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Influence of Storage on Supercooling of Rice Starch and Flour Gels

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Supercooling of amylose–amylopectin mixtures and starch or flour gels from fresh and stored rice has been studied. The results showed that the extent of supercooling of the amylose–amylopectin gels was indirectly related to the amylose content. Amylose solutions or gels did not supercool and, in the mixtures with amylopectin, inhibited supercooling. The supercooling of starch gels was influenced by oryzenin (rice storage protein). The supercooling of starch or flour gels from different varieties of postharvest and stored rice was related to the molecular weight of oryzenin and starch, oryzenin–starch interactions, and water binding, which reflected the structural changes of oryzenin and starch during rice storage.

Freezing point and various kinds of structural transitions usually occur at well-defined and reproducible temperatures. On the other hand, supercooling (an unstable cooling below the freezing point of the mixture) is more complicated and depends not only on nucleation rates but also on diffusion, glass transition, and crystallization rates as well as on other physicochemical factors (Beaman, 1952; Lauritzen and Hoffman, 1960; Peterlin, 1960, 1980; Hoffman and Lauritzen, 1961; Boyer, 1963; Hoffman et al., 1976; Michelmore and Franks, 1982; Billmeyer, 1984). Nucleation can be initiated or accelerated by traces of impurities, but simultaneously it may be inhibited by solvation, structural hindrance, or intermolecular forces.

For example, in simple water solutions of poly(ethylene glycol), the inhibition of nucleation resulted mainly from perturbation of the diffusion freedom of water molecules (Michelmore and Franks, 1982). In the mixtures and other more complex systems, like polysaccharides and/or proteins, the mechanism of supercooling is more complicated but depends basically on the same factors: water binding and diffusion freedom.

Thus, the phenomenon of supercooling should be directly or indirectly related to these factors. This might be particularly important for some food products where these factors (water binding and diffusion freedom) are important because they are related to functional properties.

In this work the supercooling of starch and/or flour gels from postharvest and stored rice grains has been studied.

EXPERIMENTAL PROCEDURES

Materials. All chemicals were obtained from J. T. Baker or Sigma Chemical Company and were of reagent grade or of the highest purity obtainable from them. Commercial amyloses or amylopectins which were less than 98% pure (for example, corn amylose) were further purified by the modified thymol method described elsewhere (Chrastil, 1990a).

Methods. All determinations were done in triplicates. The average statistical coefficients of variation of these methods are shown in references cited for each used method.

Rice Storage. Highly polished (30% removed) rice grains (less than 1 month after harvest) of two U.S. rice varieties (Lemont, long grain, and Mercury, medium grain) were stored in triplicate in closed jars at 40 °C. At the beginning of storage and after 5 and 12 months, the grains were ground to flour for subsequent tests. These triplicates were used as a starting material for all extraction and analytical experiments.

Starch Components and Oryzenin from Rice. The flour, rice storage polysaccharides, and proteins were prepared from different rice samples as described by Chrastil (1990a). Starch and its components (amylose and amylopectin) and oryzenin were 98–99% pure.

Determination of Amylose and Protein. Starch content was determined according to the method of Clegg (1956). Amylose content in starch, amylopectin, and amylose samples was determined according to the method of Chrastil (1987). Protein content in oryzenin was determined according to the method of Lowry et al. (1951).

Determination of Molecular Weights. The molecular weights of oryzenin, starch, and its components were determined according to the viscosimetric methods described by Chrastil (1990a).

Determination of Oryzenin–Starch Binding. The equilibrium binding ratio of oryzenin to starch was determined according to the method described by Chrastil (1990a).

Determination of Water Uptake by Starch or Flour. The water uptake by starches or flours was determined according to the method described by Chrastil (1990b).

Preparation of Starch or Flour Gels. In small 10-mL test tubes, 2 g of starch, flour, or amylose plus amylopectin mixture was suspended in 4 mL of 0.1 M NaOH. The suspension was kept for 15 min at 95 °C with occasional mixing on a Vortex. The mixtures of starch puls protein (oryzenin) contained 10% (w/w) protein. The gel was cooled to 4 °C in a refrigerator overnight and used for supercooling experiments.

Determination of Supercooling. After a small (2-mm diameter) thermocouple was inserted (1 cm from the bottom of the gel) into the gel in a small test tube, it was cooled in a freezer (average air temperature −30 °C). The thermocouple was connected by means of a telethermometer (YSI Model 42SC, Yellow Springs Instrument Co., Yellow Springs, OH) to a recorder (50-mV scale, 30 cm/h speed, and 0 °C in the middle of the recorder scale). The average cooling rate of the gel under these conditions was 0.2 °C/min. The samples were run in triplicate. The coefficient of variation between the triplicates was less than ±0.5 °C. The typical supercooling curve is shown in Figure 1.

RESULTS AND DISCUSSION

Mixtures of Amylose with Amylopectin. The mixtures of starch components were prepared from pure amylose (>99%) and pure amylopectin (>99%). Amylose and amylopectin from different starches (potato, corn, and rice) were either obtained from commercial sources or prepared from pure starches according to the method shown under Experimental Procedures for rice (Chrastil, 1990a). The supercooling of the gel prepared from the mixtures of amylose with amylopectin was measured as described above with starch. It is apparent from Figure 2 that supercooling of the gels made from amylose plus

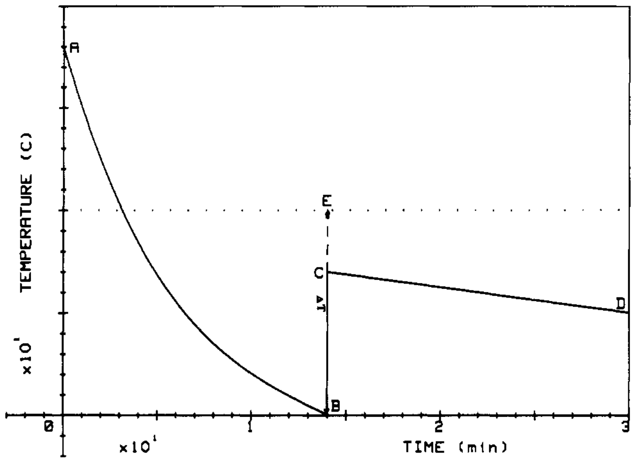

Figure 1. Example of supercooling profile. Dotted line, 0 °C; A–B and C–D, cooling curves; B–E, extent of supercooling; E–C was not a freezing point and depended on experimental conditions (for example, sample geometry and quantity or cooling rate). The sudden increase of temperature at the supercooling point, B, was caused by the heat of crystallization of water.

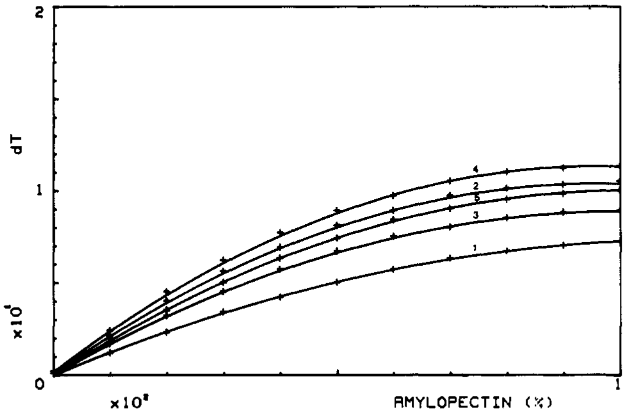

Figure 2. Supercooling (ΔT) of amylose–amylopectin mixtures from different starches. (1) Potato amylose with potato amylopectin; (2) corn amylose with corn amylopectin; (3) potato amylose with corn amylopectin; (4) rice amylose with rice amylopectin (from fresh rice); (5) rice amylose with rice amylopectin (from rice stored for 1 year at 40 °C).

Table I. Supercooling of Starch from Fresh and Stored Rice[a]

rice variety	storage, months	starch MW ($\times 10^6$)	amylose, %	amylopectin, %	water uptake, %	supercooling ΔT, °C
Mercury	0	2.59	17.0	83.0	93	6.2
	12	2.68	17.7	82.3	113	7.2
Lemont	0	2.37	26.0	74.0	77	5.0
	12	2.44	27.0	73.0	101	6.0

[a] Fresh rice was used about 1 month after harvest; stored rice was stored for 12 months at 40 °C.

Table II. Supercooling of Amylose plus Amylopectin Mixtures from Fresh and Stored Rice[a]

		mixture					
		amylose		amylopectin			
rice variety	storage, months	%	MW ($\times 10^5$)	%	MW ($\times 10^6$)	water uptake, %	supercooling ΔT, °C
Mercury	0	17.0	1.15	83.0	3.10	120	9.0
	12	17.7	1.03	82.3	3.23	144	10.1
Lemont	0	26.0	1.55	74.0	3.15	106	8.2
	12	27.0	1.10	73.0	3.30	130	9.2

[a] Fresh rice was used about 1 month after harvest; stored rice was stored for 12 months at 40 °C.

Table III. Influence of Oryzenin on Supercooling of Rice Starch[a]

rice variety	storage, months	oryzenin %	oryzenin MW ($\times 10^5$)	$n{:}m$	ΔT, °C
Mercury	0	0	1.34	2.43	6.2
	0	5	1.34	2.43	4.2
	0	10	1.34	2.43	4.1
	12	0	1.85	1.53	7.2
	12	5	1.85	1.53	5.2
	12	10	1.85	1.53	5.2
Lemont	0	0	1.75	1.71	5.0
	0	5	1.75	1.71	4.0
	0	10	1.75	1.71	3.8
	12	0	2.10	1.09	6.0
	12	5	2.10	1.09	4.5
	12	10	2.10	1.09	4.4

[a] Fresh rice was used about 1 month after harvest; stored rice was stored for 12 months at 40 °C. $n{:}m$, oryzenin–starch equilibrium binding ratios.

amylopectin mixtures increased with the amylopectin content. Pure amylose did not show any supercooling.

At the same ratio of amylose:amylopectin the supercooling of the mixtures of rice amylose plus rice amylopectin or corn amylose plus corn amylopectin was greater than the supercooling of potato amylose plus potato amylopectin. The supercooling of the mixtures of potato amylose plus corn amylopectin was located between the values for supercooling of corn amylose plus corn amylopectin and potato amylose plus potato amylopectin. The supercooling of these mixtures showed some additive properties, but it was also influenced by other structural factors (except amylose:amylopectin ratio) which were characteristic for different plant varieties.

Starch. The supercooling of starch gel from fresh (postharvest) rice was always less than the supercooling of starch gel from stored rice of the same variety (Table I). This agreed with the increase of water binding in stored rice grains (Chrastil, 1990b). Bound water limits the diffusion freedom, and thus the probability of ice formation is greatly reduced.

The amylose plus amylopectin mixtures in Table II had intentionally the same composition as the starch from the same source. The supercooling of the amylose plus amylopectin mixture with the same ratio of amylose:amylopectin as the purified starch from the same variety was greater than the supercooling of starch. This could be explained partially by the greater water binding by these mixtures than by starch. Additionally, we have to keep in mind that the physicochemical structures of starch and of the mixture of its isolated components are similar but not identical.

Influence of Protein. Although the rice storage protein (oryzenin) itself did not show any significant supercooling in dilute alkaline solutions (not shown here), the addition of oryzenin to the rice starch gels decreased the supercooling (Table III). This change was not directly related to the amount of oryzenin added to the starch (in the 5–10% limits, w/w of dry mixture). However, the supercooling of rice starch in the presence of oryzenin was related to the molecular weight of oryzenin and to the binding of oryzenin to starch.

The binding of oryzenin to starch was much greater in postharvest rice than in stored rice than in stored rice (Chrastil, 1990a), and the relative decrease of supercooling in the mixtures of starch with oryzenin was related to the binding ratio of oryzenin on starch ($n{:}m$). Both oryzenin–

Table IV. Supercooling of Flour from Fresh and Stored Rice[a]

rice variety	storage, months	starch amylose, %	starch amylopectin, %	starch water uptake, %	supercooling ΔT, °C
Mercury	0	17.0	83.0	75	3.3
	12	17.7	82.3	94	5.0
Lemont	0	26.0	74.0	64	2.5
	12	27.0	73.0	93	4.0

[a] Fresh rice was used about 1 month after harvest; stored rice was stored for 12 months at 40 °C.

amylopectin and oryzenin–amylose binding constants decreased during storage of rice (Chrastil, 1990a), but for the supercooling, the binding to amylopectin was evidently more important because of the prevailing influence of amylopectin on supercooling.

Other proteins, for example, some globulins, also decreased the supercooling of the starch gels but only when they were bound to starch (not shown here). The addition of proteins that did not bind to starch, for example, rice albumins, did not influence the supercooling of starch. Although these experiments were effected in the mixtures of oryzenin with starch, similar interactions must occur in heated alkaline flour gels, where the starch and protein bodies are disrupted and may interact with each other.

Flours. The rice flours behaved similarly as the starches. Supercooling of rice flour gels increased with storage in all studied rice varieties (Table IV), but it was less than the supercooling of starch from the same variety of rice. Except starch (about 90%), the rice flour contained oryzenin and other proteins (about 8%), and thus, from the above experiments with the mixture of starch with oryzenin, it was expected that the supercooling of flour would be lower than that of starch.

Generally, we may conclude that the supercooling of rice starch or flour gels was caused by amylopectin. Amylose solutions or gels did not supercool, and amylose inhibited supercooling of amylopectin or starches. The supercooling also increased with the molecular weight of starch or amylopectin, but this influence in rice was small because the molecular weight of starch increased only very little during storage. The addition of oryzenin to the starch gels decreased the extent of supercooling. The supercooling phenomenon was related to the water binding in stored rice, which decreased the diffusion freedom of water molecules. It was also apparent that, in the rice flours, one of the main factors influencing the water binding and thus the supercooling was the molecular weight of oryzenin and the oryzenin–starch binding.

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Registry No. Amylose, 9005-82-7; amylopectin, 9037-22-3; starch, 9005-25-8.