
Ingeniería de alimentos

Revista Mexicana de Ingeniería Química
Vol. 14, No. 2 (2015) 293-302

STRUCTURAL FEATURES OF BANANA STARCHES USING HPSEC-MALLS-RI

CARACTERÍSTICAS ESTRUCTURALES DE ALMIDONES DE PLÁTANO EMPLEANDO HPSEC-MALLS-RI

G. Méndez-Montealvo[1]*, S.L. Rodriguez-Ambriz[2], L.A. Bello-Pérez[2]

[1]*Instituto Politécnico Nacional, CICATA Querétaro. Cerro Blanco No. 141. Col. Colinas del Cimatario. C.P. 76090, Santiago de Querétaro, Querétaro, México.*

[2]*Instituto Politécnico Nacional, CEPROBI. Km. 8.5 Carr. Yautepec-Jojutla Col. San Isidro, C.P. 62731, Yautepec, Morelos, México.*

Received August 21, 2014; Accepted April 2, 2015

Abstract

An analytical method to quantify the amylose content and to characterize the structures of the banana starch molecules is reported. This study consists of two parts: Part 1 describes the individual chains present in the starch granules, as the chain-length distribution, and Part 2 characterizes the molecular structure of the starch components by high performance size exclusion chromatography coupled with multi-angle laser light scattering and refractive index detection (HPSEC-MALLS-RI). The quantification of amylose using two methods (HPSEC-RI and Concanavalin A) is in agreement with the percentage reported. In addition, the ratio Fr III/Fr. II (Fr. II represents amylopectin long B chains and Fr. III represents amylopectin A and B short chains), shows structural differences among banana starches, with more branching in Morado banana starch and less branching in Macho banana starch. A recognition that amylose has significant branching, and thus is not linear, led to the characterization of two populations of amylose: one that is highly branched and the other, which is essentially linear, as established by the HPSEC-MALLS-RI method. Morado amylose has the highest M_w of the branched starches while Macho amylose has the highest M_w and R_z values consistent with differences in the structures of banana starches.

Keywords: starch, banana, amylose, quantification, degree of polymerization (DP), weight-average molar mass (M_w), z-averaged radius of gyration (R_z).

Resumen

Se presenta un método analítico para cuantificar el contenido de amilosa de almidones de plátano y caracterizar su estructura. La investigación está en dos partes: Parte 1, estudia las cadenas individuales presentes en el almidón, como la distribución de longitud de cadena, y la parte 2, realiza la caracterización de la estructura molecular de las amilosas empleando cromatografía de líquidos de alta resolución por exclusión de tamaño acoplado a los detectores de dispersión de luz e índice de refracción (CLARET-DL-IR). La cuantificación de amilosa empleando dos métodos (CLARET-IR y Concavalina A) mostraron analogía en el porcentaje reportados. La proporción de Fr III/Fr II (Fr II representa cadenas largas tipo B y la Fr III a las cadenas cortas tipo A y B, de la amilopectina) mostrando diferencias estructurales entre los almidones de plátano, siendo el más ramificado el almidón Morado y el menos, el de Macho. Se ha descrito que la amilosa es significativamente ramificada, y no completamente linear, se estableció con el método por CLARET-DL-IR que la amilosa de Morado presentó el valor mayor de la M_w en la sección ramificada, mientras que en la parte lineal, la amilosa de Macho presentó los valores mayores de M_w y R_z.

Palabras clave: almidón, plátano, amilosa, cuantificación, grado de polimerización (GP), masa molar (M_w), radio cuadrático medio (R_z)

*Corresponding author. E-mail: : cmendez@ipn.mx
Tel.: +52 442 2290804 Ext 81004

1 Introduction

Starch is a biopolymer that consists of two major components: amylose and amylopectin. Amylose constitutes 15-35% of starch granules in most plants and is essentially a linear polysaccharide with α-(1-4)-linked D-glucose units. Some amylose molecules, particularly those of high molar mass, could have branches. Amylopectin is a highly αbranched molecule, with α-(1-4)-linked D-glucose backbones, and possesses approximately 5% of α-(1-6)-linked branches, which affect its physicochemical and biological properties (Perez and Bertoft, 2010). Clearly, it is important to understand the molecular and structural characteristics of starch components to explain their applications in different systems such as foods, cosmetics, and drugs (Rivas-Gonzalez *et al*. 2009, de la Rosa-Millan *et al*. 2014, Agama-Acevedo *et al*. 2015). However, due to the essentially linear structure of amylose, the studies of starch molecules have focused on the molecular structure of amylopectin. Traditionally, amylose content has been determined from the absorbance of an amylose-iodine complex, for which several methods have been published with small changes in sample preparation (Morrison and Lagnilet, 1983). Thermal analysis by differential scanning calorimetry (DSC) based on the formation of an amylose-fatty acid complex (Mestres *et al*. 1996) has been used to determine the amylose content in starches. From previous work, a method based on the separation of amylose and amylopectin by the formation of an amylopectin, Concanavalin A complex has been developed (Gibson *et al*. 1995). Although the quantification of amylose by enzymatic hydrolysis has been widely used (Gibson *et al*. 1997), the challenge of determining amylose content persists. Some years ago, the International Network for Quality Rice (INQR) published an inter-laboratory study indicating that five different versions of the iodine binding test are in use. The results from these different methods show high repeatability within laboratories but low reproducibility between laboratories. These results demonstrate that standardization of the procedure to determine the amylose content is necessary (Fitzgerald *et al*. 2009). In this sense and understanding that amylose is important in the functionality of starch, it is essential to establish methods to quantify amylose content and to characterize the amylose structure with high reproducibility. Previously, we published a chromatographic method to determine the fine structure of starch with different amylose/amylopectin ratios, in which the elution of both starch components is quantitative (Bello-Perez *et al*. 1998). From those data, it suggested that the methods of high-performance size exclusion chromatography (HPSEC) multi-angle laser light scattering (MALLS) and refractive index detection (RI) in combination can be used to determine the amylose content and the fine structure of both components in banana starches isolated from different varieties.

Gilbert *et al*. (2013) and Vilaplana *et al*. (2012) gave information pertaining to the quantification and characterization of amylose and amylopectin, in which an accurate quantification procedure for starch components was proposed. Vilaplana *et al*. (2012) described a method to characterize the amylose by HPSEC; in that study, the MALLS results were not reported. As they mentioned, current measurement methods of amylose content often yield different results even when cross-checked against laboratory, operator, and calibration variability. This variability was ascribed to the fact that different methods measure different physical quantities. We took this into account, and because the starch structure is complex, we divided the results into two structural levels as described by Gilbert *et al*. (2013). Level 1 pertains to individual chains from a branch point, with data normally presented as the chain-length distribution (CLD), which is applied to long chains in amylose with an average degree of polymerization (DP) typically between 10^3 and 10^4 monomers. Level 2 recognizes that amylose does not have a linear structure but has significant branches, as characterized in this study by means of HPSEC with MALLS-RI examination. From our work, we propose a simple and accurate method for structural characterization of banana starches.

2 Materials and methods

2.1 Starch samples

Starches from four different varieties of green bananas (Macho, Morado, Valery, and Enano gigante) were provided by Dr. Agama-Acevedo. The method to starch isolation from the banana varieties was reported by Agama-Acevedo *et al*. (2014).

2.2 Amylose starch content

Amylose content was determined by the method of Gibson *et al.* (1995) using a Megazyme amylose/amylopectin assay kit (K-AMYL 04/06, Megazyme International Ireland Ltd., Bray, Co. Wicklow, Ireland).

2.3 The standard calibration curve

Pullulan standards (Fluka Chemie GmbH, Steinhein, Switzerland) of different molar masses (1.3×10^3, 5.9×10^3, 2.3×10^4, 1.2×10^4, 2.1×10^5, and 7.9×10^5 g mol^{-1}) were used to establish a second order polynomial regression calibration curve. The pullulan standards were dissolved in HPLC-grade water at 25 ºC and allowed to equilibrate by standing overnight. Before use, they were filtered with 0.2 μm nylon syringe filters (Daigger & Company, Inc. Vernon Hills, IL, USA) and then injected into the HPLC system.

2.4 High performance size exclusion chromatography coupled with multi-angle laser light scattering and differential refractometric index detection (HPSEC-MALLS-RI)

Debranched starch was prepared by boiling 10 mg of a starch sample in 3.2 mL of deionized water with stirring for 30 min. After cooling to room temperature, 0.4 mL of a 0.1 M acetate buffer (pH 3.5) and 5 μL of isoamylase were added to the starch solutions. The mixtures were incubated in a water bath shaker at 45 °C at 150 rpm for 2 h. Isoamylase activity was arrested by the addition of 0.21 mL of 0.2 M NaOH in a boiling water bath for 15 min. After cooling, the samples were filtered through a 0.45-μm membrane (NYL w/GMF, Whatman, Clifton, NJ), mixed with 0.2 g of bed exchange resin IONAC NM 60 H^+/OH^- form, type I beads (16-50 mesh) (J.T. Baker, Phillipsburg, N.J. USA) for 60 s prior to injection into the HPLC system. The relative proportions of amylose and debranched amylopectin for each sample were determined from the area of their respective retention peaks by using high-performance size-exclusion chromatography with MALLS and refractive index detection (HPSEC-MALLS-RI), following the method of Kasemsuwan *et al.* (1995).

The HPSEC-MALLS-RI equipment consists of an Agilent 1100 series HPLC unit coupled with a MALLS detector. The system includes an isocratic pump with a 100-μL sample loop, inline degasser, autosampler, column thermostat, a guard chromatographic column (OHpak SB-G (6 mm x 5 cm)), Shodex OHpak SB-804 HQ (8 mm ID x 30 cm in length, Showa Denko K.K., Tokyo, Japan) analytical column, DAWN 8-angle light scattering detector (Wyatt Technology, Santa Barbara, CA, USA), and a refractive index detector. The eluent was deionized water with 0.2% sodium azide filtered with a 0.1 μm membrane supor-100 (Pall Corporation, Ann Arbor, MI, USA). The column was maintained at 30°C, with a flow rate of 0.3 mL/min through the chromatographic column. The light scattering dn/dc value (the change in the refractive index for an increment in concentration) used was 0.146. The resulting data were processed with ASTRA 5.3.4.14 software (Wyatt Technology, USA). The coefficients for the photodiode detector were calibrated using a pullulan standard of 22,500 g mol^{-1} (Showa Denko K. K., Tokyo, Japan) as the reference. The values reported below are averages of three measurements.

2.5 Statistical analysis

Data were analyzed using JMP software (SAS Institute, Cary, NC, USA). From these data, a one-way analysis of variance was calculated. Differences among mean values were calculated using the least significant difference (LSD) multiple range test and considered significant when $P < 0.05$.

3 Results and discussion

3.1 Amylose content

First, we present the results for level 1. The amylose content was studied using two methods: (1) with the use of the concanavalin A Megazyme kit (Concanavalin A effectively complexes the amylopectin component of starch but not that of the primarily linear amylose component) and (2) as debranched starch after treatment with the isoamylase enzyme. We worked with banana starches that have more than 92% purity (Agama-Acevedo *et al.* 2014); this is an important consideration in obtaining individual starch components that are free of other materials that may be present in the sample and that may produce problems during starch solubilisation. Table 1 lists the relative proportions of amylose obtained by these two methods, as well as the fractions of amylopectin designated as Fraction I (Fr. I), Fraction II (Fr. II), and Fraction III (Fr. III). Fr. I

consists mainly of amylose, Fr. II consists of a mixture of amylose and amylopectin long B- chains, and Fr. III consists of a mixture of amylopectin A-chains and short B- chains (Akai *et al*. 1971). The ratio Fr. III/Fr. II may be used as an indicator of the extent of the amylopectin branching, in which the higher the Fr. III/Fr. II ratio, the higher the degree of branching (Biliaderis *et al*. 1981). Figure 1 presents a normalized chromatogram of banana starches as eluted with the HPSEC-MALLS-RI system. The amylose content determined by both methods is similar, indicating that in addition to the determination of the fine structure of amylopectin, amylose quantification may also be obtained. As shown in Table 1, Valery starch has the lowest percentage of amylose among these four banana starches, while Morado starch has the highest percentage. Interestingly, Cameron and Wang (2005) suggested that amylose content determined by HPSEC may be more representative of the true content of amylose in rice starch. As shown in this table, there are significant differences in the level of the three fractions among these banana starches and consequently in the branching pattern. The results for the Fr III/Fr. II ratio indicate structural differences among these banana starches, with a more branching structure for Morado starch but a less branching structure for Macho starch. Hence, different banana varieties have starch with different structural characteristics and functionality. The methods to characterize starch structures can be used to understand the structure-function relationship. For example, the structures of different starches may control starch hydrolysis by digestive enzymes (Gilbert *et al*. 2013).

3.2 Structural characterization of amylose

The HPSEC method can provide structural information by the separation of starch components based on their hydrodynamic volume. In addition, data obtained from the HPSEC system may also be used to calculate the degree of polymerization (DP) of different starches using standards made with pullulans and dextrans to calibrate the system. A standard with an amylose-like structure should be used to obtain accurate calibration results which account for the use of pullulan as a calibration standard, as reported previously (Bello-Perez *et al*. 2009). However, as Ward *et al*. (2006) have mentioned, this approach presents a twofold problem: first, amylose molecules are branched and the elution profile of branched molecules does not directly translate to the molar mass distribution of amylose; and second, since pullulan standards do not have the same chemical structure as starch, their elution profile differs from that of starch. Thus, a calibration curve generated with pullulan standards should not be used for the structural characterization of amylose because an underestimation of the degree of branching of amylose results from the use of these standards.

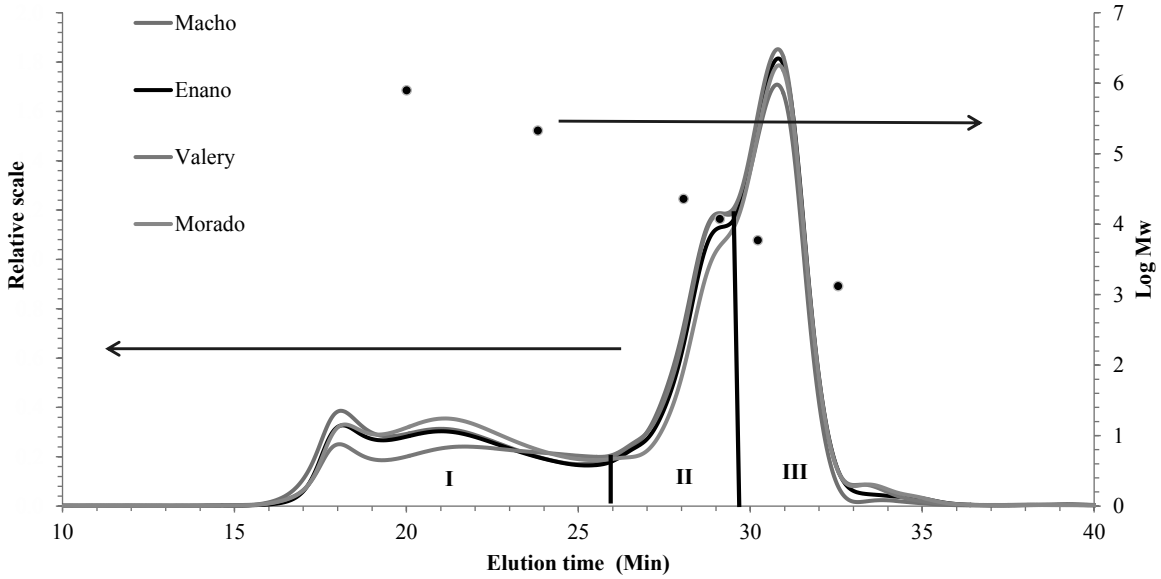

Fig. 1 Chromatograms of banana starches debranched with isoamylase (Fractions I, II, III) and analyzed by HPSEC-MALLS-RI (in secondary y axis, Log M_w for pullulan standards).

Table 1 Amylose content and molecular size distribution of debranched banana starches as resolved by HPSEC-MALLS-RI[&,*]

Sample	Amylose[✧] (%)	Fr. I[☆] (%)	Fr. II (%)	Fr. III (%)	Fr. III/Fr. II
Macho	27.0 ± 0.7^{ab}	29.5 ± 0.9^{b}	28.5 ± 0.8^{a}	42.8 ± 1.5^{b}	1.5 ± 0.0^{b}
Enano	24.6 ± 2.6^{b}	27.0 ± 0.5^{c}	26.1 ± 0.9^{b}	45.7 ± 0.7^{ab}	1.7 ± 0.1^{ab}
Morado	31.3 ± 5.0^{a}	32.8 ± 1.4^{a}	22.2 ± 1.8^{c}	44.9 ± 2.1^{ab}	2.1 ± 0.3^{a}
Valery	23.5 ± 1.4^{b}	25.8 ± 0.9^{c}	26.2 ± 1.7^{ab}	47.4 ± 1.7^{a}	1.9 ± 0.2^{a}

[&] High performance size exclusion chromatography coupled with multi-angle laser light scattering and refractive index detection
[*] Mean of three measurements ± standard deviation. Columns with the same letter are not significantly different ($p<0.05$).
[✧] Amylose content: grams of amylose/100g of starch measured with a Megazyme kit.
[☆] Fr. I amylose; Fr. II, amylopectin long B chains; Fr. III, amylopectin A and B short chains.

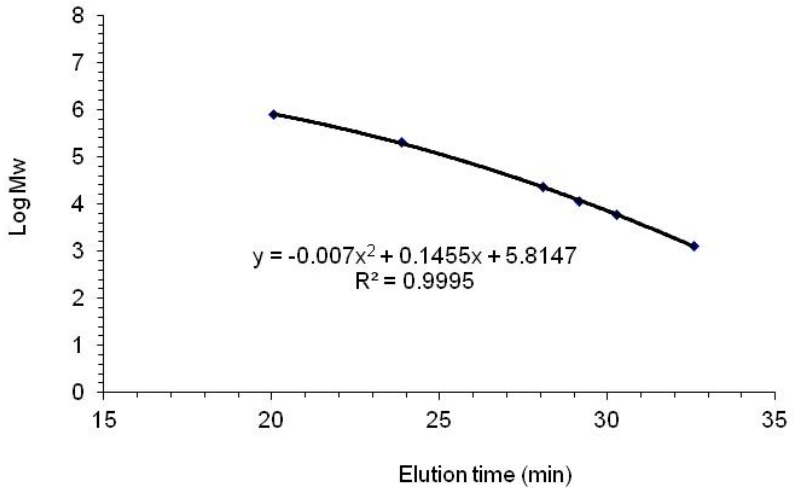

Fig. 2 Pullulan molar mass distribution and the adjustment equation

However, with the use of the MALLS detector with HPSEC, as described here, it is possible to obtain the weight-average molar mass (M_w) and the z-averaged radius of gyration (R_z) for amylose and associated branched material.

3.2.1. The standard calibration curve

A semi-logarithmic plot of molar mass versus elution volume for pullulan standards is shown in Figure 2. The correlation coefficient for the curve that relates the ratio between molar mass and elution time is 0.9995, showing that the HPSEC system used for elution of pullulan standards is reproducible. In Table 2, the molar mass of pullulan standards (given by the supplier) and the associated retention times obtained by HPSEC-MALLS-RI are presented. The theoretical DP was calculated using the expression: $DPT = M_w/162$; 162 is the molar mass of the anhydroglucose unit (AGU), considering that amylose consists of 1,4-linked-a-o-anhydroglucose units (Gallant *et al*. 1992). The calculated degree of polymerization (DP_C) was obtained using the calibration curve equation given in Figure 2 to obtain the M_{wc} at each retention time divided by 162. As shown in Table 2, there are differences between the M_w and the M_{wc}, values which may be attributed to the conditions for elution in the HPSEC system. However, these differences are not considered significant and are within the limitations of the instrumentation used in this research.

Table 3 contains values of DP and M_w calculated using the adjustment equation of the standard curve for different retention times, showing the material that may be eluted with our system, taking into account only the retention time (in accordance with the conditions of the procedures and the system used in these studies). Any material that is eluted from the HPSEC system is considered to have its M_w.

Table 2 Degree of polymerization (DP) and molar mass (M_w) estimated from the standard curve for pullulan obtained by HPSEC-MALLS-RI as described in the text

Pullulan (M_w)	Retention time (min)	$DP^{\&}_T$	DP^{*}_c	$M_{wc}{}^{\diamond}$ (g mol^{-1})
1 320	32.5	8	8	1 354.5
5 900	30.2	36	41	6 575.7
11 800	29.1	73	81	13 101.1
22 800	28.1	141	150	24 311.4
212 000	23.8	1309	1253	203 029.8
404 000	21.1	2494	3670	594 531.9
788 000	20.0	4864	5178	838 862.7

[&] The degree of polymerization expressed as glucose unit-theoretical.
[*] The degree of polymerization expressed as glucose unit-estimated.
[◇] M_w, estimated weight-average molar mass.
High performance size exclusion chromatography coupled with multi-angle laser light scattering and refractive index detection.

Table 3 Degree of polymerization (DP) and molar mass (M_w) calculated using the adjustment equation of the standard curve for different retention times as describe in the text

Retention time (min)	$DP_c{}^{\&}$	$M_{wc}{}^{*}$ (g mol^{-1})
16	13 842	2 242 332.4
18	9 040	1 464 535.8
20	5 190	840 814.1
22	2 619	424 326.4
24	1 162	188 234.8
25	737	119 453.8

[&]The degree of polymerization expressed as glucose unit-estimated.
[*]M_w, estimated weight-average molar mass.

The elution of the highest molar mass materials occurs first in the HPSEC system, with the molar mass of amylose being higher than the fractions of debranched amylopectin. Hence, for a retention time of 16 minutes, the value of the M_w is 2.2 $\times 10^6$ g mol^{-1} for amylose, with a DP_c of 13, 842. Table 3 shows the differences in DP_c for retention times between 16 and 25 minutes, as illustrated in Fig. 1, for amylose from the four banana starch varieties examined in this study (Fraction I).

The characteristics of sample preparation and the chromatographic column used in the HPSEC system led to higher values than previously reported by other authors, as mentioned by Perez and Bertoft (2010). In general, starches have a rather broad molar mass distribution of their amylose fraction with weight-average values (DPw) that are much higher than the number-average (DPn) values. The size of the polymers, more frequently given as the DP than as the molar mass values, varies among botanical species. For example, amylose from barley has been reported to have a DPn of 1570 (DPw 5580), compared to that of maize with a reported DPn of 930-990 (DPw 2270-2500). In addition, the DPn in different wheat varieties range from 830 to 1570. The size distribution obtained by HPSEC for a tapioca starch exhibits a range of DPw of 580 to 22,400, whereas in kuzu starch, the distribution is narrower, of the order of 480-12,300 (Perez and Bertoft, 2010). For banana starches, DPn and DPw values have not been reported since these values depend on elution time.

3.2.2. Structural results for amylose

The structural characterization of amylose by molar mass determination has been reported by Bello-Perez *et al.* (1998), Fishman *et al.* (1996), Suortti *et al.* (1998), and Roger *et al.* (1996), with methods that have not used calibration plots. In most of those studies, sample preparation was not as simple as the procedure used in our work, which not only yields the percentage of amylose in the starch containing material but also characterizes the structure of the debranched material. For polymers with high molar mass in particular, such as amylopectin and HPSEC in conjunction with MALLS, this method seems to be a very useful technique.

As noted above, most starches contain a mixture of linear and branched amyloses, and in the elution of the material with an HPSEC-MALLS-RI system, as shown in Figure 3, there are two trends in the

curves: the first one is attributed to the branched amylose, the first part of elution (elution time from 16.5 to 18.5 min); and the second one is attributed to the elution of the linear amylose (elution time from 18.6 to 25.5 min). However, Perez and Bertoft (2010) have suggested that most starches contain a mixture of linear and branched amyloses, and recently Gilbert *et al*. (2013) reported that amylose is not linear but has significant branching. The actual chain unit distribution in branched amyloses is not known because the branched components have not been separated from the linear components. From our work, we propose that under the conditions used for this HPSEC-MALLS-RI study, it is possible to distinguish between linear and branched amylose structures.

Table 4 presents the results for the branched amylose for the first part of the elution (between 16.5 and 18.5 minutes). Furthermore, Morado amylose has the highest M_w of the branched material, consistent with the amylose (%) results shown in Table 1, showing the higher amount of amylose. Morado starch has the highest Fr.III/Fr.II ratio and the lowest Fr. II, amylopectin long B chains, perhaps suggesting how amylose is distributed in the granules of Morado starch. The amylose for Macho, Enano, and Valery did not show significant differences in the M_w values. The R_z values showed a different pattern: Morado and Macho did not present significant differences but they were different from Enano and Valery, and the organization of the amylose was different among the samples. Enano had the lowest M_w and the highest R_z, giving the idea that this is a more open structure, and Morado had the opposite, with the highest M_w and lower R_z, showing a more branched amylose.

The plot of R_z versus M_w for the same fraction provides structural information about the amylose from the value of the exponent (v_z value) in the R_z-M_w relationship, which is linear in the double logarithmic plot and can be described by the power law behavior equation $R_{zi} = K_z M_i^v$, (M_i, and R_{zi} are weight-average molar mass and z-averaged radius of gyration of the *i*th slice, respectively). Hence, for a sphere, $v = 0.33$; for a random coil, v = 0.5-0.6, and for a rod, $v = 1$ (Hanselmann *et al*. 1996; Rolland-Sabate *et al*. 2007; Wyatt, 1993). As shown in Table 4 for v_z, branched amylose exists as dense spheres in the first part of the elution, consisting of highly branched material.

We calculated the DP_{wc} for the first part of the elution, taking into account the M_w values divided by 162 (162 is the molar mass of AGU). The resulting values were higher than those listed in Table 3 because there was an underestimation of the molar mass of material eluted under the conditions in the HPSEC-MALLS-RI system using the standard curve calibration and considering the elution time for the branched amylose.

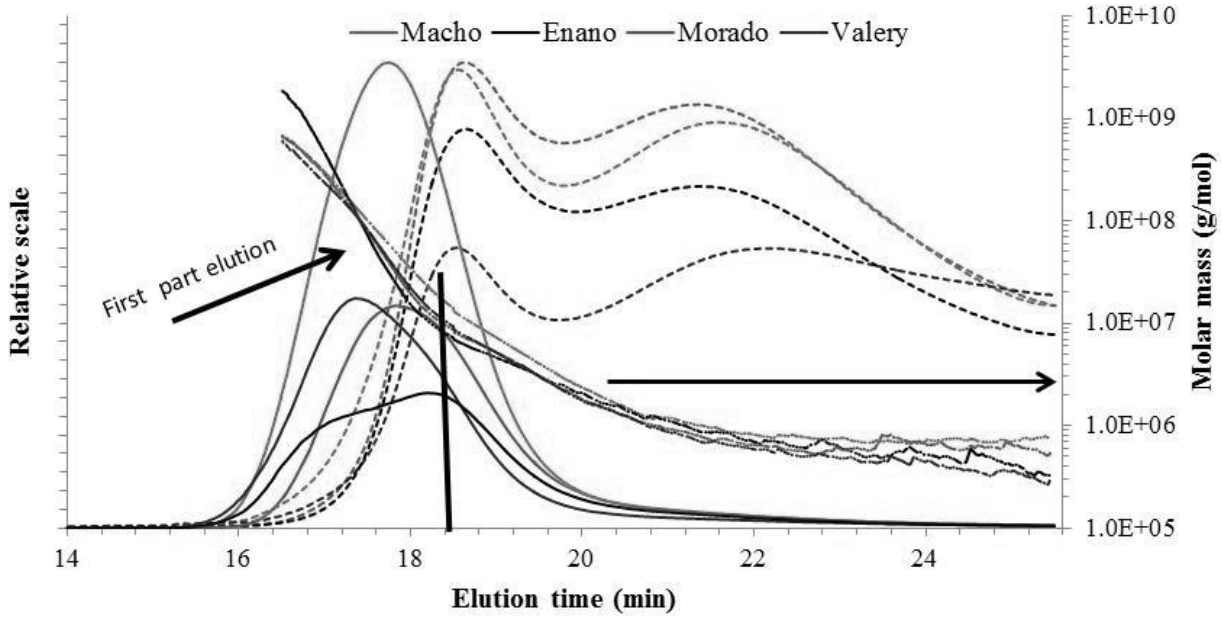

Fig. 3 Light scattering at 90^o (solid lines) and refractive index (broken lines) responses and molar masses (secondary y axis) vs elution time of debranched starches.

Table 4 Structural characteristics of branched amylose in banana starches after debranching with isoamylase[&]

Sample	M_n/M_w[*]	M_w x 10^{-7} (g mol^{-1})	R_z (nm)	ν_z	DP_{wc}[✧]
Macho	2.6 ± 0.2[b]	7.6 ± 0.4[b]	130.6 ± 5.6[b]	0.21	470 185
Enano	3.5 ± 0.4[a]	4.7 ± 0.2[b]	145.1 ± 8.5[a]	0.19	291 451
Morado	3.2 ± 0.5[ab]	12.4 ± 2.3[a]	126.3 ± 4.7[b]	0.20	767 593
Valery	3.5 ± 0.8[a]	5.5 ± 0.5[b]	116.4 ± 6.8[c]	0.20	340 926

[&]Mean of three measurements ± standard deviation. Mean values with the same letter are not significantly different ($p<0.05$).
[*]M_w, Weight-average molar mass; M_n, Number-average molar mass; R_z, *z*-averaged radius of gyration; M_n/M_w, Dispersity; ν_z, slope of M_w versus R_z. Berry adjustment: second order polynomial. Elution time is 16.5 to 18.5 min.
[✧]The degree of polymerization expressed as glucose unit-estimated.

Table 5 Amylose structural characteristics of banana starches[&]

Sample	M_n/M_w[*]	M_w x 10^{-6} (g mol^{-1})	R_z (nm)	DP_{wc}[✧]
Macho	1.9 ± 0.4[a]	2.6 ± 0.4[a]	105.1 ± 12.7[a]	15 713
Enano	2.2 ± 0.3[a]	1.4 ± 0.3[b]	73.1 ± 18.0[b]	8 326
Morado	2.2 ± 0.4[a]	1.6 ± 0.3[b]	73.9 ± 12.1[b]	9 733
Valery	2.0 ± 0.4[a]	1.4 ± 0.2[b]	79.7 ± 13.7[ab]	8 660

[&]Mean of three measurements ± standard deviation, in a column with the same letter are no significantly different ($p<0.05$).
[*]M_w, Weight-average molar mass; M_n, Number-average molar mass; R_z, *z*-averaged radius of gyration; M_n/M_w, Dispersity. Zimm adjustment: second polynomial degree. Elution time is 18.6 to 25.5 min.
[✧] The degree of polymerization expressed as glucose unit-estimated.

Table 5 shows the results for the linear component of amylose from the elution of 18.6 to 25.5 minutes. Macho amylose had the highest M_w and R_z values. These results are significantly different from the values for the other starches. Taking into account these values, of the M_w, the DP_{wc} values were calculated. These values were higher than those estimated from the calibration curve equation. As mentioned by Ward *et al*. (2006), since pullulan standards do not have the same composition as starch, their elution profile will differ from that of amylose. The underestimation is proved with these results: to characterize the structure of amylose for banana starches, this procedure is reliable and it is sensitive and easy to implement in the laboratory.

Conclusion

With this HPSEC-MALLS-RI method, it is possible to determine the relative concentration and the structural characteristics of amylose for the four banana starches studied in this work. The starch samples, even if they were from the same kind of plant (bananas, in this study) exhibited differences in structural parameters, as illustrated by the summary of M_w and R_z values given in this research. From this study, a procedure was developed that combines HPSEC with MALLS and RI detection to allow for the determination of the concentration of amylose, with accuracy as high as achievable with other procedures, such as with Concanavalin A complexation. In addition, it is possible to study the linear and branched forms of amylose. Finally, amylose is not completely linear, but consists of a mixture of both branched and linear forms.

Acknowledgements

The authors are grateful for support from SIP-IPN, COFAA-IPN, and EDI-IPN. The authors appreciate the technical support of M. C. Olga Rosales Reynoso with amylose quantification, and of Dr. Edith Agama-Acevedo for providing the banana starch samples used in this study. The authors are grateful to Dr. Paul E. Riley of the United States Peace Corps/Mexico for his help in the English revision.

Abbreviations

HPSEC-MALLS-RI	high performance size exclusion chromatography coupled with multi-angle laser light scattering and refractive index detection
Fr. I	amylose
Fr. II	amylopectin long B chains
Fr. III	amylopectin A and B short chains
AGU	anhydroglucose unit
M_w	weight-average molar mass
M_n	number-average molar mass
R_z	z-averaged radius of gyration
M_n/M_w	dispersity
DP	degree of polymerization
DPT	degree of polymerization theoretical
CLD	chain-length distribution
DSC	differential scanning calorimetry

References

Agama-Acevedo, E., Rodriguez-Ambriz, S. L., Garcia-Suarez, F. J., Gutierrez-Meraz, F., Pacheco-Vargas, G., Bello-Perez, L. A. (2014). Starch isolation and partial characterization of commercial cooking and dessert banana cultivars growing in Mexico. *Starch/Stärke 66*, 337-344.

Agama-Acevedo, E., Bello-Perez, L.A., Pacheco-Vargas, G., Evangelista-Lozano, S. (2015). Inner structure of plantain starch granules by Surface chemical gelatinization: morphological, physicochemical and molecular properties. *Revista Mexicana de Ingeniería Química 14*, 73-80.

Akai, H., Yokobayashi, K., Misaki, A., Harada, T. (1971). Structural analysis of amylopectin using *Pseudomonas* isoamylase. *Biochimica et Biophysica Acta 252*, 427-431.

Bello-Pérez, L. A., Roger, P., Baud, B., Colonna, P. (1998). Macromolecular features of starches determined by aqueous high-performance size exclusion chromatography. *Journal of Cereal Science 27*, 267-278.

Bello-Perez, L. A., Rodriguez-Ambriz, S. L., Agama-Acevedo, E., Sanchez-Rivera, M. M. (2009). Solubilization effects on molecular weights of amylose and amylopectins of normal maize and barley starches. *Cereal Chemistry 86*, 701-705.

Biliaderis, C. G., Grant, D. R., Vose, J. R. (1981). Structural characterization of legumes starches. I. Studies on amylose, amylopectin, and beta-limit dextrins. *Cereal Chemistry 58*, 496-502.

Cameron, D. K., Wang, Y. J. (2005). A better understanding of factors that affect the hardness and stickiness of long-grain rice. *Cereal Chemistry 82*, 113-119.

De la Rosa-Millan, J., Agama-Acevedo, E., Osorio-Diaz, P., Bello-Perez, L.A. (2014). Effect of cooking, annealing and storage on starch digestibility and physicochemical characteristics of unripe banana flour. *Revista Mexicana de Ingeniería Química 14*, 151-163.

Fishman, M. L., Rodriguez, L., Chau, H. K. (1996). Molar masses and sizes of starches by high-performance size-exclusion chromatography with on-line multi-angle laser light scattering detection. *Journal of Agricultural Food Chemistry 44*, 3182-3188.

Fitzgerald, M. A., Bergman, C. J., Resurreccion, A. P., Möller, J., Jimenez, R., Reinke, R. F., Martin, M., Blanco, P., Molina, F., Chen, M-H., Kuri, V., Romero, M. V., Habibi, F., Umemoto, T., Jongdee, S., Graterol, E., Reddy, K. R., Bassinello, P. Z., Sivakami, R., Rani, N. S., Das, S., Wang, Y-J., Indrasari, S. D., Ramli, A., Ahmad, R., Dipti, S. S., Xie, L., Lang, N. T., Singh, P., Toro, D. C., Tavasoli, F., Mestres, C. (2009). Addressing the dilemmas of measuring amylose in rice. *Cereal Chemistry 86*, 492-498.

Gallant, D. J., Bouchet, B., Buléon, A., Pérez, S. (1992). Physical characteristics of starch granules and susceptibility to enzymatic degradation. *European Journal of Clinical Nutrition 46*, S3-S16.

Gibson, T. S., McCleary, B. V., Solah, V. A. (1995). Con A revisited - a simplified assay to measure amylose in cereal starches. In: Williams, Y.A., Wrigley, C.W. (Eds.), Proc. *45th Australian Cereal Chemistry Conference*. Pp. 162-167.RACI: Melbourne.

Gibson, T. S., Solah, V. A., McCleary, B. V. (1997). A procedure to measure amylose in cereal starches and flours with concanavalin A. *Journal of Cereal Science 25*, 111-119.

Gilbert, R. M., Witt, T., Hasjim, J. (2013). What is being learned about starch properties form multiple-level characterization. *Cereal Chemistry 90*, 312-325.

Hanselmann, R., Burchard, W., Ehrat, M., Widmer, H. M. (1996). Structural properties of fractionates starch polymers and their dependence on the dissolution process. *Macromolecules 29*, 3277-3282.

Kasemsuwan, T., Jane, J-L., Schnable, P., Stinard, P., Robertson, D. (1995). Characterization of the dominant mutant amylose-extender (ael-5180) maize starch. *Cereal Chemistry 72*, 457-464.

Mestres, C., Matencio, F., Pons, B., Yajid, M., Fliedel, G. V. (1996). A rapid method for the determination of amylose content by using differential-scanning calorimetry. *Starch/Stärke 48*, 2-6.

Morrison, W. R., Lagnilet, B. (1983). An improved colorimetric procedure for determining apparent and total amylose in cereal and other starches. *Journal of Cereal Science 1*, 9-20.

Perez, S., Bertoft, E. (2010). The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. *Starch/ Stärke 62*, 389-420.

Rivas-Gonzalez, M., Zamudio-Flores, P.B., Bello-Pérez, L.A. (2009). Efecto del grado de acetilación en las características morfológicas y fisicoquímicas del almidón de plátano. *Revista Mexicana de Ingeniería Química 8*, 291-297.

Roger, P., Tran, V., Lesec, J., Colonna, P. (1996). Isolation and characterisation of single chain amylose. *Journal of Cereal Science 24*, 247-262.

Rolland-Sabate, A., Colonna, P., Mendez-Montealvo, M. G., Planchot, V. (2007). Branching features of amylopectins and glycogen determined by asymmetrical flow field flow fractionation coupled with multiangle laser light scattering. *Biomacromolecules 8*, 2520-2532.

Suortti. T., Gorenstein, M. V., Roger, P. (1998). Determination of the molecular mass of amylose. *Journal of Chromatography A 828*, 515-521.

Vilaplana, F., Hasjim, J., Gilbert, R. G. (2012). Amylose content in starches: Toward optimal definition and validating experimental methods. *Carbohydrate Polymers 88*, 103-111.

Ward, R. M., Gao, Q., de Bruyn, H., Gilbert, R. G., Fitzgerald, M. A. (2006). Improved methods for the structural analysis of the amylose-rich fraction from rice flour. *Biomacromolecules 7*, 866-876.

Wyatt, P. J. (1993). Light scattering and the absolute characterization of macromolecules-Review. *Analytica Chimica Acta 272*, 1-40.