

Influence of Annealing on Gel Properties of Mung Bean Starch

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ABSTRACT

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Mung bean starch gels (8% solids) were prepared after annealing at 45–60°C for 1–24 hr, and the relationship between the physical properties of gels and the swelling power (SP) and solubility of starch was investigated. The SP and solubility decreased with increasing annealing temperature and time, mostly in the first 6 hr. The solubles were mainly composed of amylose. Gel hardness at a 5 mm depth of annealed starch was larger than that of native starch, and gel hardness increased as SP decreased ($r = -0.94$). Upon continued compression, the yield force of gel showed a different function. Above SP of ≈ 12.5 , the yield force of annealed starch

gels decreased, but at <12.5 the yield force increased with increasing SP. Both granular rigidity and extent of packing appeared to determine the yield force. Although annealing increased the gel hardness, α -amylase digestibility of gel was not affected. Pasting analysis in the Rapid Visco Analyser (RVA) revealed that annealing increased pasting temperature. A pasting peak was found only in 45 and 50°C annealed starches. Overall paste viscosities of the starches annealed at $>55^\circ\text{C}$ were lower than that of the control starch. Final viscosities in RVA were correlated with the yield force of gel ($r = 0.99$).

Starch granules heated in excess water undergo an order-disorder phase transition called gelatinization (Hoover 1995). This phase transition is associated with the diffusion of water into the granule, hydration and swelling of the starch granules, loss of crystallinity, and amylose leaching. As a consequence, swollen granules become embedded in a continuous matrix of entangled amylose molecules. On cooling, the complex composite sets as a viscoelastic gel when starch concentration is $>6\%$. This change is called retrogradation. In this stage, amylose gel network develops relatively fast and remains unchanged during storage. Meanwhile gelation of amylopectin within the swollen granules is slow and progresses slowly during storage, resulting in increasing gel hardness (Biliaderis and Zawistowski 1990, Morris 1990, Biliaderis 1992). Hoover (1995) reviewed the factors affecting starch retrogradation such as starch concentration, temperature, sugar, lipids, salts, and chemical and physical modification.

Annealing is a process in which starch granules are heated in excess amounts of water at a temperature slightly below gelatinization temperature for a relatively long time. Tester et al (1998) reported that annealing was restricted unless the moisture content exceeded 60% by weight of the mixture. Annealing decreases swelling power and solubility of starch (Eerlingen et al 1997), delays gelatinization (Fisher and Thompson 1997, Kim 1991, Knutson 1990, Krueger et al 1987, Seow and Teo 1993, Stute 1992), increases susceptibility to amylase (Wang et al 1997), and changes pasting curves (Jacobs et al 1995, Stute 1992).

Mung bean starch, along with acorn and buckwheat starches, is widely used to make a gel food called *mook* in Korea. Mook is prepared by cooking at 8–10% solids followed by cooling the paste in a rectangular or circular plate with a depth of ≈ 10 cm. Since mook is served with chopsticks, it requires a certain gel hardness (less breakability) for easy handling. The objective of this investigation was to determine how various annealing conditions influence the physical properties and the degree of gelatinization of mung bean starch gels. In addition, Rapid Visco Analyser (RVA) and DSC (differential scanning calorimetry) analyses were performed with annealed starches.

MATERIALS AND METHODS

Preparation of Mung Bean Starch

Mung bean starch was isolated from commercial mung beans purchased in a local market by an alkaline steeping method. Mung beans were split and the splits were soaked in tap water for 3–4 hr. The hulls were removed by hand and the dehulled cotyledons were blended (1:4) with 0.02N NaOH solution using a Waring blender for 1 min. The slurry was filtered through the No. 400 sieve and the starch suspensions were stored at 4°C overnight. The supernatant and residue above the starch layer were discarded. The starch slurry was washed with distilled water until no alkali was detected and dried at 40°C in a drying oven. The starch passing through the No. 100 sieve was used in this work; its crude protein content ($N \times 6.25$) was 0.32% on dry basis.

Annealing of Mung Bean Starch

All starch slurries for annealing were prepared using water containing 0.02% sodium azide to prevent microbial contamination. Starches for gel preparation and measurements of swelling power and solubility were annealed at 45–60°C for 1, 6, or 24 hr just before each experiment. For RVA testing, mung bean starch (2.24 g, dry weight) was added to water (20 mL) in a capped tube and the tubes were placed in a 45–60°C water bath for 24 hr. After cooling to room temperature, a starch suspension was transferred to the aluminum container of the RVA and water was added to make 28 g before measurement. For DSC analysis, mung bean starch (5 g) was added to 50 mL of water and annealed at 45, 50, 55, and 60°C for 6 hr. Starch slurry was added to 1 L of anhydrous ethanol. The precipitated starch was collected on a G 3 glass filter pad, washed twice with anhydrous ethanol and once with acetone, and dried in a vacuum desiccator.

Gel Properties

Gels of the control and annealed mung bean starches were prepared by mixing starch with water containing 0.02% sodium azide to make 8% (w/w) slurries (350 g of total weight). The slurries were cooked in a boiling water bath for 30 min. To prevent sedimentation of starch granules during heating, a starch slurry was stirred for the first 5 min, then covered with aluminum foil and heated further. After heating, a starch paste was placed in a vacuum chamber for 20 sec to remove air bubbles. Hot excess paste was poured into a container (diameter 4 cm, height 2 cm) whose edge was extended >0.5 cm using tape. Each starch paste was covered with a double layer of polyethylene wrap to prevent drying and then stored at 25°C for one day.

After storage, excess gel above the container edge was cut off carefully with a knife and the gel was removed from the container and subjected to texture measurement. A Tensilon universal testing in-

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strument (model RTM-500, Orientec Corporation, Tokyo, Japan) equipped with a cylindrical probe (diameter 2.5 cm) with a spherical shape at one end was used to measure hardness at 5 mm compression. In addition, yield force and depth to yield point were measured as compression was continued beyond 5 mm depth.

Swelling Power, Water Solubility, and Color of Iodine Complex of Solubles (λ_{max})

Swelling power (SP) and solubility of the control and annealed starches were measured at 85°C by the method of Schoch (1964). For λ_{max} , 0.2 mL of iodine solution (2% potassium iodide + 0.2% iodine) was added to 10 mL of supernatants diluted 100-fold obtained after measuring solubility. Scanning was performed with a Milton Roy 1201 spectrophotometer.

Degree of Gelatinization of Gel

Degree of gelatinization of gel was measured by an α -amylase iodine method (Tsuge et al 1990). Samples for enzymatic hydrolysis were prepared. After measuring texture, a portion of a gel (10 g) was blended with 200 mL of anhydrous ethanol using a Waring blender for 1 min. The precipitate was collected on a G 4 glass filter pad, washed twice with anhydrous ethanol, once with acetone, and dried in a vacuum desiccator.

RVA Pasting

Pasting curves were recorded on a Rapid Visco Analyser (RVA) (model 3D, Newport Scientific, Sydney, Australia). The temperature-time conditions for a starch slurry (8%, w/w; 28 g total weight) were an equilibration step at 50°C for 1 min, a heating step to 95°C at 12°C/min, a holding stage at 95°C for 2.5 min, a cooling step to 50°C at 12°C/min, and a holding stage at 50°C for 2 min.

DSC Analysis

DSC analysis was performed using a calorimeter (model DSC-120, Seiko Instruments, Kawasaki Kanagawa, Japan). Temperature and enthalpy of the instrument were calibrated using indium. Starch (≈ 3 mg) was weighed into aluminum pans and distilled water was added to make 66.7% moisture content. The sample pans were sealed and equilibrated for 1 hr at room temperature before heating. All samples were heated from 30 to 130°C at a rate of 5°C/min.

Statistical Analysis

All measurements were done at least in duplicate and the Statistical Analysis System (SAS Institute, Cary, NC) was used to analyze data and to calculate Fisher's least significant differences ($\alpha = 0.05$).

TABLE I
Physical Properties of Annealed Mung Bean Starch Gels^a

Annealing Condition	Hardness at 5-mm Depth (Kg _f)	Yield Force (Kg _f)	Depth to Yield Point (mm)
Control (8%)	0.084 ± 0.006	1.24 ± 0.06	14.8 ± 0.4
45°C - 6 hr	0.084 ± 0.004	1.32 ± 0.11	14.9 ± 0.5
24 hr	0.088 ± 0.004	1.45 ± 0.09	14.8 ± 0.2
50°C - 6 hr	0.087 ± 0.003	1.48 ± 0.04	15.1 ± 0.4
24 hr	0.102 ± 0.004	1.56 ± 0.06	14.8 ± 0.4
55°C - 1 hr	0.112 ± 0.007	1.52 ± 0.12	14.6 ± 0.4
6 hr	0.113 ± 0.004	1.42 ± 0.04	14.6 ± 0.3
24 hr	0.119 ± 0.009	1.38 ± 0.06	14.1 ± 0.4
60°C - 1 hr	0.120 ± 0.007	1.02 ± 0.07	13.0 ± 0.3
6 hr	0.118 ± 0.006	1.03 ± 0.05	13.3 ± 0.3
24 hr	0.124 ± 0.004	0.91 ± 0.07	12.8 ± 0.3
LSD ^b	0.006	0.08	0.4
Native (9%)	0.102 ± 0.008	1.40 ± 0.10	13.8 ± 0.3

^a Mean value of eight measurements ± standard deviation.

^b Least significant difference ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Relationship Between Physical Properties of Gel and SP and Solubility of Starch

The hardness at 5 mm depth (surface hardness), yield force, and depth to yield point of the control and annealed mung bean starch gels are shown in Table I. The hardness of all annealed starch gels was equal to or greater (0.084–1.124 Kg_f) than that of the control starch (0.084 Kg_f). But the yield force and the depth to yield point depended on the annealing conditions. The yield forces of annealed starches ranged from 0.91 to 1.56 Kg_f, while the control starch showed 1.24 Kg. The depth to yield point of annealed starches ranged 12.8–15.1 mm, which were statistically equal to or slightly lower than the control starch (14.8 mm).

Because a starch gel is formed by the leached amylose retrograding during cooling and the swollen granules being embedded within the amylose network, it can be stated that gel properties are related to the SP and solubles of a starch. The SP of mung bean starches annealed at 45, 50, 55, and 60°C for 1, 6, or 24 hr are shown in Fig. 1A. As annealing temperature and time increased, SP decreased. The decrease was prominent in the first 6 hr, especially in 1 hr. Control starch had a SP of 15.92, but for those annealed at 45°C, SP values were 14.83 and 14.50 for 6 and 24 hr, respectively. At 60°C, SP values were 11.02, 10.27, and 9.90 for 1, 6, and 24 hr, respectively. The solubility of mung bean starch during annealing showed a trend similar to SP (Fig. 1B). Control starch had a solubility of 15.37%, yet solubility values at 45°C were 14.06 and 13.09% for 6 and 24 hr, respectively. At 60°C, solubility values were 11.74, 10.68, and 9.89% for 1, 6, and 24 hr,

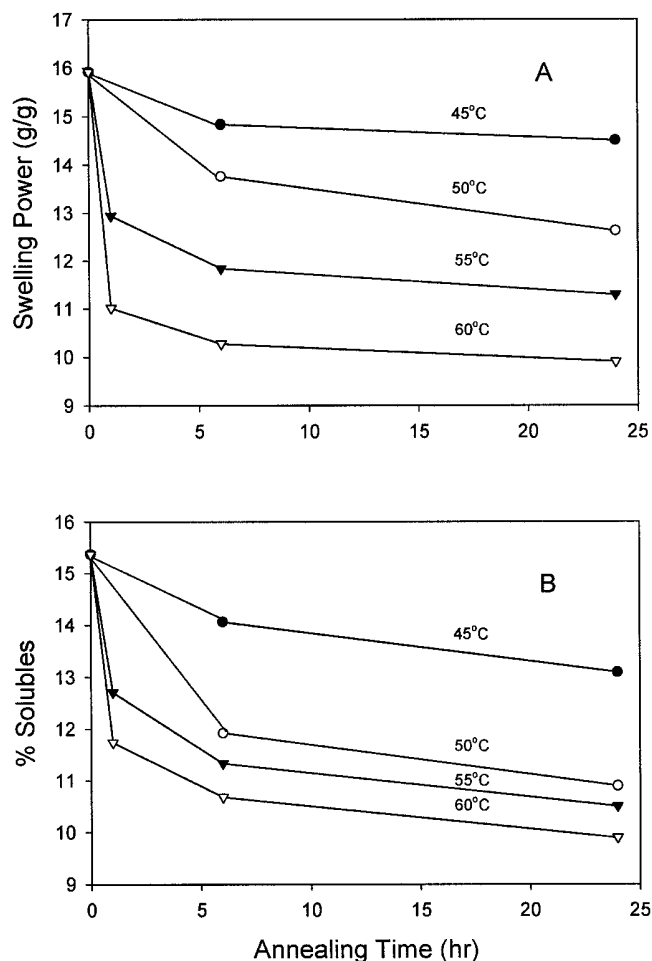


Fig. 1. Swelling power (A) and solubility (B) of mung bean starches annealed at 45–60°C for 1–24 hr.

respectively. The soluble fractions of all samples were considered to be essentially amylose because λ_{max} values of iodine complexes were 646–660 nm (data not shown). Decreases in SP and solubility were maybe due to the ordering rearrangement of starch molecules in the granules during annealing. Eerlingen et al (1997) proposed that the decrease in SP and the increase in gelatinization temperature were caused by: 1) transformation of amorphous amylose into a helical form; 2) an increase in interactions between amylose chains or between amylose and amylopectin chains in the amorphous regions; and 3) alteration in the interaction between crystallites and the amorphous matrix during annealing.

To elucidate the relationship between physical properties of gel and SP and solubility of starch, these two parameters were plotted against the gel properties tested. The plot of SP versus hardness at 5 mm depth (Fig. 2A) showed that the hardness increased as SP decreased ($r = -0.94$). The increase may be caused by the increase in granular rigidity with decreasing SP. Lii et al (1996) reported that the rigidity of starch granules is inversely proportional to the swelling degree of granules. Likewise, in the plot of solubles versus the hardness (Fig. 2B), as the solubles decreased, hardness increased. But the correlation coefficient was smaller ($r = -0.79$) than in Fig. 2A. It was expected that when the leached solubles increased, the gel would become harder because the leached amylose forms the continuous gel matrix. But the result was the opposite. Lii et al (1996) and Tsai et al (1997) reported that the rigidity of swollen starch granules was the major factor determining the rheological properties of a starch paste or gel. It is hypothesized that even though the leached-out component forms the gel network, the lower rigidity of the swollen granules reduced surface hardness.

The plot of SP versus yield force showed that samples were separated into two groups at a juncture of ≈ 12.5 (Fig. 3A). At SP

>12.5 , as SP increased yield force decreased (right line), but at <12.5 , as SP increased yield force increased (left line). Also in the plot of solubility versus yield force (Fig. 3B), samples could be grouped into two types, but the relationship was not so clear as in Fig. 3A. Similar relationships were found in plots of SP or solubility versus depth to yield point (Figs. 4A and B). But the depth to yield point appeared to be same when SP and solubles exceeded a certain level.

Plots of both SP versus yield force and SP versus depth to yield point showed the crossover phenomenon at $SP \approx 12.5$. This value is the reciprocal of the starch concentration (8%) tested. In the starch gel with SP 12.5, the volume of the swollen granules was just sufficient to occupy the total volume of the gel, resulting in the highest yield force. At >12.5 (when annealed at low temperature or for short time), as the starch granules swelled more, the swollen granules are less rigid and slid past each other easily, and they could be deformed more in the system. As a consequence, the gel was weak. But at <12.5 , yield force was proportional to SP. In this region, the interaction between the swollen granules might play an important role rather than the granular rigidity. As the granules exist closely, the interaction will be strong, resulting in large yield force. These results are in agreement with the report of Eerlingen et al (1997). They also observed, in the 6.6% annealed potato starch gel, a decrease in G' (storage modulus) with increasing SP in the concentrated regime (high SP region) and an increase in G' with increasing SP in the dilute regime (low SP region).

Effect of Annealing on Degree of Gelatinization of Gel

Annealing increased gel hardness. To know whether annealing affects retrogradation of gel or not, the degree of gelatinization was measured by α -amylase hydrolysis. The results showed a little difference in some treatments, but overall the differences were small

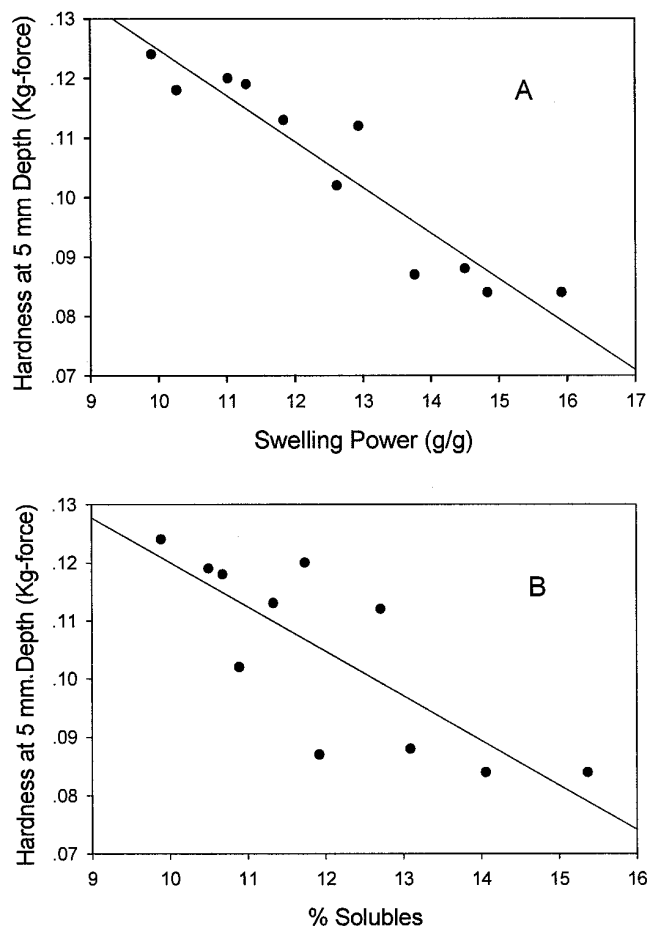


Fig. 2. Swelling power (A) and solubility (B) vs. hardness.

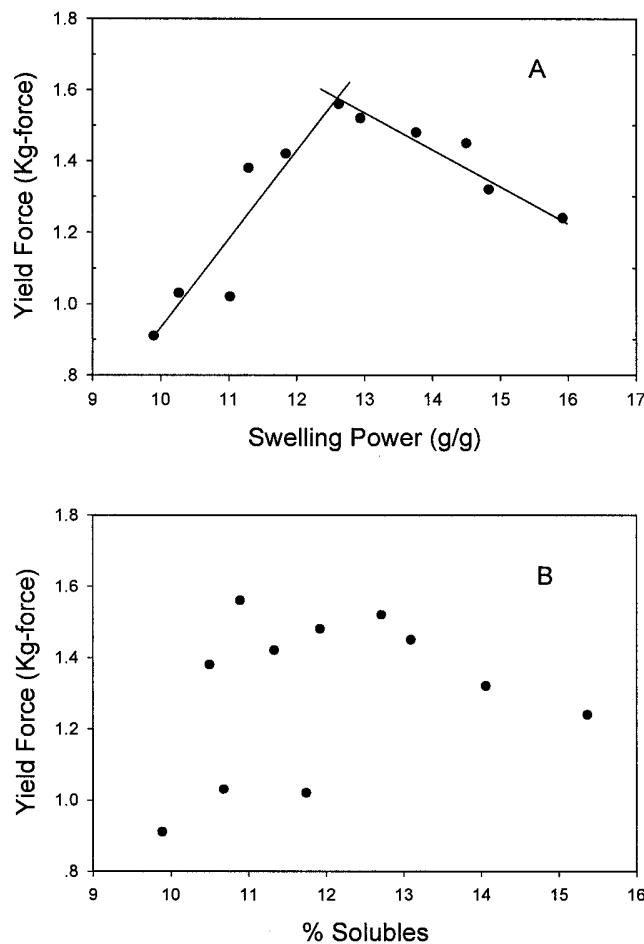


Fig. 3. Swelling power (A) and solubility (B) vs. yield force.

(Table II). Hence, it can be said that annealing did not alter the digestibility of gel even though it increased the gel hardness.

RVA Viscosity

As annealing temperature increased, initial pasting temperature increased from 71.9°C for the native starch to 80.4°C for 60°C annealed starch (Table III and Fig. 5), which was related with SP decrease. Peak viscosity and holding strength were found only in the control starch and in the starches annealed at 45 and 50°C. They all had almost the same peak viscosities (245–251 RVU) with close peak times (4.8–5.0 min) even though SP values are quite different (as shown in Fig. 1A). Also, the viscosities at first 95°C (245–250 RVU) were not significantly different in these starches. According to Jacobs et al (1995), both the formation of a tightly packed array of swollen and deformable granules and the leaching of amylose can contribute to viscosity development in starch paste during heating. Very similar peak viscosities of native, 45 and 50°C annealed starches may be explained. First, as annealing temperature increases, the amount of soluble component decreases (Fig. 1B). This should give lower viscosity to paste. Second, as annealing temperature increases, SP decreases or rigidity of granule increases due to insufficient gelatinization. This should give higher viscosity to paste because the rigid granules are more resistant to shearing. A combination of these negative and positive effects may induce the same peak viscosity in these three samples.

At >55°C of annealing, the viscosities at 95°C decreased remarkably to 192 or 112 RVU. Here the swollen granules with low SP did not occupy sufficiently the 8% paste system in RVA analysis, resulting in relatively low viscosity. Furthermore, the leached fraction was small, which gave low viscosity. Final viscosity increased from

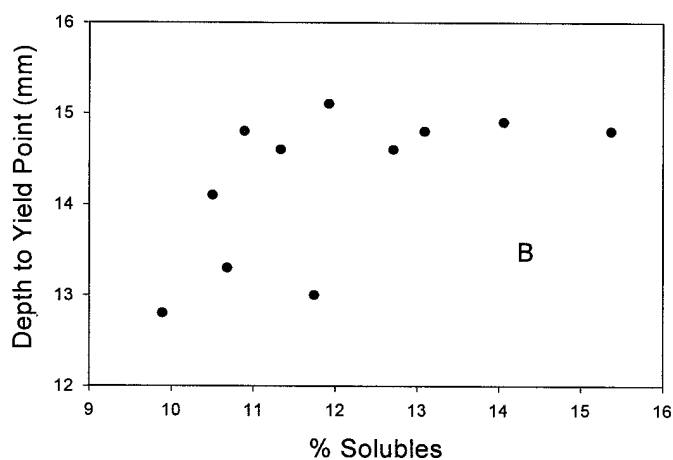
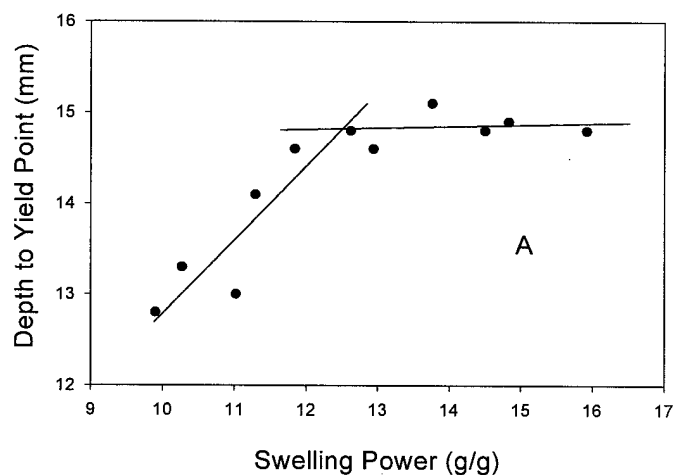


Fig. 4. Swelling power (A) and solubility (B) vs. depth to yield point.

TABLE II
Degrees of Gelatinization of Annealed Mung Bean Starch Gels Stored at 25°C for One Day^a

Annealing Condition	Degree of Gelatinization (%)
Control	73.4 ± 0.6
45°C – 6 hr	72.1 ± 0.5
24 hr	74.0 ± 0.7
50°C – 6 hr	74.3 ± 3.0
24 hr	74.3 ± 2.5
55°C – 1 hr	72.1 ± 0.4
6 hr	74.7 ± 1.4
24 hr	74.5 ± 2.9
60°C – 1 hr	74.0 ± 1.4
6 hr	76.0 ± 1.0
24 hr	73.6 ± 0.7
LSD ^b	2.2

^a Mean value of four measurements ± standard deviation.

^b Least significant difference ($\alpha = 0.05$).

TABLE III
Rapid Visco Analyser (RVA) Parameters of Annealed (24 hr) Mung Bean Starches^a

Annealing Temp. (°C)	Pasting Temp. (°C)	Peak Time (min)	Peak Viscosity (RVU)	Viscosity at 95°C (RVU)	Final Viscosity (RVU)
Control	71.9 ± 0.2	4.9 ± 0.1	249 ± 1	248 ± 1	260 ± 5
45	73.6 ± 0.5	4.8 ± 0.0	251 ± 3	250 ± 3	303 ± 2
50	75.4 ± 0.5	5.0 ± 0.1	245 ± 5	245 ± 5	342 ± 15
55	78.4 ± 0.6	– ^b	–	192 ± 1	312 ± 13
60	80.4 ± 0.7	–	–	112 ± 3	194 ± 6
LSD ^c	1.1	0.1	8	6	22

^a Mean value of two or three measurements ± standard deviation.

^b Not found in RVA.

^c Least significant difference ($\alpha = 0.05$).

TABLE IV
Differential Scanning Calorimetry (DSC) Data of Annealed (6 hr) Mung Bean Starches^{a,b}

Annealing Temp. (°C)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (mJ/mg)	$T_c - T_o$ (°C)
Control	52.0 ± 0.6	66.5 ± 0.2	85.5 ± 0.5	7.07 ± 0.55	33.5
45	55.9 ± 0.2	65.9 ± 0.3	85.7 ± 0.8	10.03 ± 0.15	29.8
50	59.4 ± 0.1	66.7 ± 0.0	85.0 ± 0.3	10.00 ± 0.26	25.6
55	62.8 ± 0.5	68.8 ± 0.1	84.6 ± 0.7	10.43 ± 0.42	21.8
60	66.3 ± 0.1	71.8 ± 0.2	83.2 ± 0.4	8.90 ± 0.26	16.9
LSD ^c	0.7	0.2	1.0	0.65	...

^a Mean value of three measurements ± standard deviation.

^b T_o = onset temperature, T_p = peak temperature, T_c = completion temperature, ΔH = enthalpy.

^c Least significant difference ($\alpha = 0.05$).

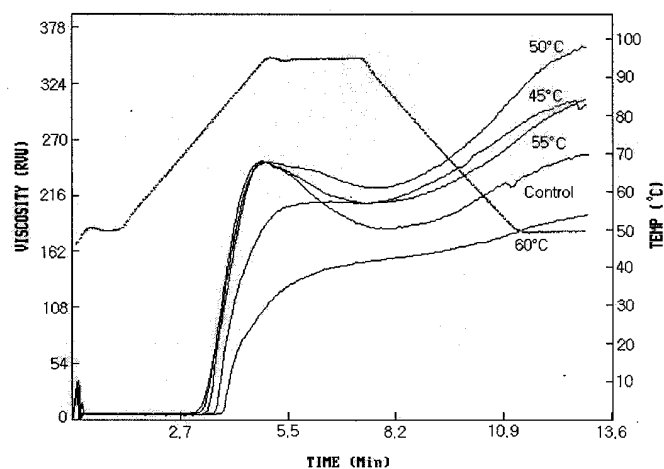


Fig. 5. Viscosity profile of mung bean starches annealed at 45–60°C for 24 hr.

260 to 342 RVU as annealing temperature increased up to 50°C, after that it decreased with increasing annealing temperature. When the final viscosity of the pastes was plotted against yield force of the gels, an excellent correlation ($r = 0.99$) was found between them.

Thermal Properties

Native mung bean starch showed onset temperature (T_o) of 52°C, peak temperature (T_p) of 66.5°C, and completion temperature (T_c) of 85.5°C (Table IV). The gelatinization temperature range ($T_c - T_o$) was 33.5°C. As the annealing temperature increased from 45 to 60°C, T_o increased markedly from 55.9 to 66.3°C, meanwhile T_c decreased slightly from 85.7 to 83.2°C, which means the endothermic peak became sharper. T_p also increased slightly from 65.9 to 71.8°C. Stute (1992) suggested that the narrow peaks indicate melting of the crystallites and swelling and hydration of the granules are more homogeneous in an annealed starch.

Mung bean starches annealed at 45, 50, and 55°C had higher gelatinization enthalpies (ΔH) (10.00–10.43 mJ/mg) than the control starch (7.07 mJ/mg). The increase in enthalpy with annealing implies that the molecular order in the treated starch granules had increased. Starch annealed at 60°C also had a higher enthalpy value (8.90 mJ/mg) than the control, but a lower value than those of the starches annealed at <55°C, indicating increasing molecular rearrangement and partial melting may have occurred simultaneously.

CONCLUSIONS

Annealing increased the surface hardness of mung bean starch gels, and the hardness was inversely proportional to the swelling power. Yield force increased and the depth to yield point remained unchanged when starches were annealed under moderate conditions (at <50°C or at 55°C for 1 hr), after that they decreased; gels became weak and fragile. Thus, annealing can be used to improve the texture (firmer) of the gel food *mook*. By annealing starch (8% solids) at 55°C for 1 hr, gels had a 33% larger surface hardness and 23% larger yield force than the native starch, and they were even better than those of a 9% native starch gel (Table I). Although gel became harder by annealing, α -amylase digestibility was not affected.

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