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AGRICULTURAL AND FOOD PROCESSING WASTES AS POTENTIAL SUBSTRATES IN MICROBIAL PROTEIN PRODUCTION: CHEMICAL ANALYSIS

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ABSTRACT

A number of agricultural and food processing wastes were analyzed in order to determine their potential as substrates for microbial cell production. Potential was evaluated in terms of the following parameters: % crude protein, % neutral detergent fiber, % cellulose, % crude fat, % lignin, and gross energy value. The high lignin content of a good number of these materials indicates that the lignocellulose complex may have to be broken down in a chemical step before these can effectively function as a carbon source during the fermentation process leading to the production of microbial proteins.

INTRODUCTION

The prohibitive cost of imported fish meal and other protein sources for use in animal feeds has greatly hampered meat and poultry production in the Philippines. As a result, the prices of these food products have been rising. The search for other sources of proteins is therefore of utmost importance to the development of the local livestock industry. Microbial proteins (MBP) are one such source. These are obtained by allowing microorganisms to grow on the inexpensive, non-proteinaceous materials contained in agricultural and food processing wastes.

There is an abundance of agricultural wastes in the Philippines. Annually, approximately 500 x 10^3 metric tons of hull and straw are discarded in connection with rice production (1). Corn stalks comprise the wastage of corn production (3,249 x 10^3 metric tons/annum), and huge amounts of bagasse are left of the approximately 22,352 x 10^3 metric tons of sugarcane milled annually (2). In the production of coconut coir fiber, 8 tons of dust are produced for every ton of fiber manufactured. Then there are the leaves and stems of the water hyacinth plants that grow abundantly in many localities, clogging rivers and other waterways.

A waste product readily available to the livestock owner is manure. Daily, 10 hogs can yield approximately 26 gallons of liquid (3.5 cu ft solid) manure while 10 chickens can produce approximately 300-500 grams (3).

In addition, innumerable waste products can be obtained from food processing plants. It has been reported that one food-preserving plant alone annually discards 100-200 kilos of mango peels (4). While no figures are currently available, it is known that coconut husk, coconut expeller residues (*sapal*) and coconut water abound in any market area. In Mindanao, banana fruit rejects make up 215,123 metric tons (5). Pineapple processing plants discard the shell, cuts, and pressed pulp which constitute 40% of the fruit. Dole Philippines, Inc. alone generates aproximately 155,000 tons of pineapple wastes (6). Manufacturers of monosodium glutamate (*Ajinomoto*) and soybean noodles (*Canton*) discard processing liquors.

Reported here are the partial results of an investigation of the potential of agricultural and food processing of wastes as substrates for microbial cell production.

EXPERIMENTAL SECTION

Sample preparation

Solid samples were air-dried and rotor-milled to 60 mesh in a Retsch Rotor Beater Mill before analyses.

Analysis

Liquid samples were analyzed both as received and as residues obtained after the removal of water. Moisture was determined with an Ohaus Moisture Determination Balance. A 10-g sample was used for each determination. The dried samples were then divided into four parts for subsequent assays.

Crude protein was determined by the Kjeldahl method (7) in a Buchii 425 automatic nitrogen analyzer and Buchii 320 distillation unit with HgO as catalyst.

Fat (ether extract) was analyzed in a Rafatec Extractor. For fat analysis of liquid substrates, the residues left after moisture determination was used.

Fiber content was determined by use of a Fibertec hot extractor model 1020 and Fibertec cold extractor model 1021 according to the method of Van Soest (8). In this method, acid detergent fiber (ADF) of a one-gram sample was first determined. Lignin, cellulose and silica content were successively determined following repeated extractions of another one-gram sample with appropriate solvents.

Gross energy was determined in a Parr plain jacket oxygen bomb calorimeter model 1341. The values obtained were not corrected for sulfur content.

Calcium was measured by atomic absorption spectrophotometry with a Varian model AA-275 atomic absorption spectrophotometer. The samples were digested in HNO_3-$HClO_4$ prior to analysis (9).

Phosphorus was determined photometrically by use of a Perkin Elmer UV-Vis spectrophotometer model 551. Samples were prepared by ashing at 600°C (10).

RESULTS AND DISCUSSION

The results of the chemical analysis of the various agricultural and food processing wastes are summarized in Table 1. It should be recognized that total nitrogen content is of prime importance in the selection of substrates for the production of microbial cells. The crude protein assay gives a fair estimate of total nitrogen content. However, it should be noted that crude protein content can be correlated only with the amino nitrogen present in the sample. It can be seen that among the agricultural wastes, water hyacinth leaves and chicken manure have the highest crude protein content. The stems of water hyacinth are moderately rich in protein, while corn stalk, coir dust, and rice straw contain moderate amounts of this nutrient. Nevertheless, the latter are promising substrates because of their abundance while the rest of the agricultural wastes, particularly rice hull and bagasse, are rather poor protein sources and would therefore need high nitrogen enrichment before they can be utilized as substrates. Of the food processing wastes, *ajinomoto* waste liquor has the highest crude protein content. This is to be expected because *ajinomoto* is essentially monosodium glutamate, an organic nitrogen compound. Pineapple wastes, soybean extracts, banana rejects and mango peels contain fair amounts of crude protein, in view of which their potential as substrates for microbial protein production should be further investigated.

Except for coconut expeller residue (*sapal*) which has a high oil content, the fat content (expressed as ether extract) of all substrates is low. Low fat content is a factor considered in the selection of

TABLE 1. CHEMICAL ANALYSIS OF AGRICULTURAL AND FOOD PROCESSING WASTES.[a]

Substrate	Crude Protein %	Ether Extract %	NDF[b] %	Lignin %	Cel-lulose %	Silica %	Ca %		Gross Energy Kcal/kg	Moisture %
A. Agricultural										
Water hyacinth leaves	28.61	2.49	53.17	5.00	16.18	0.58	1.91	0.10	3.55	87.92
Fermented chicken manure	22.97	1.12	33.78	5.96	16.04	2.03	1.06	1.86	2.88	14.95
Water hyacinth	8.37	1.36	55.00	5.20	27.90	1.81	2.08	0.69	2.89	93.25
Corn stalk	5.15	2.50	57.61	5.27	25.12	4.94	0.85	0.31	3.65	48.50
Coir dust	5.09	0.18	58.02	21.91	15.94	30.02	0.91	0.15	2.28	8.70
Rice straw	4.19	0.84	58.93	7.69	31.80	20.11	0.35	0.08	3.01	8.90
Camote, peels only	3.16	1.58	47.80	5.89	9.04	1.67	1.64	0.27	3.21	84.40
Coconut husk	3.13	0.36	78.24	16.02	28.81	0.51	0.37	0.13	4.15	8.37
Cassava, peels only	2.77	1.23	40.78	8.86	10.75	8.25	1.64	0.07	3.18	72.63
Cassava	2.60	1.33	48.25	2.02	4.14	0.29	0.48	0.15	3.62	60.80
Camote	2.42	1.64	52.20	1.23	3.15	0.51	0.20	0.20	3.97	65.83
Rice hull	2.37	0.16	68.65	6.14	33.98	15.59	0.28	0.03	3.30	6.20
Sugarcane bagasse	1.26	0.48	77.55	9.44	39.18	1.82	0.40	0.02	3.47	9.12
B. Food Processing										
Ajinomoto processing waste[c]	34.06	0.62	0.13	0.09	-	0.08	2.91	0.27	-	35.00
Sapal	13.76	35.49	75.24	22.70	23.83	0.39	0.38	0.13	6.45	64.46
Banana rejects	7.06	2.17	31.21	3.66	5.88	0.47	0.22	0.10	3.32	87.00
Coffee grounds	7.01	0.87	37.72	11.20	16.22	0.23	0.63	0.18	3.41	15.73
Peanut shell	6.80	0.30	74.98	11.19	33.72	6.16	0.23	0.08	3.78	8.30
Pineapple waste	6.76	2.32	42.50	3.02	21.45	1.19	0.43	0.14	3.69	16.70
Soybean extract[c]	4.80	2.84	6.06	1.13	2.51	0.13	0.10	0.04	0.84	84.80
Mango peels	4.34	1.27	35.42	8.26	34.25	1.78	0.50	0.15	3.72	83.70
Coconut water[c]	0.08	0.04	1.82	0.29	0.11	0.012	0.03	0.008	0.23	94.28

[a] All analyses were carried out on a dry basis.
[b] NDF refers to Neutral Detergent Fiber.
[c] Liquid samples; data pertain to analysis of dry residue

appropriate strains for microbial cell production.

True dietary fiber consists of a carbohydrate portion made up of such structural polysaccharides as cellulose, hemicellulose and pectin and a noncarbohydrate portion consisting mainly of lignin and cutin. The determination of crude fiber content was not carried out because the acid/alkali treatment involved destroys 80% of the hemicellulose, 50-90% of the lignin and 20-50% of the cellulose. Instead, neutral detergent fiber (NDF) was determined. NDF values are better estimates of true dietary fiber. In feed formulations, these values are more significantly correlated with digestible energy and metabolizable energy. At present, however, only the gross energy values of the substrates can be obtained and these cannot be related to NDF.

Lignin content is of utmost importance in the selection of substrates. A high lignin content constitutes a major drawback in cellulosic waste recycling: it retards the microbial degradation of cellulose. Of the waste materials investigated, coconut waste products have the highest lignin content. However, lignin is also present in considerable amounts in most of the other materials. In view of this problem, chemical pretreatment (i.e., prior to fermentation) may be necessary in order to break down the lignocellulose complex.

Cellulose content is a rough estimate of the carbohydrate content of the various waste materials. It also represents the amount of carbon (in the form of glucose, its hydrolytic product) available for microbial use. The results of cellulose analysis show that practically all of the substances have a high cellulose content. Cellulose degradation into glucose may be carried at by chemical or microbial processes.

The presence of silica in feedstuffs is objectionable. While ruminants reportedly can tolerate a small amount of silica, other animals cannot. Thus, microbial cells grown on the substrates under investigation should contain a minimal amount of silica if these are to be used as animal feeds. In this context, the large amounts of silica present in coir dust, rice hull and rice straw must be removed by chemical pretreatment prior to fermentation. Such pretreatment processes should remove the silica, possibly as soluble silicates, in the most economical way.

Gross energy was determined as the heat of combustion per gram substrate. The energy values of the various substrates may later be compared with the heats of combustion of the fermented end products which are to be used as animal feeds. These figures may be used in the assessment of food value, but only if they are considered in relation to digestible, metabolizable, and ultimately, net energy.

Coir dust is an interesting substrate. Huge amounts of this waste material are readily available. It contains fair amounts of crude protein. However, its lignin and silica content are quite high, a factor that could impede microbial degradation. To ascertain the feasibility of using coir dust in the production of microbial cells, preliminary fermentation studies were conducted. Manure was added as microbial source. The fermented product was then subjected to chemical analysis, the results of which are given in Table 2. The observed increase in crude protein content indicates that coir dust might be a suitable fermentation substrate.

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TABLE 2. CHEMICAL ANALYSIS OF FERMENTED COIR DUST.

Sample	Moisture %	Ash %	Fat %	CF[a] %	CP[b] %	NFE[c] %	Ca %	P %	Energy kcal/kg
1	24.2	17.68	0.53	33.50	10.10	38.19	0.64	1.93	3427.92
2	26.2	20.98	0.76	33.92	12.42	31.92	1.36	2.91	3516.74
3	22.9	28.03	0.44	27.48	14.98	29.09	2.07	2.78	-
4	20.2	29.35	0.54	26.80	17.76	25.55	2.85	4.71	-
Unfermented	8.7	30.02	0.18	25.04	5.09	39.67	0.91	0.15	2280.00

[a] CF = Crude Fiber

[b] CP = Crude Protein

[c] NFE = Non-Fat Extract

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