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Simultaneous determinations of the molecular weight distributions of amyloses and the fine structures of amylopectins of native starches

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Abstract

Native A (wheat and waxy rice), B (potato), and C (cassava and sweet potato) types of starches were each debranched with isoamylase, and separated into amylose and amylopectin fractions by HPLC on size exclusion columns coupled on-line to multi-angle-laser-light-scattering and differential refractometer detectors. The absolute molecular weights of amyloses and chain length distributions of amylopectins were determined simultaneously, and pre-isolation of the amylopectin was not necessary. The molecular weights of debranched amylose from starches that have not been fractionated to separate amylose and amylopectin are significantly higher than published values for the undebranched fractionated amylose. The polymodal profiles of the refractive index chromatograms showed the complexity of the amylopectin structure of starches. The chain length distribution of amylopectin depends critically on the method for analysing the broad chromatogram when determined by either noting the minima/inflections or deconvoluting the overlapping amylopectin fraction into numerous normal/Gaussian distributions. Although the results from the former (conventional) method of analysis were comparable with the literature values, they did not appear to be as sensitive a technique for detecting differences as the multiple Gaussian approach. Overall, the study suggested that the amylopectin chain units might be more complex than originally envisaged and that different degrees of chain packing for the molecules can be inferred from this multiple component analysis.

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1. Introduction

Pre-separation of starch.—The molecular weights of amylose (AM) and amylopectin (AP) depend on the botanical origin of the starch, the method of isolation employed for AP or AM from the starch granule, and the technique used to determine the molecular weight, e.g., ultracentrifugation, light scattering [1] or chemical methods [2]. Klingler and Zimbalski [3] showed that the various methods for molecular weight determination almost invariably were associated with the incomplete isolation of starch components, which naturally invalidated their results. The fractions isolated by different methods were found to be contaminated by AP and/or the sub-fraction of AM. The classic method of separating AM and AP is by selective precipitation, followed by six or more recrystallization steps of AM as an inclusion complex with 1-butanol or other small guest molecules, and finally by ultracentrifugation to remove traces of AP [4]. Concanavalin A has been used as a precipitant for AP [5], but this method has not been widely tested [6]. Separation of starch by such methods is very often susceptible to contamination by the intermediate and/or the anomalous AM or AP components. In fact, complete isolation of AM and AP does not seem to be possible [3]. This study reports the simultaneous separation and characterization of AM and AP by an HPLC (size exclusion) system.

Fine structure of amylopectin.—Enzymic hydrolysis followed by size exclusion chromatography has proved a valuable method for investigating the fine structure of AP [7]. The debranching enzymes, isoamylase and/or pullulanase have been very widely used for such structural analyses. The debranching enzymes specifically hydrolyse the α-(1 $\rightarrow$ 6)-D-glycosidic inter-chain linkages, but have no action on the major α-(1 $\rightarrow$ 4)-D-glycosidic linkages. The concentration and the purity of these enzymes, the debranching temperature, and the pH are all critical factors in ensuring rapid and complete debranching. Contamination with any alpha- or beta-amylase or the presence of the residual amylases in the native starch will inevitably give spurious results.

The debranched components of starch can be determined by chemical or physical means based on the glucose residues or the molecular weights. The total sugar content has often been measured by the phenol–sulfuric acid method [8], and the total reducing end groups by the modified Park–Johnson method [9]. These chemical assays can be very laborious, time-consuming, and susceptible to errors arising from contamination [10,11]. There is also considerable concern over hazards associated with some of the chemicals used. A more advanced and efficient technique is to use a molecular weight detector, namely low- or multi-angle laser-light-scattering and concentration (RI) detectors coupled on-line downstream from the HPLC columns [12–14]. The use of such a technique in the analysis of starch was introduced by Lee [15], but was subsequently refined by Takagi and Hizukuri [16] who used a series of PW (polymer/water) aqueous HPLC columns to determine the molecular weight of lily AM. A low-angle (5°) laser-light-scattering detector was used, since AM has a high molecular weight

(e.g., 10^5–10^6). The molecular weights of other AM samples were subsequently reported by Takeda et al. [17] and Asaoka et al. [18].

Hizukuri [19] applied the same technique, but with different HPLC columns, to determine the weight-average chain lengths of 17 AP specimens debranched by isoamylase. Corn, rice, and potato products were reported to show a bimodal distribution, whereas those from wheat, tapioca, and tulip gave a trimodal distribution. With different types of columns (SW, silicon/water, rather than PW), Hizukuri [20] showed a polymodal distribution for AP specimens from waxy rice, potato, tapioca, and kuzu. The chromatogram was divided into five fractions (i.e., A, B1–B4) at the minima, with A and B4 chains being the shortest and longest chains, respectively [20]. In his study, A and B1 chains were grouped into a single cluster, and B2 and B3 into two and three separate clusters. Based on this method of analysis, Hizukuri and co-workers [20,21] also evaluated the relationship between the A, B, and C types of starches with chain lengths of AP. Hizukuri and Maehara [22] further proposed categorization into Ba and Bb chains, according to whether the A chains were bound (Ba) or not bound (Bb) to the B chain.

In most of these studies of the AM or AP of the non-waxy starches, the AM was first isolated by the method of Lansky [23] or as modified by Takeda [17], and the AP was separated from AM by a series of alcohol precipitations [19,24]. A trimodal distribution of chain lengths of barley AP was reported earlier, following the analysis of fractions eluted from a Bio-Gel P-6 conventional GPC system at 70°C [25]. In fact, Hizukuri [19,20] has demonstrated that the bimodal or polymodal profiles of AP can result from the use of different types of columns. Even with the same HPLC method, the use of different sample preparations, column eluent, or temperature and various types of HPLC column (aqueous or organic) and packing material (PW or SW) may lead to different findings [19,26–28].

The broad profiles of the AP fraction point to the presence of various chain lengths of AP. This chain population of AP has long been construed as consisting of the long and short chains ($\overline{dp}$ ca. 50 and 20, respectively). However, Hizukuri [20] proposed a refined structure with more than four populations of chain lengths in the clusters of chains. Other researchers, e.g., Yuan and co-workers [28], divided the AP chromatogram into three fractions with low, intermediate, and higher molecular weights ($\overline{dp}$ ca. 10, 20, and 50). The chain distributions of AP from these studies were mainly derived by dividing at the minima/inflections in the chromatogram. Recently, it has been suggested that these chromatograms may be deconvoluted into a number of components each of which may be described as a normal (Gaussian) distribution [6]. This seems appropriate as the AP chains are biological derivatives. Although this approach is new to the structural analysis of starch, it has been applied widely in studies of other polysaccharides, e.g., pectins, alginate, and gums [29–31]. These studies demonstrated that a single but broad chromatogram from a high-pressure size-exclusion system consisted of at least five components and that the number of components increases with the multiple modal distributions.

This paper, therefore, records measurements of the absolute molecular weight distribution (MWD) of AM and the chain length distribution of AP simultane-

ously, i.e., without prior isolation of AM and AP from the starches. The absolute MWD of AM was determined directly by the light-scattering detector and the chain length distribution of AP was relative to the molecular standards detected by the refractive index detector. The unresolved AP peaks on the chromatogram were analysed by the conventional division analysis, and the results were compared with those obtained from a multiple Gaussian curve-fitting program.

2. Experimental

Materials.—Unmodified wheat and potato native starches were purchased from the Sigma Chemical Co., St. Louis, USA. Waxy rice and cassava starches were obtained from Cairn Foods, Chesham, UK, and Ajinomoto, Malaysia, respectively. The sweet potato starch, which originated from Peru, was kindly provided by Dr. J.E. Rickard (Natural Resources Institute, Chatham, UK). Crystalline isoamylase (glycogen 6-glucanohydrolase, EC 3.2.1.68, isolated from *Pseudomonas amylodermosa*) was purchased from the Hayashibara Biochemical Laboratory Inc., Japan. Mono-disperse pullulan standards included: P800 (853 000; 1.14), P400 (380 000; 1.12), P200 (186 000; 1.13), P100 (100 000; 1.10), P50 (48 000; 1.09), P20 (23 700; 1.07), P10 (12 200; 1.06), P5 (5 800; 1.07) (figures in parenthesis correspond to the values of average molecular weight and polydispersity, respectively, supplied by Polymer Laboratories, UK). Sugar standards were purchased from the Sigma Chemical Co.: maltoheptaose (molecular weight: 1153), maltopentaose (828), maltotriose (504), maltose · H_2O (360), and D-glucose (180). Ethanediol (molecular weight: 62) was obtained from Fisons Scientific Equipment, Loughborough, UK. All other chemicals used were of Analar grade.

Methodology.—A schematic diagram of the GPC-MALLS-RI (GPC with a multi-angle laser-light-scattering detector and a differential refractometer) system is shown in Fig. 1 and a brief outline of the theory was given by Wyatt [14]. Starch (75 mg) was heated in water (3710 μL) for 6 minutes and cooled. Acetate buffer (1 M, 250 μL, pH 3.5) and isoamylase (55 μL) were added and incubated for 2.5 h at 45°C. The concentration of the debranched sample was adjusted by using sodium phosphate buffer with 0.1% sodium azide (0.5 M, 1000 μL) and the sample was again heated for 3 min before centrifugation. The sample solution of 100 μL was filtered through a 0.45-μm syringe filter (Whatman, 13 mm, PVDF) and injected into a sample loop (100 μL) via a rheodyne injection valve (Rheodyne 7125) fixed on the column oven (Anachem, Luton, UK). The sample with eluent was then pumped (Waters 590 HPLC pump) into a guard column (TSK GEL PWXL: 6 × 40 mm) followed by 5 main columns (TSK GEL G3000 PWXL, Asahipak GS-320H (2 ×), TSK G2500 PWXL, and G-Oligo PWXL) connected in that order, in the column oven at 40°C. [All the TSK GEL main columns (7.8 × 300 mm) were supplied by Anachem, UK, whilst the two Asahipak columns (7.8 × 250 mm) were purchased from Rhône-Poulenc, UK.] The eluate was first examined with a Dawn F multi-angle laser photometer (Wyatt Technology Inc., Santa Barbara, USA), with a He–Ne laser operating at 632 nm and equipped with 15 detectors at angles

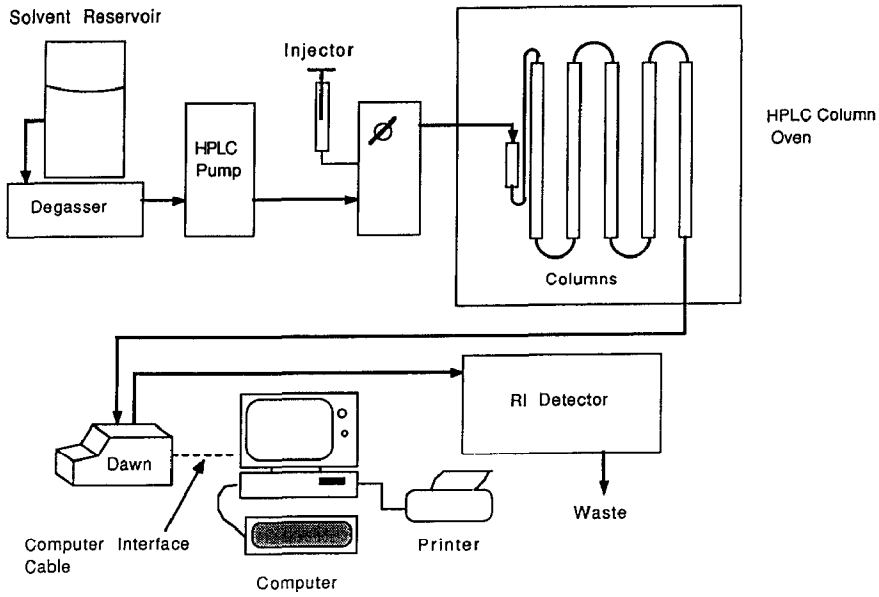

Fig. 1. The components of the GPC-MALLS-RI system (Dawn is the multiple laser photometer).

ranging from 22 to 160°, and subsequently (on-line) by an RI concentration detector (Waters 410 differential refractometer with the RI increment, $dn/dc = 0.15$ mL/g [1]). The responses from the detectors were transmitted to a personal computer, and the light-scattering and RI chromatograms were displayed on the screen during fractionation. Because of the high dilution during elution before entering the light-scattering cell (≤ 0.1 mg/mL), correction for non-ideality effects was deemed not necessary, and A_2 was taken as ca. 0 (see Horton et al. [32]). The column eluent, composed of Na_2HPO_4 (0.1 M), NaH_2PO_4 (0.05 M), and NaN_3 (0.02%) at pH 8.6, was filtered under vacuum through a 0.2-μm nylon membrane filter (Gelman Sciences Inc., 47 mm) in a glass Millipore solvent filtration system, and degassed with an on-line degasser (Degasys, DG-1200, uniflow from HPLC Technology Co., UK) in the GPC-MALLS-RI system. The pump flowrate was set at 0.5 mL/min. Two complete digestions were carried out for each starch sample.

Data analysis.—The absolute MWD of AM was measured by means of laser light scattering and the results were analysed by the ASTRA 2.04 and EASI 6 Wyatt Technology software, whereas the chain population of the AP fraction, i.e., the lower molecular weight compounds, was obtained via a calibration curve constructed from the pullulan and sugar standards. The unresolved AP peaks were analysed by two methods. In the first method, the chromatogram was divided visually into divisions at chosen points (shoulders and minima) of the GPC profile, while in the second, a sum of several Gaussian components was fitted to the data by using a multiple Gaussian fitting program.

Before analysis, the AP region in the chromatogram was selected, to establish a baseline (which was then subtracted from all the points in the profile); subsequently, any doubtful regions or obvious artifacts were excluded. Since the elution volume of the component was proportional to the negative log of the molecular weight, the refractive index (I_r) for the region could be fitted to a function of the form:

$$I_r(V_e) = \sum_{i=i}^{N} H_i \exp - \left(\frac{V_e - P_i}{W_i} \right)^2 \quad (1)$$

Elution volume (V_e), rather than mass, was used as the independent variable, both in order to accommodate the wide range of masses found in each sample, and to obtain simple expressions for the $\overline{dp}_n$ and $\overline{dp}_w$. In the conventional division method, all integrations were performed (between appropriate limits) according to Simpson's rule and after the limits had been chosen by the user.

For the second technique, the number of Gaussian components to be fitted (N) was chosen by the user of the program, according to the appearance of the chromatogram. The initial estimates for the peak positions (P_i) could be assigned; otherwise evenly spaced default values were assumed by the program. The estimated widths of all the peaks (W_i) were set to be equal initially. Fitting was then carried out according to the Levenberg–Marquadt nonlinear, least-squares algorithm provided by "Numerical Recipes in Pascal" [33]. The program stopped when chi-squared had fallen by less than a given fraction (usually 0.1%) after several successive iterations (usually ten). Fitting was repeated for a lower number of peaks and the optimum number was derived from the *minimum* number of peaks for a satisfactory fit. Only the exact fit is given in the result.

The heights (H_i), peak positions (P_i), and widths (W_i) of the N peaks were reported (together with their uncertainties). The area of each peak was given as

$$A_i = \int_{-\infty}^{\infty} H_i \exp - \left(\frac{V_e - P_i}{W_i} \right)^2 dV_e = \pi^{1/2} H_i W_i \quad (2)$$

Fractional areas were obtained by dividing each of these by their sum. Finally, the average dp was obtained from the fitted parameters:

$$\overline{M}_{ni} = \frac{\int_{-\infty}^{\infty} I_r(V_e)\,dV_e}{\int_{-\infty}^{\infty} \frac{I_r(V_e)}{m(V_e)}\,dV_e} = \exp(\delta - \gamma P_i) \cdot \exp - \left((1/2)\gamma W_i\right)^2 \quad (3)$$

$$\overline{M}_{wi} = \frac{\int_{-\infty}^{\infty} I_r(V_e) m(V_e)\,dV_e}{\int_{-\infty}^{\infty} I_r(V_e)\,dV_e} = \exp(\delta - \gamma P_i) \cdot \exp\left((1/2)\gamma W_i\right)^2 \quad (4)$$

The values of α and β were derived from the slope and intercept of the

calibration line obtained using the pullulan and sugar standards; the values used in this study were: $\alpha = 0.123$ and $\beta = 7.658$.

3. Results and discussions

X-ray diffraction patterns.—The X-ray diffractograms showing the A, B, C_A, and C_B patterns of the starch samples are shown in Fig. 2.

Amylose fraction.—The light-scattering chromatogram in Fig. 3 and the first peak of the RI chromatograms in Fig. 4 were both referred to as the AM fraction from the debranched starches. The results in Table 1 show that the MWD of the AM depends on the starches; potato has the highest, whereas the waxy rice (which has only ca. 1% AM content) has the lowest, molecular weights. The $\overline{dp}$ values of AM from this study are compared with those of Takeda et al. in Table 2. It is noted that the molecular weights determined from the debranched starch without pre-separation of AM and AP in this study were higher than the literature values obtained from undebranched starch with pre-separation of AM and AP (although, as far as we are aware, the absolute values for the nonisolated AM, for example, by the light-scattering technique, are not available in the literature). This difference may suggest either that higher molecular weight material could be lost during pre-isolation of AM and AP from native starch, as reported in the literature, or that the measurement of only the linear AM chains from the debranched starch may inevitably increase the average molecular weight of AM, as compared to the average value of the branch and linear AM.

The polydispersities ($\overline{M}_w/\overline{M}_n$) of 2.6–6.9 given in Table 1 indicate the broad distribution of the AM components. The wheat, waxy rice, and potato specimens have an average polydispersity of 6.3, 6.6, and 6.9, respectively, while the polydispersities for cassava and sweet potato were 4.6 and 2.6, respectively. Comparison with the literature values has proved difficult since the molecular weights of AM determined by the light-scattering method have been confined so far to the AM isolated and purified from starches, and not on the debranched starches. Even with the same method of isolation and determination, a wide variation (polydispersities of 3.1–8.4) between the same starch (rice) has been reported by Asaoka et al. [18]. With a similar technique, Takeda et al. [17] reported lower polydispersities ranging from 1.3–3.4 for AM isolated from a variety of starches.

The results so far suggest either that the AM components in the natural form (intact molecule) are inherently heterogeneous with a high polydispersity, or that the high $\overline{M}_w$ observed in the study and its impact on the $\overline{M}_w/\overline{M}_n$ ratio is due to some experimental artefact. It is possible that the molecular weight of the long linear chain of AM was measured because the starch had been debranched. Further research is required to investigate whether the MWD of AM is best determined from the whole or debranched starch with or without preseparation of AM and AP.

Chain profiles of amylopectin.—The AP profiles of the starches debranched with isoamylase possessed characteristic RI-chromatograms which are shown in Fig. 4.

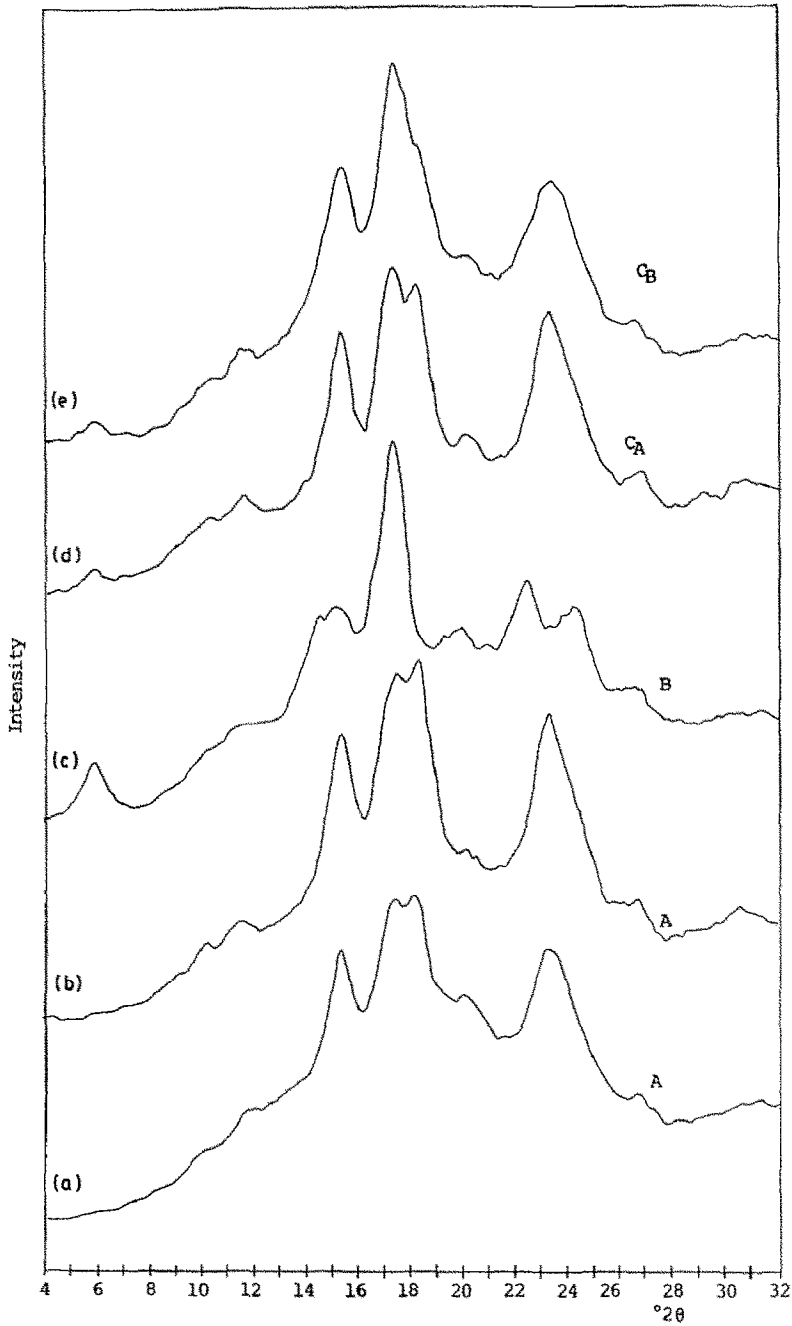

Fig. 2. Wide angle X-ray diffractograms of native starches from (a) wheat, (b) waxy rice, (c) potato, (d) cassava, and (e) sweet potato. The X-ray pattern (A, B, and C) is indicated at the right-hand side.

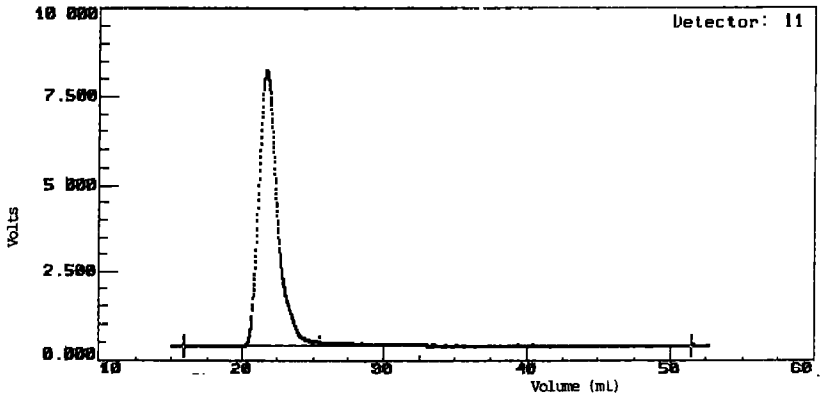

Fig. 3. An example of the light-scattering chromatogram of amylose from the GPC-MALLS-RI system.

Wheat, waxy rice, and potato gave quite different profiles, whilst cassava and sweet potato showed some similarity. The profiles clearly illustrated the polymodal chain-length distributions of the AP components, supporting the results reported by Hizukuri [20]. The wheat and waxy rice APs showed at least four distinct peaks with shoulders along the peaks. Potato, cassava, and sweet potato profiles displayed broader peaks.

As far as their super-molecular organization is concerned, wheat and waxy rice both had an A-type X-ray diffraction pattern (Fig. 2) but showed different GPC profiles (Fig. 4). There was insufficient evidence to demonstrate whether a distinctive AP profile can be associated with a specific type of starch, although the cassava and sweet potato, with C_A and C_B X-ray patterns, respectively (Fig. 2), showed some similarity in their profiles and dp distributions (Fig. 4). Potato with a B pattern was more similar to the C than the A patterns. Further research in this laboratory (to be reported elsewhere) has shown that the chain profiles of AP extracted from starches derived from the same botanical species (e.g., sweet potato, rice) can vary with the varieties and that this can be related to the functional properties of food. Each starch appears to have a specific profile due to the unique characteristic of its AP structures.

Chain length distribution of amylopectin by the conventional division analysis.—All the GPC profiles were divided at the minima/inflections into six fractions which gave four B and two A chain populations. Fig. 5 shows the division analysis for the chain length distributions of AP of wheat and potato starches, which were calibrated from the standard curve. The chain length distributions for both the number ($\overline{dp}_n$) ($\overline{dp}_w$) averages obtained from the analyses are given in Table 3. Both weight and molar (relative mole) percentages of each population of chains are given in Table 3 (the latter may be more meaningful as the values can vary with the chain lengths, especially since, from this analysis, the B chains vary from ca. 21 to 110 dp). The wheat and waxy rice (both A starches) had less long chains (B4, B3, and B2 chains) but more of the shorter (especially the A1) chains than the potato,

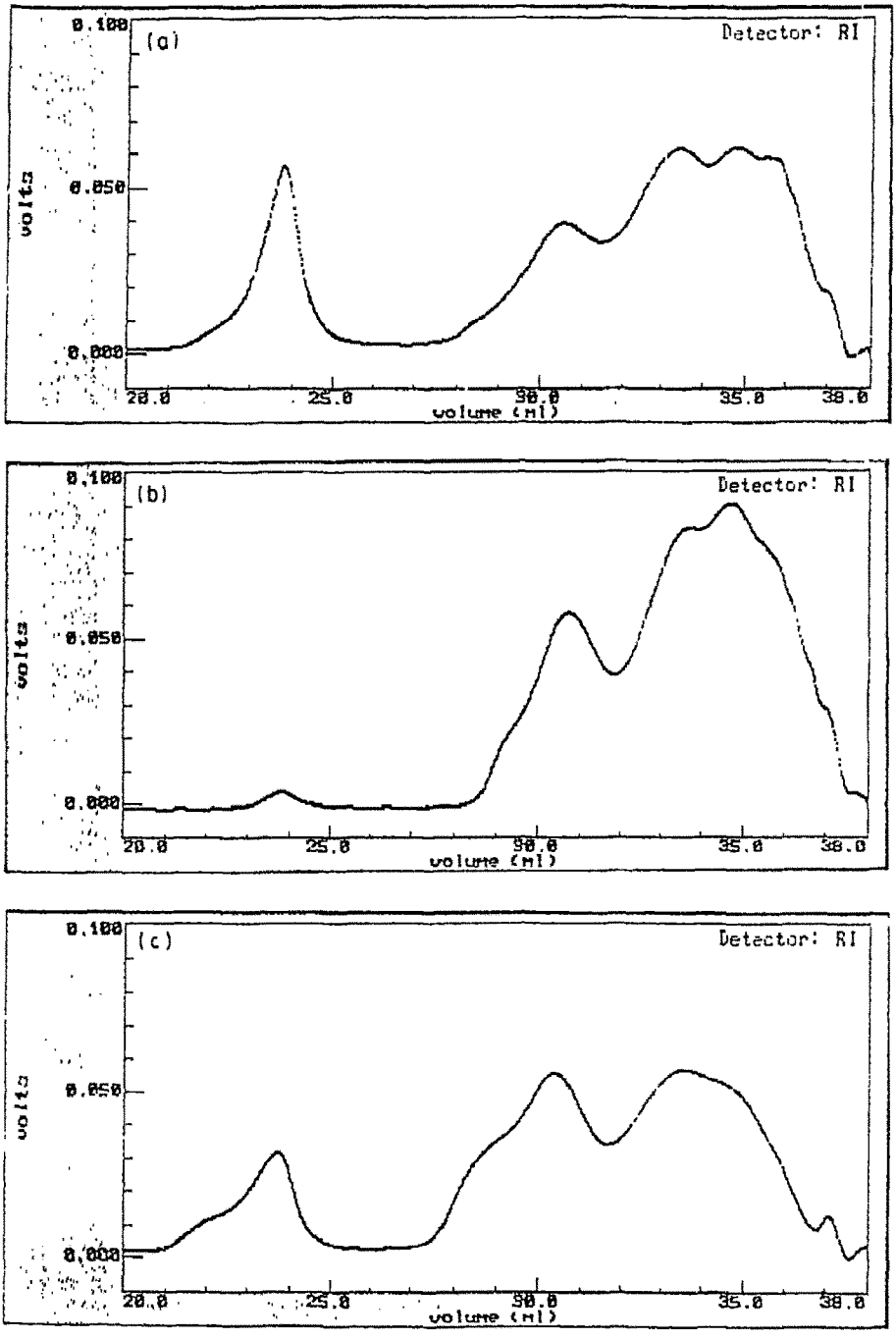

Fig. 4. RI-chromatograms showing the amylose and amylopectin fractions of the isoamylase debranched starches from (a) wheat, (b) waxy rice, (c) potato, (d) cassava, and (e) sweet potato.

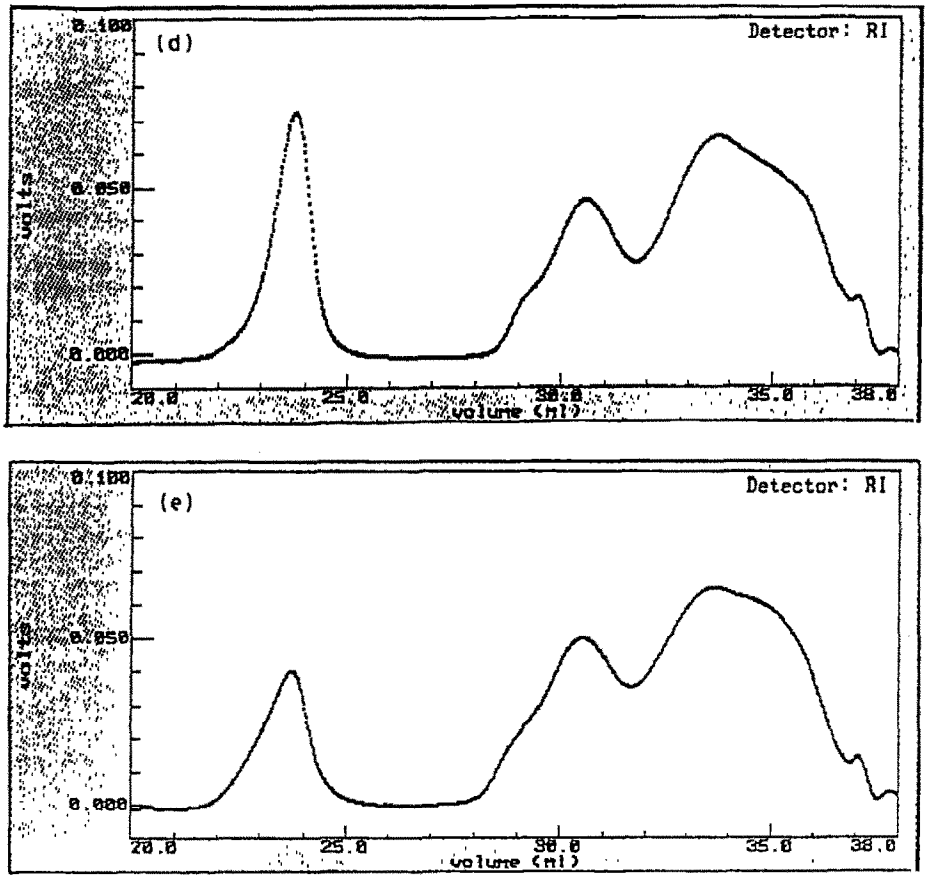

Fig. 4 (continued).

cassava, and sweet potato starches (the B and C type starches). The same findings were reported by Hizukuri [19,20]. Since B4 and B3 were present in very small amounts, the B2 chain ($\overline{dp}$ 44–49, component no. 3 in Table 3) is a good index

Table 1
Molecular weight distribution of the amylose fraction of debranched starch measured by the MALLS system

Amylose	$\overline{M}_n$	$\overline{M}_w$	$\overline{M}_w/\overline{M}_n$
Wheat	$6.2(\pm 0.8)\times 10^5$	$3.9(\pm 1.0)\times 10^6$	6.3 ± 1.0
Waxy rice	$3.5(\pm 0.7)\times 10^5$	$2.3(\pm 0.9)\times 10^6$	6.6 ± 1.0
Potato	$13.0(\pm 1.0)\times 10^5$	$9.0(\pm 0.8)\times 10^6$	6.9 ± 0.1
Cassava	$5.9(\pm 0.2)\times 10^5$	$2.7(\pm 0.5)\times 10^6$	4.6 ± 0.7
Sweet potato	$4.9(\pm 0.03)\times 10^5$	$1.3(\pm 0.05)\times 10^6$	2.6 ± 0.1

Table 2
Comparison of $\overline{dp}$ of amyloses obtained with and without pre-isolation $\overline{Dp}_n$ of amylose of starches

	No isolation (This study)	Pre-isolation [17] (Takeda and co-workers)
Wheat	3827	1290
Waxy rice	2160	
Potato	8025	4920, 6340
Cassava	3642	2660
Sweet potato	3025	3280–4400

Table 3
Chain length distribution of the debranched amylopectin components determined by the conventional division analysis

Starch	Component	Chain	$\overline{Dp}_n$	$\overline{dp}_w$	Wt%	Molar%	A:B
Wheat	1	B4	110	111	0.3	0.1	
	2	B3	84	84	2.3	0.6	
	3	B2	49	51	20.9	8.2	
	4	B1	24	25	35.6	28.3	
	5	A2	15	15	21.4	27.4	
	6	A1	10	11	18.5	35.4	1.69
Waxy rice	1	B4	86	87	0.5	0.1	
	2	B3	69	69	3.9	1.1	
	3	B2	44	46	21.9	9.1	
	4	B1	24	24	27.2	20.7	
	5	A2	15	16	26.2	32.0	
	6	A1	10	11	20.2	36.9	2.20
Potato	1	B4	119	120	0.5	0.1	
	2	B3	83	84	11.6	3.4	
	3	B2	49	51	29.5	14.5	
	4	B1	23	24	33.7	35.2	
	5	A2	14	14	18.5	31.8	
	6	A1	10	10	6.2	15.0	0.88
Cassava	1	B4	90	91	0.6	0.1	
	2	B3	74	74	2.7	0.7	
	3	B2	48	49	24.7	10.4	
	4	B1	21	22	45.6	43.6	
	5	A2	13	13	17.0	26.3	
	6	A1	10	10	9.4	18.9	0.82
Sweet potato	1	B4	99	100	0.5	0.1	
	2	B3	77	78	4.6	1.3	
	3	B2	48	50	25.4	11.2	
	4	B1	23	24	37.2	34.3	
	5	A2	14	14	24.7	37.2	
	6	A1	10	10	7.5	15.9	1.13

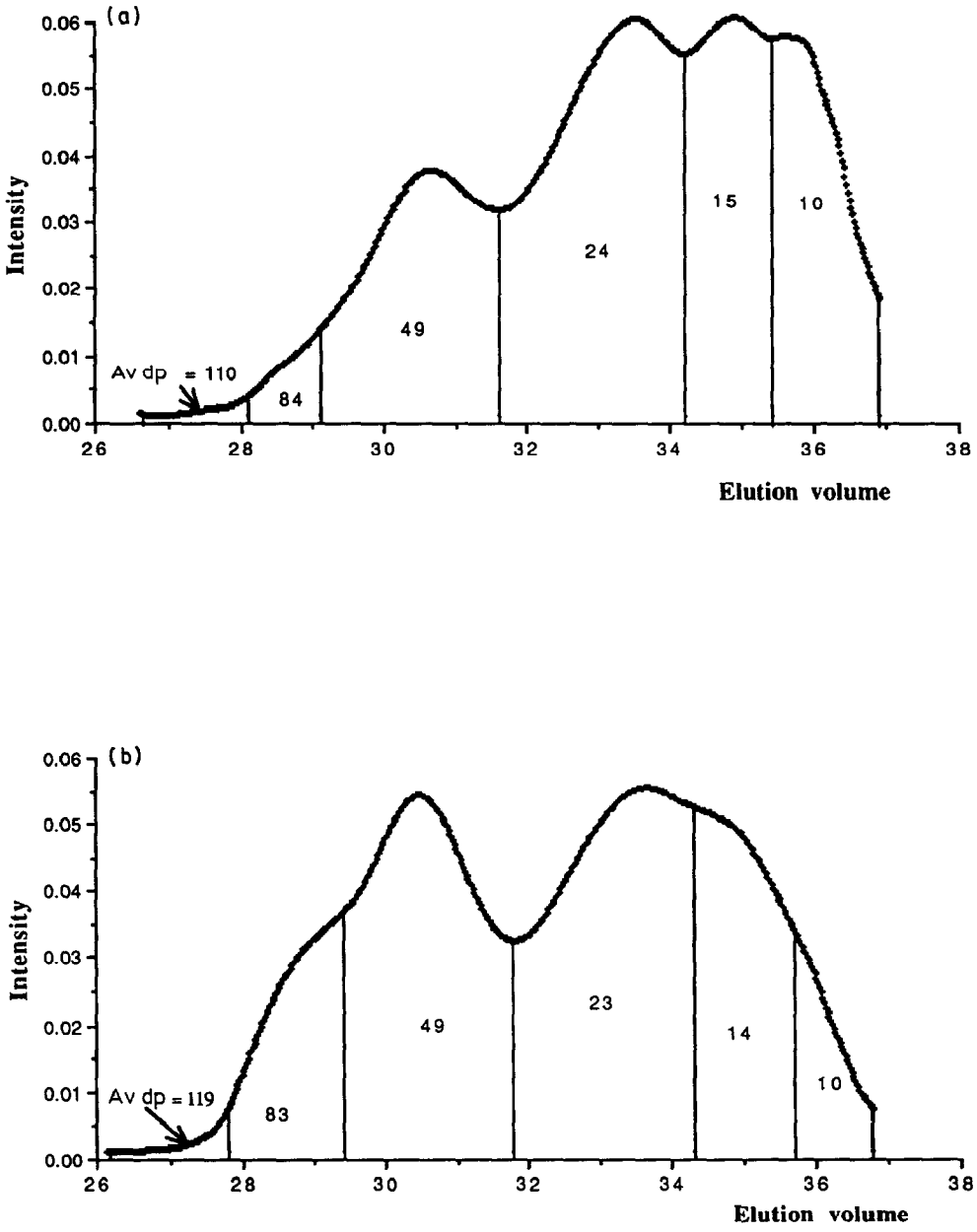

Fig. 5. Chromatograms of debranched amylopectin fractions showing the different division of chains for (a) wheat and (b) potato starches from the cluster division analysis.

against which to compare the longer chain population of the B and C starches in contrast to the A starches. For example, potato (B starch) has the highest amount of B2 chains (14.5%) and the A starches (wheat and waxy rice) have the least

Table 4
Chain length of amylopectin compared with the published values

Chain population	This study ($\overline{dp}$) (5 starches)	Hizukuri [20] ($\overline{dp}$) (4 starches)
B4	86–119	101–119
B3	69–84	69–75
B2	44–49	42–48
B1	21–24	20–24
A2	13–15	12–16 [a]
A1	10	
(A + B1)	80–90%	82–91%

[a] One A-chain population was reported.

(8.2%, 9.1%), whilst the cassava and sweet potato (C starches) have intermediate amounts (10.4%, 11.2%) of these chain populations.

Table 4 compares the results from this study with that of Hizukuri [20]. The

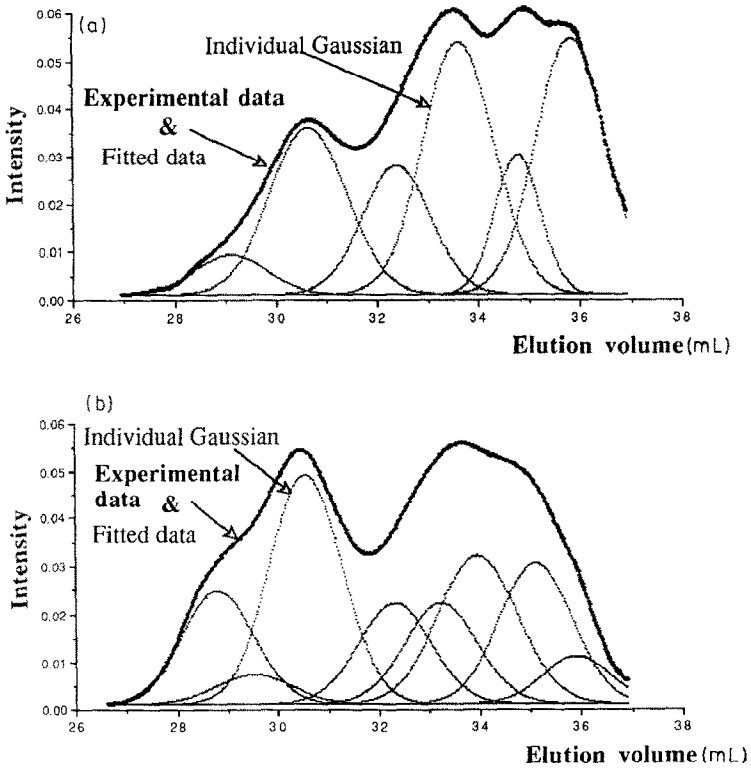

Fig. 6. Chromatograms of debranched amylopectin fractions showing the fitting of Gaussian peaks for (a) wheat and (b) potato.

division analysis revealed that the majority of the AP chains (82–91%) consisted of the B1 and the A chains, in agreement with the literature values [20]. From the Table, the chain populations were quite similar to the chain length classification introduced by Hizukuri [20], with five divisions (four B and one A chain populations) where A and B1 are formed in a single cluster; B2, B3, and B4 extend to 2, 3, or even 4 clusters, respectively. The difference in the study is that the shortest chain populations (namely the A chains) were sub-divided into two populations with $\overline{dp}_n$ ca. 15 (A2) and 10 (A1) due to a more refined resolution. The results supported the varied chain length population of the B chains, which was also demonstrated by Hizukuri [20]. All the B chains except the B1 chains are probably involved in the inter-cluster connection. The majority of the chains are the A and B1 chains which comprised a single cluster.

Chain length distribution of amylopectin by Gaussian distribution analysis.—The previous division analysis, which has been commonly used by many researchers, was found to be straightforward and the results were comparable with published values. In practice, locating the minima or (especially) the inflection points between peaks or shoulders is somewhat subjective and possibly prone to error, particularly with increasing numbers of peaks due to the better separation. Further, the Gaussian analysis might also overcome the ambiguity over the B4 chains which contributed to the onset of the AP fraction but were considered as a single component in the previous division analysis (Fig. 5).

Fig. 6 shows examples of the minimum number of components required to give a good fit between the experimental results and the computed multiple Gaussian simulations (fitted data) for the wheat and potato samples. The number of components varied with the type of starch; wheat and waxy rice each required at least six Gaussian components, cassava and sweet potato needed seven, while the potato profile required as many as eight components for a satisfactory fit (Table 5). The use of such a large number of populations may appear to be an excessive mathematical manipulation of the data; however, a smaller number of Gaussian components has been tried out but failed to give a reasonable fit. A greater number of components has also been reported for other complex polysaccharides [29–31]. The regular appearance of six fractions as observed in the division was not apparent in the Gaussian analysis. The terminologies for the four B and two/one A chain populations used previously and by Hizukuri may be only applicable for samples which have only six Gaussian populations (i.e., wheat and waxy rice). This suggests that the chain lengths within the molecule or the cluster may be more randomly or narrowly distributed with various chain populations depending on the type of starches, which may also infer a different degree of chain packing of the AP molecules.

The composition and dp distribution of each Gaussian peak are tabulated and compared in Table 5. The AP chain populations ranged from the longest chain of $\overline{dp}$ 79 to the shortest chains of $\overline{dp}$ 11. The first Gaussian peak, which consisted of the longest chain populations, varied from 62–79 dp, which is shorter than the values reported in the division analysis ($\overline{dp}$ 86–119 in Table 3). Both the division and Gaussian analyses show that the longest chain in waxy rice is shorter than in the

Table 5
Chain length distribution of the debranched amylopectin components determined by the Gaussian distribution analysis

Sample	Gaussian peak	Chain	$\overline{Dp}_n$	$\overline{Dp}_w$	Wt%	Molar%	A:B
Wheat	1	B	74	77	4.1	1.1	
	2	(″)	47	49	20.0	8.1	
	3	(″)	29	30	11.9	7.7	
	4	(″)	20	21	27.6	25.8	
	5	A	15	15	10.7	13.3	
	6	(″)	11	11	25.7	44.0	1.34
Waxy rice	1	B	62	65	7.4	2.2	
	2	(″)	45	47	13.2	5.2	
	3	(″)	30	32	14.3	8.7	
	4	(″)	21	21	23.0	19.9	
	5	A	15	15	12.1	14.6	
	6	(″)	11	11	30.0	49.4	1.78
Potato	1	B	79	82	12.3	3.9	
	2	(″)	65	68	3.5	1.2	
	3	(″)	49	51	24.1	11.8	
	4	(″)	30	31	11.3	9.2	
	5	(″)	23	24	11.2	11.8	
	6	A	18	19	17.5	23.4	
	7	(″)	13	14	15.0	27.7	
	8	(″)	11	11	5.1	11.0	1.64
Cassava	1	B	72	74	0.6	0.2	
	2	(″)	64	66	6.5	2.0	
	3	(″)	46	48	20.5	8.9	
	4	(″)	27	28	13.9	10.1	
	5	(″)	20	21	24.0	23.9	
	6	A	15	15	15.7	20.9	
	7	(″)	11	11	18.8	34.0	1.22
Sweet potato	1	B	74	77	2.7	0.8	
	2	(″)	66	68	6.7	2.1	
	3	(″)	47	49	19.6	8.6	
	4	(″)	31	32	10.7	7.2	
	5	(″)	23	24	18.8	16.9	
	6	A	16	17	22.8	29.4	
	7	(″)	11	12	18.7	35.0	1.81

Table 6
Total A+B1 chain populations ($\overline{dp} < 25$) of amylopectin

Method of analysis	A+B1 Chain populations (%)
Gaussian	74–84%
Division	82–91%
Hizukuri [20]	80–90%

Table 7
Ratios of the A:B chain populations for amylopectins [a]

Analysis	Starch				
	Wheat	Waxy rice	Potato	Cassava	Sweet potato
Gausian	1.34	1.78	1.64	1.22	1.81
Division	1.69	2.20	0.88	0.82	1.13
Hizukuri [20]		2.2	0.79	[b]	

[a] Based on the molar percentages from the division and Gaussian analyses. [b] Not included here because of a possible misprint; see ref 28.

rest of the starches; the majority of the AP chains are derived from the short chains with $\overline{dp} < 25$ (A and B1), as illustrated in Table 6.

Further comparisons of the ratios of the A/B chain population, based on the molar percentages from both the division and Gaussian analyses in Table 7, show that the ratios obtained from the Gaussian analyses differed substantially from the values calculated from the previous division analysis. However, the values from the division analysis are comparable to those of Hizukuri who used the same method of evaluating the chromatogram. When the AP fraction was deconvoluted, based on individual Gaussian distributions, the ratio of the A:B chain population of AP was found to be between 1 and 2, which falls closer to the A:B values derived from the debranching of the beta-amylodextrin of AP [11] and therefore suggests that the various A:B values could well be due to the method of analysis of the chromatogram. This study, therefore, has shown that the method of data analysis, whether conventional division or Gaussian analysis, can influence the interpretation of the results and therefore the ultimate structure of the AP molecule. The results from the literature are so far mainly confined to the so-called conventional division analysis. It would be inappropriate at this stage to try to explain the differences, but this may prompt the re-examination of the vast amount of similar results obtained by the classical method.

4. Conclusions

The MWD of AM and the chain length distributions of AP were determined simultaneously using the GPC-MALLS-RI system. The polymodal chain length distribution of AP was found to vary with types of starches and possibly to be related to their crystalline patterns. The fine structures of AP depend critically on the resolution of the chromatogram and the method of data analysis. The study demonstrated that the two methods used to analyse the unresolved AP fraction in the RI-chromatogram suggested different structures of AP. Deconvolution of the chromatogram by means of a program designed to fit multiple Gaussian components may be more meaningful when relating the AP profiles to the actual normal distribution of the chain length of native starch. The latter analysis also suggests

that the chain lengths within the AP molecule in the starch granule may be more complex than originally envisaged, and a different degree of chain packing was postulated from the multiple component analysis.

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