DOI 10.1002/star.200400279 *Starch/Stärke* **57** (2005) 134–144

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Comparative Study of Pilot Scale Rice Starch Production by an Alkaline and an Enzymatic Process

Rice starch is commonly isolated by alkaline (sodium hydroxide) extraction because this process provides high yield, high purity and is low in capital costs. This process produces an highly loaded alkaline effluent that contributes significantly to general costs of wastewater treatment. The present study attempted to develop an enzymatic procedure to isolate pure rice starch and to investigate the physico-chemical properties in comparison with that of rice starch produced by an alkaline process of comparable scale. The isolation of starch from polished rice grain was effected by application of cellulase under slightly acidic conditions in order to degrade the cellular tissue, followed by protease (Corolase 7089 or papain) under neutral conditions in order to loosen the protein bodies that are associated with starch granules. In comparison with the alkaline process, the enzyme process provided rice starch with a slightly elevated protein content, but less damaged starch. No differences were found between the two proteases used. Washing the enzyme-isolated starch with 0.2% sodium hydroxide or 0.5% sodium dodecylsulphate (SDS) solutions further improved the purity of rice starch. The physico-chemical properties of the enzymatically-isolated starches were mostly comparable with starch from the alkaline process. The developed process allows to replace the alkaline process and thus eliminates critical levels of mineral load in effluents of rice starch plants.

Keywords: Rice starch; Isolation; Protease; Protein content; Functional properties

1 Introduction

Rice starch is used as an additive in various food and industrial products. It consists of tiny granules ($< 5\ \mu m$) with a narrow size distribution. The starch is white in colour and has a neutral odour, which makes it ideally suited as a cosmetic dusting powder or a textile-stiffening agent. In addition, rice starch causes minimal allergic reactions. Furthermore, it is used in dessert and bakery products and as a fat mimetic in food, such as flavoured milk based beverages, ice cream, yoghurt, and salad dressings as well as non-dairy ice cream products [1–3]. High purity rice starch with low surface protein/lipid impurities is desired to minimize rancidity during storage and for use as a starting material in chemical modification, fermentation, or in diverse industrial applications [4, 5].

Rice starch production is limited because of its relatively high product cost, which is connected with long-term soaking and intensive washing procedures needed to remove residual sodium chloride. Rice protein in the endosperm, which accounts for 7–8% (dry substance, d.s.), is tightly associated to the surface of the starch granules and difficult to remove [6, 7]. Besides the problems associated with rice protein impurities, the tiny granules of rice starch sediment slowly in water. The retarded sedimentation results also in losses during separation and purification operations. For the indicated reasons the purification of rice starch is more costly compared with other starches. In particular, the alkaline steeping step generates large quantities of alkali and salt resulting in high costs for wastewater treatment.

To isolate rice starch, alkaline solutions, detergents or proteolytic enzymes are commonly used to remove protein from ground rice endosperm or rice flour [4, 8–11]. Alkaline solutions were commonly preferred for isolation of rice starch with good recovery and low residual protein content, because high portions of the rice protein consist of alkali-soluble, high molecular weight glutelins [6, 8,12–14]. In general, starch isolated by the alkaline steeping method with approximately 0.03–0.05 M NaOH solution

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Publication No. 7402 of the Federal Research Centre for Nutrition and Food, Location Detmold and Münster.

(0.14–0.2%, w/v) achieved 73–85% yield of starch (d. s.) having 0.07–0.42% residual protein and 0.07–2.6% damaged starch [8, 9]. Anionic detergents also removed protein and fibre from ground rice endosperm, but those treatments would cause an effluent problem, too, and probably also reduce starch paste consistency. Alkaline treatments damage in any way the fine structure of the starch to be isolated [11]. A protease digestion has also been employed to isolate starches from different sources, for instance legume starches [15]. *Lumdubwong* and *Seib* [9] applied a food-grade alkaline protease to isolate rice starch on a laboratory scale from wet-milled rice flour. The procedure resulted in a starch yield of 95% (d.s. base) with 0.52% residual protein content. The content of damaged starch reached a level of 2.1%. However, the protease digestion was still performed under alkaline conditions (pH 10) and a long-term digestion period of 12 h. The sodium hydroxide and sodium salts would still result in factory effluents requiring further treatment. *Wang* and *Wang* [10] studied the use of acidic, neutral and alkaline proteases at neutral conditions within a period of 18 h to isolate rice starch. The applied procedure resulted in high-purity rice starch with a residual protein content of 0.07–0.33% (d.s.), but still required a long process time. *Chiou* et al. [11], in contrast, applied a short-term protease treatment (15 min) for protein extraction at neutral pH and compared the isolated rice starch with a precipitate recovered from an ammonia treatment (0.2 M) and starch extracted by means of a laundry detergent. In addition to the successful protein removal the effect of damaging the starch fine structure by alkali methods was proven. Non-alkaline isolation procedures can be supported obviously by mechanically breaking partially the starch-protein matrix, in particular, by applying a screw-loop reactor [15] or high-pressure homogenisation [16].

The objectives of this study were (i) primarily to isolate rice starch from soaked rice grain by initially treating with cellulase at slightly acidic conditions to enhance cell-wall degradation, followed by two proteases (Corolase 7089 or papain) at neutral condition to reduce the protein content, and (ii) to compare the physico-chemical properties of rice starches extracted by the enzymatic versus the alkali standard method.

2 Materials and Methods

2.1 Materials

Polished long-grain rice (Indica type) of Thailandese origin were kindly donated by Rithmers Reismühle GmbH (Bremen, Germany). Cellulase derived from *Trichoderma longibrachiatum* (EC. 3.4.1.4; Röhm Enzyme GmbH, Darmstadt, Germany) was used for cellular endosperm tissue degradation. The cellulase's optimum activity was specified for a pH of 5.0 and a temperature of 65°C and indicated by 1453 CU/g of enzyme solution. Corolase 7089, a food-grade protease, derived from *Bacillus subtilis* (EC.3.4.24.28; Röhm Enzyme GmbH, Darmstadt, Germany) and organic synthesis grade papain from *Carica papaya* (Cat. No. 108 014, EC. 3.4.22.2; Roche Diagnostics GmbH, Germany) were used for degradation of the endosperm protein matrix. Both enzymes are described by an endo-protease activity reacting at pH optimum conditions of a range of 6.0–7.0 and an optimum temperature of 55–60°C. The protease activity was described as being 840 U_{Hb}/g (pH 7.0) for Corolase 7089 and 30 U/mg (pH 7.0) for papain.

2.2 Methods

2.2.1 Proximate analysis

Quantitative determinations of moisture, lipid and mineral content were performed in triplicate by applying standard procedures described by AOAC [17,18]. The protein content was measured in duplicate by applying the Dumas combustion principle. For calculation of crude protein a factor of 5.95 was used [19]. The total starch content was determined in triplicate polarimetrically [20] and the starch damage was determined in triplicate by enzyme digestion using commercial test kits described in the Megazyme test procedure (Megazyme International Ireland Ltd., Wicklow, Ireland) [21].

2.2.2 Rice starch isolation by the alkaline process

Substantial steps of the procedure of alkaline isolation are presented in Fig. 1. Polished rice (8.0 kg) was steeped under gentle stirring in the initial phase in 0.1 M (0.4%, w/w) aqueous sodium hydroxide solution (NaOH) in the ratio of 1:2 for 18 h at 5°C followed by wet milling of the grains sucked from the steeping liquor. To obtain the disintegrated rice slurry a colloid mill with a 150 μm milling slit (Type MZ 80; Fryma-Maschinenbau GmbH, Rheinfelden, Germany) was used. During milling a steady stream of tap water (approximately four times the rice weight) was admixed to prevent heating and gelatinisation of the starch. The slurry was then separated with a decanter (Type CA 150-01-33; Westfalia Separator Industries GmbH, Oelde, Germany) to recover the solids. The resulting cake was re-suspended in the steeping solution (0.4% NaOH) and kept for 1 h. After steeping the liquor was separated finally by decanting and the cake was re-suspended in water to accomplish further disintegration

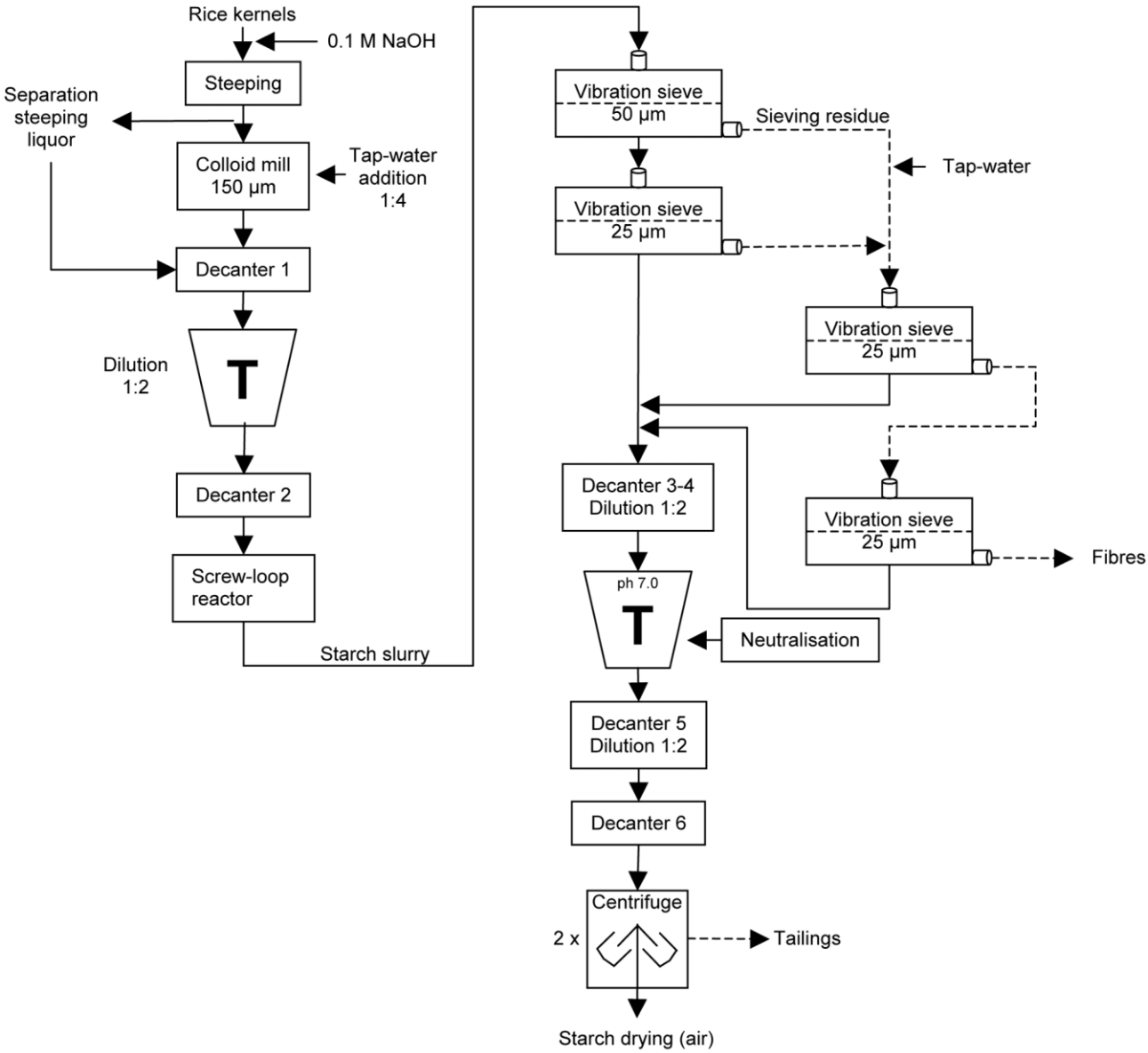

Fig. 1. Rice starch isolation process using alkaline steeping and protein extraction.

while passing through a screw-loop reactor (Type Mischer size 50; EMS Engineering Maintenance Services GmbH & Co. OHG, Buseck, Germany) at 1200 min^{-1} [15]. Following the final disintegration step the homogenised mixture was screened through 50 and 25 µm sieves, respectively, of a horizontal vibration sifter (Type SWECO LS 18S3333; Beck & Partner Verfahrenstechnik GmbH, Hamburg, Germany) to remove fine fibre material. For washing the separated solids two subsequent decanter separations were applied. The cake was then re-suspended in tap water, neutralized with 10% H_2SO_4 and separated by decanting. It was washed additionally one time and decanted again. Finally, the cake was re-suspended in tap water and centrifuged at 685 × *g* for 2 min and 4275 × *g* for 5 min in a laboratory centrifuge with 2800 mL capacity (Type Varifuge®F; Heraeus Sepatech GmbH, Osterrode am Harz, Germany). Following to centrifugation the supernatant was discarded and the dark tailing layer atop the starch carefully scraped away manually. The recovered cake was re-suspended in water and centrifuged again; purification was repeated four times in total. At the very end, the starch was dried at room temperature after spreading the cake over filter paper in thin layer. The separated tailings were discarded.

2.2.3 Isolation of rice starch by enzymatic process

The alternative procedure for isolation by an enzymatic treatment is presented in the flow chart in Fig. 2. Polished rice (8.0 kg) was steeped in tap water in a ratio of 1:2 for 18 h at 5°C followed by one time wet milling in the colloid mill as described in the alkaline procedure to obtain a slurry of ground rice. Because of the tap water addition in milling, the slurry was then concentrated by decanting and the resulting cake re-suspended in the neutral steeping liquor. The slurry was then adjusted to pH 5.0 by

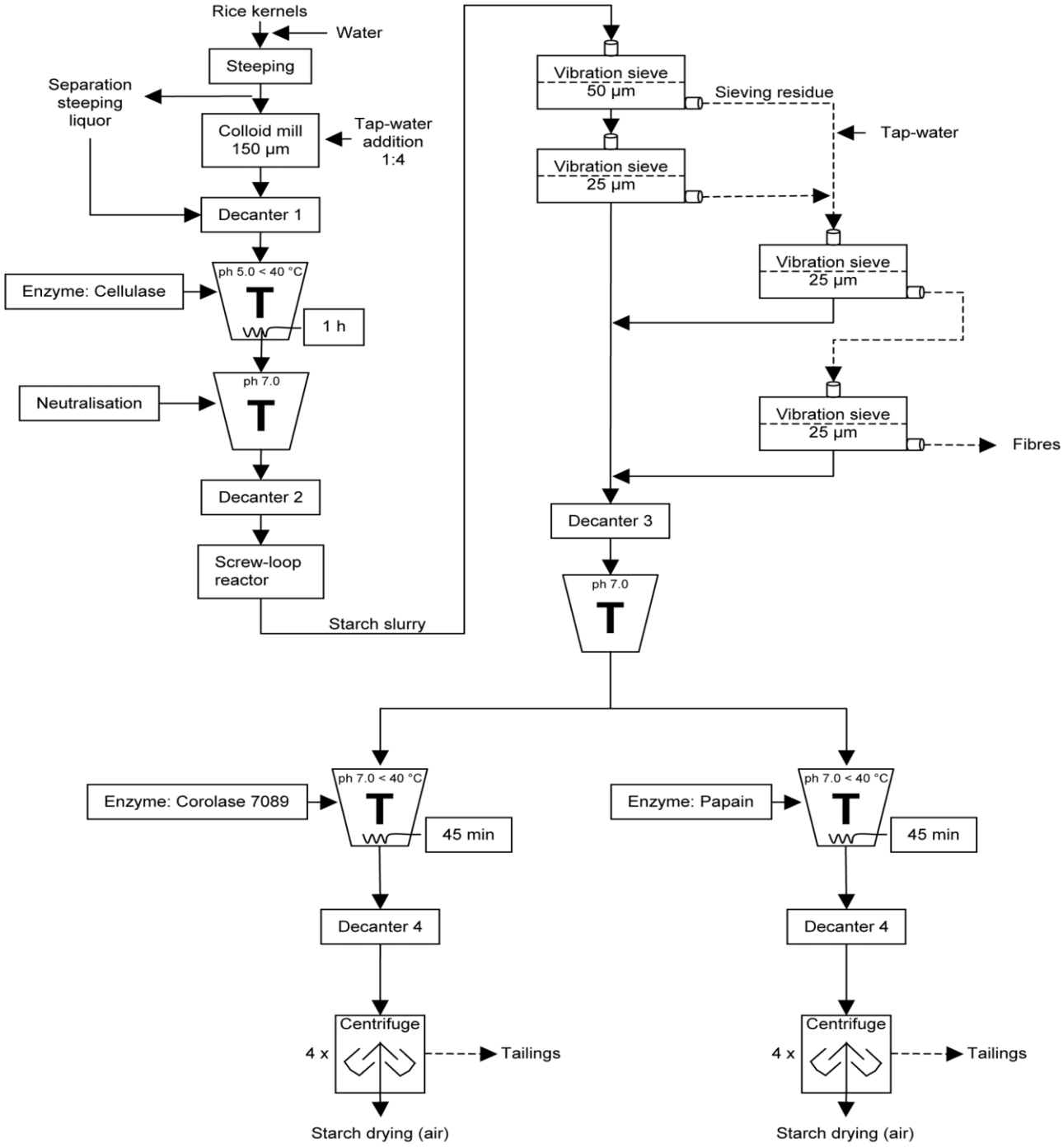

Fig. 2. Rice starch isolation process based on enzymatic treatments for grain disintegration and purification of mill starch followed by manual removal of tailings.

adding H_2SO_4 (10%) and warmed to 40°C. As soon as the temperature was reached, 7.068 g cellulase (0.1%, w/w, based on rice dry substance) were added and the mixture was stirred for 1 h. After cellulolytic degradation the slurry was neutralized with 0.8% (w/v) NaOH, passed through the screw-loop reactor [15] and screened through 50 and 25 μm vibration sieves.

The slurry of rice flour was warmed up to 40°C and was divided into two equal portions. Equivalent amounts (98 units) of Corolase 7089 (0.117 g) and papain (3.262 mg) were added to both portions, which were then stirred for 45 min. For further purification the slurry was separated with a decanter as presented at an earlier stage. The resulting cake was re-suspended in water and centrifuged the same way as described in the alkali process. The supernatant was discarded and the dark tailing layer atop the starch was carefully scraped away. The cake was re-suspended in water and centrifuged again; this operation was repeated three times. Finally, the starch was dried at room temperature. The tailings were discarded.

2.2.4 Purification of enzyme-isolated rice starch by NaOH solution and sodium dodecyl sulphate (SDS) solution

For further reduction of protein residues the rice starch isolated by enzymatic treatment was suspended in 0.2% (w/v) aqueous NaOH or in a 0.5% (w/v) solution of SDS and stirred at room temperature for 30 min. The slurry was centrifuged and the resulting new tailing layer on top was scraped off. The rice starch layer was finally washed three times with distilled water and dried at room temperature.

2.2.5 Amylose content determination

The amylose content in rice starch was determined following the iodine affinity method [22].

2.2.6 Swelling power and solubility

Swelling power and solubility of isolated starch samples were measured in five repetitions by applying the procedures described by *Schoch* [23]. A sample of dried starch (1 g) was accurately weighed and mixed in a centrifuge tube with 30 mL of distilled water taking into consideration the moisture content of the starch. The suspension was then stirred at approximately 200 min^{-1} in a water bath at increasing temperatures from 60 to 90°C and at intervals of 5°C within 30 min. The tubes were centrifuged at 1000 × *g* and 25°C for 15 min to separate paste sediment and supernatant. Tube and paste were weighed. The supernatant was then transferred into a moisture can, evaporated to dryness in an hot air oven at 130°C, cooled in a desiccator and weighed.

2.2.7 Pasting viscosity of starch by RVA

The pasting properties of the isolated starch samples were determined in duplicate by a Rapid Visco Analyser (Series 4V, New Port Scientific Pty. Ltd., Warriewood, Australia) at a constant rotation rate of the applied paddles of 160 min^{-1}. Starch (2.5 g d.s.) and 25 mL of distilled water were mixed and heated to 50°C (initial temperature); the slurry temperature was held for 2 min and then further heated to 95°C at a rate of 7.5°C/min. The hot paste was held at 95°C for 2 min and then cooled to the initial temperature at the rate indicated previously. Following cooling set back was observed by stirring another 4 min at 50°C.

2.2.8 Thermal properties

Thermal properties of starch samples were analysed by using a Differential Scanning Calorimeter (Type 444, Netzsch-Gerätebau GmbH, Selb, Germany). Approximately 18 mg of the starch sample were mixed in a stainless steel crucible with approximately 80 mg water and sealed. The samples were heated from 25° to 100°C at the rate of 3°C/min and the endothermic heat flow recorded. Water was used as reference. The data of gelatinsation (T onset = T_o, T peak = T_p, T conclusion = T_c, ΔH) were averaged on a minimum of three replicates for each starch sample.

3 Results and Discussions

3.1 Chemical composition of rice

The chemical composition of the polished long-grain nonwaxy rice (Indica type, Thailand origin) is presented in Tab. 1. On a dry basis, the rice consisted of 88.9% total starch, 6.9% protein, 0.18% lipids and 0.30% minerals. It is likely that 4% of unaccounted-for material is mostly cell-wall polysaccharides.

Tab. 1. Chemical composition of long-grain polished rice.

Composition	Content [%]
Protein (d.s.); × 5.95	6.89 ± 0.03
Lipid (d.s.)	0.18 ± 0.01
Ash (d.s.)	0.30 ± 0.01
Total starch (d.s.)	88.9 ± 1.1

3.2 Alkaline and enzymatic isolation of rice starch

This study investigated the small-scale isolation of rice starch from long-grain rice by both an alkaline process following the traditional way of manufacture and a novel enzymatic procedure. Fig. 1 presents the details of the alkaline isolation process. The first step consisted of steeping the rice in 0.4% NaOH solution at 5°C for 18 h (overnight) followed by removal of the steeping liquor via a decanter and subsequent milling of the steeped material in a colloid mill. According to general expectations a high amount of alkali-soluble proteins [7, 14, 24–26] could be dissolved during the initial phase and then removed with the liquid phase. This treatment provided ground material of rice with a residual protein content of 2.59% (Tab. 2). The material was steeped again in 0.4% NaOH solution and left for 1 h at room temperature to further extract endosperm proteins. The extended extraction produced material with only slightly higher purity (protein content: 2.36%). A much more pronounced reduction of protein bodies could be achieved when exposing the material to other separation steps as presented in Tab. 2. The final separation of protein residues was carried out by centrifugation and removal of the dark tailing layer atop the starch sediment. These tailings were scraped off manually and with accuracy to obtain rice starch of high purity. By the described process, the starch recovery calculated on dry starch content basis reached a level of 79.8%. For the rice starch isolated by the traditional alkaline procedure a final protein content of 0.48% could be achieved (Tab. 2). The minor components of the starch were 0.03% lipids, 0.12% minerals as documented in Tab. 3. The starch content reached a level of 95.7%, which does, in general, not satisfy industrial standards, where a purity of 97.5 to 98.0% is requested [27]. Residues of fibre material not fully removed by purification measures could be an explanation for the observed deficits in starch content. The portion of damaged starch was determined with 4.14%. The amylose content of 35.7% was higher than reported so far.

In an alternative process rice starch was isolated by utilising enzymes to degrade rice grain structures and to interrupt protein/starch interactions. For this purpose the process sketched in Fig. 2 was developed. Following previous experiences [15], the designed process used cellulase in preparing rice grains for disintegration and proteases of different origin to decompose the glutelins [25, 26] of ground rice (either Corolase 7089 or papain). Cellulase was used to break down the cellular tissue of endosperm whereas protease was used to digest rice protein to release pure rice starch.

In the first steps of the process, the polished rice was steeped in water instead of NaOH solution used in the alkaline process. As result a significant reduction of the protein content was not accomplished in the initial step of the process. After grinding the water-steeped rice had a residual protein content of 6.87% (Tab. 2). When the wet-ground material was digested by cellulase and the solubles removed in a decanter separator, the digested dewatered residue still contained 6.48% protein. That protein level was still similar to the original concentration in the rice kernels (Tab. 1).

Tab. 2. Residual protein content in the alkaline and the enzyme isolation process.

Process steps in isolation	Residual protein content (N × 5.95) [% d.s.]		
	Alkaline process	Enzyme process	
		Corolase 7089	Papain
Polished rice	6.89 ± 0.03[a]	6.89 ± 0.03[a]	6.89 ± 0.03[a]
Decanter 1	2.59 ± 0.10[b]	6.87 ± 0.09[a]	6.87 ± 0.09[a]
Decanter 2	2.36 ± 0.09[c]	6.48 ± 0.06[b]	6.48 ± 0.06[b]
Vibration sieve 25 μm	1.01 ± 0.05[d]	5.66 ± 0.13[c]	5.66 ± 0.13[c]
Decanter 3	0.90 ± 0.12[de]	5.38 ± 0.03[d]	5.53 ± 0.08[d]
Decanter 4	0.86 ± 0.08[ef]	–	–
Decanter 5	0.74 ± 0.12[f]	–	–
Decanter 6	0.74 ± 0.07[f]	–	–
Isolated starch	0.48 ± 0.03[g]	0.60 ± 0.02[e]	0.55 ± 0.00[e]
Further alternative purification treatments			
a) by 0.2% NaOH; 30 min	–	0.54 ± 0.00[e]	0.52 ± 0.03[e]
b) by 0.5% SDS; 30 min	–	0.50 ± 0.01[e]	0.49 ± 0.00[e]

*) The same superscript in a column represents an insignificant difference at $P < 0.05$.

These results indicate that cellulase could not assist in protein removal, however, it helped to prepare the tissue material for the subsequent disintegration in the screw-loop reactor and to separate fibres and larger particles on vibrating sieves with throughs at 50 and 25 μm mesh. After fibre removal and digestion of the material with the proteases (Corolase 7089 or papain) it did not release soluble proteins. Only a slight reduction in protein content was observed upon removing the liquid phase of the digest with the decanter. The residual protein content was reduced to 5.38% by Corolase 7089 treatment and to 5.53% by papain treatment (Tab. 2). The main part of rice protein, however, was separated when the protease digest was subjected four times to a laboratory centrifuge. Upon centrifugation tailing fractions were located atop the settled starch, and those protein-rich layers were removed manually by scraping off. That step followed the separation of purified rice starch with a recovery of 76.5%, irrespective which protease was used. The composition of the various purified starches showed only negligible differences (Tab. 3), except for the level of damaged starch at 2.65%, which was much smaller than found for the alkaline process (4.14%).

The recovery of 76.5% starch by protease digestion of rice endosperm at neutral pH is approximately the same as 79.8% recovery by the alkaline procedure, as has been shown by other investigators [10]. Although the yields were much smaller than the ones presented by *Lumdubwong* and *Seib* [9] (85% by the alkali method, 95% with enzymes; both extracted from wet-milled rice flour), they were in accordance with data published so far [10]. The yield differences between the alkaline and the enzymatic processes may be caused by the efficiency and specificity of the enzymes to digest rice proteins. The dominant portion of rice protein (ca. 80%), that is tightly attached to the granular surface, becomes preferentially soluble under alkaline conditions (above pH 10, especially glutelin), but inhibited swelling in water at neutral condition [7, 23]. Finally, starch granules coated with protein might repel each other during centrifugation [9]. This observation corresponded well with quantitative aspects of the tailing layers. In case of enzymatic isolations the tailing layers were evidently thicker than with the alkaline method. In contrast to starch recovery, the residual protein content of the enzymatically isolated rice starch was significantly higher than for the starch isolated by application of an alkaline medium. However, no significant difference could be observed between both protease enzymes.

The amount of damaged starch was significantly lower in case of the enzyme process than in the alkaline isolation procedure, which reflected the more severe treatment of the starch granules in the alkaline process. In principle, this tendency was also reported elsewhere [9, 10], when flours of unknown milling intensity were used in laboratory scale isolation procedures. Milling, in any case, can also be regarded as important source of damaged rice starch [11].

Since the required purity levels of rice starch with respect to protein content could not be fully achieved by application of enzymes in wet processing, the produced starch was extracted additionally either with NaOH (0.2% solution) or SDS (0.5% solution). As can be seen in Tab. 2 both purification treatments allowed further reductions in protein content, and the starch extracted with SDS had a final protein content similar to that isolated by the alkaline process. These final extraction procedures removed 0.06–0.10% protein, but were connected with a reduction of yield. Apart from a reduction of lipids in connection with the SDS treatment, the overall quality of rice starch was not changed significantly.

3.3 Property profiles of the isolated rice starch

Swelling profiles of isolated rice starch at different temperatures are presented in Fig. 3a. From 80°C onwards, the alkaline-isolated rice starch presented a slightly higher swelling than the enzymatically isolated one. After washing enzymatically isolated and purified starches additionally with SDS solution starch swelling nearly doubled from approximately 18% to 38%, but washing with NaOH solution was less effective.

The results of solubility investigations are presented in Fig. 3b. Between 60 and 70°C no differences were visible between the differently isolated starches, and even at 80°C the difference was small. At 90°C the solubility of starch from the alkaline isolation process was more than 10% higher than that of samples of enzyme-assisted processes. Washing the enzyme-isolated starch with SDS produced a solubility of 55 to 60% at 90°C. The NaOH treatment was less effective, but offered a small advantage to the Corolase 7089 product.

The pasting properties of the isolated rice starch samples are shown in Figs. 4 and 5. The alkaline process resulted in a starch represented by curve 1 with lower pasting temperature and greater breakdown than observed for the starches obtained from both enzymatic processes represented by curves 2 and 3, with the latter curves being similar. Since starch granules are susceptible to the alkaline medium, partial swelling may have caused partial and irreversible changes even below gelatinisation temperature. Those changes may have caused more pronounced swelling in the initial phase of the RVA diagram. Moreover, the alkaline-isolated rice starch had 0.01% less

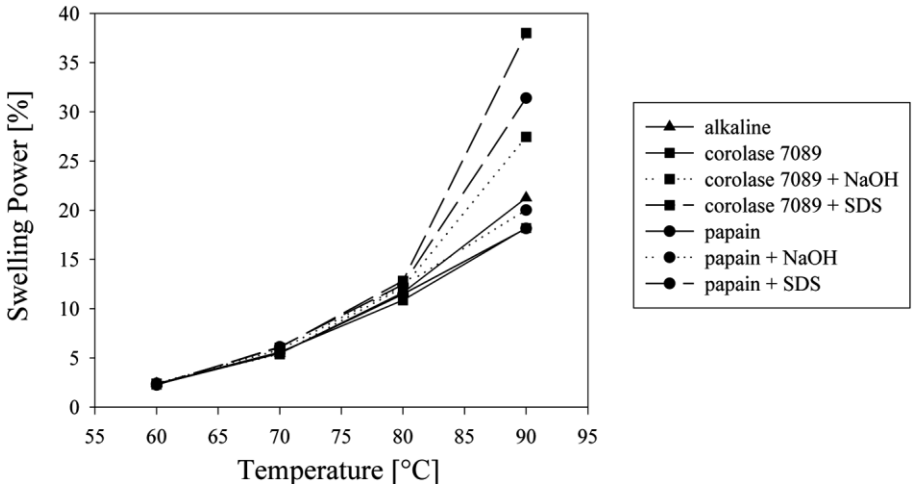

Fig. 3a. Effects of starch isolation and purification on swelling power of rice starch.

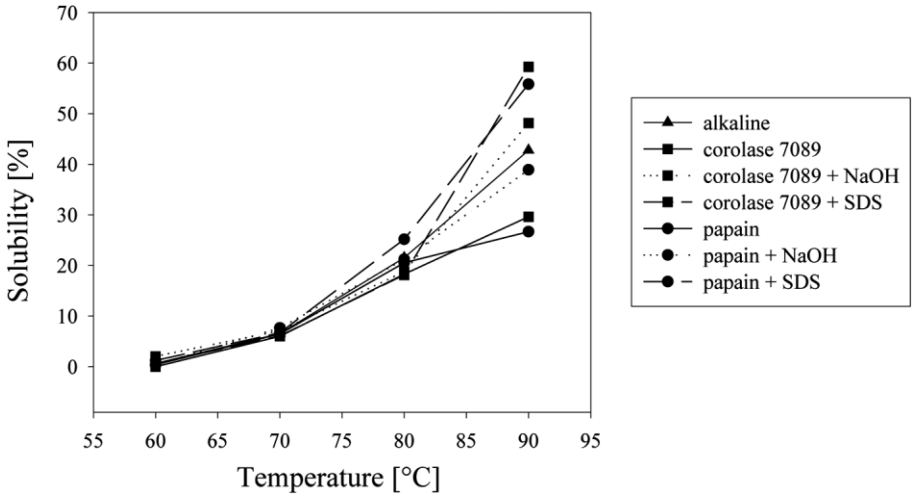

Fig. 3b. Effects of starch isolation and purification on solubility of rice starch.

lipid content than the enzyme-isolated starches. The observations made for the alkaline and enzymatically isolated starches differed from those made by *Lumdubwong* and *Seib* [8], but they used an alkaline medium (pH $\geq$ 10) in both isolation schemes.

After washing enzyme-isolated rice starches with NaOH solution, the pasting properties were changed only slightly (Fig. 5). However, when applying SDS for reduction of protein content, initial pasting was highly intensified as were breakdown and set back. This observation may be also due to the reduced lipid content in the starch treated with SDS (see also Tab. 3). Since surface lipids of intact starch granules were presumably dissolved in SDS solution, the observed effect of this investigation was not surprising. It is, for example, also well known for monoacyl lipids in cereal starches that they reduce hot swelling and solubility [28, 29]. Regarding the effects of SDS on starch granules, *Seguchi* [30] reported that 1% aqueous SDS solutions containing 1% 2-mercaptoethanol cause granule destabilisation as indicated by loss of birefringence. At both room temperature and 50°C SDS allows granules to gelatinise at lower temperature than normal, but that effect was not found here for 0.5% SDS and short-term treatment. Furthermore, it was established that SDS solutions not only enable liberation of starch granule-associated proteins (SGAPs), but also increase leaching of starch polymers. The dispersed starch polymers are mainly amylose chains as described by *Gough* et al. [31], who also reported that phospholipids, in particular, are released from granules treated with 1% (w/v) SDS. Those previous findings corresponded well with the decrease in lipid content of rice starch after washing with 0.5% SDS solution (Tab. 3). Yet, the observed effects differ, for example, in gelatinisation and pasting from results presented in a previous report by *Maningat* and *Juliano* [32].

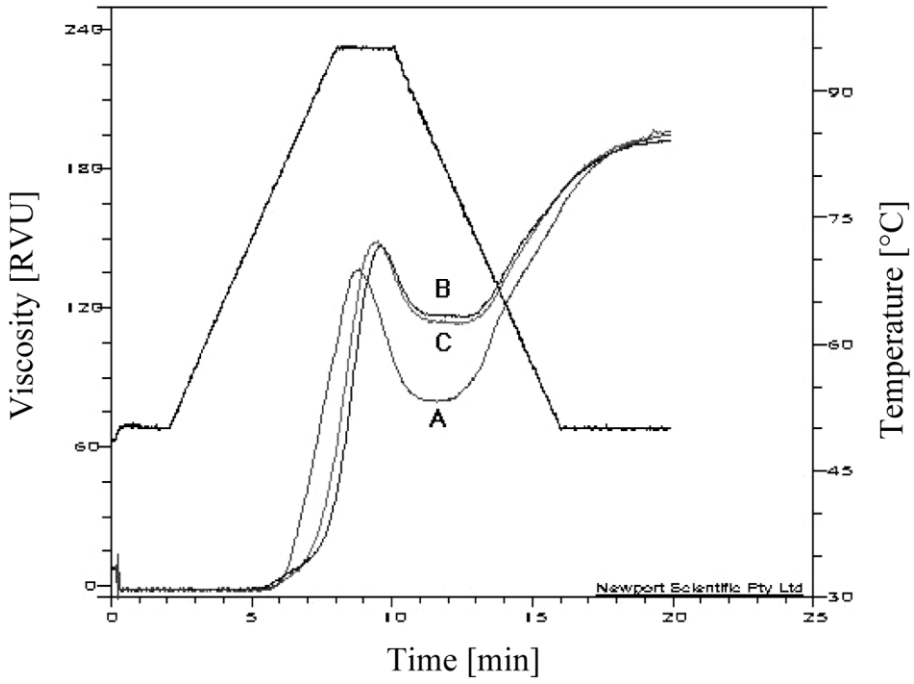

Fig. 4. RVA pasting behaviour of rice starch isolated by alkaline (curve A) and enzymatic (curve B: Corolase 7089, curve C: papain) processes.

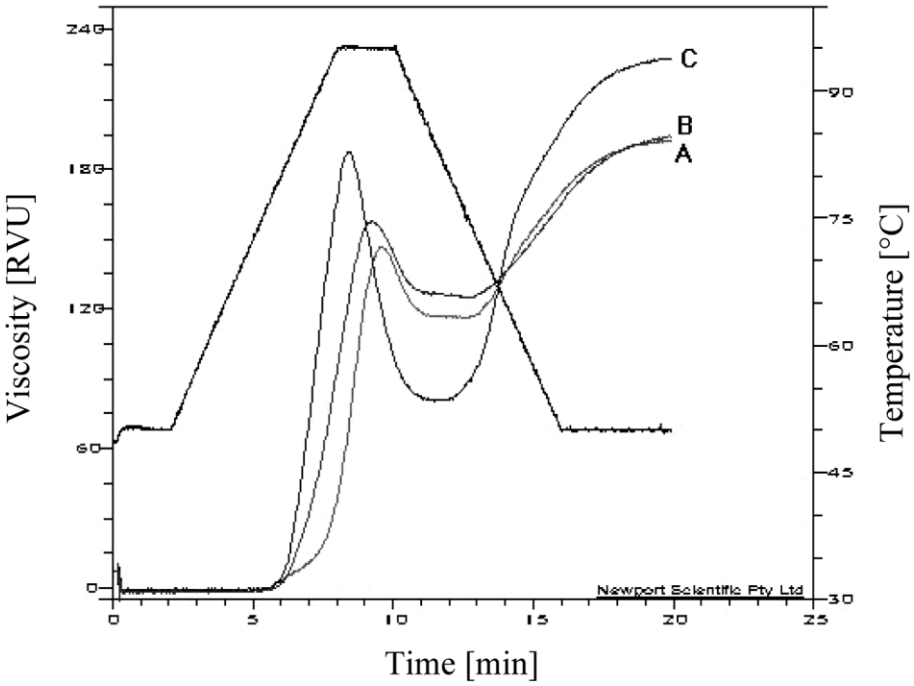

Fig. 5. RVA pasting behaviour of rice starch isolated by a Corolase 7089 based process (curve A) in combination with washing as purification procedure (curve B: 0.2% NaOH solution, curve C: 0.5% SDS solution).

The gelatinisation properties of the differently purified rice starches, as determined by DSC measurements, are presented in Tab. 4. The isolation procedures gave little to no discernable differences in thermal characteristics, in particular the relevant temperature ranges. The sample isolated with alkali presented the highest enthalpy of gelatinisation (13.2 J/g) while papain produced a clearly smaller value (11.1 J/g). Similar effects were visible for the temperature range between onset (T_o) and conclusion temperature (T_c).

Tab. 3. Recovery and chemical composition of isolated rice starches

Type of extraction	Recovery [% d.s.]	Amylose content [% d.s.]	Protein content (N × 5.95) [% d.s.]	Lipid content [% d.s.]	Mineral content [% d.s.]	Total starch [% d.s.]	Damaged starch [% d.s.]
– 0.4% NaOH	79.75	35.7 ± 0.15^{a}	0.48 ± 0.02^{a}	0.03 ± 0.00^{a}	0.12 ± 0.02^{a}	95.7 ± 0.23^{a}	4.14 ± 0.01^{a}
– Corolase	76.48	36.2 ± 0.07^{a}	0.60 ± 0.02^{b}	0.04 ± 0.01^{a}	0.10 ± 0.01^{a}	96.3 ± 1.18^{a}	2.65 ± 0.01^{b}
a) Followed by 0.2% NaOH; 30 min	76.02	36.0 ± 0.23^{a}	0.54 ± 0.01^{c}	0.03 ± 0.00^{a}	0.13 ± 0.02^{a}	96.1 ± 0.45^{a}	2.63 ± 0.00^{b}
b) Followed by 0.5% SDS; 30 min	73.94	35.9 ± 0.11^{a}	0.50 ± 0.01^{a}	0.01 ± 0.00^{a}	0.14 ± 0.03^{a}	94.0 ± 0.13^{b}	2.30 ± 0.03^{c}
– Papain	76.50	35.7 ± 0.09^{a}	0.55 ± 0.01^{c}	0.04 ± 0.00^{a}	0.12 ± 0.03^{a}	95.9 ± 0.76^{a}	2.64 ± 0.00^{b}
a) Followed by 0.2% NaOH; 30 min	75.90	35.7 ± 0.17^{a}	0.52 ± 0.03^{c}	0.03 ± 0.00^{a}	0.13 ± 0.01^{a}	95.7 ± 2.01^{a}	2.64 ± 0.01^{b}
b) Followed by 0.5% SDS; 30 min	75.21	35.7 ± 0.09^{a}	0.49 ± 0.01^{a}	0.01 ± 0.00^{a}	0.12 ± 0.02^{a}	94.7 ± 1.63^{a}	2.29 ± 0.02^{c}

*) The same superscript in a column represents an insignificant difference at $P < 0.05$.

Tab. 4. DSC characteristics*) of rice starch isolated and purified by various isolation procedures.

Type of extraction	T_o [°C]	T_p [°C]	T_c [°C]	ΔH [J/g]	T_c–T_o [°C]
– 0.4% NaOH	68.0 ± 0.4^{a}	79.3 ± 0.1^{a}	89.6 ± 0.3^{a}	13.2 ± 0.2^{a}	21.6 ± 0.6^{a}
– Corolase 7089	68.4 ± 0.4^{a}	78.7 ± 0.3^{a}	88.1 ± 1.3^{a}	12.6 ± 0.6^{a}	19.7 ± 0.6^{b}
a) Followed by 0.2% NaOH; 30 min	68.1 ± 0.2^{a}	78.6 ± 0.4^{a}	89.3 ± 0.6^{a}	15.2 ± 0.5^{b}	21.3 ± 0.8^{a}
b) Followed by 0.5% SDS; 30 min	68.1 ± 0.2^{a}	78.2 ± 0.1^{a}	88.6 ± 0.6^{a}	12.9 ± 0.2^{a}	20.5 ± 0.7^{a}
– Papain	70.5 ± 0.2^{b}	78.8 ± 0.4^{a}	88.6 ± 0.3^{a}	11.1 ± 0.4^{d}	18.1 ± 0.1^{c}
a) Followed by 0.2% NaOH; 30 min	68.7 ± 0.4^{a}	78.9 ± 0.4^{a}	89.0 ± 0.4^{a}	13.7 ± 0.6^{a}	20.3 ± 1.1^{a}
b) Followed by 0.5% SDS; 30 min	68.9 ± 0.5^{a}	78.9 ± 0.4^{a}	89.3 ± 0.3^{a}	11.4 ± 0.3^{d}	20.5 ± 0.5^{a}

*) The same superscript in a column represents an insignificant difference at $P<0.05$.

4 Conclusions

Enzymatic processes using cellulase [15] together with neutral proteases (Corolase 7089 or papain) aided in the purification of rice starch from polished rice after a short-term enzyme treatment (45 min). The enzyme process gave at least a starch recovery of approximately 76% with about 0.6% protein, which was somewhat higher than the alkaline process. The enzyme process involved the use of only low levels of acids or salts in the digestion step.

However, the study also demonstrated that the purification of starch from rice endosperm at neutral conditions was limited, and that alkali or SDS treatments were eventually needed for rice starch of higher purity. Washing of rice starches contaminated with 0.6% protein with NaOH solutions did not effectively remove protein, while SDS solution could produce a reduction to about 0.5%. Starch purity and its swelling and solubility at temperatures > 80°C were significantly affected by the SDS washing procedure. With respect to the level of starch damage inflicted by the processes, the NaOH steeping caused twice the damage compared to the enzymes (4% vs. 2%).

Acknowledgements

The authors want to express the gratitude to the Thailand Research Fund (TRF) and the German Academic Exchange Service (DAAD) for the financial support granted to Mrs. *Hatairat Puchongkavarin* during her studies at the Institute for Cereal, Potato and Starch Technology of the Federal Research Centre for Nutrition and Food, location Detmold, Germany, on the basis of the Royal Golden Jubilee Ph.D. Programme.

References

[1] T. H. Schoch: Properties and uses of rice starch, in *Starch Chemistry and Technology,* Vol. *2* (Eds. R. L. Whistler, E. F. Paschall) Academic Press Inc., New York, NY, **1967**, 79–86.

[2] R. J. Alexander: Fat replacers based on starch. *Cereal Foods World* **1995**, *40*, 366–370.

[3] J. Bao, C. J. Bergman: The Functionality of rice starch, in *Starch in Food: Structure, Function and Application* (Ed. A.-C. Eliasson) *Woodhead Publishing Ltd.*, Cambridge, **2004**, 258–294.

[4] B. O. Juliano: Rice starch: Production, properties, and uses, in *Starch Chemistry and Technology* (Eds. R. L. Whistler, J. N. BeMiller, E. F. Paschal) Academic Press Inc., Orlando, FL, **1984**, 507–528.

[5] F. Labell: Rice starch reduces fat, adds creamy texture without imparting flavor. *Food Processing*. **1991**, *3*, 95–96.

[6] Y. Tanaka, T. Sugimoto, M. Ogawa, Z. Kasai: Isolation and characterization of two types of protein bodies in the rice endosperm. *Agric. Biol. Chem.* **1980**, *44*, 1633–1639.

[7] P. S. Landers, B. R. Hamaker: Antigenic properties of albumin, globulin and protein concentrate fractions from rice bran. *Cereal Chem.* **1994**, *71*, 409–411.

[8] C. Yang, H. M. Lai, C. Y. Lii: The modified alkaline steeping method for the isolation of rice starch. *Food Sci.* **1984**, *11*, 458–162.

[9] N. Lumdubwong, P. A. Seib: Rice starch isolation by alkaline protease digestion of wet-milled rice flour. *J. Cereal Sci.* **2000**, *31*, 63–74.

[10] L. Wang, Y. J. Wang: Comparison of protease digestion at neutral pH with alkaline steeping method for rice starch isolation. *Cereal Chem.* **2001**, *78*, 690–692.

[11] H. Chiou, M. Martin, M. Fitzgerald: Effect of purification methods on rice starch structure. *Starch/Stärke* **2002**, *54*, 415–420.

[12] A. P. Resurreccion, X. Li, T. W. Okita, B. O. Juliano: Characterization of poorly digested protein of cooked rice protein bodies. *Cereal Chem.* **1993**, *70*, 101–104.

[13] F. F. Shih: Rice proteins, in *Rice, Chemistry and Technology* (Ed. E. T. Champagne) American Association of Cereal Chemists, Inc, St Paul, MN, **2004**, 143–162.

[14] Z. Y. Ju, N. Hettiarachchy, T. J. Siebenmorgen: Rice-protein preparation, thermal properties and denaturation, in *B. R. Wells Rice Research Studies* (Eds. R. J. Norman, C. A. Beyrouty) Ark. Agric. Exp. Stn., Div. Agric., University of Arkansas, Fayetteville **2000**.

[15] W. Bergthaller, H. Themeier, R. Lepa: Extraction of starch and protein from pea mutant cotyledon breaks in a pilot plant by applying neutral media and enzyme technology, in *Towards the sustainable production of healthy food, feed and novel products*. Proceedings of the 4th European Conference on Grain Legumes, 8–12 July **2001**, 386–387.

[16] H. S. Guraya, C. James: Deagglomeration of rice starch-protein aggregates by high-pressure homogenisation. *Starch/Stärke* **2002**, *54*, 108–116.

[17] AOAC, *Official Methods of Analysis: Moisture and Ash*, 15 ed., Association of Official Analytical Chemistry, Arlington, U.S.A., **1990**, 777.

[18] AOAC, *Official Methods of Analysis: Fat*, Association of Official Analytical Chemistry, Arlington, U.S.A., 15 ed. **1990**, 780.

[19] ICC standard No. 167. Determination of crude protein in grain and grain products for food and feed by the Dumas combustion principle. International Association for Cereal Science and Technology. **2000**.

[20] International Standard. ISO 10520:1997 (E). Native starch – Determination of starch content – Ewers polarimetric method.

[21] AACC Method 76–31. Megazyme: Starch damage assay procedure.

[22] C. A. Knutson: A simplified colorimetric procedure for determination of amylose in maize starches. *Cereal Chem.* **1986**, *63*, 89–92.

[23] T. J. Schoch: Swelling power and solubility of granular starch, in *Methods in Carbohydrate Chemistry*, Vol. *IV* Starch (Eds. R. L. Whistler, R. J. Smith, J. N. BeMiller, M. L. Wolfrom) Academic Press, New York – London, **1964**, 106–108.

[24] A. R. Del Rosario, V. P. Briones, A. J. Vidal, B. O. Juliano: Composition and endosperm structure of developing and mature rice kernel. *Cereal Chem.* **1968**, *45*, 225–235.

[25] G. B. Cagampang, L. J. Cruz, S. G. Espiritu, R. G. Santiago, B. O. Juliano: Studies on extraction and composition of rice proteins. *Cereal Chem.* **1966**, *43*, 145–155.

[26] B. O. Juliano, D. Boulter: Extraction and composition of rice endosperm glutenin. *Phytochemistry* **1976**, *15*, 1601–1606.

[27] unpublished results.

[28] R. F. Tester, W. R. Morrison: Swelling and gelatinization of cereal starches I: Effect of amylopectin, amylose and lipids. *Cereal Chem.* **1990**, *67*, 551–556.

[29] W. R. Morrison, R. F. Tester, M. J. Gidley, J. Karkalas: Resistance to acid hydrolysis of lipid-complexed amylose and lipid-free amylose in lintnerised waxy and non-waxy barley starches. *Carbohydr. Res.* **1993**, *245*, 259–295.

[30] M. Seguchi: Surface staining of wheat starch granule with Remazolbrilliant Blue R. dye and their extraction with aqueous sodium dodecylsulphate and β-mercaptoethanol. *Cereal Chem.* **1995**, *72*, 602–608.

[31] B. M. Gough, P. Greenwell, P. L. Russell: On the interaction of sodium dodecylsulphate with starch granule, in *New Approaches to Research on Cereal Carbohydrates* (Eds. R. D. Hill, L. Munck) Elsevier Science Publishers B.V., Amsterdam. **1985**, 99–108.

[32] C. C. Maningat, B. O. Juliano: Starch lipids and their effect on rice starch properties. *Starch/Stärke* **1980**, *32*, 76–82.

(Received: January 19, 2004)
(Revised: December 6, 2004)
(Accepted: December 6, 2004)

