopectin – not relevant in this case) and formation of lipid-amylose complexes [14]. The reassociation of starch that can occur after gelatinisation is known to decrease the potential for molecular solubilisation or dispersion of starch [15]. *Slade* and *Levine* [16] reported that the crystallisation of amylose-lipid complexes is favoured over amylose retrogradation. Also it seems unlikely that amylose retrogradation would occur at a holding temperature of 90 °C. *Nelles* et al. [1] had shown the presence of starch-lipid complexes during the formation of the second pasting peak.

Amylose complexes with most lipids are insoluble in water [17]. This is supportive of the presented results. Amylose-lipid complexes can be identified by means of their X-ray diffraction patterns. Type I complexes can be annealed into a semi-crystalline form (type II) [17]. Type II complexes and their "superstructures" [18] were originally found in starches after gelatinisation [19]. The superstructures of amylose-lipid complexes refer to the degree of crystallinity of the complex [18]. It is suggested that the decrease in soluble starch during the second pasting peak is due to the formation of type II amylose-lipid complexes and their superstructures [18], which are formed due to crystal lattice intermolecular forces. It is further suggested that the formation of type II amylose-lipid complexes and their superstructures are in fact the cause of viscosity development in the second viscosity peak in maize starch pasting. Amylose-lipid complexes are relatively large structures [14, 20], that have greater potential for entanglement during starch pasting and therefore viscosity is increased. The subsequent breakdown in viscosity is attributed to a breakdown of amylose-lipid complex superstructures due to a net shear thinning force, even as complexing of amylose seems to continue as seen by a further decrease in amylose in solution (Tab. 1). It is suggested that the alignment of molecules in the direction of stirring breaks the crystal lattice intermolecular forces supporting these complexes and their superstructures [18]. Eventually, the complexes seem to be broken down, as amylose in solution increases after a total pasting period of 95 min (Tab. 1).

4 Conclusions

During starch pasting, maize starch granules lose their integrity (i.e. break up) during the formation of the second viscosity peak. This could potentially allow greater interaction between starch molecules in solution. Starch solubilisation is not directly related to pasting time. The second pasting peak coincides with a decrease in starch in solution. This reduction of starch in solution is due to the amylose component of the starch. This supports the theory that the second viscosity peak is formed due to the formation and break down of type II amylose-lipid complexes and their superstructures.

Acknowledgements

We are particularly grateful to Dr. *Alistair Wight* for his technical input and useful discussions throughout the conduct of this work.

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(Received: January 21, 2002)

(Revision received: September 6, 2002)