PROJECT NO. ___

ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
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PROJECT TITLE: ISOLATION AND ULTILIZATION OF RICE STARCH AND PROTEIN

PROJECT LEADER Charles F. Shoemaker
UC Davis, Food Science and Technology Department
One Shields Ave, Davis, CA 95616

COOPERATORS: Wallace H. Yokoyama
USDA, ARS, WRRC, 800 Buchanan St., Albany, CA 94710

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OBJECTIVES AND EXPERIMENTS CONDUCTED, BY LOCATION, TO ACCOMPLISH OBJECTIVES:

Objective I. Selection of proteases and hydrolyzing parameters
California rices waxy grain - Calmochi-101 and medium was used as the source of rice for starch and peptide production. Different protease enzymes were evaluated for their ability to purify starch from milled rice. Degree of protein hydrolysis (DH) was tested.

Objective II. Rice Starch Characterization
Particle size and pasting properties properties of California M202 rice starch isolated by protease digestion were compared starches prepared with other chemical methods.

Objective III. Characterization of Bio-active Peptides
The bio-active properties of the peptides were evaluated.

SUMMARY OF 2005 RESEARCH (major accomplishments), BY OBJECTIVE:

Objective I. Selection of proteases and hydrolyzing parameters

Different protase enzymes were studied for their ability to remove protein from the starch in rice. M202 was used for testing of the various enzymes. The following enzymes which are commercially available were tested. Alcalase, Neutrase, Protamex, Flavourzyme (NovoNordisk, Denmark), AS1398, Fungal protease, GC710 (Genencor International, Palto Alto, CA, USA), and ProteaseN (Amano, Japan).

Protease treatment was carried out at the optimal enzyme reaction condition for each protease, pH was controlled during the treatment.. The following conditions were used for each enzyme:

Table 1: Protease treatment condition for each protease

protease	pH	Temperature (C)	Protease activity (U/mL or U/g)	Usage level (U/mL or U/g)
Acalase	8.5	50	88000	35000
Protamex	7.5	40	15000	35000
Neutrase	7.5	40	12000	35000
Flavourzyme	7	50	11000	35000
ProteaseN	7	50	1110000	35000
Fungal protease	7	40	10000	35000
GC710	7	50	15000	35000
AS.1.398	7.2	50	120000	35000

The degree of protein hydrolysis (DH) of rice protein was calculated by the pH-stat method.

$DH=B\times N_b\times(1/a)\times(1/MP)\times(1/h_{tot})\times 100$

Where,

B = the NaOH consumption (ml)

N_b = NaOH concentration (normality)

$a=10^{(pH-pK)}/(1+10^{(pH-pK)})$,

MP = total amount of rice protein

h_{tot} = mmol Amino acid/ g protein(8.13mmol/g protein)

The ProteaseN enzyme was found to be the most effective enzyme in terms of producing a rapid degree of hydrolysis, Figure 1. However, Protease N is in a purified form and is expensive. The other enzymes used were in a commercial form, i.e. less expensive. From these, Alcalase was the most efficient.

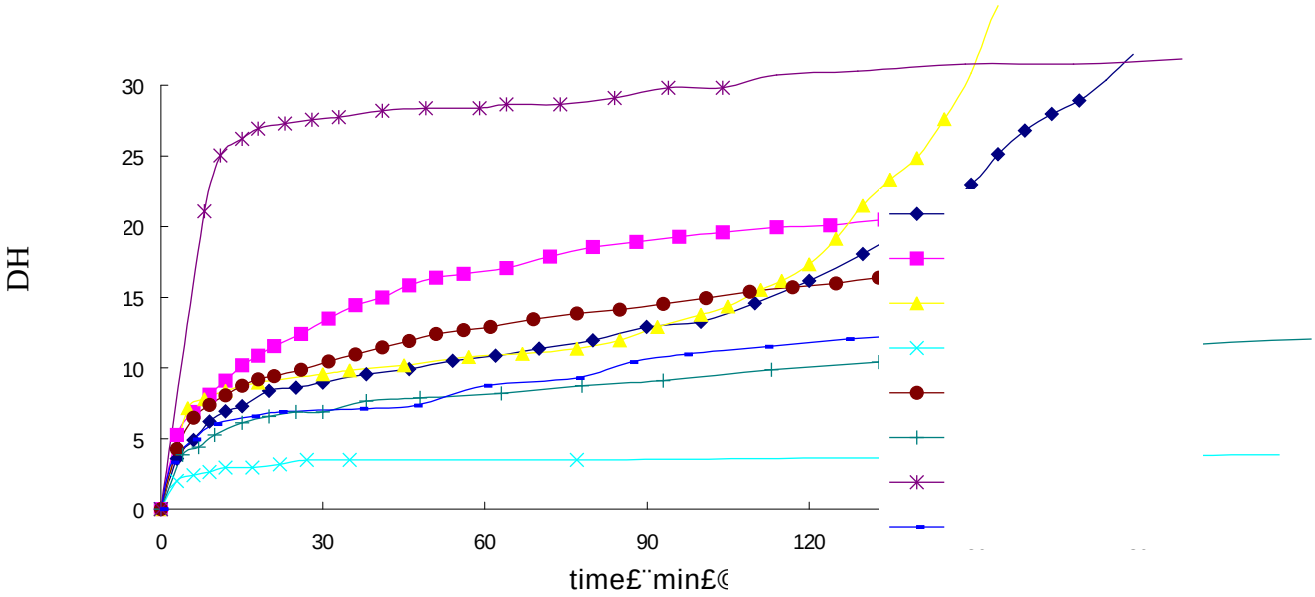

Figure 1. Hydrolysis profiles of rice protein during protease treatment

From the standpoint of residue protein left in the starch, Alcalase was found to be the most effective with the procedure used, Table 2.

Table 2: Protein[a] content of the isolated rice starch

protease	Protein content of the starch(%)
Alcalase	0.84
Protamex	3.2
Neutrase	3.51
Flavourzyme	3.26
Protease N	1.1
Fungal Protease	2.28
GC710	1.18
AS.1.398	2.4

[a] The protein content of the M202 rice used was 6.24% .

Thus Alcalase & ProteaseN were also found to be were more effective for rice protein removal from the standpoint of residue protein. The possibility of a synergistic effect with a combination of two enzymes was also investigated. Combinations of Alcalase and ProteaseN were chosen since these two enzymes were the most effective in their action alone. Different starting pH's were also used.

Table 3: The various levels of the two enzymes and starting pH's used.

Factor	Level		
	1	2	3
A. Starting pH	10.5	10	9.5
B. Alcalase usage level (mL)	0.2	0.15	0.1
C. ProteaseN usage level (g)	0.06	0.05	0.04

There was no significant gain in protein removal with any of the other combinations of the two enzymes or with different starting pH's, Table 4.

Table 4: The effect of different mixtures of Alcalase and ProteaseN with various starting pH's

A	B	C	Final protein content(%)
1	1	3	1.09
2	1	1	1.36
3	1	2	1.03
1	2	2	0.83
2	2	3	0.96
3	2	1	0.89
1	3	1	0.75
2	3	2	1.03
3	3	3	0.98

* Alcalase was added at the designated starting pH, when the pH of the system decreased to 7.2, ProteaseN was added, total reaction time was 4 hr for all cases.

Thus it appears that the use of Alcalase alone is the best choice for protein removal from milled rice. With the choice of Alcalase, its optimization was investigated next. The first parameter was the starting pH. Two pH's were chosen, 11 and 10.5. Reaction times of 4 hours were used with a level of 0.4 mL of added Alcalase. For the starting pH's of 11 and 10.5, the final protein content was found to be 0.69% and 0.61%, respectively. Although, a starting pH of 11.0 produced a slight lower residue protein content, the difference in protein between pH's of 11 and 10.5 were not significant.

The next parameter of interest that was investigated was the level of Alcalase added. Four different levels were used Table 5. With greater levels of added Alcalase there was observed a decrease in the residue protein; however, the protein different between the two extreme levels of added enzyme were not significant. Thus the lower level of Alcalase could be used.

Table 5: The effect of Alcalase usage level of protein removal from rice

Usage level of Alcalase (mL/30g rice)	Usage level of Alcalase (U/mL)	Protein content(%)
0.1	8800	0.62
0.2	17600	0.62
0.3	26400	0.61
0.4	35000	0.57

The effect of different reaction times was next investigated, Table 6. There was an expected result showing an increase in the degree of protein removal with reaction time. The difference was found to be significant between the reactions times of 2 and 4 hr but not different between 4 and 6 hr.

Table 6: The effect of reaction time on protein removal from rice (Alcalase usage level 0.1 mL/30 g rice)

Reaction time (hour)	Protein content (%)
2	0.85
4	0.62
6	0.60

An additional aid to the process was tested. This was the addition of the enzyme Cellulase (NovoNordisk) before the addition of Alcalase. Various levels of Cellulase was added to remove cell wall material, which may have retarded the action of Alcalase on the rice proteins. Cellulase was added before protease treatment for 2 hr; the starting pH of the rice dispersion was adjusted to 5. After this treatment 0.1 mL Alcalase was added with a starting pH of 10.5; the reaction time was 2 hr for this treatment was, Table 7. A significant gain in protein removal was found with a pretreatment of milled rice with levels of Cellulase equal to or greater than 0.4 mL

Table 7: The effect of a pretreatment of rice with Cellulase at different levels before the treatment with Alcalase for protein removal from milled rice.

Cellulase level(mL/30 g rice)	0.1	0.2	0.3	0.4	0.6	0.8
Final Protein content (%)	0.84	0.82	0.70	0.48	0.52	0.47

Objective II. Rice Starch Characterization

The pasting properties of rice starch prepared with Alcalase, Sodium Hydroxide, SDS (a detergent), and rice flour were compared, Table 8. The pasting behavior of the rice starch prepared with Alcalase was found to be similar to the rice flour. This suggests that the enzymatic removal of protein from the rice flour does not affect the properties of the starch. This treatment leaves the starch granules in their native state. The chemical separation of starch from the flour with either NaOH or SDS does show some changes in their pasting properties.

Table 8: The pasting properties of rice starch compared by different methods.

Treatment	**Temperature at initial point of pasting (°C)**	**Temperature at peak viscosity (°C)**	**Peak Viscosity (Pa.s)**	**Final Viscosity after cooling to 50°C (Pa.s)**
Flour	70.1	95.5	0.49	0.52
Starch Enzyme	68.7	95.2	0.48	0.51
Starch NaOH	68.1	93.8	0.51	0.76
Starch SDS	67.4	87.2	0.49	0.66

Microscopic examination of the rice flours with scanning electrode microscopy, Figure 2, showed that the starch granules prepared with Alcalase appeared to be less affected by the separation process. This would also suggest that these starch granules are more like the native starch granules in the milled rice.

Thus these experimental results suggest that the enzymatic prepared rice starch leave the starch granules more intact than the two chemical processes.

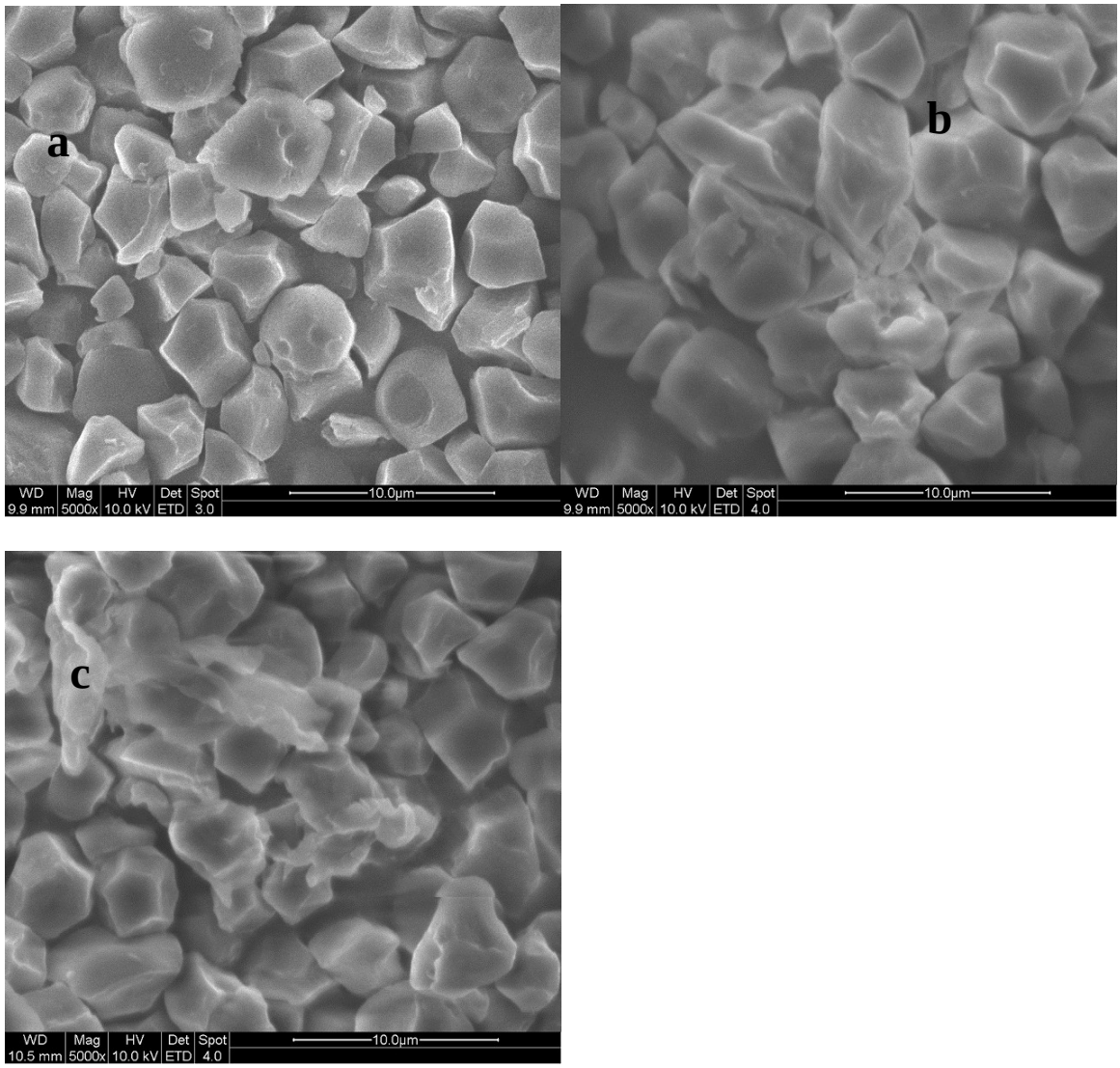

Figure 2: Scanning electrode micrographs of rice starch granules prepared by different methods. a: treatment with Alcalase, b: treatment with NaOH, and c: treatment with SDS.

Objective III. Characterization of Bio-active Peptides

In order to test for the potential of health effects of peptides which were produced form the hydolysis of rice proteins during the purification of rice starch, two tests were conducted. The first was related to blood pressure lowering effects of peptides and the second was related to the cholesterol lowering effects.

Angiotensin converting enzyme (ACE) is an enzyme whose physiologic action is related to blood pressure and fluid balance in the body. The assay of its activity is an important measure of the potential for high blood pressure. Agents, which can inhibit its activity may have the potential for blood pressure lowering. The inhibition of ACE activity by the rice peptides which were products from the preparation of rice starch with Alcalse

were evaluated, Table 9. Soy peptides have been the most extensively studied peptide for ACE inhibition and cholesterol lowering. Our results showed that rice peptides obtained with certain protease treatment are effective in inhibiting ACE. When compared to the soy peptides, the rice peptides prepared with Alcalase were effective but less so that peptides from soy protein formed with Alcalase. However, when prepared with protease Protex 6L, these rice peptides showed tremendous inhibition of ACE activity, which was more effective than soy peptides in terms of ACE inhibition and cholesterol lowering.

Table 9: The inhibition of ACE with peptides.

	% ACE inhibition
Rice Peptide from Alcalase treatment	47.1
Rice peptide from Protex 6L treatment	97.5
Soy peptide from Alcalase treatment	58.2

The peptide concentration was 0.1mg/mL.

Dietary proteins have showed the ability to influence serum cholesterol level in many studies. Many studies have been carried out with soy and milk peptides. A current hypothesis is that the hypocholesterolemic peptides derived from proteins like soy might exist and influence the serum cholesterol level. Using this hypothesis, the cholesterol lowering potential of rice peptides were measured and compared to that of soy peptides, Table 10. These results show very good potential for a cholesterol lowering effect of rice peptides as compared to soy peptides.

Table 10: The potential for the lower cholesterol by peptides.

	cholesterol lower effect (%)
Rice Peptide from Alcalase treatment	82.6
Rice peptide from Protex 6L treatment	85.0
Soy peptide from Alcalase treatment	76.3

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

The project has identified a method for the production of rice starch with a commercial enzyme Alcalase (NovoNordisk). The rice starch powder prepared in this way is a very fine powder and less coarse that other starch powders. The average size of the rice starch granules is 5.5 microns. This method of preparation also minimizes damage to the starch granules as compared to other methods based on chemical treatments. The peptide by-products from the enzymatic preparation of rice starch have also been shown to have potential for healthy ingredients for foods. Thus this process, which separates starch

from protein in milled rice, has provided some directions for the marketing of the two products. Finally, the rice used for the process could be older or broken rice grains.