

# Pasting and Crystalline Property Differences of Commercial and Isolated Rice Starch with Added Amino Acids

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**ABSTRACT:** This study compared effects amino acid structure and type on the pasting properties and crystallinity of rice starch isolated from flour. Amino acids were added at 2 and 6% of the starch (dry basis) for RVA analyses. Charged amino acids had more of an influence on pasting properties than neutral amino acids. Our results indicated that the negatively charged amino acids had a similar effect as that of the positive charged amino acids for decreasing cooking stability of the starch, but opposite effects on retrogradation tendency. Charged amino acids increased the crystallinity of the starch, potentially enhancing resistant starch nature.

**Keywords:** pasting, rice starch, amino acids, crystallinity, x-ray diffraction

## Introduction

PROTEIN AND STARCH INTERACTION HAS BEEN REPORTED DURING BAKERY product manufacture and storage (Dreese and others 1988; Martin and others 1991; Holm and others 1985; Bjorck and others 1986; Guerrieri and others 1997). Those interactions may influence the staling of the final products, the availability of starch to digesting enzymes, and the quality of the final products. Greenwell and others (1985) suggested that surface proteins in the starch granule may represent an obstacle to the access of amylolytic enzymes or may interact with them, modifying their surface distribution. High-protein content rices were generally less tender than low-protein content rices after cooking (Onate and others 1964, Juliano and others 1965). Studies from Juliano and others (1964) suggested that the protein content could influence the peak viscosity and set back values of milled rice, although those influences may vary from one variety to another.

Rice starch isolation is more difficult compared with maize and wheat and may be due to the hydrophobic properties of the proteins (prolamin and glutelin) in rice starch granules, (Lumdubwong and Seib 2000). Protein removal by solvent extraction may decrease the gelatinization temperature, increase the peak viscosity, and reduce the peak temperature of pasting (Marshall and others 1990; Yang and Chang 1999). Brown rice flours are obtained from unpolished rice kernels. These flours were reported to add a different flavor and chewy texture to baked products (Juliano 1985). A study of the pasting characteristics of brown rice flour and its isolated starch in comparison with white rice flour and starch could provide some valuable information for expanding the use of brown flour and its starch.

Proteins with disulfide bonds were found to restrict starch granule swelling during gelatinization and make the swollen granules less susceptible to disruption by shear (Hamaker and Griffin 1993). After mixing reducing agent (dithiothreitol) or various proteinases with white rice flour, Hamaker and Griffin (1990) observed that the pasting viscosity was reduced over the whole pasting temperature range, whereas no viscosity influence was observed when mixed with isolated rice starch. Based on those findings, they suggested that the hydrolyzed product of rice proteins might interact with rice

starch and result in the viscosity reduction of rice flour. However, the influence of amino acid structure and type has not been studied in terms of quality assessment. It is well known that cereal proteins vary in amino acid composition (Khan and Bushuk 1979). Studies suggested that protein and its subunit properties (such as solubility) are related to their difference in amino acid composition (Bushuk 1985). Further study is needed to examine the influence of amino acid structure and type on the pasting properties of rice for potential use of amino acids as starch modifiers.

X-ray diffraction has been used to study the crystallinity of starch. Cereal starches give an A-type pattern and V-type patterns are believed to be attributed to lipid-amylose complexes (Zobel 1988a, b; Hibi and others 1990). Yang and Chang (1999) observed that protein could interfere with the crystalline X-ray diffraction pattern of rice flour, but no one has previously studied the effects of amino acids on the crystalline nature of rice starch. Enhanced crystallinity may indicate that the starch is more resistant to enzymatic digestion and so may have fiber-like properties in the body (Botham and others 1995, 1997; Hoebler and others 1999).

The objectives of this study were: (1) to determine the lipid and protein removal effects on pasting properties of rice flours; (2) to determine added amino acids effects on pasting properties of rice starch; (3) to compare those additive effects on commercial rice starch versus rice starch isolate (4) to determine the lipid removal and protein removal effects on rice flour XRD patterns and (5) to determine the effects of added amino acids on the rice starches XRD patterns.

## Materials and Methods

### Materials

Two types of commercially available rice flour (white, brown), obtained from Riviana Foods, Inc. (Abbeville, La., U.S.A.), were used in this study. Commercial rice starch (S-7260), purchased from Sigma Chemical Co (St. Louis, Mo., U.S.A.), was used as a control. Amino acids used in this study included positive charged (arginine and lysine, both in free base form), negative charged (aspartic acid and glutamic acid, both in free acid form), and neutral ones (leucine

and alanine, in free form). The amino acids, amylose (A0512), amylopectin (A8515), and protease (P5147) were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.).

### Lipids extraction from rice flour

Defatting of rice flour was performed by the modified soxhlet extraction method (Yang and Chang 1999). A 30 g flour sample was weighed and transferred into a 80mm- high extraction thimble. The thimble was then covered with cotton and put into a Soxhlet extraction tube. One hundred ml of petroleum ether were added to a 500 ml flask, and then an Allihn condenser, the Soxhlet extraction tube, and the flask were connected and placed on a hot plate with a temperature setting of 45 °C. A cooling system was then connected to the condenser with the coolant temperature setting at -5 °C.

The petroleum ether extraction was performed for 12 h, and the methanol extraction followed with 100 ml methanol at 65 °C for another 12-h extraction. Finally, the defatted flour sample was air-dried under a vacuum hood. Duplicate samples were prepared.

### Protein removal of rice flour

The modified alkaline protease digestion method (Lumdubwong and Seib 2000) was applied to remove protein from defatted rice flour. A 40 g defatted flour sample was weighed and transferred into a 500 ml flask. Then 150 ml of 0.001 M NaOH solution was transferred to the flask and 0.2 g protease powder was weighed and added to the mixture. The mixture was then stirred and adjusted to pH 10 by adding 1 M sodium hydroxide solution. Then the flask was covered with parafilm and placed in a shaking water bath for 18 h at 55 °C.

The slurry was then centrifuged at 3000 g for 20 min. Finally, the supernatant was discarded while the sediment was washed twice with 150 ml distilled water and centrifuged at 3000 × g for 15 min. The residue was then suspended in 150 ml distilled water and adjusted to pH 7 by adding 1 M hydrochloric acid. After that, the pH-adjusted slurry was centrifuged at 10000 × g for 20 min. The supernatant was discarded, and the dark tailings layer atop the starch was carefully scraped away and discarded. The starch was finally washed 3 times with 100 ml distilled water until the tailing fraction became negligible after centrifuging. The isolated starch was dried in a convection oven at 40 °C for 48 h. Duplicate samples were prepared.

### Chemical composition analysis

Two types of rice flours (white and brown), their defatted flours, starch isolates, and commercial starch were analyzed for moisture (method 985.14, AOAC 1995), lipid (method 945.16, AOAC 1995), protein (N × 5.95) (method 992.15, AOAC 1995), and ash content (method 920.153, AOAC 1995). The amylose content of the samples above was determined using the standard iodine colorimetry method proposed by Juliano and others (1981). Duplicate samples were used for above analysis.

### Standard rapid visco analysis (RVA)

Amino acids were used as additives to test their effects at 2 levels, 2% and 6% on a starch basis. Commercial rice starch and the RSI from white flour were tested with those additives, while the defatted flours and the RSI from brown flour were used for pasting tests without additives. A rapid visco analyzer (Newport Scientific, Warriewood, Australia) was used to measure the apparent viscosity of samples as a function of temperature, time, and stirring. A modified procedure of the RVA Rice Method (1997) was followed. Each additive was carefully weighed into a RVA canister. Distilled water (25 ml) was measured and transferred into the canister. Then 2.65 g (dry basis) starch sample was weighed and transferred to the can-

ister, and distilled water was added to reach a total weight of 28 g. The weight of starch was held constant. A plastic paddle was placed into the canister and vigorously jogged through the sample up and down for 10 times. The canister with the paddle was then inserted into the instrument. The measurement cycle was initiated by lowering the motor tower of the instrument into position.

The starch suspension was stirred rapidly at 960 rpm for 10 s before the shear input was decreased and held constant at 160 rpm for the heating and cooling cycles. The suspension was heated from 50 °C to 95 °C in 3 min and 48 s, then held at 95 °C for 2 min and 30 s before cooling to 50 °C over 3 min 48 s. All pasting curves were performed in duplicate. The viscosity was expressed in RVU. Peak viscosity (PV), minimum viscosity (MV), final viscosity (FV), pasting temperature (PT), and time to peak (TP) were reported. The total set back (TSB) and break down (BKD) viscosity were derived from the following formulas: TSB = FV - MV and BKD = PV - MV.

The gelatinized sample gels, obtained from the RVA tests, were stored at refrigerator temperature for 3 d to accelerate retrogradation. Those gels were then freeze-dried and milled to 100 mesh powders using a rice miller. Samples were hydrated at 75% relative humidity (RH) in a sealed vessel using saturated NaCl for 24 h before the X-Ray Diffraction (XRD) test.

### X-Ray diffraction (XRD)

About 1 g of sample was pressed into a 10 × 25 mm pellet with a hydraulic press. X-ray diffraction pattern was obtained using a Siemens D5000 X-ray diffraction instrument. X-ray diffractograms were obtained under conditions of 40 KV, 30 mA, with the scanning angle 2θ set from 2° to 36° at a scanning rate of 0.6°/min. Relative crystallinity (RC) of the starch was determined by the method of Hermans and Weidinger (1948), as described by Nara and others (1978); that is, the area of the crystalline fraction ( $a_c$ ) is divided by the diffraction area for a 100% crystalline substance ( $A_c$ ). In this study, the area of the crystalline fraction in raw commercial starch XRD pattern was used as the value of  $A_c$  at 100% (Dragsdorf and Varriano-Marston 1980). X-ray patterns were designated according to the d-spacings and intensities given by Zobel (1988a, b). The diffraction patterns were recorded and compared.

### Statistical analysis

SAS (Statistical Analysis System) software (Version 8.0) was used for data analysis. Analysis of Variance (ANOVA), with Tukey's studentized range (HSD) test, was performed to examine the additive (amino acids) effects on the pasting characteristics (PV, MV, FV, PT, TP, TSB, and BKD) of commercial starch and starch isolate. Similarly, the lipid and protein removal effects on the pasting characteristics of flours were examined. Duplicate samples were used and a significance level of  $P \leq 0.05$  was applied.

### Results and Discussion

THE RVA VISCOSITY CURVES REFLECT THE PASTING CHARACTERISTICS of starch during processing and use (Deffenbaugh and Walker 1989). The pasting temperature (PT) is the temperature at which the viscosity starts to rise. Usually pasting temperature is higher than the gelatinization temperature, meaning the starch granules are gelatinized before the viscosity begins to rise and be detected by RVA. Lower pasting temperature means faster swelling. Peak viscosity (PV) reflects the extent of granule swelling. Most of the time, we must cook through this stage in order to obtain a usable starch paste. Time to peak (TP) indicates the time required for cooking. The drop in viscosity from a maximum value (peak viscosity, PV) to a minimum value (minimum viscosity, MV) is the breakdown value (BKD). BKD reflects the stability of the paste during cooking,

whereas the final viscosity (FV) at 50 °C indicates the stability of the cooked paste. Total setback (TSB) shows the viscosity increase on cooling to 50 °C, indicating the extent of retrogradation of the starch product. Rice flours with higher amylose contents that showed greater retrogradation properties in bread were shown to have greater total setback than other flours. Therefore, total setback was correlated with retrogradation (Juliano and others 1964; Bean 1986; Leelavathi and others 1987).

**Effects of lipids and protein removal on pasting properties of rice flours**

Brown flour contained more lipids and protein than the white flour did (Table 1). Petroleum ether and methanol extraction completely removed the lipids from both flours, whereas the alkaline protease digestion resulted in a protein residue of 1.4% in white starch isolate and 4.1% in brown starch isolate. For white rice flour, lipid removal significantly reduced the FV and decreased the TSB, resulting in a lower cold paste viscosity and retrogradation tendency (Table 2, Figure 1a). The pasting temperature of white flour was also reduced by 2 °C after defatting. Therefore, the starch granules in defatted white flour were slightly easier to cook than in white flour control.

Compared to the defatted white flour, protein removal greatly reduced the PV, MV, and FV (Table 2). In addition, the TSB and the BKD were also reduced. The proteins in the rice flour were believed to restrict starch granule swelling and reduce the amylogram viscosity (Hamaker and Griffin 1993; Yang and Chang 1999). Protein removal by solvent extraction was reported to increase the paste viscosity (Yang and Chang 1999), but we saw the opposite effect. In this study, alkaline protease digestion was used to remove proteins

**Table 1—Chemical composition of starches, flours, and defatted flours<sup>1</sup>**

Sample	Moisture (%)	Protein (%) (N × 5.95)	Lipids (%)	Ash (%)	Amylose (%)
Commercial starch	11.1	0.6	0.0	0.3	25.3
White flour	10.7	8.7	0.8	0.7	30.0
Defatted white flour	11.6	8.0	0.0	0.6	29.4
White starch isolate	7.3	1.4	0.1	0.5	29.2
Brown flour	9.1	10.0	3.3	1.8	28.5
Defatted brown flour	10.7	10.5	0.1	2.1	27.7
Brown starch isolate	6.7	4.1	0.1	1.7	28.7

<sup>1</sup>All values are calculated based on the dry weight of samples except moisture content.

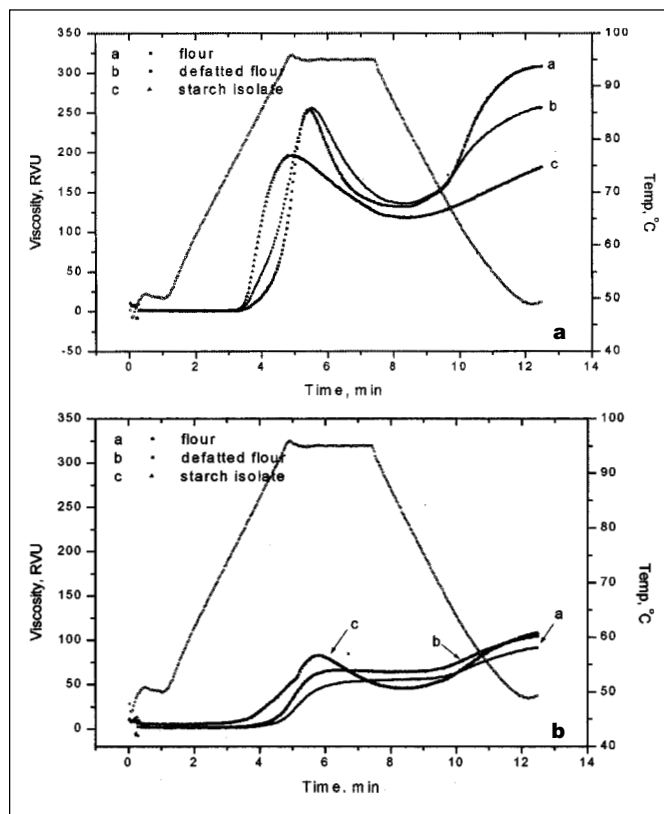
from the defatted white flour. Starch isolation by alkaline protease digestion was reported to generate amino acid salts as the co-products (Lumdubwong and Seib 2000). Therefore, the decrease in the paste viscosities (PV, MV, FV), in this study, might be due to the presence of residual amino acids in the isolated starch.

Brown flour (Table 2, Figure 1b) had a lower PV and a greater PT than white flour, which might be related to the greater protein and lipids contents in brown flour. Brown flour showed no BKD, the viscosity continued to increase after passing the initial 95 °C viscosity. This behavior reflects the greater stability of the swollen starch granule against mechanical disintegration. Defatting increased the PV, MV, FV (Table 2). The pasting temperature was reduced by 2 °C while the TSB was increased. Removing protein from defatted brown flour caused a further increase of PV, whereas the MV was reduced by 22 RVU. PT was further reduced by 9 °C, while TSB was increased by 24 RVU. BKD increased significantly after alkaline protease treatment, indicating destabilization of the granules making them more susceptible to shear during cooking. The fact the PV of the isolate from brown flour could not reach the same level as the isolated white rice starch may be due to the greater level of protein remaining and the possible presence of residual amino acids (Hamaker and Griffin 1993; Yang and Chang 1999; Lumdubwong and Seib 2000).

**Effects of amino acids on pasting properties of commercial starch**

Figure 2 shows examples of how positively charged and negatively charged amino acids affect the pasting properties of commercial starch. Compared to the commercial starch without additives, the presence of 2 and 6% aspartic acid significantly decreased the MV, FV, and TSB, but increased the BKD (Table 3, Figure 2a). Both aspartic and glutamic acids had no influence on PT. Compared to the control, arginine (Figure 2b) and lysine at both levels reduced the MV and FV, while increasing the BKD (Table 3). Arginine also increased the TSB (Table 3) and reduced the pasting temperature by 15 °C. Leucine and alanine showed minimal influence on pasting properties of commercial starch at both levels, with the main effects of increasing MV and decreasing TSB particularly for alanine (Table 3).

Among the amino acids, arginine caused the lowest MV, PT, and TP, and the greatest BKD, resulting in a starch that is easier to cook but less stable to shear. On the other hand, addition of aspartic acid resulted in the lowest FV and TSB, making the starch more stable to retrogradation. Our results indicated that the negatively charged amino acids (aspartic and glutamic acids) had a similar effect as that of the positive charged amino acids (lysine and arginine) for



**Figure 1—Effects of lipids and protein removal on pasting properties of (a) white rice flour and (b) brown rice flour**

**Table 2—Effect of lipid and protein removal on pasting properties of rice flour<sup>1,2,3</sup>**

Sample	PV	MV	FV	PT	TP	TSB	BKD
White flour	249.96 <sup>a</sup>	132.21 <sup>a</sup>	309.58 <sup>a</sup>	80.43 <sup>a</sup>	5.47 <sup>a</sup>	177.38 <sup>a</sup>	117.75 <sup>a</sup>
Defatted flour	260.58 <sup>a</sup>	137.96 <sup>a</sup>	262.21 <sup>b</sup>	78.35 <sup>b</sup>	5.54 <sup>a</sup>	124.25 <sup>b</sup>	122.60 <sup>a</sup>
Starch isolate	197.75 <sup>b</sup>	119.46 <sup>b</sup>	183.63 <sup>c</sup>	77.25 <sup>b</sup>	4.87 <sup>b</sup>	64.17 <sup>c</sup>	78.29 <sup>b</sup>
Brown flour	54.00 <sup>c</sup>	56.88 <sup>b</sup>	92.13 <sup>b</sup>	92.33 <sup>a</sup>	6.88 <sup>a</sup>	35.25 <sup>c</sup>	-2.88 <sup>c</sup>
Defatted flour	68.03 <sup>b</sup>	66.42 <sup>a</sup>	105.96 <sup>a</sup>	90.15 <sup>b</sup>	6.60 <sup>a</sup>	39.54 <sup>b</sup>	1.67 <sup>b</sup>
Starch isolate	81.83 <sup>a</sup>	44.63 <sup>c</sup>	107.42 <sup>a</sup>	81.70 <sup>c</sup>	5.85 <sup>b</sup>	62.79 <sup>a</sup>	37.21 <sup>a</sup>

<sup>1</sup>PV=Peak Viscosity, MV=Minimum Viscosity, FV=Final Viscosity, PT=Pasting Temperature, TP=Time to Peak, TSB=Total Set Back, BKD=Breakdown;  
<sup>2</sup>Units: Viscosity (RVU), Temperature (°C), Time (min).  
<sup>3</sup>Different letters within each column, for white separately from brown flours and starches, indicate values are significantly different at the level of p ≤ 0.05.

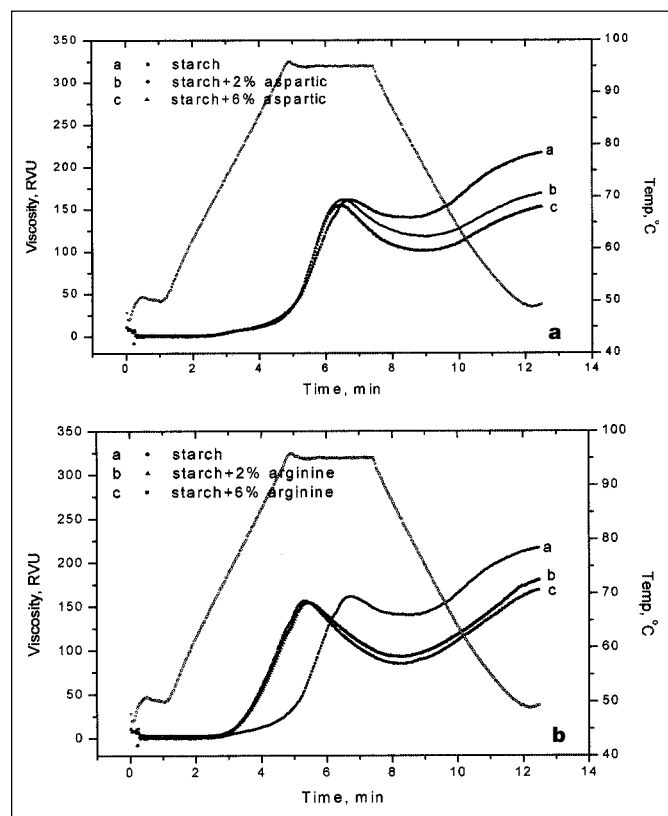
decreasing cooking stability of the starch, but opposite effects on retrogradation tendency (arginine only).

**Effects of amino acids on pasting properties of white starch isolate**

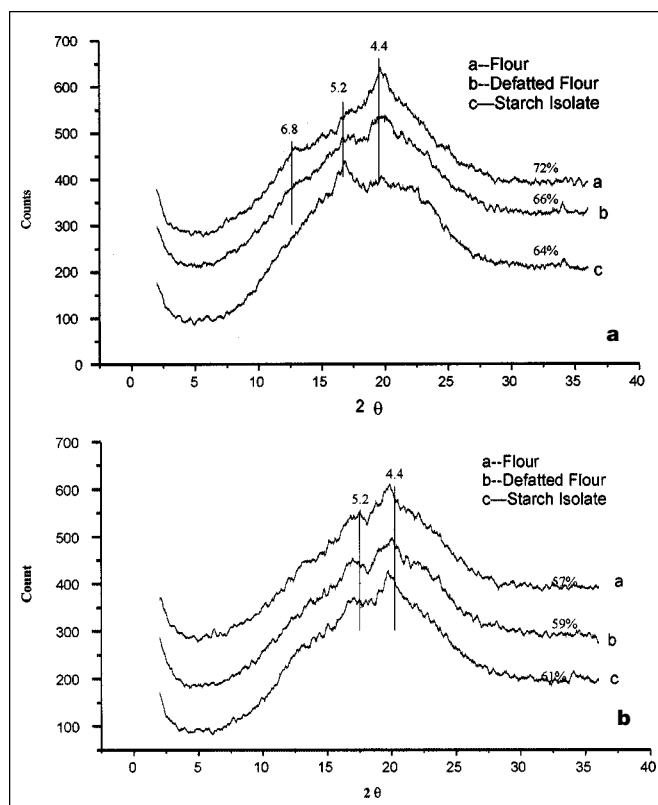
Without additives, white starch isolate contained a greater level of amylose and protein residues than the commercial starch did (Table 1). It was reported that lower amylose content starches tend to produce greater PV and BKD with lower FV and PT (Endo and others 1989; King and others 1994; Zeng and others 1997). Results from this study did not support that finding. We observed the opposite effect where PV and BKD were 35 RVU and 57 RVU lower, respectively, in commercial starch than for white starch isolate. PT was 12 °C and FV 34 RVU greater in commercial starch than the isolate. Our results indicated that not only the amylose content may influence the pasting characteristics, but other factors such as variety, starch preparation method, and minor component content

(amino acids), might also play an important role in governing the pasting properties of rice starch.

The amino acids effects on pasting properties of white starch isolate (control) were studied. Only arginine affected the PV and increased PV at both levels (Table 4). The charged amino acids tended to decrease the MV and FV. Among these amino acids, 6% aspartic acid showed the greatest reduction in MV and FV. At 6%, glutamic acid and arginine also reduced the MV and FV, whereas lysine only decreased the MV at 2% and did not affect FV. The presence of positively charged amino acids resulted in 1.5 to 2 °C increases in PT, whereas TP was not affected by any amino acid. Negatively charged amino acids decreased the tendency for retrogradation as seen by lower TSB values (Table 4), whereas 2% arginine increased TSB. Greater BKD was observed for all amino acids except for alanine, with arginine having the largest change, 50 RVU, at 6% addition. Neutral amino acids had almost no affect on



**Figure 2—Effects of amino acids on pasting properties of commercial starch (a) aspartic acid and (b) arginine**



**Figure 3—Influence of defatting and protein removal on X-ray diffraction pattern of (a) white flour and (b) brown flour (samples a, b, c were gelatinized and stored in the refrigerator for 3 d)**

**Table 3—Effects of amino acids on pasting properties of commercial starch<sup>1,2,3</sup>**

Sample	Additives <sup>4</sup> (%)	PV	MV	FV	PT	TP	TSB	BKD
Control	—	162.17 <sup>bcd</sup>	141.17 <sup>c</sup>	217.34 <sup>a</sup>	89.38 <sup>a</sup>	6.70 <sup>bc</sup>	76.17 <sup>bc</sup>	21.01 <sup>gh</sup>
Aspartic	2	162.80 <sup>bcd</sup>	117.75 <sup>ef</sup>	169.42 <sup>d</sup>	91.28 <sup>a</sup>	6.49 <sup>d</sup>	51.67 <sup>g</sup>	45.05 <sup>de</sup>
	6	157.08 <sup>def</sup>	102.63 <sup>g</sup>	154.00 <sup>e</sup>	89.68 <sup>a</sup>	6.49 <sup>d</sup>	51.38 <sup>g</sup>	54.56 <sup>c</sup>
Glutamic	2	167.04 <sup>abc</sup>	127.17 <sup>d</sup>	182.46 <sup>c</sup>	89.83 <sup>a</sup>	6.63 <sup>cd</sup>	55.29 <sup>g</sup>	39.88 <sup>f</sup>
	6	150.25 <sup>f</sup>	110.67 <sup>f</sup>	165.42 <sup>d</sup>	91.55 <sup>a</sup>	6.62 <sup>cd</sup>	54.76 <sup>g</sup>	39.59 <sup>f</sup>
Lysine	2	158.08 <sup>de</sup>	112.50 <sup>f</sup>	191.12 <sup>b</sup>	74.58 <sup>b</sup>	5.88 <sup>f</sup>	78.63 <sup>b</sup>	45.58 <sup>d</sup>
	6	160.88 <sup>cde</sup>	119.75 <sup>e</sup>	191.71 <sup>b</sup>	75.15 <sup>b</sup>	6.05 <sup>e</sup>	71.96 <sup>cd</sup>	41.13 <sup>ef</sup>
Arginine	2	154.75 <sup>ef</sup>	93.54 <sup>h</sup>	181.84 <sup>c</sup>	74.00 <sup>b</sup>	5.41 <sup>g</sup>	88.30 <sup>a</sup>	61.21 <sup>b</sup>
	6	157.17 <sup>def</sup>	84.79 <sup>i</sup>	170.96 <sup>d</sup>	74.03 <sup>b</sup>	5.33 <sup>g</sup>	86.17 <sup>a</sup>	72.38 <sup>a</sup>
Leucine	2	167.96 <sup>abc</sup>	148.88 <sup>ab</sup>	220.34 <sup>a</sup>	89.50 <sup>a</sup>	6.81 <sup>ab</sup>	71.46 <sup>d</sup>	19.09 <sup>hi</sup>
	6	168.71 <sup>ab</sup>	144.55 <sup>bc</sup>	219.54 <sup>a</sup>	90.03 <sup>a</sup>	6.70 <sup>bc</sup>	75.00 <sup>bcd</sup>	24.17 <sup>g</sup>
Alanine	2	167.13 <sup>abc</sup>	149.88 <sup>ab</sup>	216.29 <sup>a</sup>	89.25 <sup>a</sup>	6.83 <sup>ab</sup>	66.42 <sup>e</sup>	17.25 <sup>hi</sup>
	6	170.92 <sup>a</sup>	155.04 <sup>a</sup>	215.75 <sup>a</sup>	90.38 <sup>a</sup>	6.90 <sup>a</sup>	60.71 <sup>f</sup>	15.88 <sup>i</sup>

<sup>1</sup>PV=Peak Viscosity, MV=Minimum Viscosity, FV=Final Viscosity, PT=Pasting Temperature, TP=Time to Peak, TSB=Total Set Back, BKD=Breakdown;

<sup>2</sup>Units: Viscosity (RVU), Temperature (°C), Time (min).

<sup>3</sup>Different letters within each column indicate values are significantly different at the level of  $p \leq 0.05$ .

<sup>4</sup>Additive percentage was based on the starch dry weight.

pasting properties of white starch isolate, except for the presence of 6% leucine, which increased the BKD.

The charged amino acids significantly depressed the pasting viscosities (MV and FV), but increased the BKD. The neutral amino acids (alanine and leucine), on the other hand, were less effective than the charged ones. Alanine might be able to be used to decrease retrogradation tendency at the 2% level, with little other effects on starch pasting properties depending on the starch source. The influence of amino acids on starch paste viscosity has not been documented. However, other investigators found that addition of proteinase to rice flour could reduce the paste viscosity over the temperature range, and linked this phenomenon to the influence of protein hydrolyzed product on starch pasting (Hamaker and Griffin 1990). The results from our study partially supported the hypothesis of Hamaker and Griffin (1990), that amino acids may reduce the paste viscosity of starch.

### Effects of lipids and protein removal on XRD pattern of rice flours

During starch gelatinization, the double helices of amylopectin can be destroyed, whereas part of the free lipids present in cereal starches can form a helical inclusion complex with the amylose molecules (Hoover and Hadziyev 1981, Zobel 1988b). This results in the V-type X-ray diffraction pattern, which was characterized by peaks around  $2\theta$  of 4.4, 6.8, and 12 Å. Gelatinized white flour showed a strong peak at 4.4 Å and a weak peak at 6.8 Å, confirming a V-pattern crystallite structure formation between the amylose and the lipids present in white flour (Figure 3a). Lipid-removal led to an expected decrease in intensity of the 4.4 Å peak and the destruction of the 6.8 Å peak. Another peak formed at 5.2 Å, indicating that an A-type pattern characteristic of a raw starch was forming (Zobel 1988b), and the relative crystallinity (RC) was reduced by 8% in comparison to the flour before defatting. The removal of protein from defatted flour caused almost total loss of the 4.4 Å peak and increased development of the 5.2 Å peak. The RC of the isolate was reduced by 11% when compared to the white flour control.

The gelatinized brown flour showed a strong peak at 4.4 Å and a weak peak at 5.2 Å (Figure 3b). However, defatting of brown flour did not affect the 4.4 Å peak, but induced the further development of the 5.2 Å peak. The RC was slightly increased (3%) compared to the control brown flour. After protein removal, the peak at 5.2 Å was slightly weakened, whereas the 4.4 Å peak remained and the RC increased another 3%.

### Amino acids effects on commercial starch and white starch isolate XRD pattern

A relationship was established between crystallinity of starches measured by XRD their resistance to enzymatic digestion and these starches were shown to have fiber-like properties in the body (Botham and others 1995, 1997; Hoebler and others 1999). Therefore, XRD can be used to provide an indication of the resistant starch quality of starches. In raw starch, A-type X-ray diffraction patterns are characterized by clear diffraction peaks around  $2\theta$  of 3.8, 5.2, and 5.8 Å and the A-pattern was generally regarded as cereal starch crystal form (Zobel 1988a,b). Gelatinization can destroy the crystalline pattern of starch and enhance V-type pattern (Eliasson and Krog 1985, Zobel 1988b). In this study, gelatinization resulted in a 47% reduction of the RC (Figure 4a) compared to raw starch. The X-ray pattern developed a peak at 4.4 Angstroms similar to that found by Hibi and others (1990).

The presence of 6% aspartic acid did not affect the RC in commercial starch, but increased the 4.4 Å peak intensity and induced the development of 2 new peaks at 3.7 and 3.4 Å (Figure 4a). Addition of 6% arginine resulted in a 9% RC increase (compared to the gelatinized commercial starch) while the X-ray pattern was not changed. Both glutamic acid and lysine enhanced the 4.4 Å peak and caused the RC to increase by 81% and 79% respectively, compared to the gelatinized commercial starch, which potentially enhanced the resistant starch and nutritional fiber-like quality characteristics of the starch (Botham and others 1995, 1997; Hoebler and others 1999).

Addition of amino acids to white starch isolate also enhanced the potential for resistant starch quality. The presence of 6% aspartic acid in the white starch isolate increased the RC by 34%, enhanced the 4.4 Å peak, and induced the development of another peak at 3.4 Å (Figure 4b). Addition of 6% glutamic acid resulted in a 9% RC increase, while the 5.2 Å peak was enhanced, and with no change in the 4.4 Å peak. Similarly, addition of arginine and lysine did not affect the 4.4 Å peak and increased the RC by 30%, while enhancing the peak at 5.2 Å greatly.

### Conclusion

THIS STUDY SHOWED THAT INDIVIDUAL AMINO ACIDS HAVE DIFFERENT pasting property influences on rice flour starch isolate. The effect of amino acids on starch pasting property might be related to their amphiphathic characteristics and influenced by the charges that those amino acids carried. Charged amino acids usually

**Table 4—Effect of Amino Acids on Pasting Properties of White Starch Isolate** <sup>1,2,3</sup>

Sample	Additives <sup>4</sup> (%)	PV	MV	FV	PT	TP	TSB	BKD
Control	—	197.75 <sup>c</sup>	119.46 <sup>abc</sup>	183.63 <sup>ab</sup>	77.25 <sup>bc</sup>	4.87 <sup>ab</sup>	64.17 <sup>bc</sup>	78.29 <sup>h</sup>
Aspartic	2	209.13 <sup>bc</sup>	103.04 <sup>fg</sup>	153.13 <sup>d</sup>	78.05 <sup>abc</sup>	4.97 <sup>a</sup>	50.08 <sup>ef</sup>	106.08 <sup>cd</sup>
	6	211.25 <sup>bc</sup>	93.13 <sup>h</sup>	138.00 <sup>e</sup>	78.23 <sup>abc</sup>	4.86 <sup>ab</sup>	44.88 <sup>f</sup>	118.13 <sup>ab</sup>
Glutamic	2	203.54 <sup>c</sup>	113.00 <sup>cde</sup>	168.42 <sup>c</sup>	78.20 <sup>abc</sup>	4.99 <sup>a</sup>	55.42 <sup>d</sup>	90.54 <sup>efg</sup>
	6	203.42 <sup>c</sup>	106.38 <sup>efg</sup>	156.88 <sup>d</sup>	78.18 <sup>abc</sup>	4.97 <sup>a</sup>	50.50 <sup>de</sup>	97.04 <sup>de</sup>
Lysine	2	206.67 <sup>bc</sup>	105.92 <sup>efg</sup>	174.00 <sup>bc</sup>	78.45 <sup>ab</sup>	4.81 <sup>ab</sup>	68.08 <sup>abc</sup>	100.75 <sup>de</sup>
	6	207.33 <sup>bc</sup>	113.75 <sup>cde</sup>	177.04 <sup>bc</sup>	78.88 <sup>a</sup>	4.85 <sup>ab</sup>	63.29 <sup>c</sup>	93.58 <sup>ef</sup>
Arginine	2	218.54 <sup>ab</sup>	106.04 <sup>efg</sup>	176.79 <sup>bc</sup>	78.60 <sup>a</sup>	4.76 <sup>b</sup>	70.75 <sup>a</sup>	112.50 <sup>bc</sup>
	6	228.08 <sup>a</sup>	99.88 <sup>gh</sup>	169.13 <sup>c</sup>	79.00 <sup>a</sup>	4.74 <sup>b</sup>	69.25 <sup>ab</sup>	128.21 <sup>a</sup>
Leucine	2	208.54 <sup>bc</sup>	115.71 <sup>bcd</sup>	183.63 <sup>ab</sup>	77.33 <sup>bc</sup>	4.81 <sup>ab</sup>	67.92 <sup>abc</sup>	92.83 <sup>ef</sup>
	6	204.33 <sup>c</sup>	110.75 <sup>def</sup>	176.71 <sup>bc</sup>	77.03 <sup>c</sup>	4.81 <sup>ab</sup>	65.96 <sup>abc</sup>	93.58 <sup>ef</sup>
Alanine	2	206.80 <sup>bc</sup>	122.97 <sup>ab</sup>	190.00 <sup>a</sup>	77.19 <sup>c</sup>	4.92 <sup>ab</sup>	67.03 <sup>abc</sup>	83.83 <sup>gh</sup>
	6	204.58 <sup>bc</sup>	124.25 <sup>a</sup>	189.88 <sup>a</sup>	77.09 <sup>c</sup>	4.92 <sup>ab</sup>	65.63 <sup>abc</sup>	80.33 <sup>gh</sup>

<sup>1</sup>PV=Peak Viscosity, MV=Minimum Viscosity, FV=Final Viscosity, PT=Pasting Temperature, TP=Time to Peak, TSB=Total Set Back, BKD=Breakdown;

<sup>2</sup>Units: Viscosity (RVU), Temperature (°C), Time (min).

<sup>3</sup>Different letters within each column indicate values are significantly different at the level of  $p \leq 0.05$ .

<sup>4</sup>Additive percentage was based on the starch dry weight.

showed stronger influence on starch pasting than the neutral amino acids. Addition of individual amino acids may be used to influence the functionality of starch-based products, such as swelling, paste stability, and retrogradation.

The gelatinization process destroyed the double helix structure of starch granules, resulting in an amorphous starch. Defatting and deproteination resulted in loss of the V-pattern, while addition of amino acids to commercial rice starch enhanced the V-pattern. These results indicated that the 4.4 Å peak might be related to the content of protein or amino acids residues in the starch, not

just the lipid content. The 5.2 Å peak was enhanced in the starch isolate by the addition of amino acids indicating that amino acids have an influence on the A-pattern of raw starch. The increase in relative crystallinity of the starch by the addition of amino acids potentially enhanced the resistant starch characteristics and fiber-like properties. The results of this study provided new information on amino acid–starch interaction in rice flour, which may be the basis for starch modification and new functional foods ingredient development.

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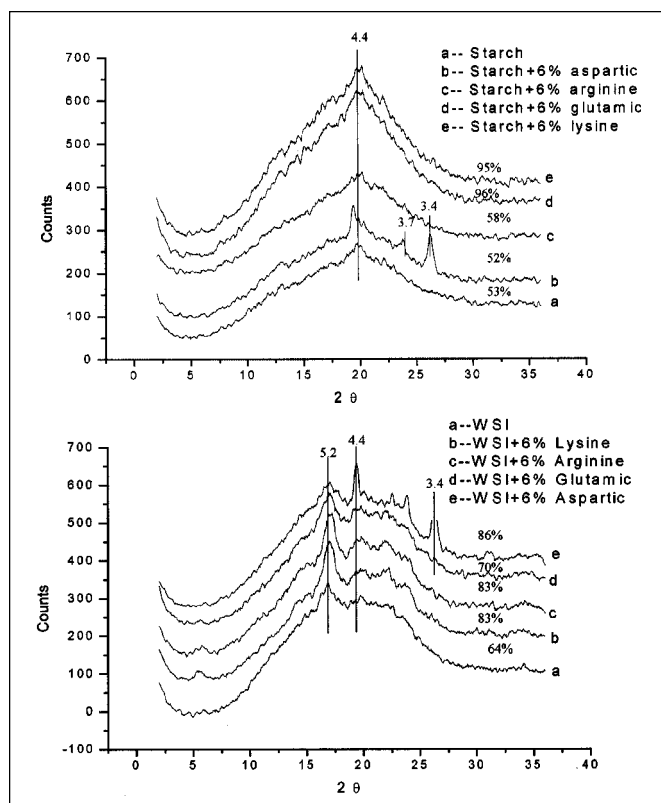
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**Figure 4—Influence of amino acids on XRD pattern of (a) commercial starch and (b) white starch isolate (WSI) (all samples were gelatinized and stored in the refrigerator for 3 d)**

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