STARCH GELATINIZATION *IN SITU*: EFFECT OF PARBOILING TREATMENTS

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ABSTRACT

Phenomena of gelatinization in the intact rice kernel were studied using Lebonnet variety obtained from Uncle Ben Co., Houston TX. The effects of temperature and time of soaking on the extent of gelatinization *in situ* of brown rice were investigated qualitatively using a polarizing microscope. The effects of commercially applied soaking and steaming treatments for producing a parboiled-milled rice conducted in a 2^3 factorial design were also investigated using Differential Scanning Calorimeter (DSC).

From microscopic examination, it was apparent that brown rice soaked at 50°C for 5 hours did not significantly change the birefringent characteristics of starch granules *in situ.* Further soaking at 80°C for 30 minutes resulted in increased granule size while the birefringent properties were still retained. Parboiling treatment by steaming the rice at 112°C for 15 minutes resulted in a gradient of gelatinization within the kernel. At the edge of the radial cross-section of the kernel, virtually no birefringence was present; however, at the center, weak birefringent characteristics were observed.

From the enthalpy determination using DSC and analyses of the factorial design applied in the commercial parboiling treatments, the order of important of variables were steaming temperature, soaking temperature, and steaming time. Based on the treatments given during parboiling, none of these conditions resulted in complete gelatinization *in situ.* The highest degree of gelatinization was obtained for the samples soaked at 71°C and steamed at 121°C for 15 minutes. The analysis of gelatinization temperatures obtained from DSC endotherms showed that increased soaking temperature, steaming temperature, and steaming time significantly lowered the gelatinization temperature. However, the effect of these treatments were not significant on the range of gelatinization temperature.

INTRODUCTION

Most of the studies on starch gelatinization phenomena have been conducted on isolated starch granules. However, *in situ* gelatinization (e.g., during parboiling of rice) is a more complex process which inolves not only starch and water but also other components such as protein or lipids (Gariboldi, 1974). It has been well-established that parboiling, due to gelatinization of starch, improves the quality of milled rice. Generally, parboiling consists of two processing operations: soaking and steaming. During soaking, rice is hydrated so that sufficient water is present to allow subsequent gelatini-

zation. During steaming, gelatinization and solubilization of starch occurs which contributes to the final quality of milled rice (Priestley, 1976b). Even though steam is the most commonly used methods to accomplish gelatinization, other means of heating such as hot sand or continuous soaking in boiling water have also been used in practice. The extent and characteristics of gelatinization *in situ*, which are affected by those processing condition, are depending on the structurs and composition of rice kernels.

Structure and Composition of Rice Kernels

A detailed diagram of the mature rice kernel is shown in Fig. 1. Generally, a rice kernel can be separated into four major parts: husk, bran, starchy endosperm, and germ (Little and Dawson, 1960).

The husk is the outermost part of a rice kernel and contributes 16—26% of the weight to the rough rice (Gariboldi, 1974). The primary function of the husk is to provide resistance to insect infestation due to the high content of

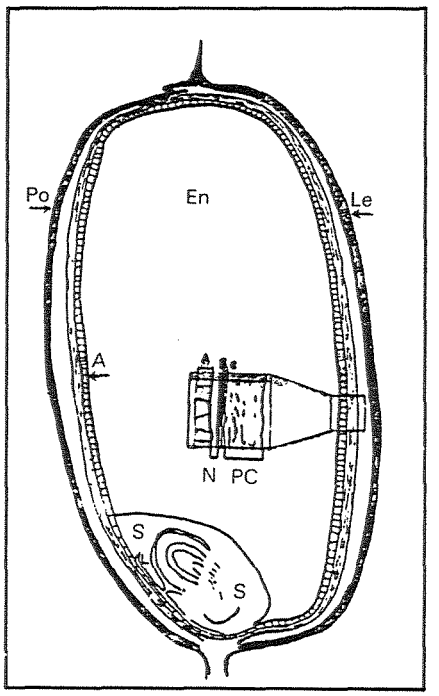

Fig. 1. Diagram of mid longitudinal section through the rice kernel (from Bechtel and Pomeranz, 1978b), where.
En = endosperm, Pa = palea, Le = Lemma, Pc = pericarp, Sc = seed coat, N = nucellus, A = aleurone, S = germ or scutellum.

silica (approximately 15% of the weight of the husk). According to Bechtel and Pomeranz (1978b), since the moisture content of the husk was low (ca. 13.90%, the husk provides protection against attack by fungi and rancidity during the storage of the paddy (Christensen and Lopez, 1965).

Rice brans is approximately 5—7% by weight of the brown rice (hulled kernel). As shown in Figure 1, the rice bran consists of four different tissues: pericarp, seed coat, nucellus, and aleurone (Bechtel and Pomeranz, 1978b). Other investigators reported that the seed coat and nucellus were actually one group called the "testa layer" (Little and Dawson, 1960). The bran is high in protein and lipid (15—20%) which provide nourishment to attacking insects and fungi. Thus, brown rice is highly susceptible to deterioration. Since the bran tissues are relatively thin, they offer little resistance to infestation and penetration into the endosperm by insects of fungi (Bechtel and Pomeranz, 1978b). Individually, each layer of the bran tissues can be characterized by its resistance to moisture migration. The pericarp is composed of crushed parenchyma cells that probably act as a sponge and readily absorb water as described by Bechtel and Pomeranz (1978b). The cell walls of the pericarp reacted positively to the test for protein, pectin, hemicellulose, and cellulose. Whereas the testa (seed coat and nucellus) and the aleurone have a positive reaction with the test for oil. Consequently, these tissues act as a water barrier (Little and Dawson, 1960). According to Bechtel and Pomeranz (1978b), prevention of water diffusion might be due to the presence of cuticle in the testa (Barber, 1972; Houston, 1972).

The portion of the rice kernal which is involved in the gelatinization process is the starchy endosperm. Based on the distribution of the size of the starch granule and the type of protein bodies, the starch endosperm can be divided into two regions: (1) the subaleurone layer, located beneath the aleurone layer, and (2) the central region, constituting the rest of the starchy endosperm (Bechtel and Pomeranz, 1980). Bechtel and Pomeranz (1978c) found three types of membrane-bound protein bodies in the subaleurone region: large spherical, small spherical, and crystalline protein bodies; whereas in the central region, only the large spherical protein bodies were present. Since the subaleurone region is only several layers thick and lies directly below the aleurone, it is removed with relative ease during milling (Bechtel and Pomeranz, 1978b). The starch granules in the subaleurone layer are small (2—4 um) and usually form tiny clusters surrounded by crowded protein bodies (Little and Dawson, 1960).

The central region is composed of large polygon-shaped, compound starch granules surrounded by proteinaceous material. The protein is found between each of the starch granules as well as around the entire compound granules (Bechtel and Pomeranz, 1978c). Little and Dawson (1960) reported that the size of starch granules was between 5 and 9 um, forming a closely

packed group, and there were several groups in each cell. They further confirmed that the proteinaceous material lined the endosperm cell walls and encased all the starch granules. The cell walls appeared fragile and their reactions to stains indicated the presence of cellulose and pectic substances.

The rice germ is located on the ventral side at the base of the grain and contributes about 1% of brown rice weight (Juliano, 1972). It contains embryonic leaves, root and stem, and is connected to the endosperm by the scutellum (cotyledon). The scutellum serves as an adsorbing and conducting organ for nutrients carried from the endosperm to the embryo during germination (Danjo and Inosaka, 1960). The germ is high in protein and lipid bodies (Bechtel and Pomeranz, 1980). During milling, the rice germ is easily removed due to the fact that the germ is not well-bonded to the endosperm. In addition, the endosperm in this region does not have well-defined walls, thus providing a structural defect that allows easy removal of the germ (Bechtel and Pomeranz, 1978b).

Factors Affecting Gelatinization and Structure of Rice During Parboiling

Parboiling commercial rice results in modification of the starch. Watson and Dikeman (1977) reported extensive modification from the aleurone layer through the center of the starchy endosperm when examined using SEM. Furthermore, some vitamins and minerals transfer from the aleurone and germ regions into the starchy endosperm, some lipids disperse from the aleurone and germ layers, and there is inactivation of enzymes and destruction of molds and insects (Gariboldi, 1974). Those changes are accompanied by reduction of chalkiness, increase in translucency of the milled rice, and improved digestibility and cooking characteristics. Parboiling strenghthens the attachment of the germ and the aleurone to the starchy endosperm and avoids separation of these parts during hulling (Bechtel and Pomeranz, 1978b). The two most important steps of processing in parboiling rice are (1) soaking, and (2) steaming.

(a). *Soaking*

It has been well-established that the purpose of soaking is to provide sufficient water necessary for subsequent gelatinization (Priestley, 1976b). Different methods are used to obtain faster hydration thus reducing soaking time including the use of hot water (above 60°C), application of vacuum and/or hydrostatic pressure, and the addition of wetting agents (Gariboldi, 1974).

Even though higher temperatures of water decrease the soaking time significantly, soaking above temperatures of gelatinization tauses rapid hydration preferentially into its surface layers leading to early bursting and leaching (Gariboldi, 1974; Bhattacharya and Subba Rao, 1966a, 1966b). Soaking at high temperatures for longer times also increases discoloration of

the parboiled milled rice, particularly at temperatures above 70°C and soaking for more than 5 hours (Jayanarayanan, 1964). It has been reported that a moisture content of 30% (w.b.) or more is necessary to ensure an even distribution of the water throughout the kernel (Mecham *et al.,* 1961; Gariboldi, 1974). Without additional treatments, i.e., vacuum or hydrostatic pressure, the optimum temperature and time of soaking are between 60—70°C and 3—4 hrs, respectively (Gariboldi, 1974). To avoid excessive discoloration during soaking, sodium bisulfite is commonly used as a bleaching agent or inhibitor of the browning reaction (Mecham *et al.,* 1961: Jayanarayanan, 1964).

(b). *Steaming*

When the starch in the endosperm has absorbed sufficient water, it must be heated above its gelatinization temperature to ensure complete gelatinization. Saturated steam is a common heating medium to accomplish this because it does not remove moisture during heating. Due to condensation and high pressure (usually above atmospheric pressure) the moisture content increases and is equilibrated throughout the kernel. Even at atmospheric pressure the steam temperature is adequate for gelatinization to occur; however, it may require a longer exposure time. Therefore, there are two parameters that can be controlled during steaming-steam temperature (pressure) and the length of steaming time (Gariboldi, 1974).

Excessive steaming causes development of dark color and increases cohesion between the germ and the aleurone layer with the starchy endosperm making these parts difficult to remove during milling. The presence of bran and germ produces a lower quality liberation of amylose during hydrolysis (Gilbert and Spragg, 1964). Viscosity measurement by Brabender amylograph, X-ray pattern using X-ray diffraction, polarizing and light microscope, and scanning electron microscope has provided qualitative information on gelatinization *in situ* (Raghavendra Rao and Juliano, 1970; Kongsuee and Juliano, 1972; Priestley, 1976a, 1976b, 1977; Hahn *et al.,* 1977; Watson and Dikeman, 1977; Bechtel and Pomeranz, 1978a). Application of enzymes to determine gelatinization *in situ* proved difficult due to nonreproducible results as stated by Varriano-Karston *et al.* (1980). Grzybowski and Donnelly (1977) reported that it was possible to obtain quantitative data from starch gelatinization *in situ* in cooked spaghetti using microscopic examination. The only drawback of this method is the preparation of thin sections for microscopic examination. As reported by several investigators (Heidemann, 1978; Wirakartakusumah and Lund, 1978; Galleti *et al.,* 1980). DSC seems to be a promising method to obtain quantitative data on gelatinization *in situ.*

The objectives of this study are: (1) to develop a technique to evaluate *in situ* starch gelatinization based on the birefringent characteristics using a

polarizing microscope, and (2) to determine the effect of commercially-applied parboiling treatments on the degree of gelatinization using differential scanning calorimetry (DSC).

MATERIALS AND METHODS

Rough rice, brown rice, and parboiled rice grains (V. Lebonnet) were obtained from Uncle Ben Co., Houston, TX. Rice kernels were hand-selected to separate the broken fractions and obtain a uniform kernel size.

Parboiling Treatments and Design of Experiments

All parboiling treatments were conducted at Uncle Ben Co. under the following conditions. The kernels were soaked for three hours at 63 and 71 °C. Following soaking, the kernels were steamed at 112 and 121 °C for 10 and 15 minutes. A 2^3 factorial experimental design was used to investigate the effect of soaking temperature, steaming temperature, and steaming time on the degree of gelatinization. All samples were air dried at 40 °C for 5 hours following the specific treatment. The specification of the experimental plan is shown in Table 1.

Table 1. Specification of 2^3 factorial experiments on commercial parboiling treatments.

Trial No.	Variable[a]		
	Soaking T (°C)	Steaming T (°C)	Steaming t (min)
1	-1	-1	-1
2	+1	-1	-1
3	-1	+1	-1
4	+1	+1	-1
5	-1	-1	+1
6	+1	-1	+1
7	-1	+1	+1
8	+1	+1	+1

	minus		plus
[a] Soaking temperatures were	63°C	and	71°C
Steaming temperatures were	112°C	and	121°C
Steaming times were	10	and	15 min.

Preparation for Sectioning the Kernel

To microscopically examine gelatinization *in situ*, the kernel was sectioned with a steel knife in a Lipshaw cryotome (Lipshaw Manufacturing Co., Detroit, MI). Prior to sectioning, the kernels were placed in a 95—100% humidity

chamber at ambient temperature for 48 hours to facilitate wetting as described by Grzybowski and Donnelly (1977). The wetted kernel was accommodated in the center of a gelatin capsule No. 000 (Eli Lilly and Co., Indianapolis, IN) which was filled with an embedding medium, Tissue-Tek II (Lab-Tek Products, Division Miles Labs, Inc., Naperville, IL). The capsule was frozen by contact with dry ice for 30 minutes or longer to make sure that the freezable water was frozen.

The cryotome was turned on and the temperature control was set to approximately -30°C. To obtain temperatures as low as -50°C, crushed dry ice could be dispersed on the bottom of the cryotome chamber.

The frozen capsule was mounted on a sample holder which was then mounted on the microtome. A desired thickness of the section (10 nm) was set according to the control provided on the cryotome. Cutting was started by turning the hand wheel from outside of the cold chamber until the cutting surface of the tissue was as smooth is possible and the desired depth was reached. A small, soft camel's hair brush was used to guide and place the sections as they came off the knife. To avoid curling of the sections, an antiroll service was provided on the instrument. The edge of the antiroll plate was positioned at or slightly beyond the cutting edge of the knife. A microscope glass slide that had been prechilled in the cold chamber was laid on the section platform and the section was transferred on to the slide using the brush. The slide was then picked up with one hand and the frozen section was thawed by warming the slide from beneath with a finger. The thawing should be done in the cold chamber to prevent the sections from curling during thawing. A drop of iodine solution was put on the thawed section and the section was subsequently covered with a cover slip that had been greased at its edges to prevent moisture loss during observation.

Microscopic Observation

For microscopic examination, the kernels were treated with a variety of conditions. After soaking at 50°C for five hours, the kernels were cooked at 80°C for 0.5 and one hour. In addition, raw rice and parboiled rice that had been processed at Uncle Ben Co. trial no. 6) were used for comparison. Observations were made with a Zeiss universal microscope (Carl Zeiss Inc, New York, NY) with a polarizing filter, and photographed using a Nikon camera with Kodak Tri X-pan, 400 ASA film.

General Procedures for DSC

A DuPont 990 thermal analyzer, cell base, and DSC cell (E. I. Dupont DeNemours & Co., Inc. Wilmington, DE) were used to determine enthalpy of transition and temperature range for gelatinization of flour. All determinations of gelatinization characteristics were run at a heating rate of 10°C/min over

the temperature range from 10°C to 110°C. For determination of enthalpy, the X-axis was run in time base at 1.0 min/in on linear graph paper. To determine the temperature range, the endotherm was recorded on a corrected temperature base paper (-120°C to +200°C scale) with the X-axis (temperature axis) on the recorder at a scale of 20°C/in and a shift of five inches. During scanning, nitrogen gas was purged through the DSC cell at ca. 5 ml/min to obtain uniform heat transfer characteristics. The difference in temperatures between reference and sample in the DuPont system is measured by chromel-constantan thermocouples. Water sealed in a pan was used as a reference and the quantity of water was adjusted according to the weight of the sample to minimize specific heat differences between sample and reference. This allowed the use of the maximum sensivity of the recorder (0.05 mcal/sec/in). Since heating the starch/water suspension over the specified temperature range could result in evaporation of the water, the aliquot of the sample and reference were placed in coated alumunium pans which could be sealed hermetically using the crimper supplied with the instrument. A sealed pan can stand pressures up to three atm.

In order to calculate the enthalpy of transition, a calibration coefficient, E, was determined using indium with a known heat of fusion. The ceel base II module provides electronic linearization of E making it independent of temperature. Approximately 15 mg of indium was weighed into a pan and the pan was sealed. The pand and sample were then heated at the rate of 10°C/min from 20°C to 200°C using an empty pan as reference. The area of the resulting endotherm was measured with a planimeter (Kauffel and Esser Co., Germany), and the calibration coefficient E was calculated from:

$$E = \frac{H.m}{60.A.B.\ \ qs}$$

where:

H = heat of fusion of indium (mcal/mg)
m = sample mass (mg)
A = peak area (in^2)
B = time base settings (min/in)
qs = y axis range (mcal/sec/in)

The temperature axis was also calibrated using indium by heating at 10°C/min through the same temperature range in the temperature base mode. The melting point of indium is 156.7°C, and the temperature axis was adjusted at this temperature.

Sample Preparation for DSC

Rice kernels were ground and its moisture content was determined using the AOAC'S procedure. After grinding, the flour was sieved through a 200 mesh (0.053 mm opening). One gram of rice flour was mixed with 10 grams

of water to get the desired 10/1 (w/w, d.b.) water to starch ratio in a screw cap, 22 ml capacity, vial. The vial was shaken in a vortex mixer (Scientific Industries Inc., Springfield, MA) for one minute. The mixture was held at ambient temperature for three hours to facilitate complete wetting of the starch granule by water. After reshaking for one minute, an aliquot of sample, between 10 ul—20 ul, was transferred to a preweighed sample pan. All weighings were performed on a Cahn Gram Electrobalance (Cahn Instruments, Paramount, CA). A drop of sample was transferred by a disposable pipette (9" long Dispo pipettes, Scientific Products, McGraw Park, IL) into the pand and, after putting the cover on, the pan was hermetically sealed using the crimper supplied with the instrument. After reweighing, the dry weight of the solid was calculated knowing its moisture content and water to starch ratio.

Determination of Enthalpy and Temperature Range for Gelatinization

Enthalpy of gelatinization was obtained using time base mode, while the temperature ranges were determined in temperature base mode. To begin the operation, usually the DSC cell was started at 10°C and ca. three minutes were allowed for system stabilization with the pen still in the up position. During this equilibration time, the range of sensitivity, slope of the base line, and position of the pen on the paper could be adjusted. The pen was then lowered resulting in a recorded scan from 40°C to 110°C. The DSC cell was cooled after each run suing a metal cooling sleeve filled with dry ice.

To calculate the enthalpy, the area under the resulting endothermic curve was measured using a planimeter, and the heat of gelatinization was calculated using the following equation:

$$H = \frac{60.A.TB.E.\quad qs}{m}$$

where:

H = enthalpy of gelatinization (mcal/mg)
A = area (in^2)
TB = time base (e.g., 1.0 min/in)
E = calibration coefficient (-)
qs = sensitivity range (mcal/sec/in)
m = mass of sample (mg. dry solid)

Onset and conclusion temperatures (T_o and T_c) were determined as the intercept of extrapolation of base lines and sides of the peak, while peak temperature (T_p) was determined by the interception of the two sides of the peak.

Development of Standard Curves

To determine the degree of gelatinization, a standard curve for the relationship between percent gelatinization and enthalpy values was devel-

oped. The unheated original rice flour represented 100% un gelatinized. To obtain zero percent ungelatinized starch, 5 grams of rice flour were heated to boiling in 250 grams of water. Gelatinization was complete when examination under a polarizing microscope showed no presence of birefringence. The gelatinized paste was dried under vacuum at 100°C for 48 hours. The dried flakes were ground using an analytical mills (Tekmar Co., Cincinnati, OH) and then sieved and equilibrated at ambient temperature for two days. Particles passing through a 200 mesh (0.053 mm opening) were used as the zero percent ungelatinized or 100% gelatinized starch. Moisture content of the gelatinized flour was determined using the AOAC method. Knowing the moisture content of the ungelatinized and gelatinized rice flour samples, various portions were weighed into DSC pans to obtain 0, 20, 40, 60, 80, and 100% ungelatinized flour. After weighing, a proper amount of water was weighed yielding a water to starch ratio of 10/1. The sealed pan was held at ambient temperature for three hours prior to DSC measurement. Linear least-squares analysis was used to determine the best fit between ΔH and percent ungelatinized starch.

RESULTS AND DISCUSSION

Microscopic Examination

Samples were prepared by soaking the rice grains in water at 50°C for 5 hours and subsequently resoaking in water at 80°C for 0.5 and 1 hour. In addition, a parboiled rice that had been processed at Uncle Ben Co. (soaked at 71°C for 3 hours, steamed at 112°C for 15 minutes) and an uncooked (raw) rice were used for comparison.

To prepare the samples for microscopic examination, the kernel was sectioned using a cryosectioning technique. A major problem of using this technique is that the rice kernel is brittle and is therefore subject to fracture while cutting. Humidification of the kernels prior to sectioning in 95—110% humidity at ambient temperature was required to facilitate sectioning. For uncooked rice kernels, however, even with prehumidification, it was impossible to obtain thin sections of 1—2 um. The major drawback of such thick sections is that less light is trasmitted through the section. Furthermore, due to the lack of integrity of the kernel structure, it is difficult to obtain an even and smooth surface on the section. This unevenness causes difficulties in focusing the object under the microscope. For further study, a method that was developed by Flint and Moss (1970) may be used. To obtain a section of desired thickness using the cryostat sectioning technique, they subjected the kernels to humidification over a saturated aqueous solution of Na_2HPO_4 at 8°C for ca. 2 days. The section was then fixed at 8°C for at least 10 days in phosphate-buffered 4.0—4.5% glutaraldehyde at pH 8.0.

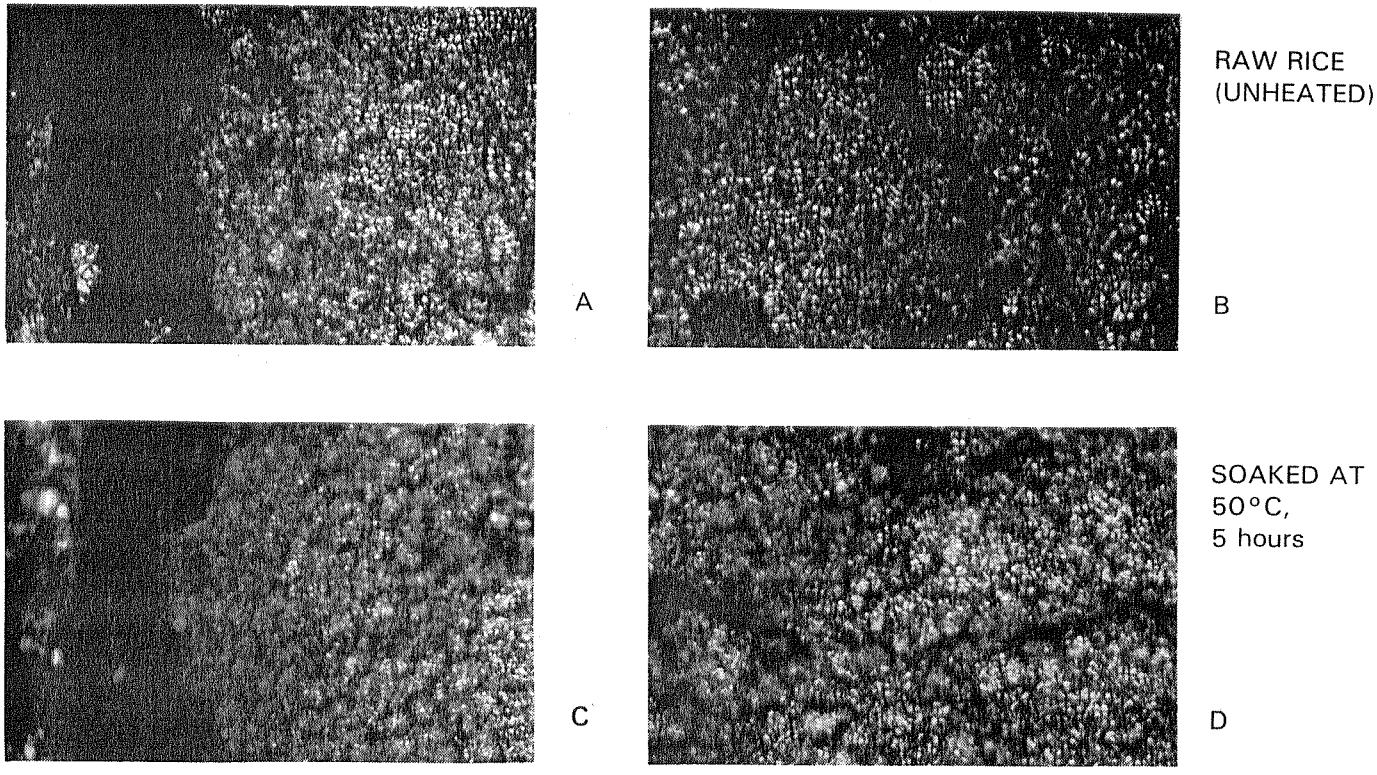

Fig. 2. Photomicrographs of sections from the edge and from the center of unheated and heated rice kernels observed under a polarizing microscope.

To improve the integrity of the kernel structure, another technique was tried by embedding the kernel with Spurr's resin as practiced in the preparation of sample for examination with transmission electron microscope (Bechtel and Pomeranz, 1978c). Using this technique, a section of about 1—2 um was obtained. However, observation under a polarizing microscope did not show any birefringent characteristics due to the fact that the plastic was immiscible to the water.

Despite the problems just described, the cryosectioning technique was employed and allowed examination of birefringent characteristics of the section to enable us to distinguish qualitatively the effect of parboiling treatments on *in situ* gelatinization. For microscopic examination, the starchy endosperm in two regions of the kernel were evaluated: (1) the edge which consists of the aleurone and subaleurone layers, and (2) the central region composed primarily of starchy endosperm. The photomicrographs of sections of the unheated brown rice are presented in Figure 2A and 2B, while Figures 2C and 2D present the photomicrographs of brown rice that was soaked at 50°C for 5 hours. In both cases, the starch granules in the subaleurone region are smaller than those in the central region. This was also observed by Little and Dawson (1960).

From Figure 2A, B, C, and D, it can be seen that there is no significant change in the size of starch granules when rice is soaked at 50°C for 5 hours. The kernel itself swelled as a result of water diffusion during soaking, and this swelling might contribute to the interaction between the water and rice components including starch. However, the interaction of the water with starch at this soaking condition did not significantly change the granule size. It can also be seen that the birefringent characteristics are uneven and slightly blurry across the kernel sections. The unevenness of the birefringence particularly as shown by the heated sample may be due to the unevenness of the section thickness which caused difficulty in focusing during photographing. Generally, there does not appear to be a change in granule size in the heated and unheated sections. Furthermore, although it is not evident in the figure, the cooked rice did not show surface fissures as a consequence of the cooking condition.

In Figure 3, it can be seen that heating to a temperature above that necessary for gelatinization results in a considerable change in the birefringent characteristics and the structure of the kernel when rice was soaked at 80°C for 0.5 hours, following soaking at 50°C for 5 hours (Fig. 3A and B), some swelling occurred causing the kernel surface to fracture. These fractures were more severe for the kernels that were soaked at 80°C for 1 hour. Because of the fissuring problem, the rice kernel was not sectioned for examination. The fractures were observed in the longitudinal direction along the ventral side. A similar observation on cooked brown rice was reported by Little and Dawson

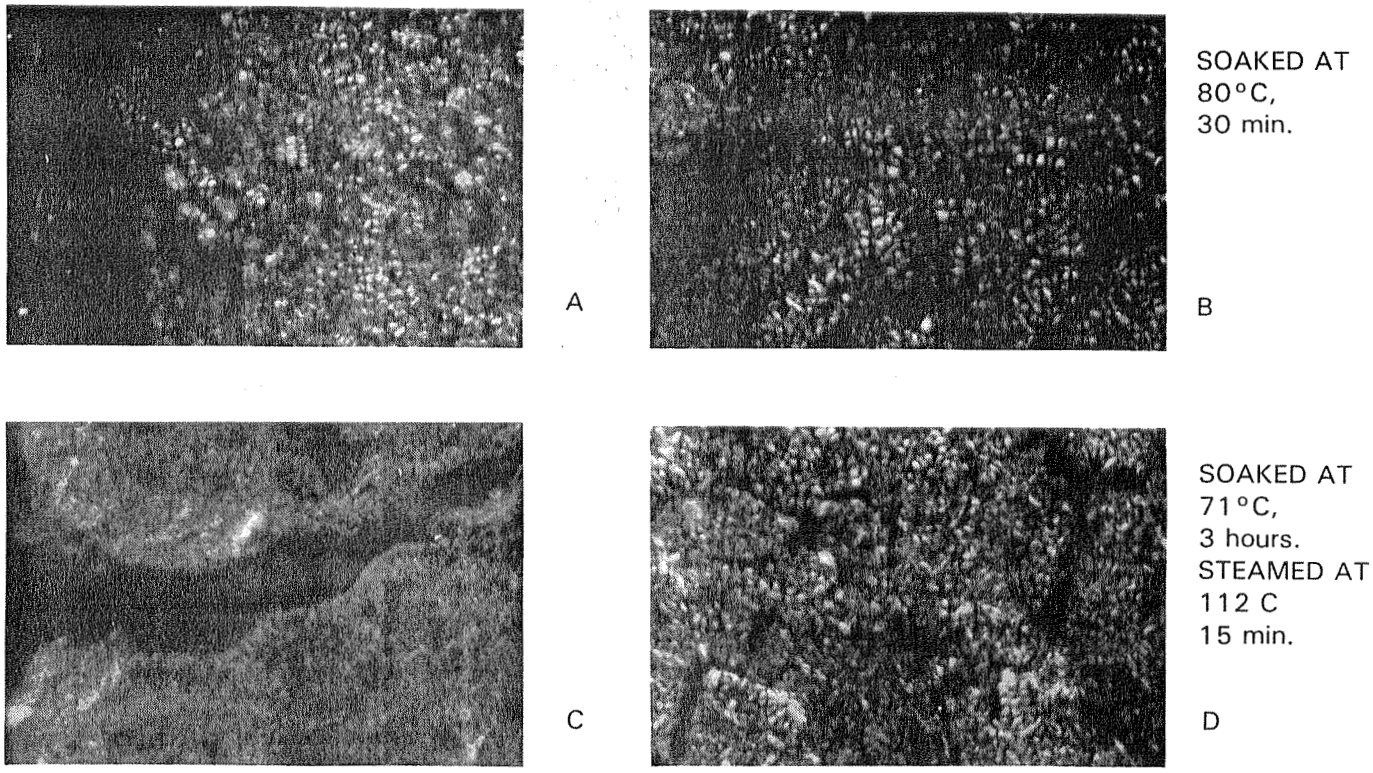

Fig. 3. Photomicrographs of sections of the rice kernels exposed to various treatments observed under a polarizing microscope.

(1960). They pointed out that longitudinal fissuring was characteristic of most long-grain varieties, while cross-wise segmentation occurred in most medium- and shortgrain varieties. Figures 3C and D are photomicrographs of sections of brown rice that received a commercial parboiling treatment (3 hours at 71°C in water followed by steaming at 112°C for 15 minutes). It can be seen that, in terms of birefringent characteristics, the process was much more severe than heating at 80°C for 30 minutes. No surface fractures were observed on the commercial samples. This is expected because soaking at 71°C for 3 hours will not result in excessive swelling due to moisture gain.

Although we do not have data on the moisture content of this commercial rice after it was subjected to steam treatment, it seems that the moisture gain from the condensed steam did not significantly sweel the rice. Priestley (1976b) reported that steaming of rice with initial moisture content ca. 55% (d.b.) at temperature 100—120°C only resulted in ca. 80% (d.b.) final moisture content. This moisture content (ca. 1/1 water to dry solid ratio) may have not reached a point to cause fracture in rice kernels. In Figure 3C, the birefringent characteristic of the parboiled rice has disappeared in the section from the edge of the kernel. The large crack in this section is an artifact created by sectioning the kernel with a steel blade. In the center section (Figure 3D), birefrigence is still evident although it is not as distinct as in Figure 3B. This would indicate that some damage has occurred to the starch granules as a result of exposure to the parboiling treatment. It is expected that there will be more heat damage to granules near the edge than in the center since the moisture content would be greater near the edge than in the central region.

The rice kernels that were exposed to these heat treatments (Figs. 2C, 2D, 3A, 3B, 3C, 3D) did not expand to the point of total rupture. Since starch can absorb up to 64 times its volume in water (Little and Hilder, 1960), this would indicate that either incomplete hydration occurred as observed earlier by Priestley (1976b), or forces are operative that hold the kernel together. Little and Dawson (1960) suggested that nonstarch components in the thick cell walls might delay water penetration, resist rupture, and restrict the expansion of starch granules. Furthermore, the composition of cell walls and the distribution of these components within the cell contribute to the cooking characteristics of rice. Bechtel and Pomeranz (1978c) reported observations on rice endosperm using a transmission electron microscope (TEM) which indicate that the subaleurone layer had more protein than the central endosperm. Furthermore, they pointed out that the type of protein in those regions were different with the subaleurone regions containing small, spherical, and crystalline protein bodies, while the central endosperm contained large, spherical protein bodies. In the sub aleurone region where the starch granules are smaller, the protein materials lined the endosperm cell walls and all the starch granules (Little and Dawson, 1960). Although there is some informa-

tion on the existence of these proteins as well as other cell wall materials (such as cellulose and pectic substances) in rice, the effect of these components on starch gelatinization *in situ* requires further investigation. To distinguish these components *in situ*, several staining techniques may be applied and the examination can be done suing a light microscope (Little and Dawson, 1960; Flint and Moss, 1970), fluorescence microscope (Fulcher and Wong, 1980), and TEM (Bechtel and Pomeranz, 1978c). The overall changes in structure of the rice kernel, as the result of the heat treatment, can be evaluated with a SEM (Bechtel and Pomeranz, 1978b).

Evaluation of the Effect of Commercial Parboiling Treatments on Gelatinization *in situ* in Rice

A two-level factorial design was used to determine the effects of soaking temperature, steaming temperature, and time on the degree of gelatinization *in situ*. The design matrix of the experimental plan was given in Table 1.

The gelatinization characteristics (the enthalpy and temperatures for gelatinization) were determined using the DSC technique. For DSC measurements entire rice kernels were ground to a flour and mixed with water to give 5/1 water to starch ratio (w/w, d.b.). The moisture content of the flour was determined in a vacuum oven (AOAC, 1975). A standard curve was developed relating enthalpy to degree of gelatinization.

The results of DSC measurements of gelatinization characteristics are presented in Table 2 and 3. Table 2 presents the results of the enthalpy determination for samples subjected to conditions of the 2^3 factorial design and their related degree of gelatinization obtained from the standard curve. Table 3 presents the gelatinization temperature of rice for various treatments.

To evaluate the effects of the variables on degree of gelatinization, the degree of gelatinization data in Table 2 were subjected to statistical analysis. The general model for a 2^3 factorial design is:

$$Y_{ijkl} = {}_{ijk} + {}_{ijkl}$$

Where:

- y = the dependent variable
- = the mean of the observation values
- = the error
- i = soaking temperature (T_{so})
- j = steaming temperature (T_{st})
- k = steaming time (t_{st})
- l = replicate

In this analysis it is assumed that errors & are independently (I) and identically distributed (ID) in a normal (N) distribution with men zero and variance q^2 (abbreviated IID, $N(O,Q^2)$; Box *et al.*, 1978).

Table 2. Degree of gelatinization *in situ* of Lebonnet variety rice as affected by various processing conditions.

Soaking Temperature (°C)	Steaming Temperature (°C)	Steaming Time (min)	H[a] (cal/g)	Degree of Gelatinization (%)
63	112	10	1.3	29.1
71	121	10	1.2	35.2
63	112	10	0.8	58.2
71	121	10	0.7	63.2
63	112	15	1.2	36.8
71	121	15	0.9	53.7
63	112	15	0.7	64.6
71	121	15	0.5	74.1
Untreated (uncooked rice)			1.9	0.0

[a] Average of two replicates; range ± 0.1 cal/g.

[b] (%G) = (100% − %G), where ungelatinized starch (UG) is obtained from H: (%UG) = 52.9 (H).

Table 3. Characteristic temperatures of gelatinization *in situ* of Lebonnet variety rice as affected by various processing conditions.

Soaking Temp. (°C)	Steaming Temp. (°C)	Steaming Time (min)	Gelatinization Temp.[a]			
			T_o (°C)	T_p (°C)	T_c (°C)	T(°C) (T_c-T_o)
63	112	10	52.2	58.1	65.5	13.3
71	112	10	53.5	60.0	66.0	12.5
63	121	10	49.7	55.9	62.0	12.3
71	121	10	49.0	56.4	61.9	12.9
63	112	15	54.1	58.1	66.5	12.4
71	112	15	49.2	55.5	61.6	12.4
63	121	15	49.3	55.8	61.5	12.2
71	121	15	49.7	56.5	62.0	12.3
Untreated (uncooked rice)			69.0	75.0	80.5	11.5

[a] Average of two replicates; range ± 0.5°C; determined by DSC at 5/1 w/s ratio and 10°C/min heating rate.

The analysis of the data by Yate's algorithm is given in Appendix N and summarized in Table 4. The estimated variance of each effect is 1.6 and the standard error is 1.3. The histogram of the residuals versus normal scores indicated that the assumption that $_{ijkl}$ IID,N(O,Q^2) is reasonable. To assist in interpreting the estimate of the magnitude of the main effects and their interactions, the data of table 4 are shown in Figure 4. The 99% confidence

interval is centered about zero with a range of 8.4. From Table 4 and Figure 4 it can be seen that all three main effects were significant while the 2 and 3 level interactions were not. To see the trends of these three main effects on the degree of gelatinization, the 2^3 factorial design is shown as a cube in Figure 5. It is apparent from these results that soaking rice at a temperature in excess of that required for gelatinization increased the ultimate degree of gelatinization. Increased steaming time and temperature also increased degree of gelatinization. Since the starch was not completely gelatinized under any experimental conditions, this would indicate that water may be limiting the process. Since these samples were prepared by a commercial company and the moisture content after various treatments was not determined. A complete analysis of gelatinization *in situ* based on the observations of experiments *in vitro* cannot be made.

Table 4. Calculated effect and standard for the 2^3 factorial design of the degree of gelatinization *in situ*.

Effect[a]	Estimate ± Standard Error
Average	51.8 ± 1.3
Main effects	
T_{so}	9.4 ± 1.3[b]
T_{st}	26.3 ± 1.3[b]
T_{st}	10.9 ± 1.3[b]
Interaction effects	
$T_{so} \times T_{st}$	2.1 ± 1.3
$T_{so} \times T_{st}$	3.8 ± 1.3
$T_{st} \times T_{st}$	-2.2 ± 1.3
$T_{so} \times T_{st} \times T_{st}$	-1.6 ± 1.3

[a] T_{so} = temperature of soaking; T_{st} = temperature of steaming; t_{st} = time of steaming.

[b] Significant difference at $\alpha = 0.01$.

Priestley (1976[b]) conducted a similar study on *in situ* gelatinization and basically reported the same observations as reported here. However, he obtained a higher degree of gelatinization (about 25% higher) for the same processing conditions. This may be because he used the iodine binding technique to measure degree of gelatinization. Priestley obtained ca. 10% gelatinization for rice that was soaked at 50°C for 4 hours. On the other hand, in our study using the DSC method, the rice was treated similarly (50°C for 5 hours) and exhibited no gelatinization. The iodine blue value method may result in a higher degree of gelatinization due to the possibility of amylose leaching during analysis.

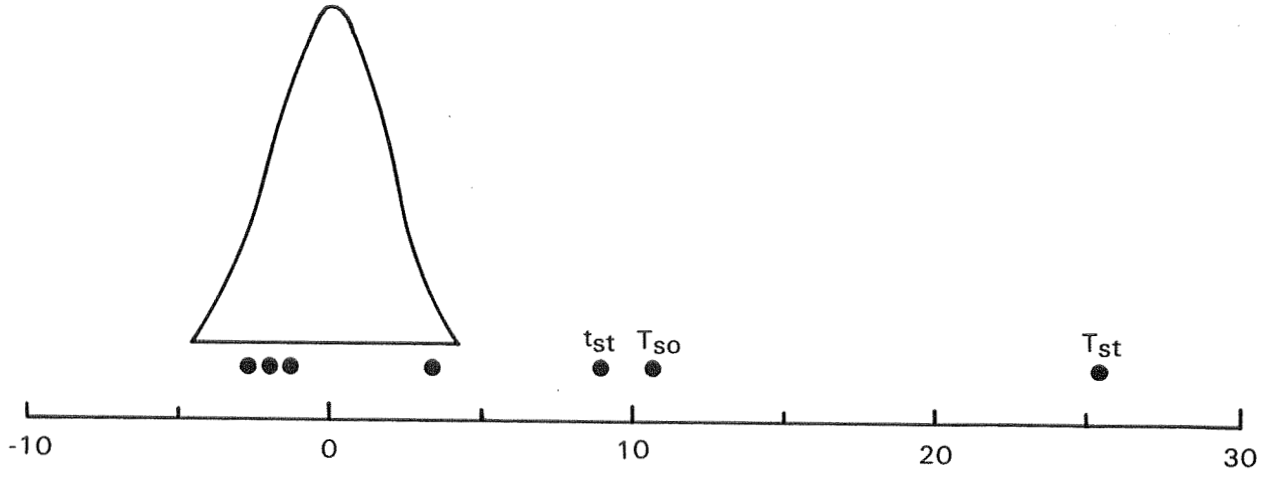

Fig. 4. Main effects and interactions of steaming temperature (T_{st}), soaking temperature (T_{so}), and steaming time (t_{st}) in relation to a reference t-distribution with 8 degrees of freedom and a standard errors of 1.3 for the degree of gelatinization *in situ* of Lebonnet variety rice.

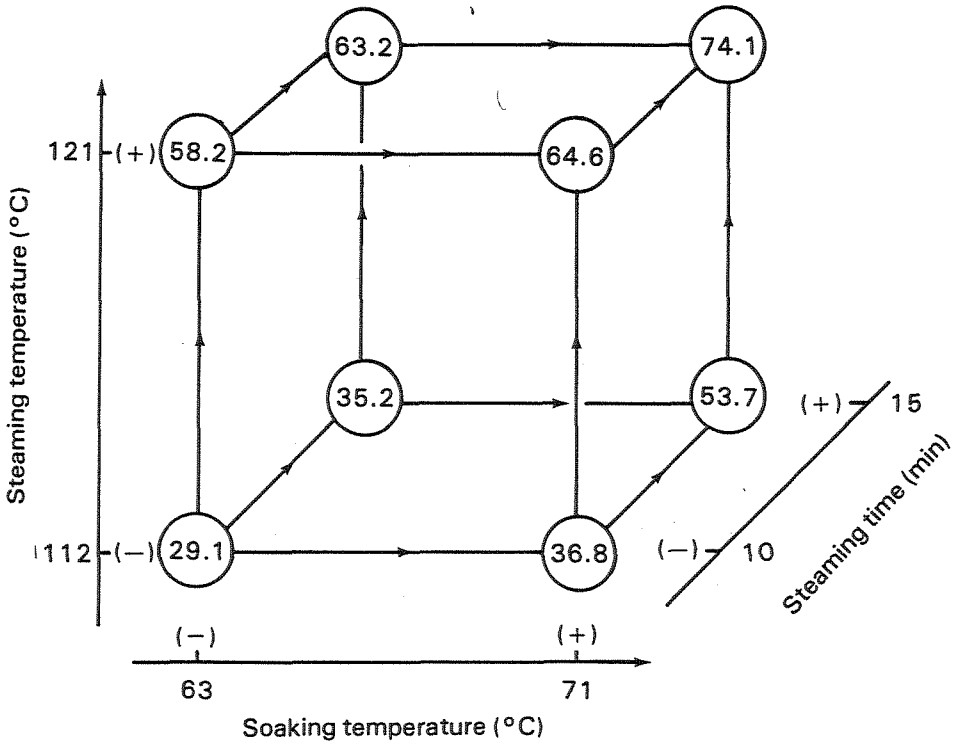

Fig. 5. Degree of gelatinization *in situ* in rice exposed to a 2^3 factorial design experiment.

Based on the data presented in Table 3, a similar statistical analysis was conducted on T_o, T_p, T_c, and T. Tables 5, 6, 7, and 8 present the calculated effect and standard errors for the 2^3 factorial design for temperatures of gelatinization by using Yate's algorithm technique.

With a reference to t-distribution (d.f. = 8) using 99% confidence interval, the effects of parboiling treatments on the characteristics of gelatinization temperature are presented in Figure 6. From Table 5, 6, 7, 8, and Figure 6, it can be seen that all three main effects were significant for T_o, T_p, and T_c, not for T. The 2 and 3 level interactions were also significant for T_o and T_c; however, for T_p the interaction between ($T_{so} \times t_{st}$) was not significant. The interaction effects were not significant for T. For T_o, T_p, and T_c, on the other hand, since the interaction effects are significant, we cannot make an interpretation about the individual main effect of the variables. Nevertheless, the soaking temperature, steaming temperature, and steaming time are statistically proven to be significant variables on gelatinization temperature characteristics.

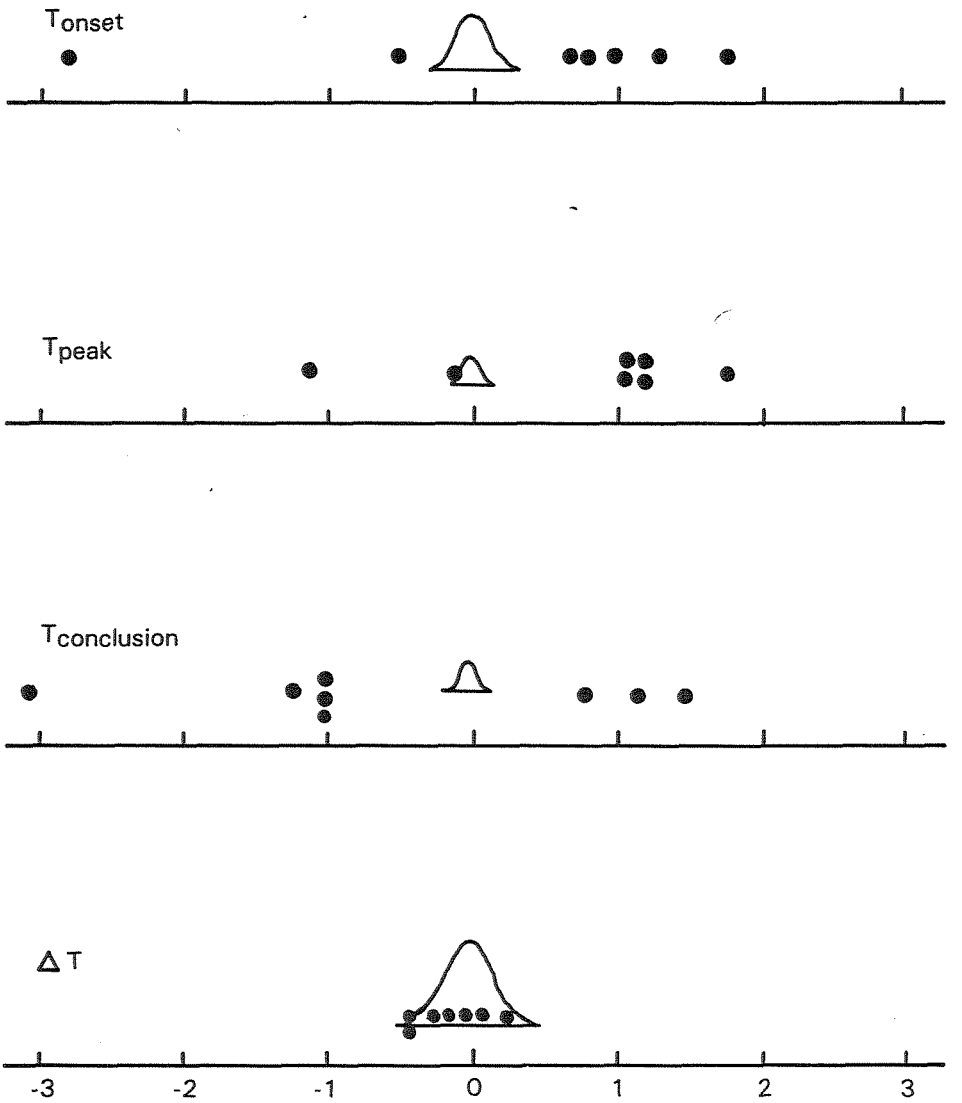

Fig. 6. Main effects and interactions of steaming temperature (T_{st}), soaking temperature (T_{so}), and steaming time (t_{st}) in relation to a reference t-distribution with 8 degrees of freedom for the characteristics of gelatinization temperature of parboiled rice (Lebonnet variety).

Table 5. Calculated effect and standard errors for the 2^3 factorial design of onset gelatinization temperature *in situ* (T_o).

Effect[a]		Estimate ± Standard Error
Average		50.8 ± 0.1
Main effects:	T_{so}	1.3 ± 0.1[b]
	T_{st}	-2.8 ± 0.1[b]
	T_{st}	-0.5 ± 0.1[b]
Interaction effects:	$T_{so} \times T_{st}$	0.8 ± 0.1[b]
	$T_{so} \times t_{st}$	1.0 ± 0.1[b]
	$T_{st} \times t_{st}$	0.7 ± 0.1[b]
	$T_{so} \times T_{st} \times t_{st}$	1.8 ± 0.1[b]

[a] T_{so} = temperature of soaking; T_{st} = temperature of steaming t_{st} = time of steaming.
[b] Significant difference at $\alpha = 0.01$.

Table 6. Calculated effect and standard errors for the 2^3 factorial design of peak gelatinization temperature *in situ* (T_p).

Effect[a]		Estimate ± Standard Error
Average		57.0 ± 0.04
Main effects:	T_{so}	1.1 ± 0.04[b]
	T_{st}	1.8 ± 0.04[b]
	T_{st}	-1.1 ± 0.04[b]
Interaction effects:	$T_{so} \times T_{st}$	1.2 ± 0.04[b]
	$T_{so} \times t_{st}$	-0.1 ± 0.04[b]
	$T_{st} \times t_{st}$	1.1 ± 0.04[b]
	$T_{so} \times T_{st} \times t_{st}$	1.2 ± 0.04[b]

[a,b] See footnotes for Table 5, above.

In general, it can be seen that the gelatinization temperatures decreased as the severity of parboiling treatment increased. It was also noticed that the temperature range became broader (T are between 12.2 to 13.3°C) than the range observed on the untreated sample (T is 11.5°C). A similar observation was made when a partially gelatinized starch with 1/1 water-to-starch ratio was rewetted and reheated in DSC. The broader temperature range and lower temperature for the endotherms have been interpreted as an indication that the starch granule was damaged and destabilized in the previous parboiling treatment but the erystalline structure had not been disordered completely. Thus, subsequent reheating with excess water resulted in a lower temperature endotherm and wider temperature due to the lower degree of erystallinity in the granules. It is important to note that we have observed an opposite phenom-

Table 7. Calculated effect and standard errors for the 2^3 factorial design of conclusion of gelatinization temperature *in situ* (T_C).

Effect[a]		Estimate ± Standard Error
Average		63.4 ± 0.04
Main effects:	T_{so}	-1.0 ± 0.04[b]
	T_{st}	-3.1 ± 0.04[b]
	T_{st}	-1.0 ± 0.04[b]
Interaction effects:	$T_{so} \times T_{st}$	1.2 ± 0.04[b]
	$T_{so} \times t_{st}$	-1.2 ± 0.04[b]
	$T_{st} \times t_{st}$	0.8 ± 0.04[b]
	$T_{so} \times T_{st} \times t_{st}$	1.5 ± 0.04[b]

[a] T_{so} = temperature of soaking; T_{st} = temperature of steaming; t_{st} = time of steaming.
[b] Significant difference at $\alpha = 0.01$.

Table 8. Calculated effect and standard errors for the 2^3 factorial design of range of gelatinization temperature *in situ* (ΔT).

Effect[a]		Estimate ± Standard Error
Average		12.0 ± 0.1
Main effects:	T_{20}	-0.1 ± 0.1[b]
	T_{st}	-0.2 ± 0.1[b]
	t_{st}	-0.4 ± 0.1[b]
Interaction effects:	$T_{so} \times T_{st}$	0.3 ± 0.1[b]
	$T_{so} \times t_{st}$	0.0 ± 0.1[b]
	$T_{st} \times t_{st}$	0.1 ± 0.1[b]
	$T_{so} \times T_{st} \times t_{st}$	-0.4 ± 0.1[b]

[a] T_{so} = temperature of soaking; T_{st} = temperature of steaming; t_{st} = time of steaming.
[b] No significant difference at $\alpha = 0.01$.

enon in the annealing experiments. In these experiments, annealing the ungelatinized starch at temperatures just below that necessary for gelatinization for 24 hours or more resulted in higher endotherm temperatures. However, the temperature range narrowed from ca. 12°C to 6°C. Banks and Greenwood (1975) and Ahmed and Lelievre (1978) suggested that these changes might be due to reordering processes of the cyrstallites from less ordered to more ordered. Therefore, examination of the annealed starch using DSC resulted in higher endotherm temperatures and narrower temperature range. On the other hand, examination of the partly gelatinized starch using DSC, such as in parboiled rice, resulted in lower endotherm temperatures and a wider temperature range due to the destabilization process of crystallites.

Another interesting point to note is that the enthalpy for the less ordered crystallites is smaller as shown by the parboiled rice samples which indicates that less energy is required to completely gelatinize the previously destabilized granules. In the case of annealed granules, however, the enthalpy remained essentially the same as the unannealed sample. This may indicate that, even though the temperature of the endotherm increased as the degree of crystallinity increased, the total energy required to gelatinize the starch did not change a great deal.

CONCLUSIONS

1. *In situ* gelatinization can be examined at least qualitatively using a polarizing microscope. Quantitative information can be obtained using DSC.

2. There may be a gelatinization gradient in rice kernels exposed to treatments sufficient for gelatinization. The edge of the kernel may be gelatinized while the center is not.

3. The water gradient and the different distribution of granule size in the edge and center regions may play a limiting role in gelatinization *in situ*.

4. Parboiling treatments resulted in an opposite characteristic of gelatinization as compared to the annealing process. Parboiling may result in destabilizing the granule structure while annealing may create a higher degree of crystalinity in starch granules.

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