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Preparation and Biological Evaluation of Weaning Food Consisting of Rice and Sludge Protein Isolate

N.M. Ahmad, N. Bhatty, K. Almas and A.R. Abid*
Department of Home Economics, *Department of Animal Nutrition,
University of Agriculture, Faisalabad-38040, Pakistan

Abstract

Stillage sludge, a by-product of sugar industry, was subjected to proximate analysis. Its protein quantity and quality was improved through fermentation with *Brevibacterium flavum*. A protein isolate was prepared from it. Its proximate analysis was carried out to know its nutritional potential. The amino acid profile shows that it is limiting in threoninc. The chemical score of protein isolate thus found to be 36.42. The quality of its protein was evaluated in terms of protein efficiency ratio (3.92), net protein utilization (71.27), digestibility (96.97) and biological value (73.00) by conducting a feeding trial on weanling albino rats.

Introduction

The role of protein in human nutrition is well established for being an essential constituent of human diet. The diet of Pakistani people is invariably unbalanced and grossly deficient in protein of animal origin. According to a survey conducted in the Punjabi, it has been estimated that per capita consumption of animal protein is 16.3 grams as against the minimum recommended allowance of 27.4 gram. (Anonymous, 1987). This may be due to uneven distribution of this nutrient among the vulnerable group. It is therefore, inevitable to increase the production of this essential nutrient. In addition to conventional sources of protein, the non-conventional sources had to be exploited to meet the ever increasing demand of it. The efforts are being made to solve this problem by using non-conventional sources such as stillage sludge. It is produced as a result of fermentation of cane molasses with *Saccharomyces carevisiae* which is rich in protein but it is limiting in lysine content (Basit, 1997). Thus it needs to enrich with respect to lysine through fermentation with *B. flavum* (Yawar, 1998).

Mother milk is considered to be the best for infants upto the age of six months. After this age the introduction of semisolid food becomes essential. Single cell yeast is a better source of protein than vegetable and can be available at lower cost.

It is therefore, exceedingly challenging and worthwhile to introduce yeast protein-isolate in weaning food with following objectives.

- Preparation of protein isolate from biomass.
- Biological evaluation of weaning food consisting of protein isolate and rice.

Materials and Methods

The present study was carried out to prepare a weaning food consisting of rice and protein isolate of fermented sludge. Stillage sludge was obtained form Shucker Gung Sugar Mills Ltd., Jhang. It was subjected to proximate analysis to know its inherent nutritional potential.

Biomass production: Enrichment of stillage sludge was carried out with lysine through fermentation with Brevibacterium flavum (Yawar, 1998). Biomass was dried in an oven at 100°C for one hour and then at 60°C to constant weight. The dried biomass was ground and subjected to chemical analysis following AOAC (1990) methods. Lysine contents of the stillage sludge and biomass was determined (Chaves *et al.*, 1988).

Protein isolation: The biomass thus produced was subjected to protein isolation by modifying the method of Basit (1997). Then it was subjected to proximate analysis. Amino acid profile (Moore and Stain, 1954) and RNA contents (Munro and Fleck, 1966) of biomass and protein isolate were determined. Nitrogen (%) of the sample was estimated by micro Kjeldahl method (Hiller *et al.*, 1948). The nitrogen (%) was multiplied with 6.25 to obtain crude protein percentage. The estimation of true protein was carried out by the method of Munro and Fleck (1966).

Preparation of weaning food: Weaning food was prepared by supplementation of protein isolate with rice to increase the protein contents. The composition of diets are shown in Table 1. The diet (A) was served as control diet which contained skimmed milk. The diet (B) was served as experimental diet. The diet C was served as protein free.

Biological evaluation: The protein quality of protein isolate was evaluated by conducting a feeding trial on weanling Albino rats following the method described by Pellet and Young (1980), for this purpose, 16 albino rats (21 days old) was weaned. They were fed on stock diet for one weak prior to the experiment and then divided at random into four groups of four rats each, with a weight difference of ±5g. Each group of rats was weighed prior to the feeding trial and thereafter daily. At the end of the feeding trial, the rats

was anaesthetised with over dose of chloroform. Their cranial and abdominal cavities was opened. Each carcass groups as weighed and dried at 110°C to a constant weight. The carcass was opened and analyzed through Kjeldahl method (Hiller *et al.*, 1984). The quality of protein of diets was evaluated in terms of digestibility percentage, net protein utilization (NPU), biological value (BV) and protein efficiency ratio (PER) following the method of Pellet and Yong (1980).

Table 1: Composition of Experimental Diet.

	Diets		
Ingredients	A	B	C
Biomass protein	-	12.0	-
Skimmed Milk	20.0	-	-
Rice	-	63.0	-
Corn Starch	55.1	-	75.0
Soybean Oil	5.0	5.0	5.0
Glucose	10.0	10.0	10.0
Potato starch	5.0	5.0	5.0
Vitamin mixture	2.5	2.5	2.5
Mineral mixture	2.5	2.5	2.5
	100.0	100.0	100.0

Results and Discussion

Chemical composition of stillage sludge: The chemical analysis of the sludge is given in Table 2. Which reveals that it contains 19.68 per cent crude protein. It contains high concentration of ash (21.50 %), a minor percentage of crude fat (0.4 %) and quite considerable level of nitrogen free extract. Percentage of crude fibre is zero.

Chemical Composition of Biomass: The chemical composition of biomass is given in Table 2 which shows that it contained 28.437 per cent crude protein, ash (22 %), crude fat and fibre 0.5, 0.0 respectively and level of nitrogen free extract decreased. RNA contents was found to be 1.955 as compared to of stillage sludges.

Estimation of lysine contents: Lysine contents of the biomass and stillage sludge are shown in Table 3. It reveals that *Brevibacterium flavum* has sufficient potential to enrich the lysine content of biomass within 3 days fermentation with 2.5 times increase of lysine concentration of the biomass, its deficiency with respect to that essential amino acid has been recovered.

Protein isolation: Biomass protein was isolated through isoelectric precipitation by adjusting isoelectric point. The per cent protein isolated was then determined using Kjeldahal method. Based on that observation and inferences the procedure for protein precipitation was then developed by solubilizing protein in alkali solution and then adjusting its pH to isoelectric pH (4.0) with 1 N H_2SO_4. By this method a protein isolate containing 40 per cent protein was obtained.

Results of present study are in line with the work of Hashmi (1987) who prepared protein isolate from biomass of rice husk and rice polishings. He obtained protein isolate containing 37.6 per cent protein.

Table 2: Chemical analysis of the stillage sludge, biomass and protein isolate on dry weight basis.

Components	Percentage (Sludge)	%age biomass	%protein Isolate
Protein	19.68	28.44	40.00
Ether extract	0.40	0.50	0.35
Crude fibre	0.00	0.00	0.00
Ash	21.50	22.00	21.00
Nitrogen free	58.42	49.06	38.65
RNA Contents	1.88	1.95	2.03
True protein		25.60	32.80

Table 3: Lysine content of stillage sludge and biomass

Ingredients	Concentration mgs/100 m
Stillage sludge	300.00
Biomass	700.00

Amino acid analysis: Amino acid profile of protein isolate shows that almost all amino acids were present in protein isolate. The chemical score of protein isolate was 36.42. However threonine was the first limiting amino acid in protein isolate.

Biological evaluation: The quality of protein isolate obtained from the biomass was evaluated by conducting a trial on feeding weanling Albino rats. Following the method of Pellet & Young (1980). The quality of protein was expressed in terms of following parameters.

Weight gained: The average weight gained per group of rat fed on skimmilk and protein isolate diet was 28 and 29. respectively after 10 days of feeding trial. There was two groups of rats on diet (B) (protein isolate) whereas protein free diet group lost 41 g during the trial.

Feed consumption: Rats fed on skimmilk consumed (329.5g) feed and while rats of protein isolate consumed (330 g and 332 g) feed respectively. While the least feed was consumed by rats fed on protein free diet.

Biological value: The data obtained on biological values of controlled and experimental diets are shown in Table The average biological value for diet A and B were 72. and 73 per cent respectively. There is difference of 6. units between the diets. The results are supported Roskhova (1979) who tested the protein quality of biomass produced with candida strains 2672 M and 2673 M grown on acetate and methanol respectively using weanling ra The biological values of strains were found to be 68.4 and 69.12 per cent respectively.

Table 4: Average digestibility, net protein utilization, biological values and protein efficiency ratio of diets with skimmilk land with rice based protein isolate.

Diets	Digestibility %	Net protein utilization %	Biological value %	Protein efficiency ratio %
Skimmilk diet (A)	96.40	76.34	79.20	4.00
Rice based protein isolate diet (B)	96.97	71.27	73.00	3.92

Protein efficiency ratio (PER): The table shows the average protein of standard and test diets shown in Table 4. Average protein digestibility skimmilk diet and rice base protein diet were 96.40 and 96.9 per cent respectively. The results of present study are in line of Enriches and Rodriquez (1983) who obtained biomass containing 58.0 per cent protein from alkali treated bagasse fermented with cellulomonas. Feeding trial on weanling albino rats gave 90 per cent digestibility.

Net Protein Utilization (NPU): The data on NPU for skimmilk diet (A) land experimental diet protein isolate (B) show the average NPU value for diet and B were 76.34 and 71.27 per cent respectively.

The results of present study are still better than Enliquez and Rodriquez (1983) who fermented alkali treated bagasse with cellulomonas and observed net protein utilization 56 per cent.

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