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Conversion of Fruit Waste into Protein-Rich Biomass by Fermentation

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Abstract. Microbial degradation of fruit waste was done to explore the potential of fruit wastes to produce digestible protein biomass for incorporation in the animal feed. Citrus waste (CW) and mango stones (MS) were selected for upgrading their nutritive values. Selected strains of the fungus (*Aspergillus oryzae*) were used to breakdown the substrate (fruit wastes) from the complex cellulosic matter into protein-rich biomass, which resulted in the production of 0.593 kg/kg biomass with 28.9% protein (MS), and 0.576 kg/kg biomass with 22.4% protein (CW). Qualitative evaluation of the fermented biomass protein showed a rich source of amino acid profile. The results show that microbially degraded fruit waste can be used as a supplement feed for animals. Nutritional and toxicological studies on monogastric animals (albino rats) showed no adverse effects on their digestive system, which exhibited 66% and 83% fertility in their teratogenic cycle.

Keywords: citrus waste (CW), mango stones (MS), normal basal diet (NBD), *Aspergillus oryzae,* fruit waste biodegradation, fruit waste fermentation

Introduction

The possibility of upgrading non-conventional agricultural sources, such as food and fruit industry wastes into protein-rich biomass has been reported (Hussain and Murtaza,1987). Cellulosic wastes, generated from the agricultural and food processing industries, have also been reported to have the potential of conversion into animal feeds and other valuable products (Aregheore, 2000). Pakistan being an agricultural country produces 5.0 million tons of fruits and 2.0 million tons of vegetables annually. Mango, citrus, apple, banana and dates are the highest yielding fruits in Pakistan (Pakistan Statistical Year Book, 2004). A number of fruit processing industries generate about 9,45,832 tons of citrus and 38,024 tons of mango waste, which is approximately 40-50% of the respective freshly harvested fruits.

A number of local cellulose decomposing fungi have been isolated and identified by the researchers of PCSIR Laboratories Complex, Karachi, Pakistan. During the screening of the fungi, *Aspergillus oryzae* Fungus was noted to be the efficient cellulose degrading fungus, and was used in during the present studies for the conversion of mango and citrus industry wastes into protein-rich biomass with considerable amount of residual sucrose and carbohydrates. Digestibility of these biodegraded wastes and their toxicological effects were also evaluated during feed trial on albino rats.

Materials and Methods

Substrate materials. Fruit waste samples of mango stones (MS) and citrus waste (CW) comprising peel/pulp of different kinds of citrus fruits were collected from the fruit processing industries. The samples were oven-dried at 55-60 °C, powdered and analyzed for chemical composition using standard methods (AOAC, 2000).

Mango stone (MS) is a hard and woody structure, therefore, the powdered sample was first treated with boiling 1 N HCl (1 : 4 ratio) for 30 min and neutralized prior to fermentation. Based on the optimum mass of the fruit wastes (10% for MS and 20% for CW), other conditions such as pH and the incubation period were standardized for maximum yield of the proteinous biomass.

Fermentation organism. Cultures of *Aspergillus oryzae* were maintained on malt extract and potato dextrose agar slants (Merck; Difco), and the fungus was used for the conversion of fruit waste cellulose into protein-rich biomass.

The standard nutrient medium (Kim and Hamdy, 1985) was modified for fruit waste fermentation by incorporating 5-10% MS and 10-20% CW as the carbon source. The other nutrient medium constituents included 1% KH_2PO_4, 0.5% NH_4Cl and 0.5% yeast extract, with pH adjusted at 5 and 6, respectively, for the MS and CW fermentation.

Batch-scale studies. Batch-scale studies were carried out in accordance with the procedure reported earlier (Radia *et al.*, 1992). One kg material each of MS and CW was separately added to 200 ml nutrient medium and autoclaved. Each batch consisting of 25 flasks was inoculated with fungal inoculum, having approximate spore count of 9000 to 10000 per ml (Feng *et al.*, 2000). Each flask was inoculated with 10 ml inoculum and incubated at 30-32 °C for 5 days. The fermented material,

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on the completion of incubation period, was drained, dried at 60 °C, powdered and chemically analyzed (AOAC, 2000). The nitrogen content was estimated by Kjeldahl method and the crude protein contents were calculated using 6.25 as the multiplying factor. Ash content was determined after the biomass incineration at 600 °C for 2 h in a muffle furnace. Fat was extracted with petroleum ether (60 °C) using Soxhlet. Fibre and organic matter were evaluated by the standard methods (AOAC, 2000; Albrechtsen and Winding, 1992). Data were analyzed according to the standard deviation method (Gorow, 1962).

Pre-pilot scale production. The observations obtained on batch-scale studies, which showed enhancement of proteinous biomass (upto 28.9% MS and 22.4% CW protein) were extended to pre-pilot scale studies of 30 litre capacity. Specially fabricated rectangular aluminium trays, 23 inch x 23 inch x 54 inch with cover having four openings for inoculation, were used for this purpose. MS and CW were separately fermented. Each batch consisted of 3 kg biomass of the respective waste material in 30 litre fermentation medium and subdivided into batches of three litre per tray in 10 trays, separately for each fruit waste. After completion of the incubation period of 5 days, the biodegraded wastes were harvested, vacuum filtered, dried, weighed and analyzed for chemical composition.

Toxicity and digestibility. Digestibility, toxicity and teratogenic effect of the fermented biomass were determined during feeding trials on normal healthy albino rats. The animals kept on normal basal diet (NBD) for one week, which comprised of bread and milk, were divided into four **groups A, B, C and D**. Each group comprised of 12 animals, weighing 92 to 120 g and categorized by sex (6 males and 6 females in each group). **Group A** was further divided into **groups A_1, A_2 and A_3**. Toxicity and digestibility were determined by feeding the CW and MS biodegraded biomass diet to these groups in three different doses of 5, 10 and 20 g per kg body weight, incorporated in the NBD. **Group B** received only NBD and served as the control. Animals of **groups C and D** were fed on 100% raw fruit wastes and their fermented biomass, respectively. The rats of all groups were kept under observation for 15 days.

Teratogenic evaluation. Virgin female and male rats were divided into four **groups A, B, C and D** as described in the toxicity and digestibility test. **Group A** animals were sub-divided in **groups A_1, A_2 and A_3** and received the experimental diet (CW and MS biodegraded diet plus NBD), comprising of three doses of 5, 10 and 20 g per kg body weight, respectively, for a period of 30 days. The male and female animals were allowed to mate during this period. After 30 days, the **group A** animals were further divided into **two groups: a and b**. The experimental diet was withdrawn from **group 'a'** and were maintained on NBD only, whereas **group 'b'** animals continued to receive the experimental diet. The animals of all the four groups were kept under observation for 42 days and were mated in the same manner.

Results and Discussion

The abundantly available fruit wastes of mango stone (MS) and citrus waste (CW) were used as the fermentation substrate and analyzed for pH, organic matter, protein, fat, carbohydrates, calories and fibre (Table 1). Due to the lignocellulosic characteristics of MS, which restricted the growth of *A. oryzae*, the MS was hydrolyzed with 1 N HCl for 30 min (Kim and Hamdy, 1985).

In each set of the batch-scale studies, 0.5 kg **(A)**, 1.0 kg **(B)**, 1.5 kg **(C)** and 2.0 kg **(D)** of MS (5 and 10 % concentration) were placed separately in 4 trays. The samples were inoculated with the specific culture medium of *A. oryzae*, having a potential for biodegradation of complex cellulosic materials into simple carbon compounds and energy, which resulted in rapid cell growth of proteinous biomass (Essien *et al.*, 2005).

Table 1. Proximate composition (%) of raw citrus waste and mango stones

	Citrus waste	Mango stone
pH	3.5	5.5
Organic matter	95.2	96.3
Protein	6.0	6.6
Fat	7.0	10.4
Carbohydrates	82.3	79.8
Fibre	13.5	19.5
Energy/100 g (kcal)	417.0	436.0

Table 2. Effect of different volumes* of media on the production of fermented biomass of mango stones** and the protein content during batch scale study

	Batch***			
	A	B	C	D
Fermented biomass obtained (kg)	0.30	0.87	0.63	1.11
Protein content (%)	32.8	29.5	30.5	28.0
Protein yield per tray (kg)	0.0984	0.263	0.191	0.311

* = volume of medium used in **batches A and C** (10 litre), and **batches B and D** (20 litre); ** = **batches A, B, C and D**, respectively, contained 0.5, 1.0, 1.5 and 2.0 kg of mango stones; *** = **batches A and B** contained 5% and **batches C and D** contained 10% mango stone

Large production of biomass and high protein contents were respectively observed in **batches A** (0.30 and 0.0984 kg), **B** (0.87 and 0.263 kg), **C** (0.63 and 0.19 kg), and **D** (1.11 and 0.311 kg) (Table 2). These encouraging results in terms of better yield of biomass, rich in protein, led to further scale-up studies upto pilot scale. During these studies, 3 kg raw material of MS and CW were spread separately in 5 trays per batch. Average production of biomass was nearly the same in two cases (1.78 kg for MS and 1.73 kg for CW). Protein enhancement was found to be 28.9% for mango stone fermented biomass (MSFB) and 22.4% for citrus waste fermented biomass (CWFB) as shown in Table 3. Worgen (1971) and Zeng (1994) reported that microbial degradation of fruit wastes by fungal strains yields a good source of protein for livestock. Biological fermentation technique has been also reported to improve the protein yield of the substrates (wastes) upto 15-20% (Forage and Righelato, 1979). The present study confirms these findings, viz., improving the protein content of fermented biomass from 6 to 22.4% for CW and 6.6 to 28.9% for MS.

Table 3. Pilot scale production of fermented* mango stones and citrus waste** for fermented biomass and protein enhancement

Mango stones	I	II	III	IV	V	Average***
Protein (%)	26.0	29.0	31.5	29.0	29.2	28.9
Total protein (kg)	0.437	0.566	0.646	0.435	0.511	0.519
Total biomass (kg)	1.68	1.950	2.059	1.500	1.759	1.780
Citrus waste	I	II	III	IV	V	Average
Protein (%)	22.8	22.2	22.0	20.9	24.2	22.4
Total protein (kg)	0.332	0.428	0.379	0.386	0.375	0.380
Total biomass (kg)	1.6	1.93	1.724	1.85	1.55	11.730

* = fermentation organism: *Aspergillus oryzae*; ** = mango stones/ citrus waste used for fermentation: 3 kg; *** = five pilot-scale trials were conducted

Table 4. Amino acid profile of protein of fermented* citrus waste and mango stones

Amino acid	Citrus waste (µmole/g of biomass)	Mango stones (µmole/g of biomass)
Aspartic acid	17.02	52.56
Threonine	18.78	22.08
Glutamic acid	191.2	90.4
Glycine	38.01	42.98
Alanine	37.78	40.19
Methionine	2.17	2.08
Histidine	26.64	29.37
Lysine	22.36	21.6

* = fermentation organism: *Aspergillus oryzae*

The comparative study for the production of CWB and its protein contents using 10% and 20% raw material was carried out in two types of fermentors, namely, conical flasks and trays. The experimental results revealed that wider and uniform surface of trays improved protein content, whereas tapered conical flasks with small surface area caused hindrance in the enzymatic reactions. No significant difference was observed in the production of CWB (0.610 kg and 0.690 kg) and their protein contents (16% and 15.6%) when using 10% and 20% CW in conical flasks. In tray type fermentors, the yield of CWB was less (0.576 kg) with high protein (27%) when 10% concentration of CW was used, whereas 20% CW yielded high biomass (0.737 kg) with low protein (19.8%). Due to the higher protein content of CWB, 10% raw CW (3 kg) was selected for pre-pilot scale studies in the tray type fermentor.

The qualitative evaluation of protein obtained from these fruit waste biomass showed a good amino acids profile (Table 4). It is documented that the deficiency of certain essential amino acids (arginine and tryptophan) affected the growth of small animals, as they were highly sensitive and prone even to the slightest change in their diet and caused adverse effects (Anon., 1977).

Nutritive value of the diet prepared from proteinous biomass of fruit wastes was evaluated on the basis of toxicity, digestibility and teratogenicity of albino rats. The studies revealed the experimental diet containing 5, 10, 20 g of MSB plus NBD per kg body weight, fed to **group A** animals **(A_1, A_2, A_3)**, was found to be non-toxic and showed 83% digestibility, almost equal to **group B** animals (control), which showed digestibility of 83.6%, while the animals of **groups C and D**, using 100% MS and MSB, exhibited low effectiveness in maintaining the nitrogen balance. They showed less digestibility, which was 70% and 75%, respectively. However, no marked changes were observed in their behavioural pattern as compared to animals of **groups A and B**. Similar studies for CW and CWB showed that the experimental diet used for **group A** animals **(A_1, A_2, A_3)** was also non-toxic. Digestibility in this experimental work was determined in terms of weight gain and weight loss of animals. About 9% weight gain was recorded in animals consuming 5 g CWB, while the increase in doses resulted in the reduction of weight, which was 4.5 and 2.5% (consuming 10 and 20 g diet), respectively. However, the animals of **groups C and D** also showed 1.5% reduction in their body weight. Animals were inactive, dull and drowsy, as compared to control animals, and also exhibited relatively high mortality (80%).

The teratogenic evaluation for the fertility of animals of **group A (A_1 and A_2**, using the experimental diet) showed 66% fertility in the case of MSB diet and 83% fertility with CWB diet, while the animals of **group A_3** exhibited 40 % and 55%

fertility using MSB and CWB diet, respectively. The only difference observed between the control and test animals was the time taken to deliver the pups, which was 27 to 30 days earlier in the test animals. Litters born in **groups C and D**, using 100% fruit wastes and their fermented biomass, were too weak as compared to controls and 80% mortality was observed in animals consuming 100% CW and CWB as their diet.

Conclusion

In the light of above discussion it is concluded that albino rats can use the degraded fruit waste (biomass) safely with NBD 20%, while feeding on unfermented biomass of the wastes as such (100%) exhibited low effectiveness in maintaining nitrogen equilibrium as required for their growth (Weisman and Cole, 1988). The deficiency of some essential amino acids may also affect the growth of small animals. As the fermented biomass, being the vegetable protein, does not generate high biological value, and thus when fed alone exerts toxicity. It is, therefore, necessary to improve its nitrogen balance by supplementing the biomass with direct inclusion of amino acids or the additional animal protein, such as milk and casein in their diet, for improvement in their nitrogen balance.

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