

## Influence of Lipid Extraction Process on the Rheological Characteristics, Swelling Power, and Granule Size of Rice Starches in Excess Water

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The influence of the lipid extraction process on both macroscopic and microscopic characteristics of nonwaxy rice starch gelatinization in excess water was examined. Surface lipids extraction did not change the thermodynamics of starch gelatinization but lead to a significant reduction (33%) in the enthalpy of starch–lipid complex melting at high temperature, resulting in less viscous dispersions. Internal lipid extraction using hot aqueous alcoholic solutions resulted in an irreversible increase in starch granule diameter (50% increase in D[4,3]) and a dramatic change in cooking characteristics of the starch. Instead of the bimodal swelling observed for native nonwaxy rice starch, only one broad transition in swelling, solubility, granule size, and viscosity was observed in the case of the totally defatted starch. While the total removal of lipids resulted in a slight increase in starch swelling at intermediate temperatures, the harshness of the process caused irreparable changes leading to notably lower swelling at high temperatures.

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**KEYWORDS:** Starch; defatting; gelatinization; rheology; swelling; granule size

### INTRODUCTION

Starch is a major component of many foods. Use of starch in the formulation of different food products, for example, as a thickener in sauces, soups, custards, mayonnaise, and beverages, depends to a large extent on the behavior of starch during gelatinization (1). Gelatinization results in irreversible swelling of granules, leaching of amylose and amylopectin, and interaction between the leached components, all of which induce an increase in viscosity (2). In recent years studies have focused on the impact of minor components of starch-like proteins (3) and lipids on starch gelatinization.

Starch lipids are predominantly monoacyl lipids with the major components being lysophospholipids and free fatty acids (4). Morrison has classified lipids into two groups based on their location on the starch granule and therefore on the processes used for their isolation (5). Starch surface lipids can be successfully extracted with low-polarity solvents such as chloroform and ether (4), while lipids inside native starch granules, called internal starch lipids, are best extracted with hot aqueous solutions of high-polarity solvents such as dioxane and ethanol as it seems both heat and water are needed to partially swell the starch granule to allow the alcohol to penetrate and extract the lipids.

In the case of rice starch, Vasanthan and Hoover (6) found that most internal starch lipids were either lysophospholipids (56%) or free fatty acids (20%) while the composition of surface

lipids was more complex and included a significant proportion of glycolipids (20%). The fatty acid composition of both lipid classes was found to be essentially the same and equally divided between palmitic (~40%) and linoleic (~50%) acids.

The influence of these lipids, either surface or internal, on the gelatinization and pasting behavior of starch is thought to be due largely to a complex formation between the fatty acids and amylose (4), but the influence of defatting on the starch properties appears quite patchy and contradictory. For example, Radosta et al. (7) found that extraction of only the surface lipids from rye starch had no effect on the swelling power, while Tester and Morrison (8) reported that extraction of surface lipids from wheat starch increased the swelling factor by 30% but had no effect on the gelatinization temperature as determined by DSC. Removal of surface lipids from oat starch did not change the swelling power to any significant extent but resulted in a substantial increase in solubility (9).

In the case of internal lipids Radosta et al. (7) found that total removal of rye starch lipids resulted in a higher temperature of gelatinization with a halving of the gelatinization enthalpy and a marked increase in solubility. These authors also reported an unexpected but small decrease in swelling power attributed to the hardening of starch granules owing to the influence of the high temperature during lipid extraction. In the case of oat starches (9), removal of internal lipids resulted in a huge increase in solubility (by a 100% or more depending on oat variety). The swelling power was only affected by this process at high temperatures (95 °C) and decreased by a comparatively small (20%) amount. In the case of potato and lentil starches,

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**Table 1.** Thermodynamic Properties of the Rice Starches

starch	gelatinization			starch–lipid complex		
	$T_o$ (°C)	$T_p$ (°C)	$\Delta H$ (J/g db)	$T_o$ (°C)	$T_p$ (°C)	$\Delta H$ (J/g db)
waxy	58.9 ± 0.7	67.1 ± 0.1	15.7 ± 0.6	ND	ND	ND
nonwaxy	55.1 ± 0.8	64.2 ± 0.2	12.8 ± 0.1	94 ± 4	101 ± 2	1.15 ± 0.09
surface defatted	55.1 ± 0.4	64.4 ± 0.4	12.4 ± 0.2	96 ± 1	101.6 ± 0.3	0.76 ± 0.04
totally defatted	55.9 ± 0.3	63.7 ± 0.2	10.6 ± 0.4	ND	ND	ND

Vasanthan and Hoover (10) found that removal of internal lipids resulted in a substantial decrease in both swelling power and amylose leaching, while this process did not affect the swelling features of the cereal starches studied (corn and wheat) much. Also, these authors found that the gelatinization temperature and relative crystallinity of both lentil and potato starch increased upon complete defatting, while no significant change was observed for the cereal starches. These authors attribute the changes to an increased order in lentil and potato defatted starch granules. The study showed that the botanical source of the starch is extremely important in determining the effects of both surface and internal lipids on swelling and gelatinization. Despite this, very little is known on the effect of defatting on the swelling and gelatinization properties of rice starches. Hibi (11) showed that internally defatted rice starch had a much higher (~86%) swelling power than nondefatted starch at 85 °C and that refatting with palmitic acid induced a return to the native starch value, even if the amount of emulsifier needed to induce such a return was 2–3 times more than the amount of lipids extracted. The effect of starch lipids on granule swelling and leaching of amylose and amylopectin also has an effect on the starch paste rheology. Hibi (11) found that defatting of rice starch made the starch pastes more elastic but less viscous. This phenomenon was explained in terms of increased amylose solubility and therefore increased gel formation. Biliaderis and Tonogai (12), however, reported that removal of internal starch lipids from rice starches resulted in a substantially increased gel elasticity and a small increase in viscosity. However, these two last studies refer to concentrated starch pastes (above 20% w/w) and are of limited value in predicting the effect of defatting on the flow behavior of starch formulations in excess water. In excess water swelling of the gelatinized starch granules appears to be a dominant variable for predicting the onset of increased viscosity in starch dispersions (13). As starch gelatinization is closely associated with the viscosity of starch dispersions, which in turn is also related to starch granule dimensions, a microscopic analysis of starch granules in relation to starch size and size distribution before and after defatting might provide a better insight to the gelatinization phenomena. The effect of partially and totally extracted lipids from starch granules on granule size and distribution is also important in relation to starch gelatinization but has not been extensively investigated.

The objective of the current study is to combine the results of macroscopic (flow behavior, swelling power, solubility), microscopic (granule size and number), and thermodynamic (DSC endotherm) studies, from which it is hoped a clear picture will emerge as to the influence of the defatting process on the gelatinization properties of rice starch.

## MATERIALS AND METHODS

**Rice Starches.** Waxy and nonwaxy (11% amylose) rice starches were obtained from Remy Industries (Leuven, Belgium).

Nonwaxy rice starch was surface defatted using diethyl ether under reflux for 4 h (14). This process yielded 0.354 mg of lipids extracted per gram of starch (dry basis, db). Removal of all lipids, including

internal starch lipids, was carried out according to Vasanthan et al. (10). Briefly, the nonwaxy starch was refluxed for 8 h with *n*-propanol–water (3:1 v/v). This process yielded a total of 0.546 mg of lipids extracted per gram of starch (db). In both cases the defatted starch was air-dried in an oven at 40 °C for 24 h.

Prior to cooking the starch suspension was homogenized at 8000 rpm for a few minutes using a Silverson High speed mixer (model L4RT equipped with a fine emulsion screen, Silverson Machines Limited, Chesham Bucks, U.K.) in order to ensure the absence of any agglomerates (15).

Cooking from 50 to 100 °C was performed in excess distilled water (maximum 4% w/w) for 30 min under mild stirring (400 rpm) to prevent excessive granule rupture. All tests were done in triplicate.

**Thermodynamics of Starch Gelatinization.** The gelatinization temperature and heat of gelatinization were determined using a differential scanning calorimeter (model DSC 2010, TA Instruments Inc., Newcastle, DE). Approximately 20 mg of 30% (w/w db) raw rice starch suspension was hermetically sealed in aluminum pans (TA Instruments). A hermetically sealed empty pan was used as a reference. The scanning temperature was increased from 10 to 140 °C at a rate of 5 °C min<sup>-1</sup>. The onset temperature ( $T_o$ ), peak gelatinization temperature ( $T_p$ ), and heat of gelatinization ( $\Delta H$ ) were determined in triplicate.

**Swelling Features.** The swelling power and percentage of leached solubles were determined according to Leach et al. (16). After centrifugation (1500 rcf, 15 min) the supernatant was dried in a hot air oven (105 °C for 16 h) to determine the amount of carbohydrates in solution. The solubility was calculated as the amount of carbohydrates in solution on a dry starch basis. The swelling power was calculated as the weight of the sedimented paste per gram of dry-basis starch, the value corrected for solubles to provide a true measure of the swelling of the insoluble starch granules.

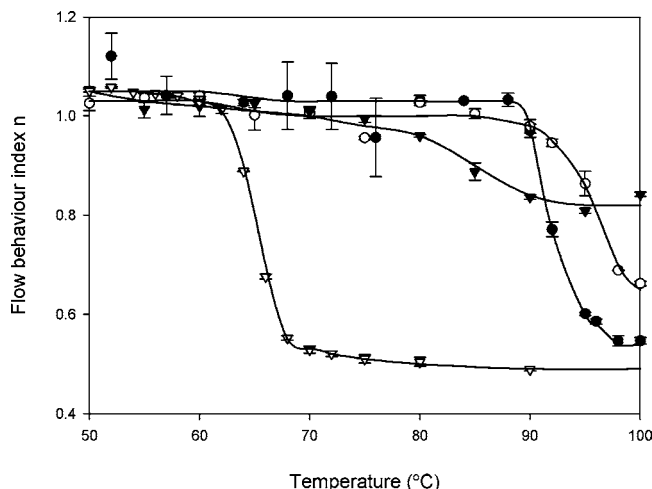
**Granule Size and Number.** The granule size distribution was determined in triplicate using a laser light scattering based particle size analyzer (Malvern Mastersizer S, Malvern Instrument Limited, Malvern, U.K.) fitted with a 300 RF range lens (0.05–900  $\mu$ m) and a small volume sample dispersion unit. The refractive indices of water and starch were taken as 1.330 and 1.5295, respectively, with an absorption of 0.1 for starch particles (17).

The volume-average diameter,  $D[4,3]$ , number-average volume  $V[1,0]$ , and volume percentage were recorded to assess both the granule size and granule number.

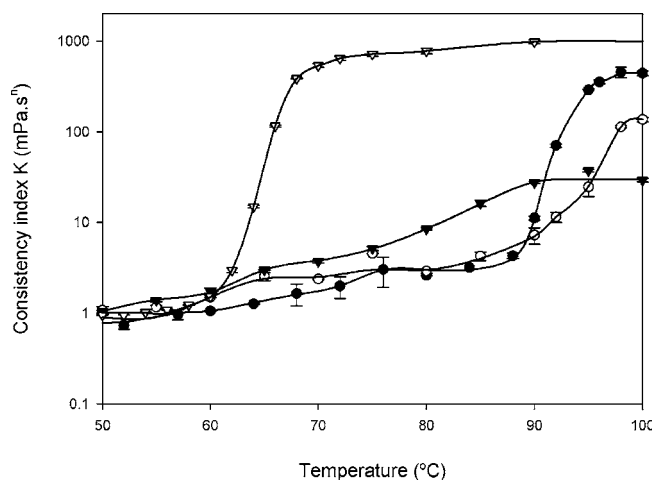
**Flow Behavior.** Steady stress sweeps of cooked starch pastes in excess water (3% w/w) were carried out using a Rheometric Scientific SR 2000 rheometer (Rheometric Scientific Inc., Piscataway, NJ) equipped with a 32 mm Couette geometry at 25 °C. Because of the relatively dilute state of these starch dispersions, no yield stress was noted; therefore, the flow curves were analyzed using the power law model (13). Both the dimensionless flow behavior index ( $n$ ) and the consistency index  $K$  (mPa s<sup>*n*</sup>) were recorded on the descending shear data in the shear range 1000–1 s<sup>-1</sup>.

## RESULTS

**Differential Scanning Calorimetry.** As gelatinization is an endothermic process, differential scanning calorimetry was used to determine the onset and peak temperatures as well as enthalpy for both the gelatinization and starch–lipid melting processes (Table 1). The peak gelatinization temperature for waxy rice starch was 67.1 °C, in line with previously published data (18), while the heat of gelatinization was 15.7 J/g (db). For this starch



**Figure 1.** Flow behavior index of 3% rice starch dispersions (2.65% db) as a function of cooking temperature: (●) native nonwaxy; (○) surface-defatted nonwaxy; (▼) internal-defatted nonwaxy; (▽) waxy. The lines are visual fits only.

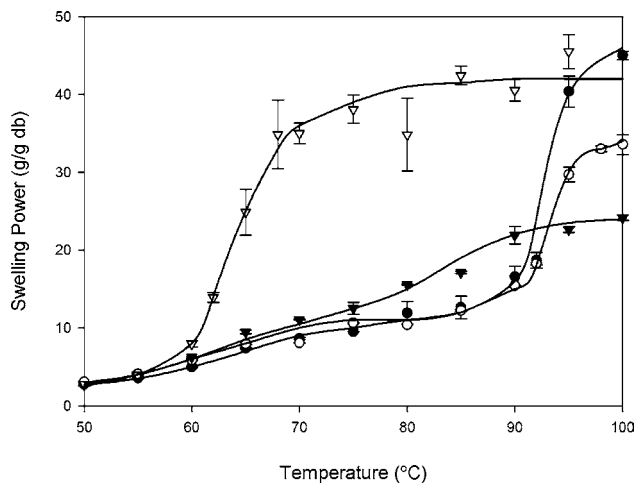


**Figure 2.** Consistency index of 3% rice starch dispersions (2.65% db) as a function of cooking temperature: (●) native nonwaxy; (○) surface-defatted nonwaxy; (▼) internal-defatted nonwaxy; (▽) waxy. The lines are visual fits only.

no melting of the starch–lipid complex was observed, probably due to the insignificant amount of lipids present. In the case of nonwaxy rice starch the peak temperature (64.2 °C) and heat of gelatinization (12.8 J/g db) were quite similar to the waxy starch values. A second endotherm was observed at 101 °C with a small enthalpy of 1.15 J/g db, most likely corresponding to melting of the starch–lipid complex (18). Surface defatting did not change significantly the gelatinization endotherm of nonwaxy starch, but the starch–lipid melting endotherm was significantly less energetic (0.76 J/g db)

Internal defatting resulted in a noteworthy loss in gelatinization enthalpy (10.6 J/g db) but did not affect the peak gelatinization temperature. This energy loss has been previously reported (10) and can be attributed to a substantial loss of crystallinity due to the combined use of heat and aqueous solvent during the defatting process.

**Flow Behavior.** Figure 1 represents the changes in flow behavior index and Figure 2 the changes in consistency index of the different starch pastes cooked in excess water as a function of temperature. For the waxy rice starch a single steep increase in the consistency index of the starch dispersion was seen from 60 to 68 °C, corresponding to the onset of shear thinning. This



**Figure 3.** Swelling power of rice starches as a function of cooking temperature: (●) native nonwaxy; (○) surface-defatted nonwaxy; (▼) internal-defatted nonwaxy; (▽) waxy. The lines are visual fits only.

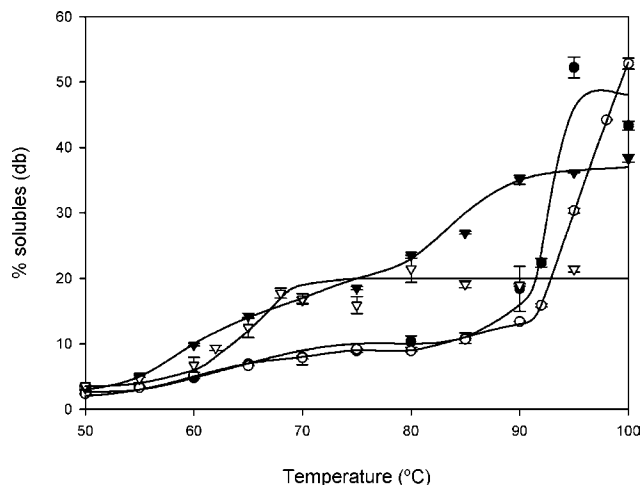
one-step increase in paste viscosity corresponds well to gelatinization of the starch as determined by DSC and is typical of waxy starches. In the case of nonwaxy rice starch a marginal increase in consistency was observed from 55 to 65 °C, while the flow behavior remained Newtonian ( $n \approx 1$ ). At temperatures above 88 °C there is a steep rise in consistency of the nonwaxy rice starch up to 95 °C, where maximum viscosity is achieved. This second transition corresponds to the onset of shear thinning, as seen by a steep decrease in the flow behavior index, and most likely corresponds to the melting of the starch–lipid complex. Surface-defatted nonwaxy rice starch had a similar flow behavior profile to the nonwaxy rice starch at intermediate temperatures (below 85 °C), but higher temperatures were needed to effect the second transition from Newtonian to pseudo-plastic behavior, and it seems that the maximum viscosity was achieved at 100 °C. Furthermore, this maximum viscosity was significantly lower than that achieved by the nondefatted starch. For internal-defatted nonwaxy rice starch the increase in consistency index was observed from 60 to 90 °C. This broad transition was repeated in the flow behavior index, and no second sharp transition was observed.

From the above observations it can be assumed that differences in the flow behavior profiles of the starch samples during gelatinization might have been partly caused by differences in the swelling properties and solubility.

Thus, an attempt was made to study the differences observed in terms of viscosity profiles for the different starches in terms of macroscopic (swelling factors) and microscopic (granule size distribution) analysis.

**Swelling Factors.** Starch swelling factors included measuring the swelling power (Figure 3) of starch and the amount of solubles (Figure 4) leached from the starch. The swelling power of waxy rice starch increased dramatically from 55 °C to a maximum value of around 40 g/g db at 70 °C and appears to remain relatively constant at temperatures above 75 °C. Tester and Morrison (8) reported similar values for waxy rice starch. Similarly, the amount of solubles leached from the waxy starch dispersions (Figure 4) increased from 55 °C to reach a plateau at 70 °C. Not surprisingly, for a waxy rice starch, the maximum solubility attained is quite small ( $\approx 20\%$ ).

In the case of the nonwaxy rice starch two phase transitions were once again seen at 65 and 95 °C. As the starch was cooked from 55 °C a gradual increase in swelling power is seen (Figure 3). At 75 °C the swelling power is  $\approx 10$  g/g db. This value



**Figure 4.** Solubility of rice starches as a function of cooking temperature: (●) native nonwaxy; (○) surface-defatted nonwaxy; (▼) internal-defatted nonwaxy; (▽) waxy. The lines are visual fits only.

remains constant, forming a plateau to 85 °C. However, as the cooking temperature was increased beyond 85 °C, the swelling power increased dramatically, forming a steep curve which appears to plateau once again at a maximum swelling power of 45 g/g db.

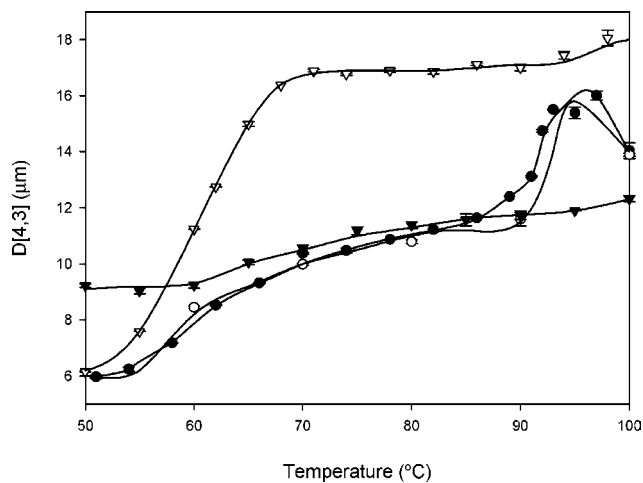
The swelling features (both swelling power and solubility) of surface-defatted rice starch were very similar to those of the native nonwaxy starch, showing a first transition at 65 °C followed by a second transition at 95 °C, this second transition happening at temperatures somewhat higher than for the native nonwaxy starch and reaching a lower value of maximum swelling power, but overall, the swelling behaviors of these two starches were very similar.

The internal-defatted rice starch exhibited the lowest swelling power as compared to the other starch samples at high temperatures. Unlike the surface-defatted rice starch and the native nonwaxy rice starch there was only one swelling transition as the swelling power increased gradually with increase in cooking temperature. Both swelling power and solubility started to increase significantly from 60 °C to reach maximum values at 90 °C. However, both these maxima were significantly lower than the corresponding values of the native and surface-defatted nonwaxy starch, indicating that this harsh defatting process has significantly altered the starch granules. Therefore, a microscopic analysis of the starch granules might provide a better understanding of the macroscopic changes observed during gelatinization in the starch samples.

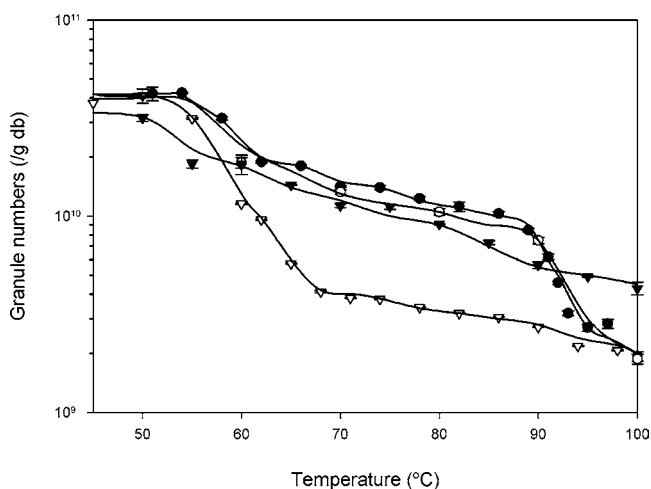
**Granule Size Distribution.** Particle size analysis of starch samples involved a study of the changes in granule size (as quantified using the D[4,3], sometimes referred to as the volume or mass moment mean or De Brouckere mean diameter) and granule numbers (Figures 5 and 6).

The D[4,3] value of uncooked waxy rice starch was  $\pm 6 \mu\text{m}$ . As cooking temperature increased, the diameter of the starch granules also increased, reaching a maximum of  $17 \mu\text{m}$  at 67 °C. Beyond this temperature no significant change was observed in granule size. This granule swelling was concomitant with a dramatic decline in granule numbers (Figure 6), indicating rupture of the starch granules upon swelling. Above 67 °C only  $\pm 10\%$  of the granules was still intact.

In the case of the native nonwaxy rice starch, once again, a two-step phenomenon was seen in the granule diameter with cooking temperature. From 55 to 65 °C a relatively small increase in granule diameter from 6 to  $10 \mu\text{m}$  was observed,



**Figure 5.** Volume-average granule diameter of rice starches as a function of cooking temperature: (●) native nonwaxy; (○) surface-defatted nonwaxy; (▼) internal-defatted nonwaxy; (▽) waxy. The lines are visual fits only.



**Figure 6.** Granule numbers of rice starches as a function of cooking temperature: (●) native nonwaxy; (○) surface-defatted nonwaxy; (▼) internal-defatted nonwaxy; (▽) waxy. The lines are visual fits only.

with a concomitant large decrease in granule numbers. Until 90 °C the granule size effectively leveled off and a significant proportion of granules was still intact. Upon further heating above 90 °C the granules reached a maximum diameter of  $16 \mu\text{m}$  along with a second concomitant loss in granule numbers. At very high temperatures the volume-average granule diameter seems to decrease, but the concentration of granules at these temperatures is so low (less than 5% intact granules) that the volume distribution might become biased toward smaller granules (13).

When compared to the native nonwaxy starch, surface defatting did not significantly affect either the starch granule size (Figure 5) or the number of granules (Figure 6) during cooking. In the case of internal defatted starch, however, major changes occurred. The granule diameter of the uncooked internal defatted starch was appreciably larger than its native counterpart ( $\approx 9 \mu\text{m}$ ), corresponding to nearly four times its original volume. Upon cooking from 50 to 100 °C only one broad continuous but small increase in size was observed, as the maximum diameter observed at 100 °C was still appreciably lower than the native nonwaxy diameter at the same temperature (12 and  $14 \mu\text{m}$ , respectively). The granule numbers drop at 58 °C, but no second drop in granule numbers was observed at higher



temperatures, just a steady decline across the temperature range 50–100 °C. Thus, 20% of intact defatted granules remained at 100 °C compared to only 5% for the native nonwaxy starch.

## DISCUSSION

**Waxy Rice Starch.** At the transition temperature of 67 °C, corresponding to the gelatinization peak temperature of the DSC endotherm, the waxy rice starch granules swell to their maximum size and start to rupture. This coincides with a macroscopic gel swelling and increase in solubility, together with a peak in the paste viscosity and onset of shear-thinning behavior. These results tend to indicate that, for this starch, complete cooking is obtained at this relatively low temperature and are characteristic of waxy starches.

**Native Nonwaxy Rice Starch.** In the case of the native nonwaxy rice starch the increase in swelling power and solubility at the same gelatinization peak temperature of the DSC endotherm (64 °C) is relatively small and accompanied by a relatively small decrease in granule numbers, indicating partial swelling of the starch granules with slight rupture. Complete cooking, characterized by a large increase in granule size and rupture, together with a large increase in paste viscosity and onset of shear-thinning behavior was only achieved when heat treatment was increased to 95 °C, corresponding to melting of the starch–lipid complex. This bimodal behavior is thought to originate from amylose–lipid complexes preventing granule swelling and rupture at relatively low temperatures. To verify this assumption, this starch has been surface defatted.

**Surface-Defatted Nonwaxy Rice Starch.** The surface defatting of the nonwaxy rice starch resulted in no significant change in granule size in the uncooked state, thereby validating the assumption that no solvent had penetrated the interior of the granule and extracted internal lipids. Even if this process resulted in a substantial removal of lipids (60% of total lipids removed), the surface-defatted starch behaved mainly as its native counterpart. At the same gelatinization peak temperature of 64 °C the granules partially swell and start to rupture, leading to a small increase in swelling power and leaching of solubles, a modest increase in consistency but without loss of Newtonian flow behavior until 90 °C. It is worth noting here that removal of surface lipids did not cause a greater swelling of the starch granules or a substantial decrease in granule numbers compared to the native starch at these intermediate temperatures, thus negating the hypothesis that surface lipid-bound amylose forms an immiscible shell around the starch granule and prevents swelling and leaching of solubles. The only appreciable difference between the surface-defatted nonwaxy starch and its native counterpart was seen at high temperatures (>90 °C), where the second transition corresponding to starch–lipid melting takes place. This second transition leads to the near complete rupture of starch granules, maximum swelling to the same granule diameter as for the native starch, and similar values of solubility. The only appreciable differences reside in a noticeably lower swelling power and viscosity and higher flow behavior index after cooking at 100 °C. While one cannot rule out the fact that the second transition is not fully effected at this temperature in the case of the surface-defatted starch, it seems more plausible that removal of surface lipids is responsible for such behavior. In fact, after cooking at these high temperatures all lipids are leached into the solution. Upon cooling, the lack of solubility of these lipids will reinforce their ability to form complexes with leached amylose and thus increase the gellike structure of the paste. Removing the surface-bound lipids, even if not responsible for any significant change in the starch gelatinization

process, will result in a substantial amount of lipids being removed from the cooked paste, therefore leading to a less structured and less viscous solution once cooled. This assumption is in line with several studies showing the structure building of starch pastes due to added insoluble emulsifiers. (11).

**Internal-Defatted Nonwaxy Rice Starch.** In the case of totally defatted starch the gelatinization behavior is drastically changed. Soxhlet reflux of nonwaxy starch with propanol/water allows for near-complete extraction of the starch-bound lipids due to partial swelling of the starch granules during the extraction process. Incidentally, this partial swelling is irreversible, as noted by the increase in raw granule diameter (from 6 to 9  $\mu\text{m}$ ) and the decrease in gelatinization enthalpy ( $\approx 10\%$ ) due to a substantial decrease in granule crystallinity. Upon heat treatment the bimodal behavior of both native and surface-defatted nonwaxy starches is lost and a broad transition is seen above 60 °C. However, this transition is considerably wider than that for the two other starch samples, spanning some 30 °C as indicated by the slow rise in macroscopic swelling and solubility, slow rise in granule size and rupture, and slow increase in paste viscosity with temperature. It is noteworthy to mention here that the granule diameter of internal-defatted starch never reaches the values of its native equivalent and that swelling power, solubility, and viscosity achieved at high temperatures are also significantly lower. It is difficult to interpret such a radical change in cooking behavior other than to note that the harsh conditions necessary for complete defatting are responsible for a substantial irreversible swelling of the starch granules and possibly a structural rearrangement of amylose and amylopectin. This release of amylose from amylopectin clusters toward the amorphous regions of the granule and the re-ordering of amylopectin clusters into close-packed arrangements as seen by Vasanthan and Hoover (10) in the case of potato and lentil starches, leading to a significant increase in crystallinity, is a plausible explanation. Nevertheless, contrary to these authors' findings, no sizable increase in peak gelatinization temperature was observed in the case of defatted rice starch, which runs contrary to the assumption of increased crystallinity.

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