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Effects of cross-linking and low molecular amylose on pasting characteristics of waxy corn starch

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ABSTRACT

The action of amylose within the granule of normal corn starch is investigated by changes in pasting characteristics of waxy corn starch in a Rapid Visco Analyzer (RVA), using addition of soluble amylose (DP = 18) and cross-linking with epichlorohydrin. Although waxy corn starch, containing no amylose, did not show an effect of addition of amylose on pasting characteristics, by cross-linking with epichlorohydrin, the pasting peak viscosity and breakdown were greatly enhanced and set-back (viscosity increased in the cooling process after gelatinization) was generated. The cross-linking depressed the disintegration of starch granules in the swelling process, with amylose interaction, resulting in RVA pasting characteristics similar to those seen with normal corn starch containing amylose. Set-back was essentially caused by rearrangement among modified amylopectins. Addition of sodium dodecyl sulfate (SDS) to the RVA more efficiently enhanced the effect. This indicated that amylose in normal corn starch interacts with amylopectin through locally strong linkages.

1. Introduction

Starch granules are mainly composed of amylose and amylopectin. The amylose has a linear structure with α-1,4-linkages, and a content of 20–25% w/w in starches of cereals and tubers. Amylopectin is a macromolecule with branching and α-1,6-linkages and has a crystalline region formed by short linear side chains. Starch is swollen by heating in a suspension in water and the resulting physicochemical properties are useful in foods. Swelling of starch is attributable to expansion of amylopectin; disruption of the crystalline region is caused by an expansion of the amorphous region, resulting in enhancement of the interaction of starch molecular chains with water (Donovan, 1979). Amylose is leached from starch granules to water in the heating process. The leaching amylose interacts with molecular chains in the amylopectin of swollen starch granules, forming a three-dimensional network, and characterizes the resulting physicochemical properties (Doublier, 1981; Eliasson, 1985; Morris, 1990; Tester & Morrison, 1990). The swollen starch is converted to a rigid structure by a decrease in the temperature. This phenomenon, retrogradation, results from the rearrangement of amylose (Gidley, 1989; Ottenhof & Farhat, 2004). Thus, amylose and amylopectin characterize the physicochemical properties of starch. Their structures, distribution, and composition vary, depending on the origin of the starch.

The interaction between amylose and amylopectin has been studied and synergism has often been observed (Klucienec & Thompson, 2002; Russell, 1987; Lu, Duh, Lin, & Chang, 2008; Jane & Chen, 1992; Lee, Swanson, & Baik, 2001). The behaviour of amylose is an important factor because of its higher mobility and lower molecular size compared to amylopectin. The location and role of amylose within the starch granule must be investigated to clarify the physicochemical properties. In general, cross-linking has been well used to prepare modified starch for thickening, stabilizing, and limiting retrogradation (Delville, Joly, Dole, & Bliard, 2002; Evans & Haisman, 1979; Lloyd, 1970; Simkovic, 1996). This also provides information on the structural characteristics of amylose and amylopectin in starch granules. Jane, Xu, Radosavljevic, and Seib (1992) investigated the location of amylose in normal corn and potato starches, using epichlorohydrin as a cross-linking reagent. As a result, amylose was found to be associated with amylopectin outside of the starch granule.

In this study, effects of addition of soluble amylose and cross-linking with epichlorohydrin were investigated in relation to pasting characteristics of waxy corn starch in a Rapid Visco Analyzer (RVA) and the action of amylose within normal corn starch granules was investigated. Waxy corn starch essentially consists of amylopectin alone and is often studied as a model of amylopectin. Insertion of soluble amylose into waxy corn starch granules was done by heat-treatment with the water–ethanol. The mixture causes rearrangement among the molecular chains within the starch granule as with heat-moisture treatment (Kurakake, Noguchi, Fujioka, & Komaki, 1997).

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Addition of the SDS (sodium dodecyl sulfate) surfactant during the pasting process is used to analyze the degree of interaction among molecular chains in starch (Kurakake, Noguchi, Fujioka, & Komaki, 2008). In normal corn starch, SDS improves the swelling of starch granules through the formation of complexes with starch molecular chains and negative-charge repulsion (Kurakake, Hagiwara, & Komaki, 2004). In cross-linking of waxy starch, the effect of SDS on the pasting properties was investigated. Although SDS is also used to investigate the interaction between amylose and lipids in starch granules (Evans, 1986), waxy starch has no amylose or lipids.

2. Materials and methods

2.1. Materials

Normal and waxy corn starches were supplied by Sanwa Cornstarch Ltd. (Nara, Japan). Normal corn starch contains about 26% amylose and 0.6% lipids, which exist within starch granules, whereas waxy corn starch contains amylopectin alone. Amylose A (soluble low molecular amylose, corn starch origin) was purchased from Wako Ltd. The mean molecular weight was 2900, corresponding to the degrees of polymerization (DP) of 18.

2.2. Treatment of maize starch with water–ethanol mixtures

Ten grammes of maize starch and 50 ml of a water–ethanol mixture (water:ethanol 30:70 by weight) were placed in a 100 ml capped-flask (Kurakake et al., 1997). After heating at 70 °C for 10 min with shaking, the sealed flask was autoclaved at 120 °C for 20 min. After cooling to 98 °C, the treated starch was washed with 80 ml of acetone by decantation, immersed in acetone (80 ml) for 24 h to replace the water in the starch, filtered, washed with ether, and finally dried at room temperature *in vacuo*. In the following sections, the treated starches are designated EW 30.

In the case where Amylose A was added, 0.23 g was dissolved in water before adding ethanol and used for heat-treatment of starch. The amylose content was adjusted to 2.2% in a starch base with waxy corn starch. Although this value is very low in comparison with amylose content of normal corn starch (26%), the prepared content is not too low to observe interaction with amylopectin chains on surface layers of the waxy starch granules.

2.3. Cross-linking reaction of waxy corn starch

Waxy corn starch (10 g) was suspended in 50 ml of solvent (20% distilled water and 80% ethanol) containing 0.4 M NaOH. Epichlorohydrin (0.1 ml) was added, and the final concentration in the mixture was 0.8%. The suspension was incubated for the cross-linking reaction by stirring at 40 °C for 1, 2, 3, 5, and 10 h. At each given time, the suspension was neutralized by 2 M HCl and the cross-linked waxy starch was collected by filtration, washed with water, acetone and methanol in turn, and dried at room temperature *in vacuo*.

2.4. Determination of swelling power and solubility

Cross-linked waxy corn starch and untreated starch were dispersed in water (2.5% w/w) and incubated at 98 °C for 30 min, followed by centrifugation at 1500*g* for 30 min. The volume of supernatant, the concentration of solubilized starch and the weight of sedimented starch were measured to calculate the swelling power and solubility of the starches. The concentration of dissolved starch was determined by the method of Dubois, Gilles, Hamilton, Rebers, and Smith (1956). Swelling power was determined by dividing the wet weight of sedimented starch by the dry weight of starch in the sediment, in which amount of dissolved starch was subtracted from the dry starch.

2.5. Pasting properties from Rapid Visco Analysis

An RVA (RVA-3D, Foss Electric Japan Ltd., Tokyo, Japan) was used to measure changes in viscosity upon heating and cooling. The sample was added to the RVA vessel to make a 10% suspension in 25 g of deionized distilled water. The sample was stirred at 960 rpm for 10 s and at 160 rpm by a plastic paddle in the vessel. After incubating for 1 min at 30 °C, the sample was heated at 5 °C/min to 95 °C, maintained at 95 °C for 6 min, cooled at −5 °C/min to 50 °C, and held for 10 min at 50 °C. RVA parameters, the gelatinization onset temperature, pasting peak viscosity and temperature, breakdown and set-back were obtained from RVA viscogram data.

SDS (sodium dodecyl sulfate) was used to investigate the interaction among molecular chains in starch. In this case, 25 g of 0.2% SDS aqueous solution were added to make a 10% starch suspension.

3. Results and discussion

Fig. 1 shows RVA profiles of normal and waxy corn starches. In waxy starch, the pasting peak and its temperature were sharper and lower than those of normal starch, respectively. The major difference is the value of set-back in the cooling process. Waxy starch shows much less set-back than does normal starch. This finding is attributable to an absence of amylose, because amylose interacts with amylopectin in the swelling (Doublier, 1981; Eliasson, 1985; Morris, 1990; Tester & Morrison, 1990). Furthermore, when SDS (sodium dodecyl sulfate) was added to the starch suspension in the RVA, the effects were very different between normal and waxy corn starches. In normal starch, the pasting peak, breakdown and set-back were enhanced by SDS. Interaction of amylose with swelling starch reduces disintegration of starch granules by forming a three-dimensional network through amylopectin molecular chains. On the other hand, in waxy starch which has no amylose, an effect of SDS was not observed. The action of amylose on the pasting characteristics of starch granules was investigated using waxy starch as the basic amylopectin model.

First, the effect of rearrangement with low molecular amylose was examined in waxy corn starch. Amylose A (DP = 18) was dissolved in water and ethanol was added to adjust the water content to 30% before heat-treatment of waxy starch. This heat-treatment with the water–ethanol mixture causes rearrangement among the molecular chains within the starch granule, as with heat-moisture treatment (Kurakake et al., 1997). Permeation of a slight amount of water into the starch granule causes the rearrangement. In heat-treatment with the 30% water/70% ethanol mixture of normal corn starch, the swelling power was decreased because of a change in molecular structure within the starch granule. In this experiment, it was expected that soluble Amylose A would invade the waxy starch granule and interact with the molecular chains of amylopectin during heat-treatment, which allows insertion of amylose into waxy granules without the gelatinization and disintegration. Table 1 shows the viscosity parameters in RVA for untreated waxy starch, EW 30 without Amylose A, and EW 30 with Amylose A. There was little effect of addition of Amylose A on viscosity in the RVA. The difference in RVA viscosities was almost within the range of error. Enhancement of pasting peak viscosity and set-back, seen in normal corn starch, was not detected for waxy starch. The effect of SDS on RVA was also not observed (data not shown). These results suggest that it is very difficult to make

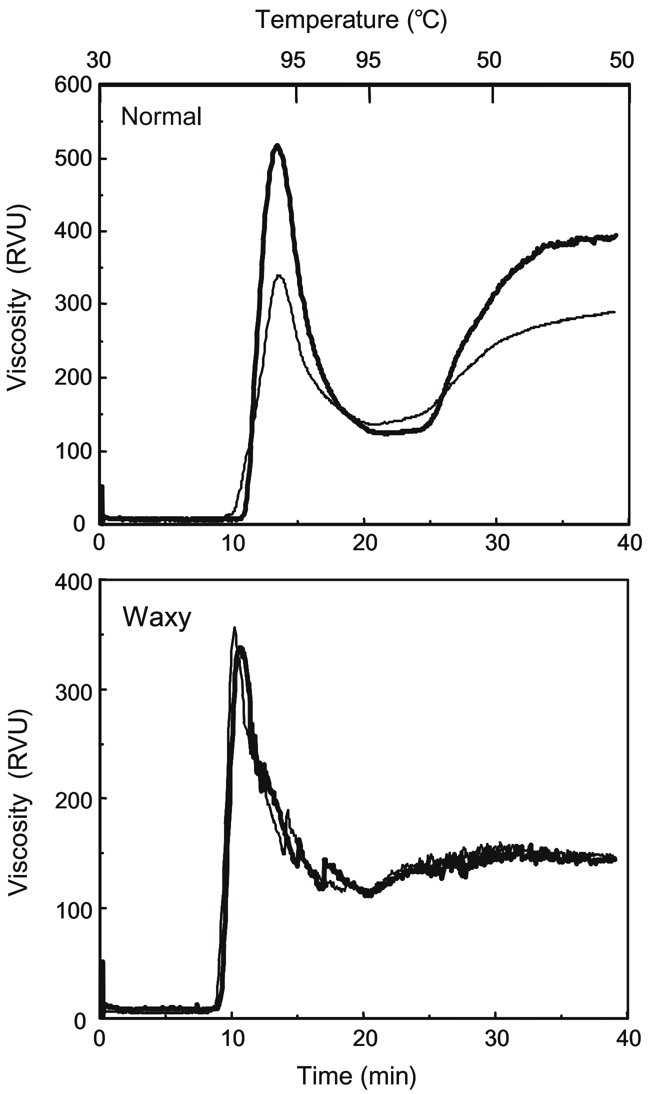

Fig. 1. RVA viscograms of normal and waxy corn starches and their changes by addition of SDS. Bold line shows RVA viscograms in the case of addition of SDS.

amylose insert itself into the starch granule, even with its low molecular size. In many reports relating to viscosity using an amylose and amylopectin mixture, separated amylose and amylopectin or separated amylose and native starch granules were prepared in a given composition (Jane & Chen, 1992; Leloup, Colonna, & Buleon, 1991; Lu et al., 2008). Large size amyloses over DP = 500 were used and these led to enhancement of viscosity. The relationship between amylose and amylopectin, however, do not present a real pasting change during the swelling of the starch granule, because they were mixed after separation from the gelatinized starch. The designed composition of amylose and amylopectin does not correspond to that of native starch. Essentially, it is impossible to insert large molecular weight amylose into the waxy starch granule.

Table 2
Changes in solubility and swelling power of waxy starch by cross-linking reaction.

Cross-linking time (h)	Solubility (%)	Swelling power (g/g)
Untreated	2.41	39.8
1	1.62	15.4
2	1.21	12.2
3	0.934	10.8
5	0.704	9.51
10	0.572	8.78

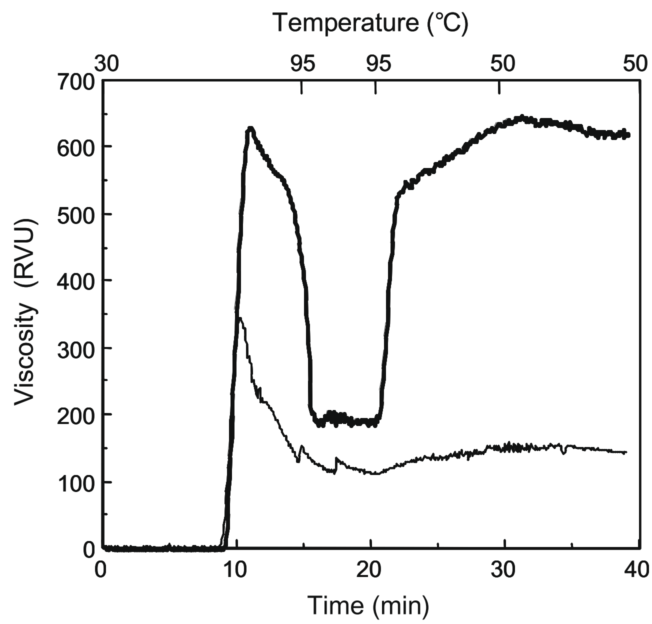

Fig. 2. Change in RVA viscograms of waxy corn starches by cross-linking. Bold line shows RVA viscograms of waxy starch cross-linked for 2 h.

Next, it was expected that covalent linkage by a cross-linking reagent would show an effect similar to the network of amylose in swelling starch granules. Epichlorohydrin was selected because of its small molecular size, which is inserted as deeply as possible into the starch granule. In order to see an effect of the long chain of amylose, larger molecular-size reagents may be required as spaces to obtain distance between the molecular chains. This requirement might be satisfied by restricting the degree of cross-linking in the reaction condition. In this experiment, 0.8% epichlorohydrin (epichlorohydrin/starch, w/w) was used and the degree of cross-linking reaction was depressed by adding ethanol and controlling the reaction time.

Table 2 shows the effect of cross-linking time on the swelling power and solubility of the modified waxy starch. Both values were decreased as the cross-linking progressed. This is a typical property of the cross-linked starch. In analysis in the RVA for 2 h-cross-linked waxy starch, the pasting peak viscosity and breakdown were greatly enhanced and set-back in the cooling process was generated, as shown in Fig. 2. Cross-linking depressed the disinte-

Table 1
Effect of addition of Amylose A on viscosity parameters of waxy starch.

Modified waxy starches	Viscosity (RVU)				Temperature (°C)	
	Pasting peak	Break down	Set-back	End	To	Tp
Untreated	356	242	33	147	68.2	75.3
EW30	322	204	32	138	72.6	78.1
EW30 + Amylose A	324	223	39	130	73.9	79.8

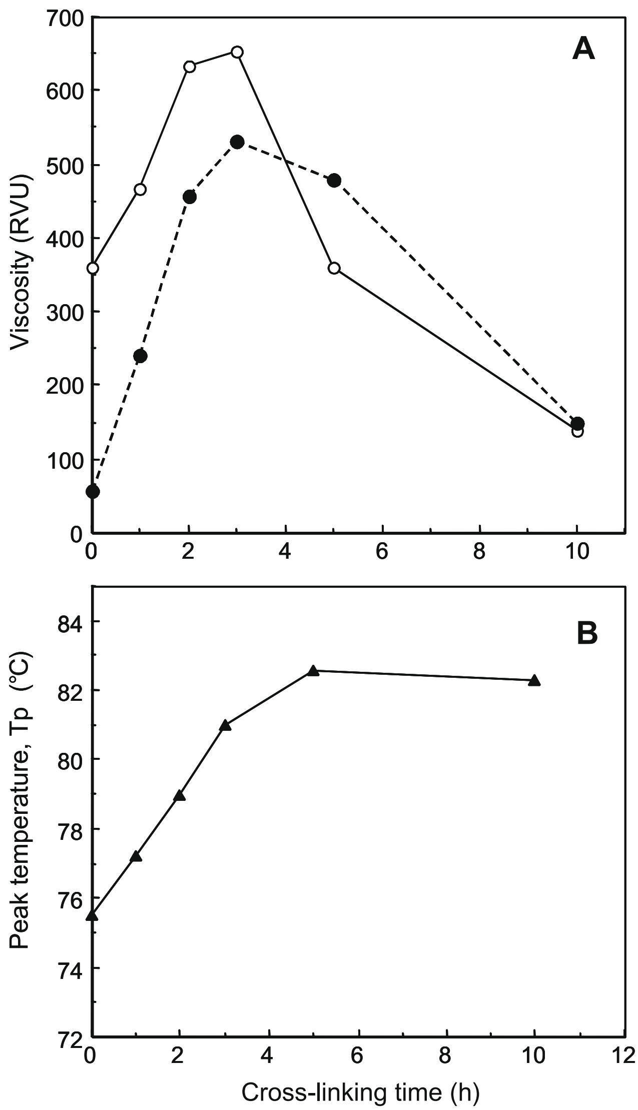

Fig. 3. Change in each viscosity and temperature measured on RVA as a function of cross-linking time. Symbols: (A) pasting peak viscosity (○) and set-back (●), (B) peak temperature (▲).

gration of starch granules and resulted in high viscosity. The high pasting peak viscosity and appearance of high set-back are attributable to amylopectin in the swollen starch granules because waxy starch has no amylose. Cross-linking reagent showed the effect, as in an amylose network.

Fig. 3 shows changes in pasting peak viscosity and set-back as a function of cross-linking time. Both viscosities have maximum values in the 2–3 h cross-linking reaction and decrease to a smaller value than that of the untreated starch over 4 h. Tp (pasting peak temperature) was increased with cross-linking time and was almost constant over 4 h. This profile corresponds to the degree of cross-linking. Occurrence of maximum values, in characteristic viscosities, is controlled by the degree of the cross-linking. At an early time, waxy starch granules could swell to a larger size without disintegration by cross-linking, resulting in high viscosity. As the degree of cross-linking was enhanced, the swelling of starch granules was depressed and viscosities were decreased.

The effect of addition of SDS on RVA was also investigated. Table 3 shows summaries of viscosity and temperature analyzed on RVA for 2 h and 10 h-cross-linked waxy corn starch and untreated waxy starch. After 2 h-cross-linking, pasting peak, breakdown, and set-back were increased by 1.74-, 1.79-, and 13.4-fold, respectively, in comparison with those of the untreated waxy starch. Furthermore, addition of SDS to RVA enhanced these by about 1.3- to 1.4-fold. Cross-linked waxy corn starch showed a SDS-effect similar to that of normal corn starch. SDS forms a complex with helical molecular chains in amylopectin and increases the swelling of starch granule (Kurakake et al., 2004). In 10 h-cross-linking, each viscosity was smaller than that of the untreated starch, and onsets of gelatinization and peak temperatures increased. The increment of viscosity by SDS was, however, much higher than that after 2 h-cross-linking, in which pasting peak, breakdown and set-back were increased by 3.2-, 10.9- and 4.0-fold, respectively. The increase in SDS effect shows the expansion of the rearranged structure, leading to high viscosity. The formation of rigid starch granules through the rearrangement was enhanced as the cross-linking progressed.

From these findings with cross-linked waxy corn starch, the effect of amylose on pasting characteristics of normal corn starch must be revisited. Although waxy starch has no amylose network interacting with swelling starch, characteristics similar to those of starch containing amylose were induced by cross-linking. This suggests that amylose chains do not form so wide a network among swelling starch granules. Many reports indicate the significance of an amylose network in experiments with amylose-amylopectin mixtures (Klucienec & Thompson, 2002; Russell, 1987; Ru et al., 2008; Jane & Chen, 1992; Lee et al., 2001). In these experiments, the mixtures were incubated at temperatures below 25 °C for long periods in order to achieve complete rearrangement between amylose and amylopectin (Klucienec & Thompson, 2002; Jane & Chen, 1992). This does not correspond with pasting changes of starch granules in the RVA. Amylose has an interaction with amylopectin within the starch granule and restricts its disintegration. Although many amylose chains were leached from starch granules in the heating process for swelling, a small amount of amylose remains within the starch granule and act as a cross-linking reagent. Amylose is in a local amorphous region or is widely distributed in amylopectin (Blanshard, Bates, Muhr, Worcester, & Higgins, 1984; Jane et al., 1992). Even if the amylose chain is located partially in the crystalline region in amylopectin, it is doubtful whether amylose shows the same effect as does a cross-linking reagent, because the crystalline region is easily melted in the gelatinization process (Donovan, 1979). The orientation between the

Table 3
Effect of SDS on viscosity parameters of cross-linked waxy starches in RVA.

Modified waxy starches	SDS (%)	Viscosity (RVU)				Temperature (°C)	
		Pasting peak	Break down	Set-back	End	To	Tp
Untreated	0	356	242	33	147	68.2	75.3
	0.2	339	225	31	145	69.5	77.2
2 h	0	632	442	456	619	70.3	79.0
	0.2	804	632	630	810	66.9	74.7
10 h	0	140	36	150	254	71.8	82.3
	0.2	447	394	594	642	70.1	78.4

single long chain amylose and short chains in the crystalline region in amylopectin is not as strong as covalent linkage by cross-linking. It is considered that some amylose chains are linked covalently with amylopectin. In fact, it is reported that amylose is not linear and has slight branching (Cura, Jansson, Krisman, & Aires, 1995; Takeda, Hizukuri, Takeda, & Suzuki, 1987). Amylose of normal corn starch has 2–3% of α-1,6-linkage sites on the basis of methylation analysis.

In biosynthesis of starch in plants, amylose is extended by granule-bound starch synthase and amylopectin is mainly synthesized by a combination of soluble starch synthase and starch branching enzyme, resulting in formation of a cluster structure (Myers, Morell, James, & Ball, 2000; Denyer, Johnson, Zeeman, & Smith, 2001; Nakamura, 2002). It is considered that α-1,6-linkage sites on the amylose chain must be rarely formed by the starch branching enzyme. The non-reducing terminal of this branch on amylose cannot cross-link with other molecular chains of amylopectin in the starch synthesis enzyme system. If a small cluster structure is formed from the branches of amylose, interaction of amylopectin with the cluster would be caused through a double helical structure. More detailed analysis is required on the structure of amylose.

4. Conclusions

Waxy corn starch containing no amylose did not show an effect of addition of small molecular weight amylose (DP = 18) on the pasting characteristics in the RVA. By cross-linking with epichlorohydrin, waxy corn starch showed characteristics similar to those of normal corn starch containing amylose. The cross-linking depressed the disintegration of starch granules in the swelling process, as in amylose interaction, resulting in pasting characteristics by RVA similar to those seen with normal corn starch. Set-back was essentially caused by rearrangement among the modified amylopectins. It is indicated that amylose interacts with amylopectin through locally strong linkages.

References

Blanshard, J. M. V., Bates, D. R., Muhr, A. H., Worcester, D. L., & Higgins, J. S. (1984). Small-angle neutron scattering studies of starch granules structure. *Carbohydrate Polymers, 4*, 427–442.

Cura, J. A., Jansson, P. E., Krisman, C. R., & Aires, B. (1995). Amylose is not strictly linear. *Starch/Starke, 47*, 207–209.

Delville, J., Joly, C., Dole, P., & Bliard, C. (2002). Solid state photocrosslinked starch based films: A new family of homogeneous modified starches. *Carbohydrate Polymers, 49*, 71–81.

Denyer, K., Johnson, P., Zeeman, S., & Smith, A. M. (2001). The control of amylose synthesis. *Journal of Plant Physiology, 158*, 479–487.

Donovan, A. M. (1979). Phase transitions of the starch water system. *Biopolymers, 18*, 263–275.

Doublier, J. L. (1981). Rheological studies on starch-flow behavior on the wheat starch paste. *Starch/Staerke, 33*, 415–420.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*, 350–356.

Eliasson, A. C. (1985). Starch gelatinization in the presence of emulsifier. A morphological study of wheat starch. *Starch/Staerke, 37*, 411–415.

Evans, I. D., & Haisman, D. R. (1979). Rheology of gelatinized starch suspensions. *Journal of Texture Studies, 10*, 347–370.

Evans, I. (1986). An investigation of starch/surfactant interactions using viscosimetry and differential scanning calorimetry. *Starch/Starke, 38*, 227–235.

Gidley, M. J. (1989). Molecular mechanisms underlying amylose aggregation and gelation. *Macromolecules, 22*, 351–358.

Jane, J. L., & Chen, J. F. (1992). Effect of amylose molecular size and amylopectin branch chain length. *Cereal Chemistry, 69*, 60–65.

Jane, J., Xu, A., Radosavljevic, M., & Seib, P. A. (1992). Location of amylose in normal starch granules. I. Susceptibility of amylose and amylopectin to cross-linking reagents. *Cereal Chemistry, 69*, 405–409.

Klucienec, J. D., & Thompson, D. B. (2002). Amylopectin nature and amylose-to-amylopectin ratio as influences on the behavior of gels of dispersed starch. *Cereal Chemistry, 79*, 24–35.

Kurakake, M., Noguchi, M., Fujioka, K., & Komaki, T. (1997). Effects on maize starch properties of heat-treatment with water–ethanol mixtures. *Journal of Cereal Science, 25*, 253–260.

Kurakake, M., Hagiwara, H., & Komaki, T. (2004). Effects of various surfactants on the rheological properties of maize starch granules. *Cereal Chemistry, 81*, 108–114.

Kurakake, M., Noguchi, M., Fujioka, K., & Komaki, T. (2008). Pasting characteristics of maize starch heat-treated with different water–ethanol mixtures. *Cereal Chemistry, 85*, 566–570.

Lee, M. R., Swanson, B. G., & Baik, B. K. (2001). Influence of amylose content on properties of wheat starch and breadmaking quality of starch and gluten blends. *Cereal Chemistry, 78*, 701–706.

Leloup, V. M., Colonna, P., & Buleon, A. (1991). Influence of amylose–amylopectin ratio on gel properties. *Journal of Cereal Science, 13*, 1–13.

Lloyd, N. E. (1970). Starch esters. U.S. patent 3,539,551.

Lu, T. J., Duh, C. S., Lin, J. H., & Chang, Y. H. (2008). Effect of granular characteristics on the viscoelastic properties of composites of amylose and waxy starches. *Food Hydrocolloids, 22*, 164–173.

Morris, V. J. (1990). Starch gelation and retrogradation. *Trends in Food Science and Technology, 1*, 2–6.

Myers, A. M., Morell, M. K., James, M. G., & Ball, S. G. (2000). Recent progress toward understanding biosynthesis of the amylopectin crystal. *Plant Physioloy, 122*, 989–997.

Nakamura, Y. (2002). Towards a better understanding of the metabolic system for amylopectin biosynthesis in plants: Rice endosperm as a model tissue. *Plant Cell Physiology., 47*, 718–725.

Ottenhof, M. A., & Farhat, I. A. (2004). Starch retrogradation. *Biotechnology & Genetic Engineering Reviews, 21*, 215–228.

Russell, P. L. (1987). The ageing of gels from starches of different amylose/amylopectin content studied by differential scanning calorimetry. *Journal of Cereal Science, 6*, 147–158.

Simkovic, I. (1996). One-step quaternization/cross-linking of starch with 3-chloro-2-hydroxypropylammonium chloride/epichlorohydrin in the presence of NH_4OH. *Carbohydrate Polymers, 31*, 47–51.

Takeda, I., Hizukuri, S., Takeda, C., & Suzuki, A. (1987). Structures of branched molecules of amyloses of various origins and molar fractions of branched and unbranched molecules. *Carbohydrate Research, 165*, 139–145.

Tester, R., & Morrison, W. R. (1990). Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose and lipids. *Cereal Chemistry, 67*, 551–557.