

Physicochemical Factors Affecting Cooking and Eating Qualities of Rice and the Ultrastructural Changes of Rice during Cooking

Young-Eun Lee¹ and Elizabeth M. Osman*

Natural Science Research Institute, KAIST, Teajon 305-701, Korea

*Dept. of Food Science and Nutrition, University of Illinois, Urbana, IL, USA

Abstract

Physicochemical factors affecting cooking and eating quality of rice and their mechanisms were investigated. The stickiness of cooked rice was negatively correlated with amylose content ($r = -0.58$, $p < 0.05$) and protein content ($r = -0.72$, $p < 0.01$), but not affected by crude fat content of rice. The ultrastructure of cooked rice grain showed the progressive gelatinization of starch from the periphery toward the center of the endosperm as water and heat energy diffused into. The rate of water diffusion appears to be dependent on the cell arrangement in the endosperm and the protein content of milled rice. Once water and heat reach the starch granules, the rate of *in situ* gelatinization of starches appears to be dependent on their own gelatinization temperature range and amylose content. Protein acts as a barrier for the swelling of starch and water diffusion in two ways: 1) by encasing starch granules in the starchy endosperm, and 2) by forming a barrier between the subaleurone layer and the starchy endosperm. Therefore, the separation and fragmentation of the outermost layers of the endosperm occurred more easily in the low-protein content rices, and was associated with increases of solids lost in cooking-water at 95°C and stickiness of cooked rice.

Key words : stickiness, amylose content, protein content, gelatinization temperature, cell arrangement

INTRODUCTION

Rice (*Oryza sativa* L.) has been cultivated for centuries and produced in many countries. Rice is the only cereal whose primary use is through cooking and consumption of the whole grain. The cooking of rice results primarily in the gelatinization and swelling of the starch in the rice endosperm, with absorption of water. Although rice starch may increase as much as 60 times in volume when cooked in excess water¹⁾, the rice kernel swells no more than 4 times even in excess water, the nonstarch constituents obviously suppressing this swelling²⁾. Since there have been observations of different endosperm cell arrangements of rice grains, loosely-packed and compactly-packed ones³⁾,

⁴⁾, it appears that the physical structure of rice grain might be important, too.

It is now generally believed that the amylose to amylopectin ratio and gelatinization temperature is the most important determinants affecting the cooking and eating qualities of milled rice. Since these differences do exist among varieties with similar amylose content and gelatinization temperature, some additional indices are needed to differentiate among them, especially in breeding program.

Further studies are required to elucidate the physicochemical changes during cooking and their relationship to the texture of cooked rice. The use of scanning electron microscope may help to clarify the role of starch and nonstarch constituents and the effect of rice grain structure on determining cooking and eating qualities of rice.

¹To whom all correspondence should be addressed

MATERIALS AND METHODS

Materials

Twelve varieties of milled rice differing in physicochemical properties were examined.

All the varieties except Kokuho Rose were obtained from the National Rice Research Laboratory, Beaumont, TX. Kokuho Rose was obtained from the local oriental store, Ames, IA.

Milled rice was ground to flours in a burr mill type grain grinder, passed through a No. 70 sieve (212 μ m), and stored at -20°C until used. Rice starches were isolated by alkali extraction of protein using cold 0.2% sodium hydroxide solution to minimize the damage of starch granule²¹.

Physicochemical properties of rice

Moisture, protein and crude fat of rice were measured according to the AOAC procedures⁶¹. Amylose content of rice starch was measured by the potentiometric iodine titration method described by Schoch⁷. The gelatinization temperature range was determined by measuring the percent loss of birefringence (2%~98%) with an electrically heated hot stage and a polarizing microscope (Leitz Wetzlar, Germany) using the method described by Watson⁶¹. The 98% loss-point was taken as the birefringence end point temperature (BEPT).

Water uptake and solids lost in cooking-water of rice during cooking was determined basically in the same way that measures the swelling power and solubility of starch described by Schoch⁹. The viscosity pattern of a 10% (as-is basis, w/w) flour suspension was determined using the Visco/amylo/Graph (C.W. Brabender Instruments, Inc., NJ) as described by Tipples¹⁰¹.

Scanning electron microscopy

Rice was cooked to various temperatures in a Visco/amylo/Graph to control the heating rate (1.5 °C/min). The cooked rice was taken out at each temperature and frozen quickly by immersion in melting trichlorodifluoromethane (Freon 113, TED Pella, Inc., CA) in liquid nitrogen (m.p. -195.8°C) to avoid the insulation effect of nitrogen bubbles.

Frozen samples were freeze-fractured transversely with a precooled sharp razor blade and then freeze dried.

Samples were examined with a JSM-35 scanning electron microscope (Jeol Ltd., Japan).

Stickiness of cooked milled rice

Stickiness of cooked milled rice was determined with the Instron Universal Testing Instrument (model 1122, Instron Engineering Co., MA) based on the method of Mossman et al.¹¹¹ and the results from the preliminary examination, because of the different sensitivity of the instrument.

Eight grams of rice (as-is basis) were placed in a 30ml beaker to which 12ml of distilled water were added.

The beaker was then covered with a watch glass and steamed at the precalibrated heating rate. After steaming 20 minutes, the pan was removed from the heat and the samples were held in the pan an additional 10 minutes. Each beaker was then removed and inverted on its watch glass to cool for 40 minutes at room temperature before testing. Without mixing, 4g of rice from the center were placed on the stationary plate. A 500kg Tension/Compression cell was lowered at 5mm/min, while the recorder chart moved at 200mm/min. At 80% of compression pressure, the crosshead movement was stopped for exactly 20 seconds, during which time the sensitivity was increased to maximum.

After the relaxation period, the crosshead was moved upward at the same speed, causing the pen to move to the negative field and return. The total area, which was converted to work unit (g · cm) was taken as representing stickiness value.

RESULTS AND DISCUSSION

Physicochemical properties of rice

Physicochemical properties of rices from twelve different varieties are presented in Table 1. Amylose content, BEPT, protein content, crude fat content varied significantly among the varieties tested ($\alpha=0.01$).

Stickiness values ranged from 17.51 to 85.34g · cm (Table 1) showed significant varietal differences ($\alpha=0.01$). Stickiness is the tendency of the cooked rice to adhere to itself and to other objects. With bulk samples, Instron stickiness was more sensitive when measured after compression to constant

Table 1. Physicochemical properties of rices^a

Variety	Amylose %, d.b.	GT range ^b °C	Protein %, d.b.	Crude fat %, d.b.	Stickiness g · cm	Water	Uptake ^c	Cook-water	Loss ^c
						75°C	95°C	75°C	95°C
Newrex	27.8	61.5~74.8	8.92	0.18	17.51	37	389	0.68	6.36
Lebonnet	23.2	60.5~74.7	10.16	0.24	28.22	51	344	0.48	5.09
Pecos	23.0	55.7~67.3	7.83	0.18	69.73	85	386	3.70	7.52
Bellemont	21.2	58.5~75.1	8.62	0.22	21.70	39	373	0.93	5.95
Labelle	20.5	59.3~75.8	7.76	0.17	35.53	35	379	1.13	5.36
S-6	19.7	55.0~64.7	8.42	0.21	42.64	115	356	3.59	8.03
Kokuho Rose	18.2	55.0~73.5	7.12	0.25	54.35	114	383	5.32	9.53
Brazos	15.2	58.0~68.0	8.21	0.14	65.07	64	358	1.49	6.30
Lemont	14.1	61.2~74.0	10.48	0.20	25.13	38	358	1.27	6.09
Early Colusa	12.8	56.2~68.8	8.45	0.29	44.96	71	348	1.75	5.31
Vista	11.2	59.7~71.2	8.32	0.18	61.29	51	339	1.22	5.81
Century Patna 231	10.4	65.2~78.8	6.68	0.21	85.34	38	385	2.17	10.68

^a : Mean value of replicates

^b : 2% birefringence loss-98% birefringence loss (BEPT)

^c : %, d.b.

Table 2. Brabender Visco/amylo/Graph characteristics of rice flours^a

Variety	Pasting temp, °C	Peak(P) B.U.	95 °C, 15min. hold(H) B.U.	50 °C(C) B.U.	Breakdown (P-H), B.U.	Setback (C-P), B.U.	Consistency (C-H), B.U.
Newrex	76.5	850	745	1420	105	570	675
Lebonnet	75.0	735	520	1150	215	415	630
Pecos	69.0	1000	610	1060	390	60	450
Bellemont	73.5	695	520	1090	175	395	570
Labelle	73.5	975	575	1120	400	145	545
S-6	64.5	770	465	900	305	130	435
Kokuho Rose	72.0	765	490	920	275	155	430
Branzos	67.5	1045	550	980	495	-65	430
Lemont	72.0	710	515	1175	195	465	660
Early Colusa	69.0	990	520	1000	470	10	480
Vista	71.3	1210	650	1120	560	-90	470
Century Patna 231	78.3	1150	500	750	650	-400	250

^a : 10% (as-is basis, w/w) paste ; 1.5 °C/min heating and cooling rate ; 75 rpm

pressure than after compression to constant clearance¹¹). Indeed, hardness of cooked rice influences the obtained stickiness. Juliano et al.¹²) reported that stickiness values showed significant negative correlation with hardness values.

Water uptake and solids lost in cooking-water were determined as an indication of cooking quality. Water uptake at 95 °C ranges from 339 to 389% (db) and solids lost in cooking-water at 95 °C from 5.09 to 10.68% (db) (Table 1). Water uptake at 75 °C ranges from 37 to 115% (db) and solids lost in cooking-water at 75 from 0.48 to 5.32% (db) (Table 1).

Brabender Visco/amylo/Graph characteristics of rice flours

Amylography is viewed as a measure of cooking characteristics of rice¹³). Amylograph pasting characteristics of rice flours are tabulated in Table 2.

Correlations among physicochemical properties of rice

Cooked rice stickiness was significantly affected by protein content ($r = -0.72$, $p < 0.01$) and amylose content ($r = -0.58$, $p < 0.05$), but not by crude fat content. It has been known for a long time that the ratio of amylose and amylopectin is the major

factor governing the eating quality of rice¹⁴. But there have been contradictory results about the effect of protein on cooked rice stickiness^{15,16}. Generally, when samples with a wide range of amylose content were employed, the effect of protein content seemed to be outweighed because the difference in amylose content was far larger than that of protein content.

From these results, it might be concluded that the ratio of amylose to amylopectin is the primary factor affecting the cooked rice stickiness, but the effect of protein on the cooked rice stickiness becomes more apparent among the rices of narrow ranges of amylose content.

Cooked rice stickiness was significantly correlated with amylograph viscosities of rice flours (Table 3).

Table 3. Correlation coefficients of milled rice and starch properties with cooking and eating quality indexes among nonwaxy rices (n=12)

Property	Amylose	Protein	Crude fat	Stickiness	BEPT
Amylose	1.00				
Protein	0.25	1.00			
Crude fat	-0.12	0.07	1.00		
Cooked rice stickiness	-0.58*	0.72**	-0.18	1.00	
Water uptake at 75 °C	0.04	-0.31	0.28	0.29	-0.69**
Water uptake at 95 °C	0.42	-0.47	-0.36	0.08	0.35
Cook-water loss at 75 °C	-0.06	-0.56*	0.21	0.47	-0.38
Cook-water loss at 95 °C	0.23	-0.68**	0.04	0.62*	0.12
Amylograph Peak	-0.55	0.55	-0.35	0.75**	-0.06
Breakdown	-0.76**	-0.61*	-0.15	0.87**	-0.10
Setback	0.71**	0.74**	0.09	0.93**	0.13
Consistency	0.58*	0.82**	0.00	-0.91**	0.15

* : $p < 0.05$

** : $p < 0.01$

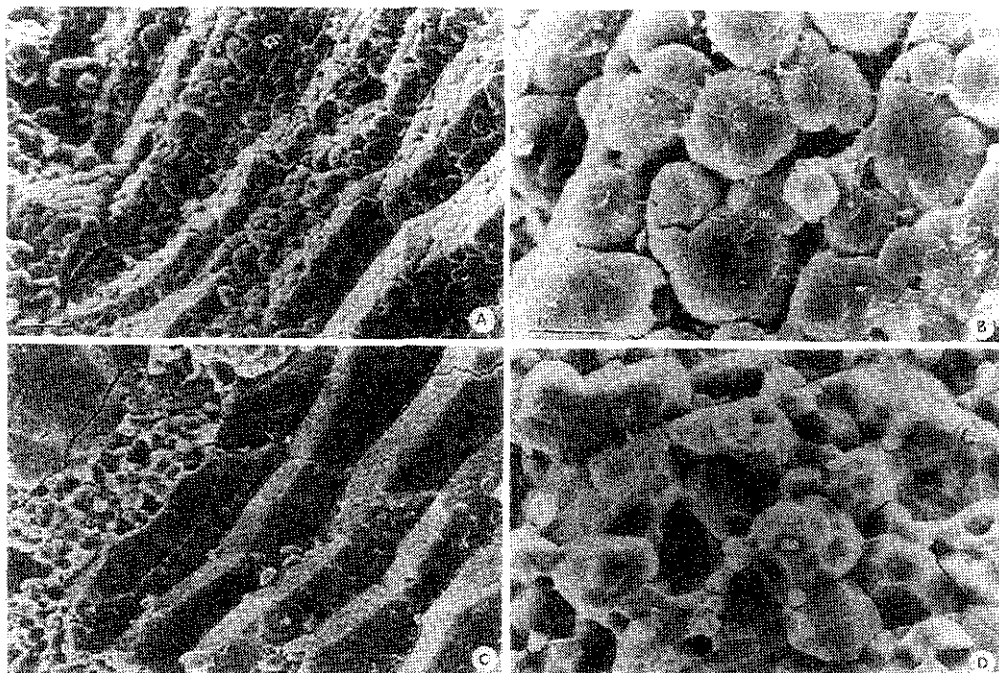


Fig. 1. Cell surface and compound starch granule of loosely-packed Early Colusa (A, B) and compactly-packed Lebounet (C, D) cs=compound starch granule, dm=demarcation.

Especially, cooked rice stickiness showed strong correlation with breakdown ($r=0.87$, $p<0.01$), setback ($r=-0.93$, $p<0.01$) and consistency ($r=-0.91$, $p<0.01$) of rice flour. These strong correlations are due to the synergistic effects of amylose content and protein content of rice. Therefore, amylograph viscosities of rice flour can be used as good indices to differentiate among rices within the narrow range of amylose content.

Ultrastructural changes of rice during cooking

The starchy endosperm consists of thin walled parenchyma cells which are elongated radially on cross-sectional view and filled with compound starch granules and some protein bodies.

The fracture faces of crumbly or soft-endosperm nonwaxy rice showed mainly intercellular cleavage. The cell boundaries in crumbly rices were relatively rough in appearance, indicating the presence of less matrix material between compound starch granules and parenchyma cells (Fig.1). The cell walls were composed of cellulose microfibrils, a matrix phase of hemicellulose, pectic substances

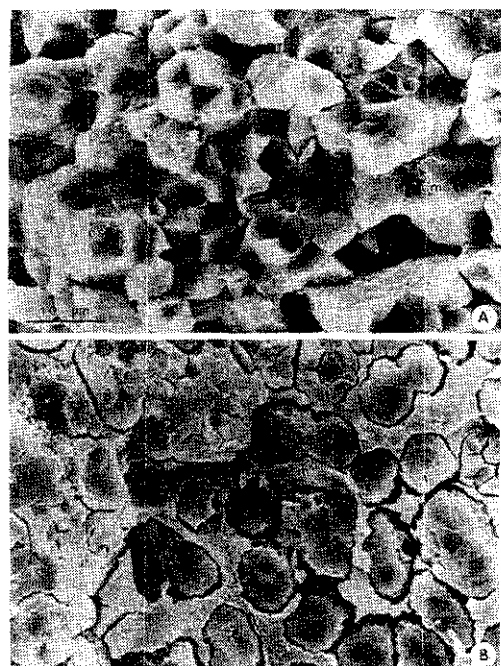


Fig. 2. Starch granules in the center (A) and the peripheral cells of the endosperm of rice grain cm=cell matrix, cs=compound starch granule, is=individual starch granule, p=protein body.

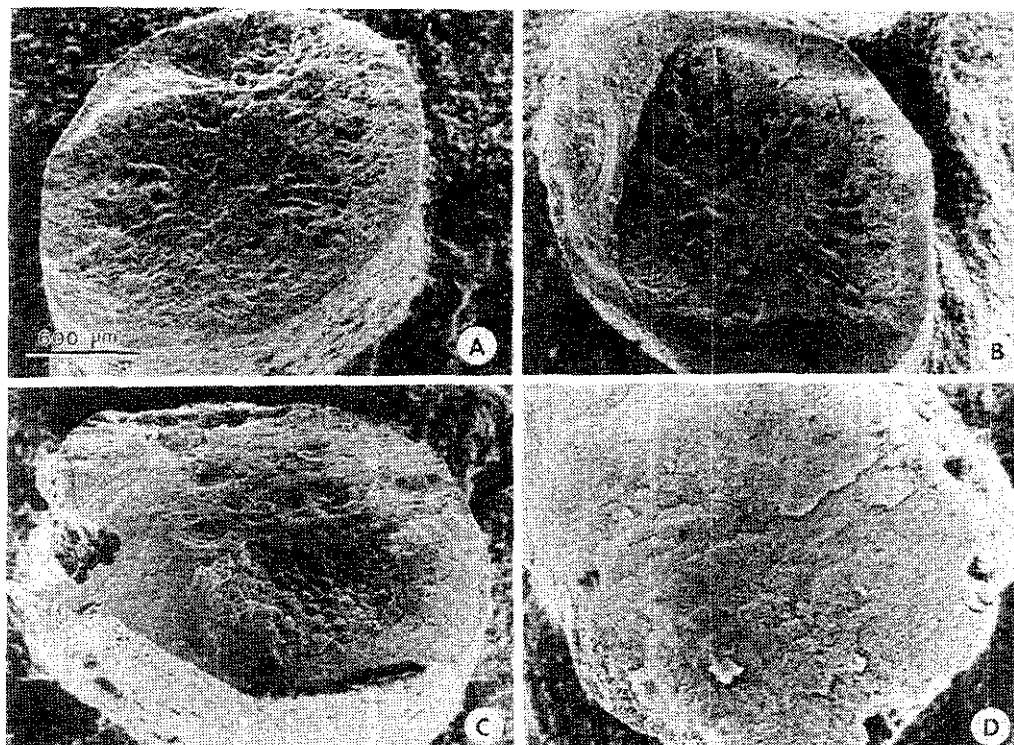


Fig. 3. Progressive changes from the periphery toward the center of the endosperm during cooking at 75°C (A), 85°C (B), 90°C (C), 95°C, 10min(D).

and 3% protein¹⁷. In the center, individual starch granules were shown through intracellular cleavage and the loose packing among individual starch granules was most marked.

The fracture faces of hard-endosperm nonwaxy rice showed mainly intracellular cleavage. The cell boundaries were smooth, and angular and starch granules in parenchyma cells compact, and the demarcation between individual granules and compound granules were not apparent (Fig. 1). This difference in packing state affected the shape of compound granule markedly, and also that of individual granules; some of the starch granules from loosely-packed rices had one or more faces well rounded.

Starch granules in the peripheral cells of the endosperm were smaller than those in the major central portion, confirming previous observations made by Little and Dawson¹¹. The compound granules in the peripheral cells were separated by electron-dense proteinaceous material^{11,17}, whereas those in the central portion were closely packed without any apparent intervening material (Fig. 2).

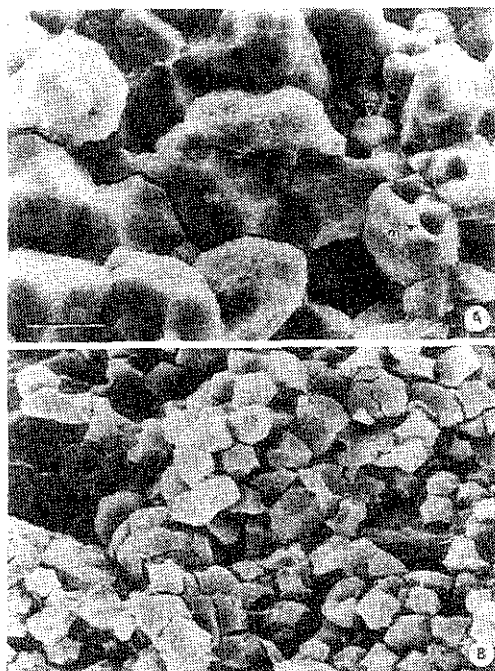


Fig. 4. Differences in water diffusion rate between compactly-packed (A, Lebonnet) and loosely-packed (B, Brazos) endosperm at 75°C cs= compound starch granule, is=individual starch granule, m=missing granule.

All the rice samples studied showed a similar pattern of structural changes during heating, regardless of variety. The variations were dependent upon the rate of water and heat diffusion, properties of starch (amylose content and BEPT) and protein content of the rice. Progressive changes from the periphery toward the center of the endosperm were evident in both sticky and less sticky rices (Fig. 3).

The water diffusion rate seems to be affected greatly by the packing state of rice grain during the initial cooking period (75°C). At the same temperature and time, water diffused into the central portion more easily in loosely packed rices than in compact ones (Fig. 4).

As water and heat penetrated into the endosperm with temperature increase, the cell matrix started to loosen up, admitting enough water and heat for gelatinization of starch. Above the gelatinization temperature, starch granules gradually started to gelatinize and swell further without losing their

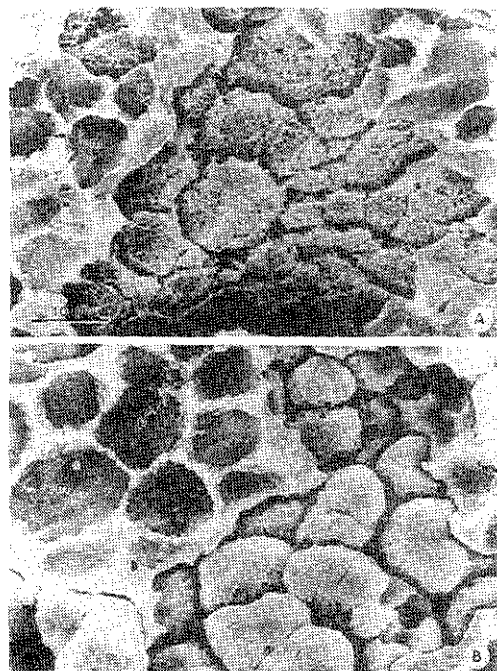


Fig. 5. Differences in the extent of gelatinization of starches between loosely-packed and low amylose rice (A, Early Colusa) and compactly-packed and high amylose rice (B, Newrex) at 85°C cs=compound starch granule, cm= cell matrix, i=indentation by protein body, p=protein body.

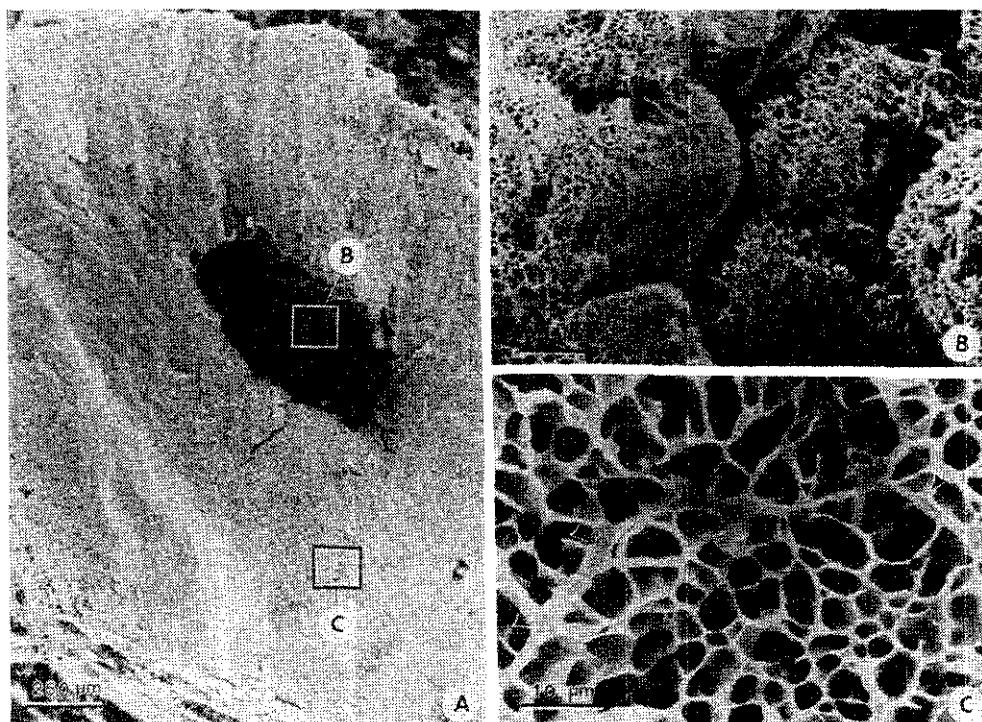


Fig. 6. Fracture surface of fully cooked rice grain (A, Lemont, 95°C, 10min) and enlarged view of the center (B) and the middle region (C) of endosperm (cell boundary is shown as arrow heads).

shape. At this time (85°C), cell matrix materials and protein bodies have not been affected by heat and water, even though the starch granules have already started to gelatinize (Fig. 5). As more water is absorbed, the size of parenchyma cells increased markedly and starch granules lost their original shape and structure. The resistance to loss of structure may be dependent on amylose content as previously described¹⁸.

After being heated for 10 minutes at 95°C, starch granules were fully gelatinized and milled rice grains were fully cooked (Fig. 6). The fractured surface showed starch gelatinization *in situ* was somewhat limited inside the cell and cell boundaries were still maintained. The enlarged view of the fully cooked rice grain showed the homogeneous gel matrix structure with cell boundaries.

Separation of the subaleurone layer from starchy endosperm occurred during heating (Fig. 7). Once this outermost layer of endosperm was separated from starchy endosperm and even removed, water and heat penetration and solids lost in cooking-water could be increased rapidly, and this assisted gelatinization of starches to a great extent. This fragmentation of the outer layers of grain seems to be greater in low-protein rices (Fig. 8). This suggested that protein may act as a barrier for water

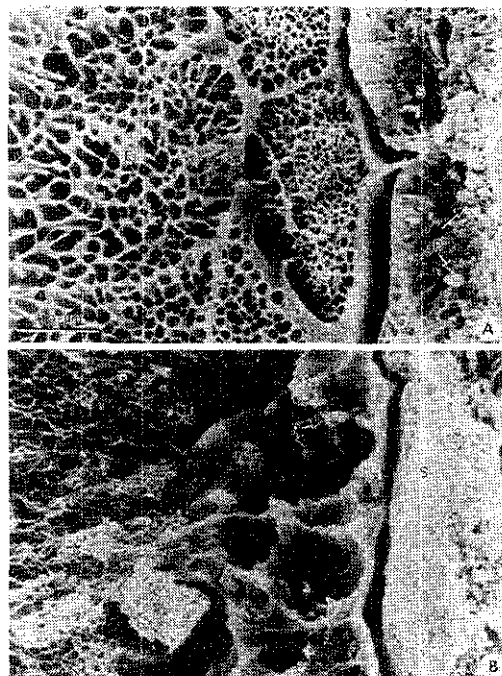


Fig. 7. Separation of the subaleurone layer of Centruy Patna 231 (A) and S-6 (B) at 95°C. E=endosperm, P=protein body, S=subaleurone layer.

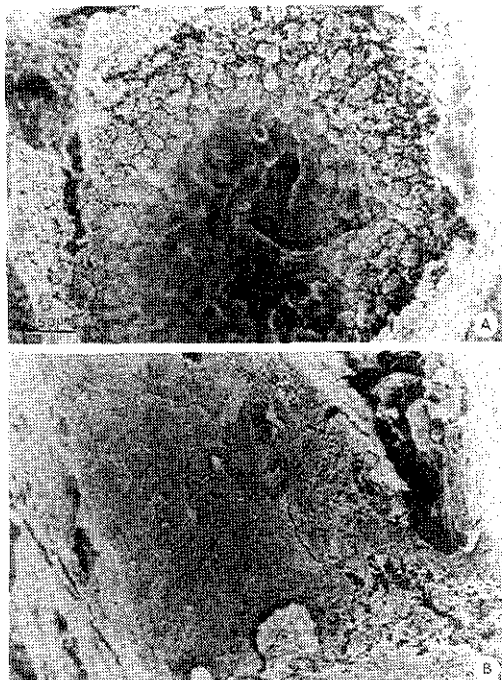


Fig. 8. Fragmentation of the outer layer of Century Patna 231(A) and Kokuho Rose(B) at 95°C, 10min.

diffusion from the subaleurone layer toward the center of starchy endosperm. Increase in solids lost in cooking-water by separation of the subaleurone layer and/or fragmentation may affect the stickiness of cooked rice. This is supported by the correlation coefficients between solids lost in cooking-water at 95°C and protein content ($r = -0.68, p < 0.01$), solids lost in cooking-water at 95°C and stickiness ($r = 0.62, p < 0.05$), and protein content and stickiness ($r = -0.72, p < 0.01$) (Table 3).

So far, the effect of the physical structure of milled rice and the role of protein on the cooking and eating qualities of rice have been underestimated. This study suggests that differences in cooking and eating qualities of rice seem to be due to several factors: endosperm cell arrangement and protein content of rice, and gelatinization temperature range and amylose content of starch.

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쌀의 취반 및 식미특성에 영향을 주는 요인들과 취반 시 쌀의 배유 조직의 변화

이영은 · 오스만 엘리자베쓰 엠*

한국과학기술원 자연과학연구소

*미국 일리노이 주립대학교 식품영양학과

요 약

쌀의 품종에 따른 취반 및 식미 특성들의 차이에 영향을 주는 요인들을 검토하여, 그 원인 및 기작을 분석하였다. 밥의 중요한 식미 특성인 끈기도는 아밀로오스($r=-0.58$, $p<0.05$) 및 단백질 함량($r=-0.72$, $p<0.01$)과 부의 상관관계를 보였으나, 조지방 함량에 의해서는 영향을 받지 않았다. 쌀의 취반시 물과 열 에너지가 쌀 알의 가장자리에서부터 중앙 쪽으로 확산되어 들어감에 따라 배유세포내에 들어 있는 전분 입자들을 점진적으로 호화시켜 주었다. 이때 물과 열 에너지의 확산 속도는 배유세포의 충전 상태와 단백질 함량의 영향을 받았다. 이는 전자 현미경에 의한 배유세포내 구조적 변화와, 95℃에서 밥물에 빠져 나온 물질의 양과 밥의 끈기도($r=0.62$, $p<0.05$) 및 단백질 함량($r=-0.68$, $p<0.01$)과의 상관관계에 의해 설명되어진다. 단백질은 배유 세포 내에서 전분 입자를 둘러싸 줌으로써, 또한 하위 호분층(Subaleurone)층에서 물의 확산을 막아주는 역할을 함으로써 취반 속도 및 식미 특성에 영향을 주는 것으로 생각되어 진다.