NUTRITIONAL EVALUATION OF MANNANASE TREATED COPRA MEAL BY THE RELATIVE NUTRITIVE VALUE (RNV) METHOD

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INTRODUCTION

The full utilization of coconut has not been achieved by the coconut industry in coconut-producing countries. As the main product from copra is coconut oil, the residual cake after oil extraction (called poonac or copra meal) is basically used only as animal feed or regarded as waste.

Efforts to extract the highly nutritive protein from copra meal and fresh coconut have been the main thrusts of various researches. A significant finding on the carbohydrate composition of copra meal is its high mannan content as shown by a very high percentage of mannose (70%), followed by glucose (20%) and small amounts of arabinose and galactose.

An attempt was also made to utilize mannan from copra meal using mannanase enzyme from Actinomycetes with plausible results. This enzyme (or enzyme system) has also been reported in various microorganisms.

This study primarily attempts to investigate the hydrolysis of mannan from copra meal without prior isolation and purification of the polysaccharide, using mannanase enzyme extract from *Streptomyces* and to compare the relative nutritive value of the hydrolysate with that of untreated copra meal using *Tetrahymena pyriformis* W as test organism.

REVIEW OF LITERATURE

Butterworth and Fox (1963), Krisnamurthy et al. (1958), Lanchance and Molina (1974), Rao and Indira (1964) and Rao et al. (1965), in separate studies pointed out the high nutritive value of proteins in copra meal. Studies on protein extraction from copra meal or fresh coconut meat include those of Alcantara et al. (1978), Chandrasekaran and King (1967), Chelliah and Baptist (1969), Hagenmaier et al. (1971). Such studies attempted to utilize the protein from copra meal. That of Rao et al.(1965) involves metabolic studies in children. The main carbohydrate found in copra meal is mannan which is significant in that it is a rare occurrence to find highly concentrated mannan in nature (Takahashi et al., 1983). The same authors described the physico-chemical properties of purified mannanase from *Streptomyces* sp. and separated the enzyme into four activities, with mannanase IV accounting for 64.4%of the total mannanase activity.

Tetrahymena pyriformis W has been shown to digest proteins and requires the same essential amino acids as higher animals. Guggenheim (1970) mentioned comparable results of relative nutritive values (RNV) of various protein sources using T. *pyriformis* with gross protein value (GPV) and net protein utilization (NPU). The same organism was used by Helmes and Rolle (1970) to study the relative nutritive value of mixed diets, by Gewan (1982) in evaluating the nutritive quality of fermented mungbeans and white kidney beans, and by Siopongco (1982) in a similar study using corn.

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MATERIALS AND METHODS

Production of Mannanase Enzyme

An agar slant culture of *Streptomyces* sp. was obtained from the culture collection of the Microbiology Laboratory of the University of the Philippines at Los Baños and inoculated into two 250 ml flasks containing 120 ml of Seed Culture Medium. These were incubated in a reciprocating shaker kept at a constant temperature of 35°C and with a speed of 125 oscillations/minute for 3 days.

Nine ml. of the seed culture were inoculated into each of the two 250 ml flasks containing 120 ml of Enzyme Production Medium. The flasks were then shaken with the same incubation conditions as described above for 5 days. The medium was then filtered using a sterile Whatman No. 2 filter paper into a sterile fermentation flask. The filtrate was used as the source of crude mannanase enzyme. Enzyme production was verified by determining the presence of reducing sugars.

Figure I shows a schematic diagram in the production of mannanase enzyme.

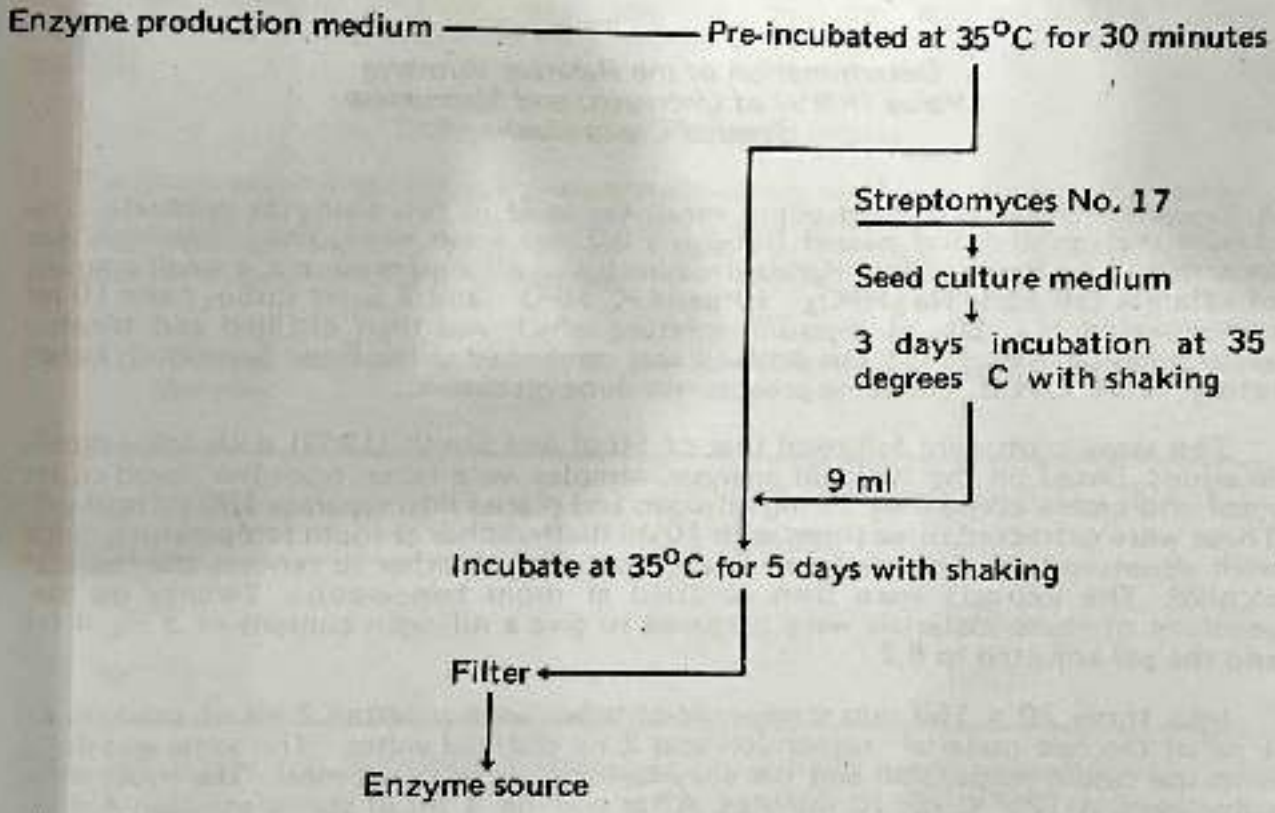

Fig. 1. Schematic diagram for the production of mannanase enzyme.

Determination of Reducing Sugars Mannanase Activity

Somogyi's method of determining reducing sugars was followed. The reaction mixture consisted of 129.4 mg. of copra meal that had been passed through a standard 0.2 mm mesh sieve (instead of pure mannan), 40 ml Mc Ilvaine buffer(pH6.8)

and 5 ml distilled water placed in a fermentation flask. The mixture was pre-incu-bated at 45°C for 10 minutes in a shaker.

One ml of this enzyme-reaction mixture combination was then transferred to a test tube containing 5 ml of Somogyi's reagent and 4 ml of distilled water and boiled for 20 minutes. Four ml of 2.5% KI solution and 1.5 ml 2N H_2SO_4 were added. The solution was titrated using N/200 $Na_2S_2O_2 * 5H_2O$. A blank consisting of 5 ml Somogyi's reagent and 5 ml distilled water was also run. When the test solution turned yellow, a few drops of starch solution was added and titrated to completion until a colorless endpoint was reached. The amount of reducing sugars was expressed in mg mannose/10 ml reaction mixture/ml enzyme solution/30 minutes (also an expression of mannanase activity). The formula used for the calculation was.

(ml N/200 $Na_2S_2O_3 \bullet 5H_2O$ (Blank) - ml N/200 $Na_2S_2O_3 \bullet 5H_2O$ (Test)) x Somogyi factor x $Na_2S_2O \bullet 5H_2O$ factor = mg mannose/10 ml reaction mixture/ml enzyme solution/30 minutes.

Determination of the Relative Vutritive Value (RNV) of Untreated and Mannanase-Treated Copra Meal

A Franklin–Baker processed copra meal was used in this study as substrate. The sample was ground and passed through a 0.2 mm mesh sieve. The % nitrogen was determined by the modified Kjeldahl method using a 0.2 gram sample, a small amount of catalyst (10 parts Na_2HPO_4, 10 parts K HPO and 2 parts $CuSo_4$) and 10 ml concentrated H_2 SO_4 as digestion mixture which was then distilled and titrated accordingly (AOAC, 1975). The analysis was conducted at the Food Sanitation Laboratory, IFST, UPLB. The same process was done on casein.

The assay procedure followed that of Stoot and Smith (1963) with some modifications. Based on the Kjeldahl analysis, samples were taken from the sieved copra meal and casein containing 15 mg nitrogen and placed into separate 100 ml beakers. These were extracted three times with 10 ml diethylether at room temperature, once with denatured ethyl alcohol and finally washed with ether to remove the residual alcohol. The extracts were then air-dried at room temperature. Twenty ml suspensions of these materials were prepared to give a nitrogen content of 3 mg/4 ml and the pH adjusted to 8.2.

Into three 20 x 160 mm screw-capped tubes were pipetted 2 ml of solution E, 4 ml of the test material suspension and 2 ml distilled water. The same was done with the casein suspension and the enzyme-hydrolyzed copra meal. The tubes were autoclaved at 121° C for 10 minutes. After cooling, 1 ml of sterile solution A diluted ten times, and 1 ml of sterile 15% glucose were aseptically added to each tube and inoculated with three drops of a 3-day old broth culture of *T. pyriformis W* ATCC 10542. These were incubated at 25° C for 4 days. The screw caps were left loose during this period and the tubes were inclined at 15 from the horizontal axis to provide sufficient aeration for rapid growth. Prior to this, an initial count was made on the 3-day old broth culture.

After 4 days, the cultures were shaken for 30 seconds in a test tube shaker and one ml of culture was transferred to a 16 x 160 mm screw-capped culture tube containing one ml preservative (90 ml distilled water, 20 ml 36% formaldehyde and 10 ml stock solution D).

Organisms were counted in a Tiefe Thoma hemacytometer. The organisms in 8 alternate one square mm were counted and the mean number per square mm multiplied by 2 gave the final population in units of 10 organisms/ml. The RNV's were then calculated using the formula derived by Helmes and Rolle (1970) expressed in percentages as follows:

$$RNV = \frac{\log (\text{count for test protein} \times 10^4) - \log (\text{count for inoculum} \times 10^2)}{\log (\text{count for casein} \times 10^4) - \log (\text{count for inoculum} \times 10^2)}$$

RESULTS AND DISCUSSIONS

Production of Mannanase Enzyme

Sufficient mannanase enzyme was produced by *Streptomyces* sp. in the Enzyme Production Medium which was verified by its activity. This shows that whole copra meal could be used instead of purified mannan for enzyme production.

Determination of Reducing Sugars

The crude mannanase extract produced above was used to hydrolyze the mannan in copra meal following the method of Somogyi. Table I shows the titration data and the computation of reducing sugars in terms of mg mannose/10 ml of reaction mixture/ml enzyme solution/30 minutes.

Table I. Average volumes of titrant at end point following titration of blank and sample.

SOURCE	VOLUME (ml) $Na_2S_2O_2 . 5H_2O$ USED
Blank	239.2
Sample	224.8

Note: (ml. Blank - ml. Sample (0.1399) (1.412) = 2.8445 mg mannose/10 ml reaction mixture/ml enzyme solution/30 minutes

Results showed a lower activity from *Streptomyces No.* 17 compared with that obtained by Takahashi et al. (1983) using extracted mannan from copra meal as substrate where they obtained a mannanase activity of 8.3 for *Streptomyces* No. 17. Since the substrate used was a whole copra meal, it may be that there was less mannan per volume of reaction mixture when copra meal was used than when pure mannan was utilized and therefore, lower concentration of substrate for the enzyme. Consequently, a lower amount of reducing sugars was produced. Another possible reason was that the mannan was bound to other organic substances in the copra meal hindering enzymatic hydrolysis.

Determination of the Relative Nutritive Value of Untreated and Treated Copra Meal with Mannanase

The % nitrogen of the copra meal and casein was first determined using the mo
fied Kjeldahl Method. Table 2 shows the titration data of the distillates with 0.0
N HCl.

Table 2. Average titration values for copra meal and casein by Kjeldahl analy

SOURCE	VOL. OF HCl USED (ml)	MEAN VOL. USED (m
Blank	0.05	0.05
1	0.05	0.05
2		
Poonac	0.35	0.325
1	0.30	
2		
Casein	0.50	0.50
1	0.50	
2		

From Table 2 the % nitrogen of copra meal and casein was computed based c
the following formula:

$$\% N = \frac{(\text{ml Sample} - \text{ml Blank})\ (\text{Normality of HCl})\ (0.014)}{(\text{g. Sample used})\ (\text{ml Digest/ ml after dilution})}$$

$$\%N \text{ Casein} = \frac{(0.50 - 0.05)\ (0.0776)\ (0.014)}{0.2\ (5/100)}$$

$$= 4.8888$$

$$\%N \text{ Poonac} = \frac{(0.325 - 0.05)\ (0.0776)\ (0.014)\ \times 100}{0.2\ (5/100)}$$

$$= 2.9876$$

From these figures, the weights of copra meal and casein were computed which
contained 15 mg of nitrogen to be used in the RNV experiment as follows:

Copra Meal

2.9876% of 0.2 g = 0.0059752 g N/0.2 g poonac

5.9752 mg N/200 mg poonac

15 mg N/5.9752 = 2.5103

2.5103 x 200 mg = 502.0752 mg poonac containing 15 mg N

Casein

4.8888% of 0.2 g = 0.0097776 g N/0.2 g casein

= 9.7776 mg N/200 mg casein

15 mg N/9.7776 = 1.5341

1.5341 x 200 mg = 306.8238 mg casein containing 15 mg N

Table 3 shows the hemacytometer counts of *T. pyriformis* W in the 3-day old broth inoculum, with casein, untreated copra meal and enzyme-treated copra meal as test materials.

Table 3. Mean hemacytometer counts of *T. pyriformis* W.

SOURCE	FINAL COUNT 2mm x 10^4 *
Inoculum	1.08
Casein	4.25
Untreated copra meal	2.00
Enzyme-treated copra meal	2.08

From Table 3, the RNV's for the untreated and enzyme treated poonac were computed as follows:

$$\text{RNV Untreated Copra Meal} = \frac{\log (2.00 \times 10^4) - \log (1.08 \times 10^2)}{\log (4.25 \times 10^4) - \log (1.08 \times 10^2)}$$

$$= 46.48\%$$

$$\text{RNV Enzyme Treated Copra Meal} = \frac{\log (2.08 \times 10^4) - \log (1.08 \times 110^2)}{\log (4.25 \times 10^4) - \log (1.08 \times 10^2)} \times 100$$

$$= 49.27\%$$

*. 10^2 for the inoculum

Results showed that the RNV of the enzyme-treated copra meal was higher by 2.79% than that of the untreated copra meal. As observed during the count, many cells in the tube containing the enzyme-treated poonac were lyzed by still undetermined factors. No lysis was observed in other tubes.

SUMMARY AND CONCLUSION

In summary, mannanase enzyme was produced by *Streptomyces* sp. grown in the Enzyme Production Medium. The amount of mannose produced per 10 ml of reaction mixture/ml of enzyme solution/30 minutes was 2.8445 mg from 129.4 mg of copra meal analyzed by the Somogyi method. The tentative nutritive value of untreated copra meal using *T. pyriformis* W was 46.48% and that of the enzyme-treated was 49.27% which showed an increase of 2.7% after enzyme treatment. This may be due to the release of bound protein that serve as additional protein source for the post organism.

Although a low mannanase activity was obtained, this study is significant in that it is shown that whole copra meal could be used as direct substrate for mannanase. This level of activity was comparable to that of *Streptomyces No.* 319 and 386 as determined by Takahashi et al.(1983) using copra mannan as substrate. The RNV results are significant in that mannanase treatment improved the nutritional quality of copra meal. The RNV of enzyme-treated copra meal would have been higher had not some *T. pyriformis W* cells lyzed. A means of eliminating the causative factor(s) should therefore be devised to prevent lysis of cells if a similar study will be conducted in the future.

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