Basic Studies on Cooking Potatoes. II. Effect of Potato Extract on the Interrelation of Gelatinization-Retrogradation of Potato Starch

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Cereal Chem. 56(4):257-261

ABSTRACT

The effect of the hot water-soluble components of potato on the gelatinization and retrogradation of potato starch and the interrelation between them was studied. The state of gelatinization of the starch paste was quantitatively determined by glucoamylase digestion and by iodine affinity. The extract inhibited the gelatinization and promoted the retrogradation and the heat-moisture modification mainly by its interaction with amylose. The pastes became gelatinization-resistant in the presence of the extract depending on the conditions of gelatinization and retrogradation. It was suggested that in the pastes of low (40%) and high (100%) degree of gelatinization (estimated by glucoamylase digestion), the gelatinization-resistant parts were formed through the cooperative effect of heat-moisture treatment and retrogradation and sole effect of retrogradation, respectively.

The behavior of starch in the processing and preservation of starchy foods is not fully understandable through knowledge of the behavior of refined starch alone, and the interactions of starch and other food components should be studied.

Previously (Suzuki et al 1969), we reported that the heated water-soluble extract of various plants, such as potato tubers, sweet potato roots, and wheat grains, promoted the precipitation of amylodextrin from aqueous solutions and, in addition, these extracts shifted the resulting crystalline structure toward the A-type. Furthermore, potato extract promoted the retrogradation and crystallization of potato starch in aqueous paste (Suzuki and Hizukuri 1974) as revealed by the estimation of the degree of gelatinization by the methods of glucoamylase digestion (Toyama et al 1966), and iodine affinity (Hizukuri et al 1971), and X-ray diffraction. These facts imply that potato extract contains materials that affect the retrogradation and crystallization of starch.

We reported (Suzuki et al 1971) also that when cube-cut potatoes were precooked (for example, heating under 90° C for 20 min) and cooled below room temperature for a short time (1 hr), the starch in the potato was no more fully gelatinized by reheating under usual cooking conditions (at 100° C for 20 min).

The purpose of this study was to clarify whether this was due to the starch itself or to interaction with other components of the potato, that is, the hot water extractable materials, since these materials promote retrogradation of starch as mentioned above and some inorganic ions do similarly (Hizukuri et al 1960, Khairy et al 1963).

The experiments were performed following the process of first heating (gelatinization), cooling (retrogradation), and second heating (gelatinization), which is involved in potato flake manufacture (Eskew 1967). The effect of potato extract on this process and the interrelations of gelatinization and retrogradation were examined.

MATERIALS AND METHODS

Potato Starch

Commercial potato starch was used after washing several times with distilled water and drying at room temperature.

Potato Extract

The extract was prepared by the method described in a previous paper (Suzuki et al 1969). Namely, 200 g of peeled potatoes was homogenized with 200 ml of distilled water in a home electric blender for 1 min. After removal of fibrous material and tissue debris by squeezing the homogenate through two layers of gauze, the resulting juice was centrifuged at 10,000 × g for 10 min. The supernatant was heated in a boiling water bath for 5 min to inactivate the enzymes. The insoluble materials produced were removed by centrifugation at 10,000 × *g* for 10 min and the supernatant was used. The extract contained 1.6% solid materials and 0.65 mg N/ml and the pH was 6.1. Nitrogen was analyzed by an Antek 703 digital nitrogen system using sodium nitrite as standard.

A concentrated extract was prepared also by the above procedure without using water.

Experimental Procedures

Potato starch suspended in potato extract or water was treated by following the process of first heating, cooling, and second heating and the degree of gelatinization (DG) of the resulting paste was estimated.

First Heating. Potato starch (1 g) suspended in 19 ml of water or the extract was heated at the indicated temperature in a water bath with stirring for 15 min.

Cooling. The hot paste was immediately cooled to 0 or 30° C for 1 hr by immersing in a water bath.

Second Heating. After the cooling or immediately after first heating, the paste was reheated and stirred in a boiling water bath for 15 min. The raw potato starch suspended in the extract was fully gelatinized under the conditions.

Estimation of Degree of Gelatinization

Pretreatment. The paste was poured into a blender that contained 100 ml of 99% ethanol and was mixed at a high speed for 1 min, thus rapidly dehydrating the paste. The resulting starch precipitate was collected on a glass filter. The dehydrated starch was stored in a desiccator over calcium chloride under reduced pressure for at least 24 hr. The DG of the treated starch was estimated by the following two methods.

Digestion with Glucoamylase. The method described by Toyama et al (1966) was used with minor modifications to simplify the procedure and to improve the accuracy as follows. Forty milligrams of dry, powdered starch was placed in a Potter homogenizer with a loosely fitted pestle and was dispersed in 4 ml of water by moving the pestle up and down 20 times. One-milliliter portions of the dispersed solution were transferred to two 10-ml test tubes. Three milliliters of 0.4 *M* acetate buffer, pH 4.8, was added to one of the test tubes (dispersed sample) and 0.1 ml of 10 *N* NaOH was added to the other to dissolve the dispersion completely, and the samples were stirred well at room temperature. Water (1.3 ml) was added to the dissolved sample, which was then acidified to pH 4.8 with 1 *M* acetic acid (approximately 1.6 ml, predetermined). The test tubes were warmed in a water bath at 37° C for a few minutes, 1 ml of glucoamylase solution (2.64 units) (Toyama et al 1966) was added, and the mixture was incubated further at 37° C with stirring

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by hand at 15 min intervals. After incubation for 1 hr, 0.5 ml of the reaction mixture was transferred into a centrifuge tube containing 0.5 ml 1*N* HCl and the mixture was diluted to 10 ml with water. The mixture was centrifuged at 3,000 rpm for 10 min and the supernatant (0.5 ml) was diluted twofold with water. The reducing power of the diluted solution was determined by the colorimetric Somogyi method (Somogyi 1952). The degree of gelatinization (DG-g) was calculated from the reducing power by the following formula:

$$\text{DG-g (\%)} = \frac{\text{Reducing power of dispersed sample}}{\text{Reducing power of dissolved sample}} \times 100$$

Amperometric Titration with Iodine. The DG by this method (DG-i) (Hizukuri et al 1971) was given by the following formula:

$$\text{DG-i (\%)} = \frac{\text{Iodine affinity of dispersed sample}}{\text{Iodine affinity of dissolved sample}} \times 100$$

Iodine affinity was determined by amperometric titration. Since iodine affinity is dependent on amylose, DG-i is specific for the gelatinization of amylose (Hollo et al 1960, Hizukuri et al 1971).

The dried specimen (50 mg) was dispersed in 5 ml of water in a Potter homogenizer as described above. To the dispersion, 10 ml of 1*N* HCl, 5 ml of 1*N* KOH, and 5 ml of 0.4*M* KI were added and the mixture was made up to 100 ml with distilled water. The mixture was titrated at intervals with 0.2-ml portions of 0.01*N* KIO_3 at 30° C and the electric current was read 1.5 min after each addition. Other procedures were the same as described by Larson et al (1953). The iodine affinity was determined from the titration curve. The iodine affinity of the dissolved specimen was determined similarly by titration of the following mixture. The specimen (50 mg) was moistened with several drops of ethanol and dissolved in 5 ml of 1*N* KOH. To the solution were added 10 ml of 1*N* HCl and 5 ml of 0.4*M* KI and the mixture was made up to 100 ml with distilled water.

Amylogram

Twenty-five grams of potato starch (moisture content 17.2%) was suspended in 450 ml of water or potato extract in a cup of a Brabender amylograph (Type DC 3, 700 CM/GRS). Temperature was raised from 30 to 92.5° C at the rate of 1.5° C per min and was maintained at 92.5° C for 15 min.

TABLE I
Relationship between Heating Time (at 100° C) and Degree of Gelatinization (DG-g and DG-i) of the Potato Pastes

	Heating Time (min)					
	5		10		15	
	Water	Extract	Water	Extract	Water	Extract
DG-g (%)	100	91	100	93	100	100
DG-i (%)	97	86	97	91	97	95

RESULTS

Heating Conditions and DG

Table I gives the DG of potato starch pastes prepared by heating the starch suspension in water (water-paste, WP) or extract (extract-paste, EP) at 100° C for 5, 10, and 15 min. After 5 min of heating, the resulting WP gave 100% DG-g and 97% DG-i, which did not increase with further heating. Therefore, it was concluded that heating at 100° C for 5 min was enough to fully gelatinize potato starch in water. The resulting EP gave 91% DG-g and 86% DG-i after heating for 5 min. The slower gelatinization of the starch in the presence of the extract was apparent and it was found that heating at 100° C for 15 min was necessary to fully gelatinize potato starch in the extract.

Figure 1 shows the temperature dependence of gelatinization of potato starch in water and in extract. Gelatinization was scarcely observed under 50° C in both the WP and the EP (raw potato starch give less than 3% DG-g). At 60° C, the WP gave 40% DG-g, while the EP gave nearly the same value as that at 50° C and little progress of the gelatinization was noted. Thus, there was a large difference between the EP and the WP under these heating conditions. At 65° C, although the gelatinization of the EP progressed to 80% DG-g, the value was slightly lower than that of the WP (85%). Gelatinization of amylose in the EP estimated by DG-i was 20% lower than that of the WP. At 100° C, as already described, the EP and the WP were assessed as fully gelatinized. These results imply that the starch is stabilized against gelatinization by the soluble materials in the extract. Gelatinization is appreciably inhibited by the extract below 72° C. To prepare EP and WP of the same DG-g, it was necessary to heat the EP at 2–3° C higher than the WP (Table II).

Retrogradation

Retrogradation, which occurred in cooling the paste at 30 and 0° C for 1 hr immediately after first heating, was estimated by decrease of DG (Table III). The greater retrogradation was found

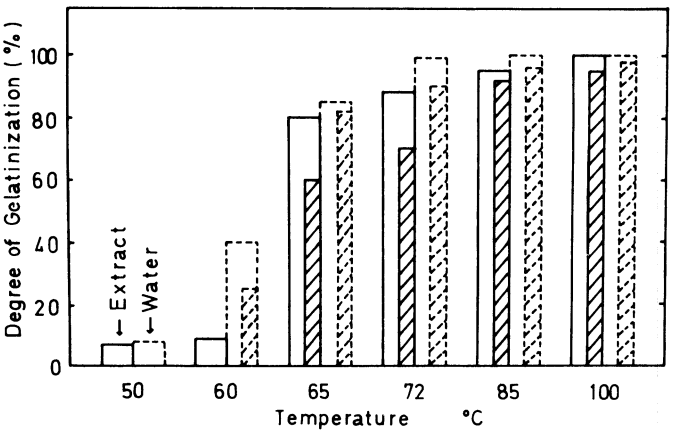

Fig. 1. Relation between heating temperature of potato starch suspension in water or potato extract and the degree of gelatinization of the resulting pastes. The starch was heated for 15 min at the temperature indicated. **Open bar** = DG-g; **shaded bar** = DG-i.

TABLE II
Heating Temperature (for 15 min) for the Preparation of Pairs of Pastes and Their Degree of Gelatinization

	Pastes[a]									
	WP-40	EP-40	WP-60	EP-60	WP-80	EP-80	WP-99	EP-88	WP-100	EP-100
	60° C	63° C	61° C	64° C	63° C	65° C	72° C	72° C	100° C	100° C
DC-g (%)	40	40	60	60	80	79	99	88	100	100
DG-i (%)	21	22	39	41	74	60	90	71	97	95

[a]WP and EP: Paste prepared with water and potato extract, respectively.

at 0°C than at 30°C, and in EP than in WP in the respective pairs of pastes. An exceptional case was a pair of WP-99 and EP-88 of which DG after first heating differed too much to compare reasonably. All the EPs except EP-40 showed definite decrease of DG-g (more than 5%), and EP-100 showed the highest retrogradation. These results confirm our previous findings (Suzuki and Hizukuri 1974) that potato extract promotes the retrogradation of potato starch. In most cases in which definite retrogradation was observed, the decrease of DG-i was greater than that of DG-g, suggesting the preferential retrogradation of amylose.

Second Gelatinization

The above cooled pastes were regelatinized by heating (second heating, 100°C for 15 min), which was enough to fully gelatinize raw potato starch. The results are shown in Table IV. In these experiments a series of pastes was heated again immediately after the first heating to examine the effect of the first heating alone on the second heating. By having the second heating immediately after the first heating, all WPs and some EPs were fully gelatinized, but EP-40 and EP-60 remained a little below full gelatinization. It therefore appears that strong molecular associations were formed in EP-40 and EP-60 during the first heating by the similar effects of the heat-moisture treatment. The treatment usually heats starch in a pressure cooker at 100% relative humidity or heats moist starch at 90–110°C in a sealed container for several hours (Sair 1964) and changes the pasting characteristics of potato starch into that similar to cereal starch by producing strong molecular association. Interestingly, the heat-moisture treatment with lower heating for a shorter time than usual, that is, heating at 63°C for 15 min, caused the evident effect similar to the treatment in the presence of potato extract. According to Sakai et al (1976), raw corn and wheat starches suspended in water give 100% DG-g by heating at 100°C for 15 min but EP-40 and EP-60 gave 95 and 92% DG-g, respectively, by the second heating immediately after the first heating (Table IV). This indicates that stronger molecular associations are formed in EP-40 and EP-60 than those in raw corn and wheat starches during the first heating. The EPs prepared at a higher temperature than EP-80 gave 100% DG-g by the second heating immediately after the first heating so that the more advanced gelatinization was unsuitable for the formation of the strong molecular association. Therefore, it is suggested that the formation of the strong molecular association during first heating is due to the heat-moisture treatment that is promoted by the extract.

The pastes, especially their amylose components, became gelatinization-resistant more or less by the cooling and were hard to be gelatinized fully by second heating as seen in Table IV. This was notable in all the EPs that gave 83–96% DG-g by second heating after cooling. The cooled EP-100 at 0°C gave the least DG-g (83%) and DG-i (68%) by the second heating. The paste cooled at 0°C was less gelatinized than that cooled at 30°C, suggesting that greater retrogradation causes stronger gelatinization-resistant structure. The structure was apparently produced by retrogradation and not by heat-moisture modification, since the paste was fully gelatinized by second heating without cooling. EP-40 gave as low as that of EP-100 by second heating after cooling. The gelatinization-resistant structure, in this case, was considered to be brought about by the combination of heat-moisture modification, as described, and retrogradation. Although almost no retrogradation was observed in both WP-40 and EP-40, only EP-40 gave appreciably lower DG values by second heating after the cooling than by that before the cooling. Therefore, the extract appears to be involved in the formation of the gelatinization-resistant structure during the cooling. It is supposed that the DG estimation method used here may not be good enough to reveal accurately the process of retrogradation. EP-60 had less gelatinization-resistant structure than EP-40 after the cooling and gave the same DG values by second heating before and after the cooling. However, by another experiment described later, it was suggested that retrogradation was concerned with the formation of the gelatinization-resistant structure. Confusing results were noted also with EP-80, which retrograded considerably by the cooling, but recovered the highest level of DG among EPs by second heating although the recovery of DG-i was a little low (87 and 88%). After the cooling, EP-88 showed a gelatinization-resistant nature between EP-80 and EP-100, which was considered to be formed by retrogradation as in EP-100.

Effect of Addition of Extract to Pastes

The foregoing data suggest that potato extract promotes retrogradation and forms strong molecular associations by the retrogradation. It is not entirely clear, however, whether these actions are possible to be brought about by the cooling alone or are connected somewhat with first heating. To clarify this, an equal volume of concentrated potato extract or water was added to WP-60 immediately after first heating and the retrogradation and

TABLE III
Retrogradation (Decrease of Degree of Gelatinization) of Pastes by Cooling at 30 and 0°C for 1 hr

		Pastes									
		WP-40	EP-40	WP-60	EP-60	WP-80	EP-80	WP-99	EP-88	WP-100	EP-100
0°C	DG-g (%)	0	1	3	6	10	10	6	21	6	17
	DG-i (%)	4	5	1	6	14	20	13	6	14	27
30°C	DG-g (%)	0	0	2	5	3	7	2	5	4	14
	DG-i (%)	3	3	1	1	13	12	12	3	3	12

TABLE IV
Degree of Gelatinization Attained by Second Heating (100°C for 15 min) after First Heating and Followed by Cooling at 30 and 0°C

		Pastes									
		WP-40	EP-40	WP-60	EP-60	WP-80	EP-80	WP-99	EP-88	WP-100	EP-100
No Cooling	DG-g	100	95	99	92	100	100	100	98	100	100
	DG-i	98	92	97	88	96	91	98	98	97	95
Cooling at 0°C	DG-g	100	85	100	92	96	96	98	91	95	83
	DG-i	96	82	97	86	89	88	87	84	83	68
Cooling at 30°C	DG-g	100	89	99	92	96	94	97	91	99	90
	DG-i	98	81	95	88	94	87	82	86	97	88

subsequent second gelatinization were examined. By addition of water, the paste did not retrograde at 30° C and only amylose retrograded at 0° C, whereas by addition of the extract, the paste retrograded definitely at 30 and 0° C as shown in Table V. By second heating of these cooled pastes, the water-added paste was gelatinized fully but the extract-added paste, expecially amylose, did not attain full gelatinization (Table V). These results imply that the extract is possible to promote retrogradation and to contribute to the formation of strong molecular associations independently of the promotion of the heat-moisture modification during first heating

Effect of Extract on Amylographic Behavior

Figure 2 compares the amylograms of the starch with water and extract. The maximum viscosity was high in pasting with water but lowered to about one-sixth with extract. Even when the starch was gelatinized with the extract diluted with 2 vol of water, its viscosity was fairly low. The gelatinization temperature was 64° C with water and 77.5° C with extract. Thus the amylograms also indicated that the extract inhibited the gelatinization and swelling of the starch.

DISCUSSION

EPs gave lower DG values than WPs under the same heating conditions and the greater difference between DG-g and DG-i (Fig. 1, 65 and 72° C), suggesting that the extract inhibits the gelatinization of amylose preferentially.

It was shown previously(Suzuki and Hizukuri 1974) that potato extract promoted the retrogradation and crystallization of fully gelatinized potato starch. The present results revealed that the extract promoted retrogradation more markedly in pastes of higher DG than in less gelatinized pastes. Furthermore, it was found that when the pastes were partially retrograded by cooling at 30 or 0° C for a short period (1 hr), it was hard to gelatinize fully by heating, which fully gelatinizes raw starch. This is probably due to retrogradation involving amylose for the following reasons: 1) EPs gave considerably lower DG-i values than DG-g after second heating; 2) amylose retrogrades rapidly and retrograded amylose does not usually dissolve in hot water; and 3) amylopectin retrogrades slowly and protects amylose from retrogradation (Cornell 1963, Whistler 1953). We found that the pastes of potato amylopectin and waxy rice starch, which were prepared by heating the 5% starch suspensions in the potato extract at 100° for 15 min, did not retrograde at 0° C at least 24 hr (keeping 100% DG-g). Therefore, the effect of extract on the retrogradation of starch paste is considered due mainly to the interaction with amylose.

Previously, we reported that when cube-cut potatoes were precooked at 55–90° C for 10 min and then cooled at 20° C for 1 hr, starch in the potato could not be gelatinized fully (86–88% DG-g) by reheating at 100° C for 10 min or more, under which conditions the starch in raw potato could be fully gelatinized (Hizukuri et al 1972). These facts indicate that strong molecular associations are produced in the starch during the precooking and the cooling of the potato. The data in Table IV suggest that this strong molecular association occurrs through the interactions between starch and the hot water-soluble components of potato, which promote heat-moisture modification and retrogradation.

Inorganic ions in the extract are thought to play an effective role in the formation of strong molecular associations, since the ions promote retrogradation (Khairy et al 1963, Whistler 1953). In preliminary tests, however, effective materials were found in both the outer and inner solutions after extensive dialysis of the extract by cellulose tubing (Visking Co.). The extract contained 0.41% total carbohydrate as glucose but was not stained with iodine. Therefore, the possibility that amylose in the extract may play the role was ignored.

One process of manufacturing potato flakes involves preheating chipped potatoes at 72° C for 20 min to partially gelatinize the starch and cooling at 24° C for 15 min before boiling (Eskew 1967). If the potato is cooked by boiling without preheating and cooling, the starch swells greatly and destroys cell walls so that the product has an undesirable, pasty texture. The present data suggest that the hot water-soluble components of potato play an effective role in the preheating and cooling process by inhibiting extreme swelling of starch granules. From the technological viewpoint of the processing of starchy materials, retrograded starch has strong molecular associations that are not found in native starch (except high amylose content starch). Generally, retrogradation is an undesirable phenomenon, but we can gain an advantage by modifying the properties of starch. The process of potato flake manufacture is one example. Harusame (starch noodle) manufacture also involving the process of gelatinization and cooling, which is considered to be important to give the heat-resistant nature to the noodles, is another example.

The retrogradation is controlled by many factors such as temperature (Hizukui et al 1972), paste concentration (Sakai et al 1976), pasting conditions, molecular structure, and interactions with other materials (Collison 1968, Whistler 1953). The proper modification of starch properties by the control of retrogradation is a subject for the future use of starch and starchy materials.

TABLE V
Effect of Addition of Potato Extract to WP-60 on Retrogradation and Second Gelatinization[a]

	Addition of			
	Extract		Water	
	DG-g (%)	DG-i (%)	DG-g (%)	DG-i (%)
Retrogradation at 30° C	8	8	0	−1
Retrogradation at 0° C	8	14	0	8
Second gelatinization				
Cooled paste at 30° C	87	77	99	92
Cooled paste at 0° C	93	83	97	95

[a] An equal volume of the concentrated potato extract or water was added to WP-60 immediately after first heating and the paste was subjected to cooling and second heating.

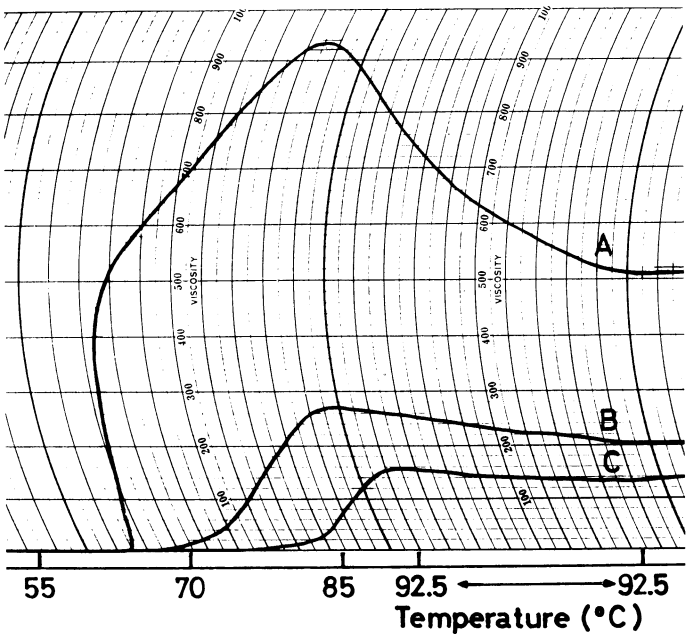

Fig. 2. Effect of potato extract on the amylogram of potato starch. Potato starch was heated with (**A**) water, (**B**) extract diluted with 2 vol of water, and (**C**) extract.

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[Received December 7, 1977. Accepted November 7, 1978]