BARLEY STARCH. VI. A SELF-LIQUEFYING WAXY BARLEY STARCH[1]

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ABSTRACT

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A new, short awn, hull-less waxy barley (Washonupana) has been developed which retains sufficient active α-amylase-like activity on the starch granules to liquefy not only itself but four times its weight of either waxy corn or normal unmodified corn starch when heated in the Brabender amylograph. Since this liquefaction occurs without cooking or the addition of enzyme, this starch should be ideal for paper and sizing operations and possibly could result in the replacement of corn syrup in brewing applications. Preliminary data suggest the untreated grain might also replace substantial amounts of malt in the brewing industry.

Waxy Compana barley was developed as a new crop source of waxy starch and its properties have been described previously (1). Since this crop has industrial potential and because the hulls are of little value and create special problems in processing, it was decided to breed a hull-less variety.

It has been observed that short awned varieties may have certain agronomic advantages and that they also tend to have larger starch granules (2). For these reasons, the short awn gene was introduced together with the hull-less gene to produce a new, short awn, hull-less waxy Compana (SAHW Compana) named Washonupana.

This investigation was designed to examine the starch from this new variety to see if it maintained the desirable waxy characteristics.

MATERIALS AND METHODS

SAHW Compana (Washonupana) barley was developed by selection of the waxy endosperm, short awn, hull-less caryopsis (*wxwx nn lk2lk2*) genotype from the cross waxy Oderbruker/7*Compana 12/short awn hull-less/7* Compana.

SAHW Compana barley was grown in two locations: one at Mesa, Ariz., and the other at Bozeman, Mont. Both were harvested under dry conditions which should have eliminated any chance for sprouting in the field. A careful examination of the seeds substantiated this assumption. Starch was isolated from composite samples obtained from both locations by the wet-milling technique described previously (1).

The corn starch was unmodified corn starch from CPC International obtained through the courtesy of Jiun Keng. The waxy corn starch was Amioca Waxy Maize Starch obtained from American Maize-Products Co., Roby, Ind.

Protein content was determined by the Kjeldahl method (3, p. 16) (conversion factor 6.25). The samples were ashed according to the usual procedure (3, p. 123). The total free fat was determined by ether extraction (3, p. 128) and crude fiber by the usual technique (3, p. 129). The iodine affinity was determined by the technique of Schoch (4), modified by the use of dimethyl sulfoxide (DMSO) as solvent. Brabender viscosities were determined using the procedure described by Smith (5). For the controls, the α-amylase-like activity associated with the

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granular starch was inactivated by dissolving 200 mg of $HgCl_2$ in the 420 ml of water used to suspend the starch sample. To determine the effect of temperature on the SAHW Compana enzyme, three runs were made in the Brabender amylograph in which the heating cycle was interrupted and the starch paste was held for 1 hr at 70°, 75°, or 80° C before continuing the heating cycle to 92.5° C. The samples were cooked for 15 min at 92.5° C and then cooled to 50° C.

TABLE I
Chemical Composition of Barley Starches

Starch Variety	Ash %	Fat %	Fiber %	Protein %	Iodine Affinity %
Compana[a]	0.21	0.29	...	0.30	5.7
Waxy Compana[a]	0.19	0.27	...	0.31	0.35
SAHW Compana Mesa	0.20	0.05	0.1	0.39	0.8
SAHW Compana Bozeman	0.20	0.07	0.1	0.80	1.0

[a]From ref. 1.

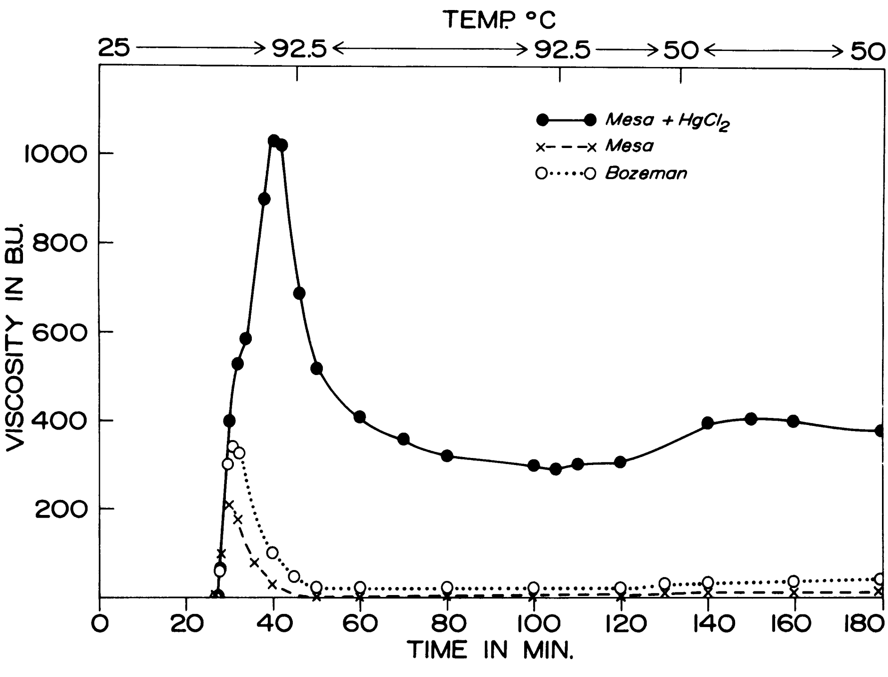

Fig. 1. Brabender amylograms at the 8% level on SAHW Compana starch with and without amylase inhibitor.

RESULTS AND DISCUSSION

The chemical composition of the SAHW Compana, waxy Compana, and normal Compana starches is given in Table I.

Apparently, SAHW Compana starch has a higher iodine affinity than the waxy Compana from which it was derived. The same observation was made on a sample from the previous crop. At this date, we are not sure if the high iodine affinity is caused by contaminating amylose, an intermediate chain length fraction, or long chain amylopectin. The Brabender curves and other properties of this starch certainly do not indicate any substantial amylose content. The low fat content of the SAHW Compana starch is a desirable feature, as the starch does not appear to become rancid on long storage. Waxy Compana starch, on the other hand, became rancid on storage.

The results of the first few Brabender viscosity curves were rather unexpected, so new starch samples were prepared to eliminate any possibility of modification during their preparation. These gave the same results, namely, after peaking at slightly over 200 BU, the viscosity rapidly dropped until at 85° C it was down to 20 units. The viscosity continued to drop during the heating and cooking stage. After cooling to 50° C and holding for 1 hr, it was only 15 BU. On the assumption that the observed results were attributable to the binding of barley α-amylase to the granule, runs were made using $HgCl_2$ as an inhibitor. To examine the effect of

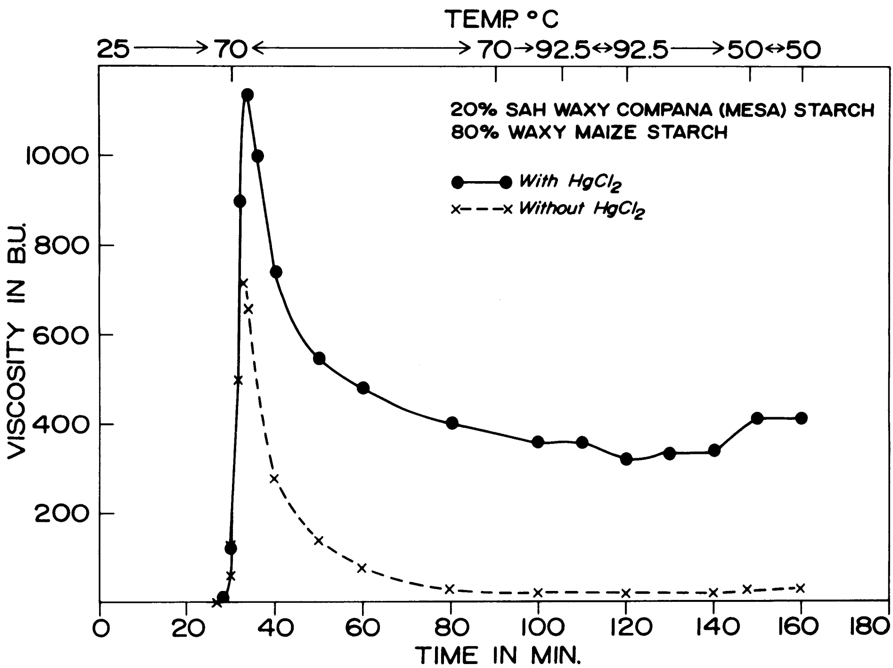

Fig. 2. Brabender amylograms on 20% SAHW Compana starch and 80% waxy maize starch at the 8% level with and without amylase inhibitor, held at 70° C for 1 hr before cooking.

environment, starch samples prepared from seeds grown at both Mesa, Ariz., and Bozeman, Mont., were used. These results are shown in Fig. 1.

It is apparent from Fig. 1 that the results observed were not due to environment, although the α-amylase activity of the Arizona sample appeared to be slightly greater than that observed in the Bozeman sample. Since the curve obtained by the use of $HgCl_2$ was essentially identical to that obtained from the parent waxy Compana (1), it must be concluded that the low viscosities obtained for the SAHW Compana are owing to adhering α-amylase.

Cereal α-amylases are known to be heat-labile and, on the assumption that a starch with a low pasting temperature might be liquefied by the SAHW Compana amylase before the enzyme was inactivated, a series of experiments was run in which the heating phase was interrupted by holding for 1 hr at 70°C before completion of the heating cycle. These samples were then cooked at 92.5°C for 15 min and cooled to 50°C. Quite arbitrarily, a ratio of 20% SAHW Compana and 80% waxy maize was used with the mixture at the 8% concentration level for these experiments. To again examine the effect of the inhibition of α-amylase, a control was run with added $HgCl_2$. These results are shown in Fig. 2.

Figure 2 demonstrates that the SAHW Compana starch has sufficient enzyme

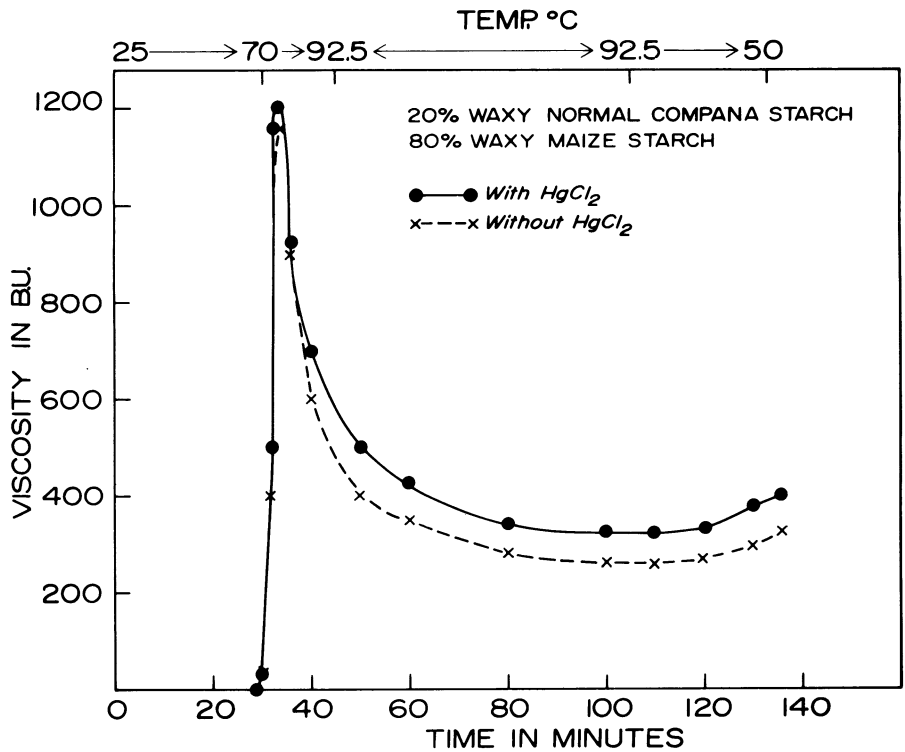

Fig. 3. Brabender amylograms on 20% normal waxy Compana starch and 80% waxy maize starch at the 8% level with and without amylase inhibitor.

to liquefy itself and four times its weight of waxy maize. A shortage of starch prevented determining the minimum amount of SAHW Compana necessary to achieve this effect.

To substantiate that the effects observed in Fig. 2 were not artifacts of the $HgCl_2$ treatment, Brabender viscosity curves were run on a starch mixture containing 80% waxy maize and 20% normal waxy Compana with and without $HgCl_2$. These results are shown in Fig. 3. Addition of $HgCl_2$ caused a slight increase in viscosity. A similar observation has been reported on corn starch (6).

Although 70°C is not high enough to completely paste corn starch (7), it was decided to try duplicating the results in Fig. 2 using unmodified corn starch in place of the waxy maize. On the assumption that higher temperatures would more effectively gelatinize corn starch and that this effect might offset the more rapid denaturation of the enzyme, holding temperatures of 75° and 80°C were used in addition to 70°C. Again a control sample was run using $HgCl_2$ as an amylase inhibitor. These curves are shown in Fig. 4.

Apparently, a 20% SAHW Compana mixture will also liquefy itself and four times its weight of unmodified corn starch. This amylase appears to be heat-sensitive and, like other barley amylase, probably has a temperature optimum in the range of 60°–65°C. However, since normal starches do not paste at this temperature, its effectiveness at this temperature could not be examined by the above procedure.

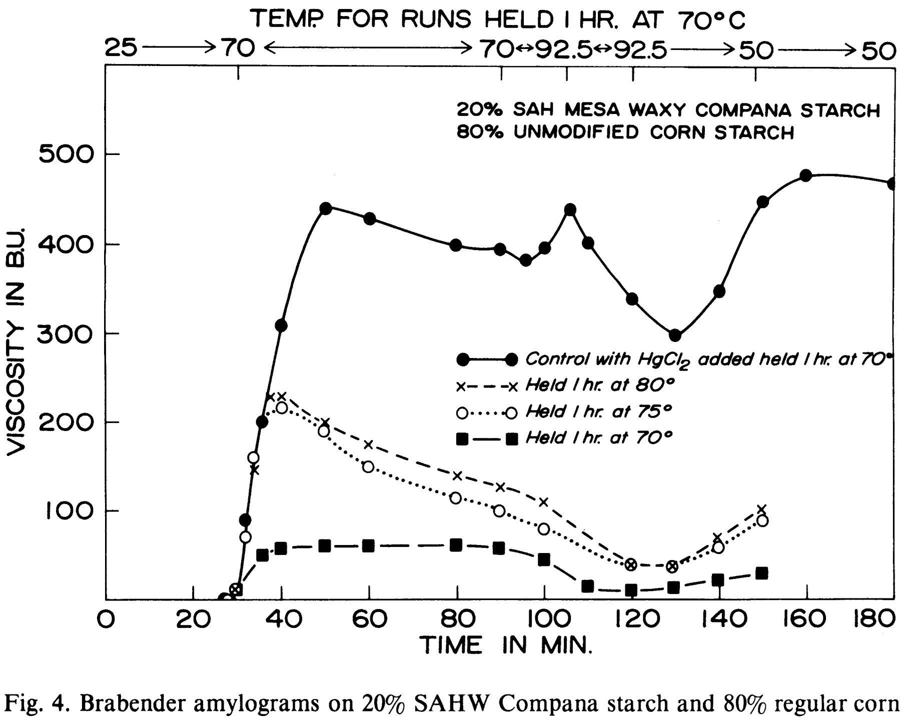

Fig. 4. Brabender amylograms on 20% SAHW Compana starch and 80% regular corn starch at the 8% level with different holding times before cooking and with amylase inhibitor.

The control sample in Fig. 4 shows two pasting peaks. The first is, in all probability, primarily due to the waxy barley starch. The second peak is probably a result of the pasting of the more resistant fraction of the normal corn starch. The rapid drop of viscosity during the cooking cycle is a normal property of waxy starches.

Fermentation tests on the thinned pastes recovered from the Brabender amylograph indicated minimal sugar production, confirming the α-amylase-like activity of this enzyme system.

This new starch has very interesting possible applications. For paper applications, pastes could be prepared by mixing 20% SAHW Compana with normal corn starch without the usual cooking, cooling, and enzyme addition. It would only be necessary to mix the two starches and heat until the proper viscosity was obtained and then denature the enzyme. This would also apply for the preparation of size. The ability of SAHW Compana starch to liquefy other starches suggests that a mixture of this starch with normal corn starch would be an economical replacement for corn syrup in brewing.

At the present time, we do not know how this amylase remains bound to the starch granule through the complex operation involved in the starch purification. In the more than 50 barley starches isolated from different varieties, this is the first case where we have found amylase associated with the isolated starch. This suggests that this amylase has an unusual ability to bind to starch granules.

Since much α-amylase was expected to be lost in the starch separation regardless of binding, one would expect a rather high concentration of this enzyme in the initial seed. This is interesting because very little α-amylase is supposed to be present in ungerminated cereals (8). Preliminary experiments have shown the ground grain to be capable of thinning starch mashes. Experiments are in progress to study the efficiency of this grain as a saccharifying agent since it is conceivable it could replace substantial amounts of the malt used in the brewing operation.

Acknowledgments

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