ELSEVIER

Food Chemistry 50 (1994) 411–417
1994 Elsevier Science Limited
Printed in Great Britain.
0308-8146/94/$7.00

Amylopectin: Structural, gelatinisation and retrogradation studies

O. Paredes-López, L. A. Bello-Pérez & M. G. López

Dept. de Biotecnología y Bioquímica, Centro de Investigación y de Estudios Avanzados del IPN, Apartado postal 629, 36500 Irapuato, Gto, Mexico

(Received 20 October 1993; revised version received and accepted 6 December 1993)

Amaranth, waxy corn, and commercial corn amylopectins were enzymically debranched and their fractions separated by gel filtration. In all cases a bimodal distribution of chain lengths was found, containing a high proportion of short chains. Amaranth, waxy corn starches and commercial corn amylopectin displayed an A-type X-ray diffraction pattern, which is characteristic of cereal starches, whereas amaranth amylopectin did not show a well-defined pattern, suggesting that the former samples have granular structure. Gelatinisation and retrogradation data, by differential scanning calorimetry, showed a good agreement between starches and amylopectins of the same source. Amaranth starch and its amylopectin presented a lower tendency to undergo these changes compared to the remaining tested materials. The temperature effect on retrogradation was more drastic for amaranth and waxy corn amylopectins.

INTRODUCTION

Starch, the major storage polysaccharide of higher plants, is a polymeric mixture of essentially linear (amylose) and branched (amylopectin) α-D-glucan molecules. Starch is deposited in the form of granules, partially crystalline, whose morphology, chemical composition, and supermolecular structure are characteristic of each particular plant species. Starch owes much of its functionality to two major high-molecular-weight carbohydrate components, amylose and amylopectin, as well as to the physical organization of these macromolecules into the granular structure (French, 1984).

Amylopectin is one of the biggest molecules in nature; it is the principal component in the majority of starches and perhaps the most important in terms of their functional properties. Substantial progress in investigating the fine structure of amylopectin has become possible by the use of highly purified amylolytic enzymes (e.g. pullulanase, isoamylase, β-amylase) (Manners, 1989).

Research on the fine structure of amylopectin has been achieved using different methodologies. Three distribution modes have been published up to now; these are bimodal (Hizukuri, 1985; Bertoft, 1991; Jane & Chen, 1992), trimodal (MacGregor & Morgan, 1984; Kobayashi *et al.*, 1986) and polymodal (Hizukuri, 1986; Koizumi *et al.*, 1991; Suzuki *et al.*, 1992) distributions. It is also known that all these different distributions are consistent with the cluster model, which best explains the amylopectin structure. This model applies to all amylopectins, irrespective of their botanical source (Manners, 1989). Some investigators (Kobayashi *et al.*, 1986; Enevoldsen & Juliano, 1988) have reported that there are no differences in the structure of amylopectin of the same source independently of the amylose and amylopectin levels. However, other authors have reported differences in the amylopectin structure even within the same source (Taki *et al.*, 1988; Hizukuri *et al.*, 1989; Sanders *et al.*, 1990); this could partially explain the diverse functional properties of these starches.

Starches display characteristic X-ray diffraction patterns, which are given by the short chains of the amylopectin component (Robin *et al.*, 1974). There are three diffraction patterns for starches (A, B and C). The A-type is typical of cereal starches; tubers yield B-type; and certain root and seed starches give C-type (Zobel, 1988). Studies done on the structure of amylopectins from different sources showed that the average chain length of the polymer is the principal determinant of the crystalline polymorphism of granular starches (Hizukuri *et al.*, 1983; Hizukuri, 1986).

Since gelatinisation and retrogradation are endothermic processes, thermal analysis methods, and differential scanning calorimetry (DSC) have been used in studies of phase transitions of aqueous starch systems to evaluate the effect of water upon such systems (Donovan, 1979; Tester & Morrison, 1990; Paredes-López & Hernández-López, 1991).

Amaranth is an ancient crop with outstanding agronomic traits and food nutritional properties; its

seeds contain a waxy type starch (Paredes-López & Hernández-López, 1992). Corn is one of the most important crops used for starch production. Thus, to comply with the following objectives, all amylopectin and starch samples were derived from amaranth and corn. The objectives of this work were to study the structure, and the gelatinisation and retrogradation behaviour of amylopectin isolated from different starch sources. Whenever possible, the amylopectin behaviour was compared with that of the starch used to isolate such amylopectin.

MATERIALS AND METHODS

Starch isolation

Mature seeds of *Amaranthus hypochondriacus*, Mercado type, were harvested at a local experimental farm. After harvest the seeds were cleaned and stored at 4°C in sealed containers until used. Amaranth starch was prepared by the method of wet-milling (Paredes-López *et al.*, 1989; Paredes-López & Hernández-López, 1992). Waxy and normal corn starches were a gift from Industrializadora de Maíz, S.A. de C.V. (Guadalajara, Jal., Mexico). Waxy and normal corn starches were used to assess the effect of different amylopectin levels in the samples under study.

Amylopectin isolation

Amylopectins were isolated from amaranth and waxy corn starches using the methodology reported by Banks and Greenwood (1967). Corn commercial amylopectin was purchased from Sigma (Sigma Chemical Co., St Louis, MO, USA); no information was provided on the type of corn used to isolate this amylopectin. Amylopectins from amaranth and waxy corn were incorporated in this study, as indicated above, as well as the corresponding starches from which they were isolated. The commercial sample was here included only for comparison purposes.

Debranching with pullulanase

Samples (100 mg) of amylopectins were debranched with 30 U of activity/mg of pullulanase (200 μl) (Boehringer Mannheim, Germany) in 5 ml of solution (20% DMSO in 0·05 M acetate buffer, pH 4·7) at 37°C for 24 h (Biliaderis *et al.*, 1981). The digested samples were subsequently heated in boiling water for 15 min to inactivate the enzyme, diluted first with 1 ml DMSO (while mixture is heated) and then with 4 ml of acetate buffer (0·05 M, pH 4·7). Insoluble material was removed by centrifugation (8000 × g for 20 min) and the supernatant analysed by gel chromatography.

Gel filtration chromatography

A column (1·25 × 200 cm) was packed with Sepharose CL-2B (Sigma). Debranched amylopectin solutions (5 ml) were applied to the column by the descending procedure and eluted at 22°C with acetate buffer (0·1 M, pH 4·7) containing 0·02% sodium azide, at a constant flow rate of 26 ml/h. Fractions of 4 ml were collected and assayed for total carbohydrate (Dubois *et al.*, 1956). The degree of polymerisation (DP) of each eluted fraction was determined by dividing the total carbohydrate concentration by its reducing capacity (Nelson, 1944). The molar ratio of chain populations was calculated as suggested by Biliaderis *et al.* (1981).

X-ray diffraction

X-ray diffraction patterns were obtained with the following operating conditions: CuK radiation; voltage, 40 kV; chart speed, 10 mm/2θ; running rate, 2θ/min (Jovanovich *et al.*, 1992).

DSC measurements

DSC-measurements were performed on a Dupont calorimeter model 9000 with a model 910 pressure DSC (E. I. Dupont de Nemours and Co., Inc., Wilmington, DE, USA) cell base. The instrument was calibrated with indium. Gelatinisation of starch and amylopectin samples was estimated (Paredes-López & Hernández-López, 1991) as follows. The samples (2 mg) were weighed directly into DSC aluminium pans and deionised water was added with a microsyringe to make a solid suspension with 65–75% (w/w) water content (on dry basis). After sealing, the pans were left to equilibrate 1 h at room temperature and scanned at a rate of 10°C/min from 30 to 180°C, with a sensitivity of 0·005 mcal/seg. In all measurements an empty pan was used as reference. For retrogradation studies, the samples were weighed in the same manner as above. The pans were heated in an oven at 105°C for 15 min and stored for 1, 2, 3, 7, 14 or 21 days at 4°C (Gudmundsson & Eliasson, 1992). After this storage time, the pans were left to equilibrate 1–2 h at room temperature and scanned under the same previous conditions. In another experiment the effect of temperature on retrogradation was evaluated. The samples were weighed as for retrogradation but were not heated; they were only stored during 15 days at room temperature and at 4°C, and thereafter were analysed as mentioned above for gelatinisation (Hoseney *et al.*, 1986; Zeleznak & Hoseney, 1986).

RESULTS AND DISCUSSION

Gel filtration chromatography

Pullulanase-debranched profiles of amylopectins from amaranth, waxy corn, and commercial (corn) are shown in Fig. 1. A bimodal distribution was observed in all cases; a similar behaviour has been previously reported by other authors (Biliaderis *et al.*, 1981; Jane & Chen, 1992; Wang *et al.*, 1993). This pattern has

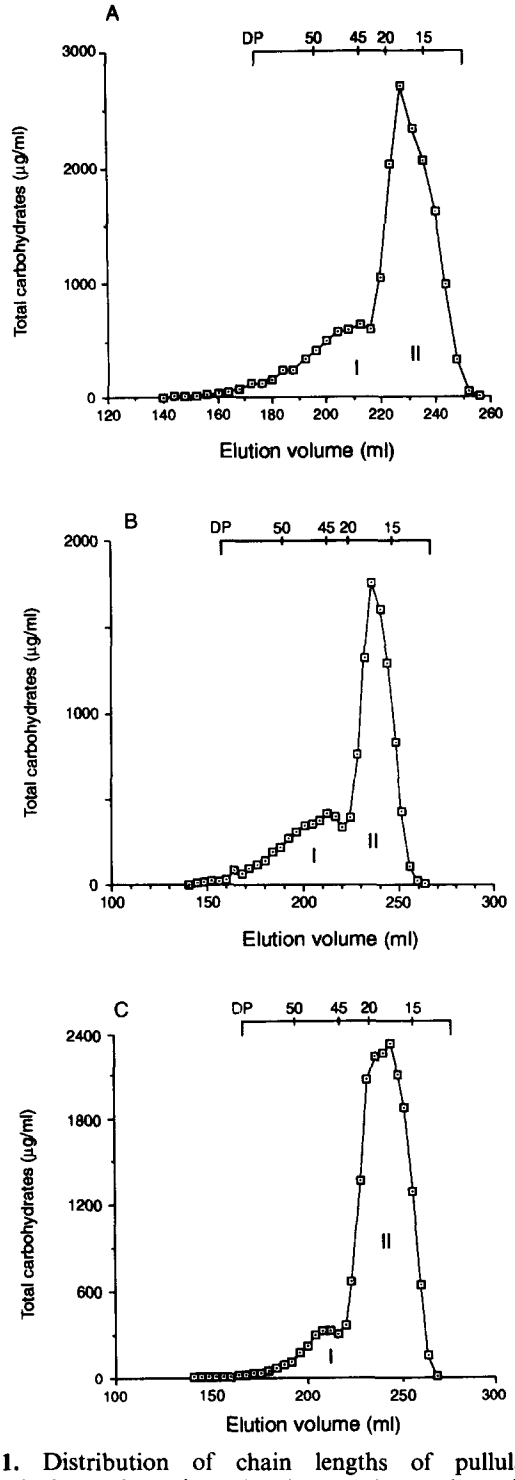

Fig. 1. Distribution of chain lengths of pullulanase-debranched amylopectins. A, Amaranth amylopectin; B, waxy corn amylopectin; C, commercial (corn) amylopectin. DP, degree of polymerisation; peaks I and II are chain fractions.

been observed even with different gel chromatography conditions. Hizukuri (1985), and Hizukuri *et al.* (1989), Sanders *et al.* (1990) and Yuan *et al.* (1993) reported a bimodal distribution using high-performance liquid chromatography (HPLC). The latter authors studied amylopectins from waxy maize genotypes and suggested that each of the two peaks was apparently made up of more than one component.

The amylopectins used in this study presented two principal fractions with DP of 45–48 (peak I) and 15–18 (peak II) for that from amaranth (Fig. 1(A)), 45–49 (peak I) and 17–19 (peak II) for that from waxy corn (Fig. 1(B)), and 47–50 (peak I) and 18–19 (peak II) for that from a commercial (corn) source (Fig. 1(C)). These two fractions represent B and A chains, respectively, which is the same nomenclature used in the cluster model (Robin *et al.*, 1974). In all cases a larger proportion of A chains was observed with a chain molar ratio of 15, 16 and 18 for waxy corn, commercial (corn) and amaranth amylopectins, respectively; this behaviour is in agreement with the classical distribution of cereal amylopectins (Yuan *et al.*, 1993). However, these ratios are the highest values that have been reported on a chain molar basis. The type of column and support used in this study may be responsible for the high resolution. The different A and B chain distributions could be an important factor in the functional properties of these amylopectins, although further studies on this topic need to be carried out.

X-ray diffraction study

Figures 2(A) and 2(C) show a typical A-type diffraction pattern for amaranth and waxy corn starches; this is a characteristic pattern for cereal starches (Zobel, 1988). The same A-type was reported by Gorinstein and Lii (1992) for amaranth starch. This diffraction pattern is attributed to starches that have amylopectins with high proportions of short chains (Hizukuri *et al.*, 1983) as can be seen in Fig. 1 (peak II). Amaranth amylopectin (Fig. 2(B)) did not show the characteristic pattern for starches. This could be due to the used isolating method; earlier reports (Hoseney *et al.*, 1986; Zeleznak & Hoseney, 1986) have suggested that the destruction of crystalline structure or the presence of imperfect or relatively small crystallites (Cooke & Gidley, 1992) may cause these types of anomalies. Commercial corn amylopectin (Fig. 2(D)) showed a characteristic A-type diffraction pattern, almost identical to the pattern displayed by waxy corn starch (Fig. 2(C)). This result suggests that commercial corn amylopectin still has a granular structure. Waxy corn amylopectin (isolated during the experiment) is not shown here due to the development of a gel phase during the analysis.

DSC measurements

DSC was used to study the gelatinisation phenomena in starches and amylopectins. Amaranth starch presented the lowest gelatinisation temperatures and enthalpy values (Table 1). These values agree with those reported previously by Paredes-López and Hernández-López (1991) when the same water levels were used. Gorinstein and Lii (1992) also reported values very close to those of Table 1. Waxy and normal corn starches showed very similar T_o and T_p temperature values but the difference between them is evident in T_c and ΔH values. Perhaps this behaviour could be partially explained by the different amylose levels present in those materials, although some authors have

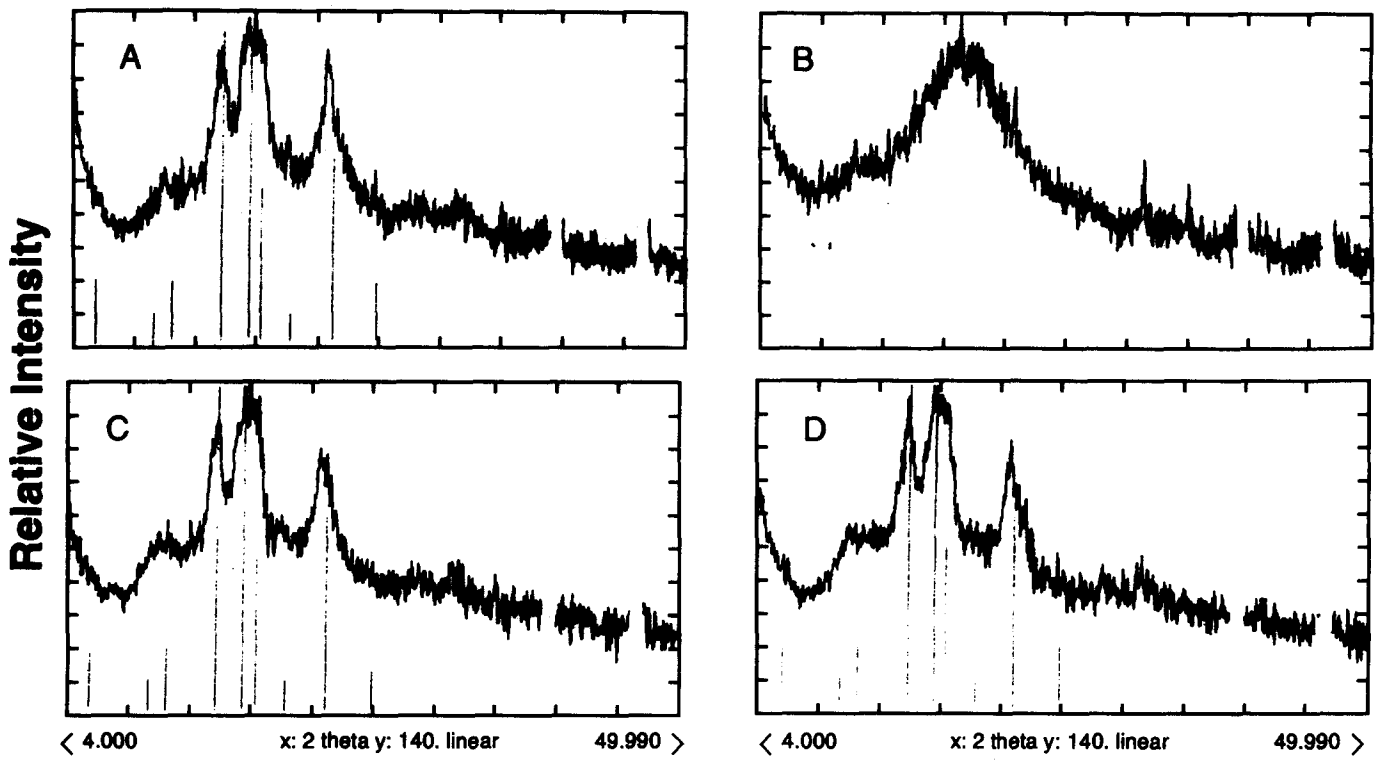

Fig. 2. X-ray diffraction patterns. A, Amaranth starch; B, amaranth amylopectin; C, waxy corn starch; D, commercial (corn) amylopectin.

reported different behaviour in these values even among maize starches from waxy genotypes (Sanders *et al.*, 1990; Yuan *et al.*, 1993).

Amaranth starch presented a low tendency to retrograde (Fig. 3(A)); after only 7 days storage an endotherm was observed. However, the enthalpy values increased, finally reaching higher values than those of the other starches. Waxy and normal corn starches (Figs 3(B) and 3(C)) showed similar behaviour in retrogradation; from storage day 1, both presented this phenomenon but waxy corn always had higher enthalpy values that normal corn. Normal corn showed a small endotherm at high temperatures due to an amylose–lipid complex characteristic of this type of starch (French, 1984). In all cases of retrogradation, the endotherms were wide, perhaps due to the formation of large and imperfect crystals and began at temperatures lower than that for gelatinisation (Cooke & Gidley, 1992; Yuan *et al.*, 1993). After 7 and 14 days of storage, amaranth starch showed the lowest ΔH values; after only 21 days the amylopectin-rich starches had the highest ΔH values. The amylopectin may play an important role in retrogradation (White *et al.*, 1989), but this role is not so clear as judged by the experiments of Figs 3(A)–3(C)).

Table 1. Thermal analysis of gelatinisation for starches and amylopectin using DSC[a]

Sample	T_o	T_p	T_c	ΔH (J/g)
Starch				
Amaranth	61·7	68·3	76·6	2·30
Waxy corn	65·4	71·6	77·2	2·45
Normal corn	65·0	71·2	82·0	5·06
Amylopectin				
Commercial corn	66·3	72·9	85·4	6·54

[a] T_o, T_p and T_c are onset, peak, and conclusion temperatures, respectively, in °C. Δh is the enthalpy of gelatinisation.

Amaranth and waxy corn amylopectins (Figs 4(A) and 4(B), respectively) did not show gelatinisation endotherms. The absence of endotherms may be due to structural changes during the isolating step (Hoseney *et al.*, 1986; Zeleznak & Hoseney, 1986). However, commercial corn amylopectin exhibited an endotherm, similar to that of starches, having gelatinisation temperatures slightly higher and an enthalpy value much higher than those of the starches analysed in this research (Table 1 and Fig. 4(C)). This behaviour suggests the existence of granular structure in this material and supports the X-ray diffraction data presented above. Amaranth amylopectin presented a low tendency to retrograde (similar to its starch) since after only 21 days storage a small endotherm was observed (Fig. 4(A)). In relation to this sample, waxy corn amylopectin showed a higher tendency to retrograde, since an endotherm was present after 14 days storage (Fig. 4(B)). These results are consistent with the data obtained for retrogradation studies of the corresponding starches (Figs 3(A) and 3(B)). Commercial corn amylopectin (Fig. 4(C)) presented the highest tendency to retrograde; an endotherm was observed after 1 day of storage; the enthalpy value was larger at longer storage times with a tendency to arrive at a plateau after storing 14 days. The structural differences between amylopectins may play an important role in this behaviour. However, more studies are needed on this topic.

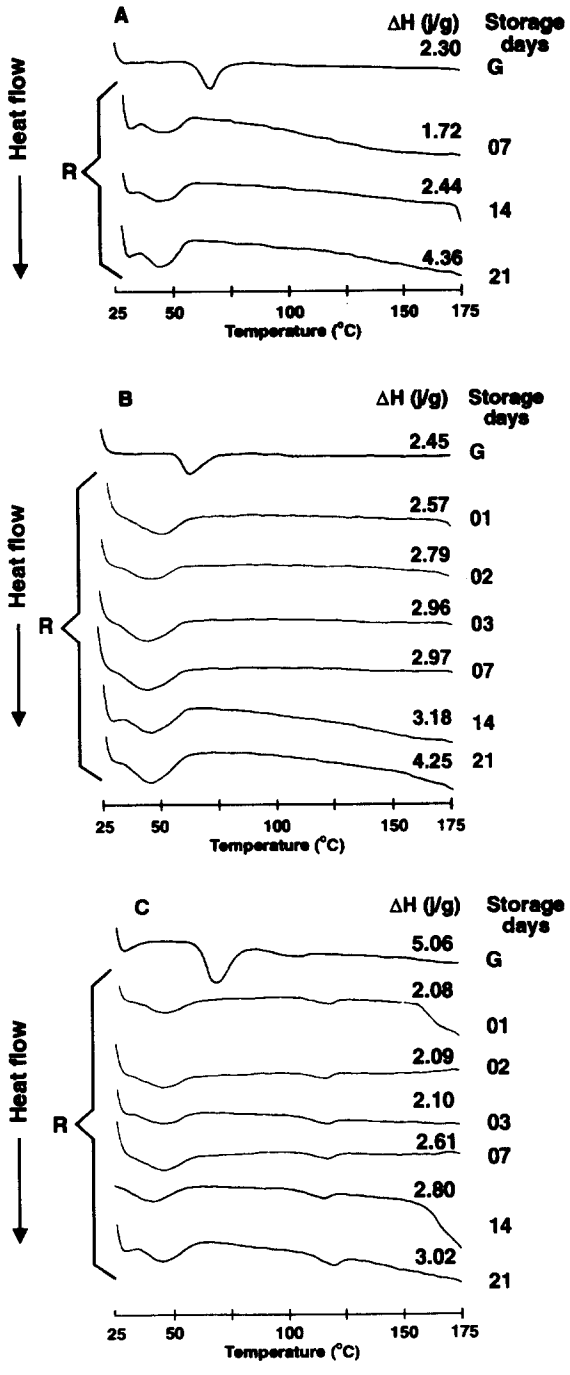

Fig. 3. Gelatinisation and retrogradation DSC thermograms for starches. A, Amaranth starch; B, waxy corn starch; C, normal corn starch; G, gelatinisation; R, retrogradation.

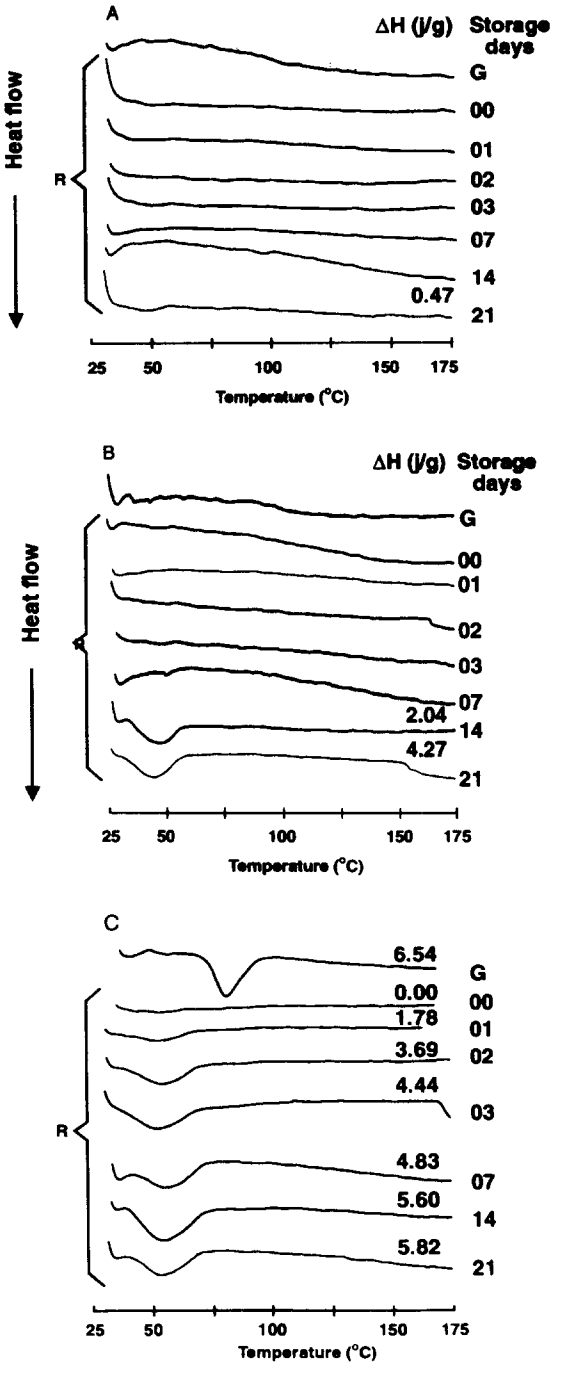

Fig. 4. Gelatinisation and retrogradation DSC thermograms for amylopectin. A, Amaranth amylopectin; B, waxy corn amylopectin; C, commercial (corn) amylopectin. G, gelatinisation; R, retrogradation.

To determine the effect of temperature on amylopectin retrogradation, all amylopectins were stored at two different temperatures as indicated previously. This effect was more drastic for amaranth and waxy corn amylopectins at room temperature since no endotherms were observed for either one, although at 4°C amaranth amylopectin showed a lower retrogradation level than waxy corn amylopectin (Fig. 5); this difference may be due to the different chain length distributions of the amylopectins (Shi & Seib, 1992; Yuan *et al.*, 1993). Commercial corn amylopectin displayed retrogradation endotherms at both storage temperatures (Fig. 5), retrogradation ΔH being smaller at 4°C than at room temperature. Yuan *et al.* (1993) mentioned that temperature and concentration may play relevant roles in the retrogradation phenomenon. Other authors (Hoseney *et al.*, 1986; Zeleznak & Hoseney, 1986) have studied the retrogradation of amylopectins stored at room temperature. Their results suggest that the amylopectin type, chain length and size are also important factors to consider in understanding the retrogradation phenomenon.

In conclusion all amylopectins presented larger proportions of A chains than B chains with a molar ratio of 15, 16, and 18 for waxy corn, commercial (corn) and

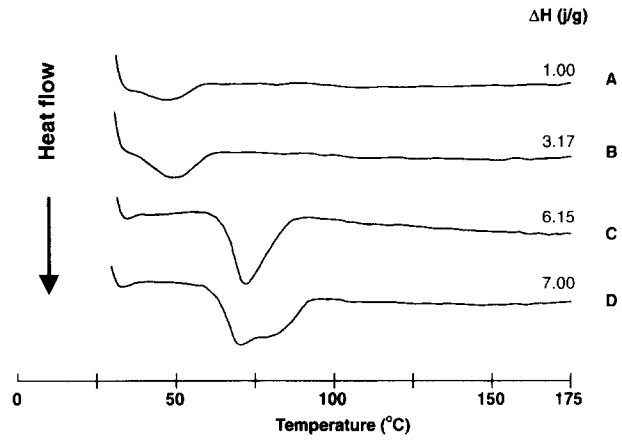

Fig. 5. Retrogradation DSC thermograms for amylopectins stored 15 days at two different temperatures. A, Amaranth amylopectin, 4°C; B, waxy corn amylopectin, 4°C; C, commercial (corn) amylopectin, 4°C; D, commercial (corn) amylopectin, room temperature. (Note: no endotherm was produced when amaranth and waxy corn amylopectins were stored at room temperature.)

amaranth amylopectins, respectively. Amaranth amylopectin did not present a characteristic X-ray diffraction pattern whereas commercial corn amylopectin showed an A-type pattern, which suggests that the latter sample still had a granular structure. Of all tested starches amaranth starch presented the lowest gelatinisation temperatures and ΔH values, as well as a low tendency to retrograde. Waxy and normal corn starches showed very similar T_o and T_p values and also similar behavior in retrogradation, but waxy corn had lower T_c and ΔH values than normal corn. Amaranth and waxy corn amylopectins did not show gelatinisation endotherms but during retrogradation the former sample presented a lower tendency to retrograde than the latter. On the other hand, commercial corn amylopectin exhibited a gelatinisation endotherm and presented the highest tendency to retrogradation. Finally, according to these experiments the effect of temperature on amylopectin retrogradation was more drastic for amaranth and waxy corn amylopectins than for commercial corn amylopectin. Further studies are required on the role of amylopectin in the cited phenomena.

ACKNOWLEDGEMENTS

Financial support from the Consejo Nacional de Ciencia y Tecnología-México is gratefully acknowledged. One of the authors (L.A.B.-P.) also acknowledges the leave of study from the Instituto Tecnológico de Acapulco.

REFERENCES

Banks, W. & Greenwood, C. T. (1967). The fractionation of laboratory-isolated cereal starches using dimethyl sulphoxide. *Starch/Stärke*, **19**, 394–8.

Bertoft, E. (1991). Chains of intermediate lengths in waxy-maize amylopectin. *Carbohydr. Res.*, **212**, 245–51.

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

Cooke, D. & Gidley, M. J. (1992). Loss of crystalline and molecular order during starch gelatinisation: origin of the enthalpic transition. *Carbohydr. Res.*, **227**, 103–12.

Donovan, J. W. (1979). Phase transitions of the starch–water system. *Biopolymers*, **18**, 263–75.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, **28**, 350–6.

Enevoldsen, B. S. & Juliano, B. O. (1988). Ratio of A chains to B chains in rice amylopectins. *Cereal Chem.*, **65**, 424–7.

French, D. (1984). Organization of starch granules. In *Starch: Chemistry and Technology*, eds. R. L. Whistler, J. N. BeMiller & E. F. Paschall. Academic Press, New York, USA, pp. 183–247.

Gorinstein, S. & Lii, Ch.-Y. (1992). The effects of enzyme hydrolysis on the properties of potato, cassava and amaranth starches. *Starch/Stärke*, **44**, 461–6.

Gudmundsson, M. & Eliasson, A.-C. (1992). Comparison of thermal and viscoelastic properties of four waxy starches and the effect of added surfactant. *Starch/Stärke*, **44**, 379–85.

Hizukuri, S. (1985). Relationship between the distribution of the chain length of amylopectin and the crystalline structure of starch granules. *Carbohydr. Res.*, **141**, 295–306.

Hizukuri, S. (1986). Polymodal distribution of the chain lengths of amylopectins, and its significance. *Carbohydr. Res.*, **147**, 342–7.

Hizukuri, S., Kaneko, T. & Takeda, Y. (1983). Measurement of the chain length of amylopectin and its relevance to the origin of crystalline polymorphism of starch granules. *Biochim. Biophys. Acta*, **760**, 188–91.

Hizukuri, S., Takeda, Y., Maruta, N. & Juliano, B. O. (1989). Molecular structures of rice starch. *Carbohydr. Res.*, **189**, 227–35.

Hoseney, R. C., Zeleznak, K. J. & Yost, D. A. (1986). A note on the gelatinization of starch. *Starch/Stärke*, **38**, 407–9.

Jane, J.-L. & Chen, J.-F. (1992). Effect of amylose molecular size and amylopectin branch chain length on paste properties of starch. *Cereal Chem.*, **69**, 60–5.

Jovanovich, G., Zamponi, R. A., Lupano, C. E. & Añon, M. C. (1992). Effect of water content on the formation and dissociation of the amylose–lipid complex in wheat flour. *J. Agric. Food Chem.*, **40**, 1789–93.

Kobayashi, S., Schwartz, S. J. & Lineback, D. R. (1986). Comparison of the structures of amylopectins from different wheat varieties *Cereal Chem.*, **63**, 71–4.

Koizumi, K., Fukuda, M. & Hizukuri, S. (1991). Estimation of the distributions of chain length of amylopectins by high-performance liquid chromatography with pulsed amperometric detection. *J. Chromatogr.*, **585**, 233–8.

MacGregor, A. W. & Morgan, J. E. (1984). Structure of amylopectins isolated from large and small starch granules of normal and waxy barley. *Cereal Chem.*, **61**, 222–8.

Manners, D. J. (1989). Recent developments in our understanding of amylopectin structure. *Carbohydr. Polym.*, **11**, 87–112.

Nelson, N. (1944). A photometric adaptation of the somogyi method for the determination of glucose. *J. Biol. Chem.*, **153**, 375–80.

Paredes-López, O. & Hernández-López, D. (1991). Application of differential scanning calorimetry to amaranth starch gelatinization–Influence of water, solutes and annealing. *Starch/Stärke*, **43**, 57–61.

Paredes-López, O. & Hernández-López, D. (1992). Food properties of amaranth seeds and methods for starch isolation and characterization. In *Modern Methods of Plant Analysis*, eds. H. F. Linskens & J. F. Jackson. Springer-Verlang, Berlin, Germany, pp. 217–39.

Paredes-López, O., Schevenin, M. L., Hernández-López, D.

& Cárabez-Trejo, A. (1989). Amaranth starch—Isolation and partial characterization. *Starch/Stärke*, **41**, 205–7.

Robin, J. P., Mercier, C., Charbonniere, R. & Guilbot, A. (1974). Lintnerized starches. Gel filtration and enzymatic studies of insoluble residues from prolonged acid treatment of potato starch. *Cereal Chem.*, **51**, 389–406.

Sanders, E. B., Thompson, D. B. & Boyer, C. D. (1990). Thermal behavior during gelatinization and amylopectin fine structure for selected maize genotypes as expressed in four inbred lines. *Cereal Chem.*, **67**, 594–602.

Shi, Y.-Ch. & Seib, P. A. (1992). The structure of four waxy starches related to gelatinization and retrogradation. *Carbohydr. Res.*, **227**, 133–45.

Suzuki, A., Kaneyama, M., Shibanuma, K., Takeda, Y., Abe, J. & Hizukuri, S. (1992). Characterization of lotus starch. *Cereal Chem.*, **69**, 309–15.

Taki, M., Suzuki, K., Taki, A., Hisamatsu, M. & Yamada, T. (1988). Gel chromatography of residue and extract obtained from starch granules by hot aqueous 1-butanol solution treatment. *Starch/Stärke*, **40**, 177–81.

Tester, R. F. & Morrison, W. R. (1990). Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. *Cereal Chem.*, **67**, 551–7.

Wang, Y.-J., White, P., Pollak, L. & Jane, J. (1993). Characterization of starch structures of 17 maize endosperm mutant genotypes with Oh43 inbred line background. *Cereal Chem.*, **70**, 171–9.

White, P. J., Abbas, I. R. & Johnson, L. A. (1989). Freeze–thaw stability and refrigerated-storage retrogradation of starches. *Starch/Stärke*, **41**, 176–80.

Yuan, R. C., Thompson, D. B. & Boyer, C. D. (1993). Fine structure of amylopectin in relation to gelatinization and retrogradation behavior of maize starches from three *wx*-containing genotypes in two inbred lines. Cereal Chem., **70**, 81–9.

Zeleznak, K. J. & Hoseney, R. C. (1986). The role of water in the retrogradation of wheat starch gels and bread crumb. *Cereal Chem.*, **63**, 407–11.

Zobel, H. F. (1988). Starch crystal transformations and their industrial importance. *Starch/Stärke*, **40**, 1–7.