APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1985, p. 388–393
0099-2240/85/020388-06$02.00/0

Vol. 49, No. 2

Solid-State Fermentation: an Alternative to Improve the Nutritive Value of Coffee Pulp

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Received 6 August 1984/Accepted 5 November 1984

Coffee pulp was subjected to a solid-state fermentation process, using *Aspergillus niger*. The initial moisture content of the pulp, as well as the fermentation time and temperature, had a significant effect on the increase in total amino acid content of the material. The increase in total amino acids showed a significant correlation with the dry matter recovered ($r = -0.98$) and the increase in pH during the process ($r = 0.98$). With a moisture content of 80%, a pH of 3.5, a temperature of 35°C, and an aeration of 8 liters/min per kg as fermentation conditions, it was found that the maximum concentration of total amino acids was attained after 43 h. The fermented product had a higher total amino acid content and a lower cell wall constituent value (primarily cellulose and hemicellulose) than the original pulp. A growing chicken's ration containing 10% of the fermented product had a feed efficiency (2.14) similar to that of the standard ration (2.19) and was significantly better than that of the diet containing 10% of the original pulp (2.53). The difference observed in feed intake and weight gain between the standard diet and that with 10% of the fermented product is considered to be due to palatability factors which should be studied further.

Coffee pulp represents the most abundant waste produced during the pulping operation of the coffee cherry needed to separate the coffee grain or coffee seed (1, 7). In Central America, as well as in other coffee-producing regions, coffee pulp is barely utilized and, therefore, it is considered the most abundant pollutant material of lakes and rivers located near the coffee-processing sites (11).

The utilization of coffee pulp as an animal feed has been mentioned as an attractive possibility; however, such utilization is limited by antiphysiological factors naturally occurring in the material (5). Present evidence relates the antiphysiological effects of the pulp to its relatively high caffeine, polyphenolic, potassium, and fiber content. The degree in which each of these factors contributes to the antiphysiological activity of coffee pulp as observed in both monogastrics and ruminants is still unknown (5).

Alternative technologies to detoxify coffee pulp have been investigated. Decaffeination has proved to be an alternative process to detoxify the material for animal feeding (17). However, it is considered a relatively high-cost technology to be implemented at the coffee-processing sites. Ensiling of coffee pulp, as well as treatments of the material with calcium hydroxide or potassium bisulfite, has proved to be inefficient in reducing its toxicity (4). Due to the high-quality essential amino acid profile detected by analysis in coffee pulp, efforts have been carried out to obtain a protein concentrate from the material; however, these have been unsuccessful (6).

Solid-state fermentation has been reported to be a relatively low-cost appropriate technology for the upgrading of amylaceous materials as a protein source in animal feeding (19, 22). Since coffee pulp is a solid material relatively rich in soluble sugars (12), the present work was undertaken to evaluate its possible use as a substrate in solid-state fermentation and to determine the possible benefits of this alternative on the antiphysiological effects of the material in growing chickens.

(This work represents part of the M.S. thesis of W.P.)

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MATERIALS AND METHODS

The mold used in the present study was *Aspergillus niger* strain 10 (from the Office de la Recherche Scientifique et Technique Outre-Mer, Paris, France).

The organism was maintained on potato dextrose agar (Difco Laboratories, Detroit, Mich.). The same medium was used for production of the spores, using an incubation temperature of 28°C. Spore suspensions for further experimentation were prepared following a technique described by other authors (18, 20). The number of viable mold spores in the spore suspension was determined by a germinating spore count in petri dishes, using potato dextrose agar with a pH of 3.5 and incubating at 28°C for 48 h.

The coffee pulp used in the present study was obtained from a coffee-processing plant near Guatemala City. All of the pulp was freeze-dried before the experimentation. The proximate composition of the raw material and that of the fermented product were determined according to the Association of Official Analytical Chemists (2). Tannins were estimated following the Association of Official Analytical Chemists method (2), caffeine was determined according to Ishler et al. (16), and the coffee pulp cell wall fractionation was carried out according to the Van Soest methodology (25–27).

The solid-state fermentation trials were carried out on a laboratory scale, using a unit similar to that described by Raimbault and Alazard (20), in which a constant temperature can be attained through a forced-convection water bath and the air flow desired in each fermenting column can be set by independent air flow valves. The fermentation temperatures studied were 30 and 35°C, using an air flow rate of 8 liters/min per kg as recommended by other authors (19). In all cases, air saturated with moisture was used for the aeration of the columns (20). To each fermenting column, 10 g of the freeze-dried, milled (to 60 mesh in a hammer mill) coffee pulp was added. Monopotassium phosphate, urea, and ammonium sulfate were added to the dried coffee pulp in proportions of 5.0, 3.5, and 7.5%, respectively. The inoculum consisted of 2×10^7 spores per g of dried coffee pulp. The moisture of the coffee pulp was adjusted to three

TABLE 1. Effect of different fermentation variables on the dry matter recovered, pH, and total amino acid content of the final product

Fermentation variables			Characteristics of the product (± SD)		
Fermentation time (h)	Fermentation temp (°C)	Initial moisture content of substrate (%)	Total amino acids (%, dry basis)[a]	Dry matter recovered (%)	pH
24	30	60	8.59 ± 0.23	99.03 ± 0.08	3.51 ± 0.11
		70	8.97 ± 0.31	95.18 ± 0.31	3.55 ± 0.06
		80	9.87 ± 0.38	93.88 ± 0.41	3.80 ± 0.10
	35	60	9.38 ± 0.47	94.87 ± 0.83	3.63 ± 0.11
		70	9.62 ± 0.21	94.08 ± 0.52	3.64 ± 0.07
		80	10.88 ± 1.07	86.59 ± 0.45	4.96 ± 0.06
67	30	60	9.54 ± 1.17	94.37 ± 0.61	3.60 ± 0.14
		70	12.15 ± 1.08	82.42 ± 0.83	5.60 ± 0.28
		80	13.21 ± 1.43	79.06 ± 1.13	6.20 ± 0.56
	35	60	10.31 ± 0.98	93.98 ± 0.72	3.80 ± 0.10
		70	13.33 ± 1.58	78.75 ± 1.85	6.30 ± 0.26
		80	14.55 ± 1.71	75.60 ± 1.73	6.80 ± 0.38

[a] The total amino acid content (dry basis) of the original pulp was 8.44 ± 0.19%.

different levels, 60, 70, and 80%. In all cases, the pH of the substrate was adjusted to 3.5 with diluted HCl. The fermented products were air dried in a forced-convection drier, using an inlet air temperature of 40°C, before any further analysis.

Total amino acids were determined through paper electrophoresis (15). Amino acid analysis was carried out with the same acid hydrolysate used for the total amino acid determination and an amino acid autoanalyzer (Technicon TSM, Tarrytown, N.Y.), following the method of Spackman et al. (24).

Biological protein quality evaluations were carried out in chickens through growth studies. For this purpose, 3-day-old animals were used. Three groups of six animals each, with a similar average weight (45 g), were used per diet. Both the diet and water were supplied ad libitum, and the weight gain and feed consumption data were recorded weekly. The general experimental conditions used for the chicken feeding trials were similar to those reported previously (8, 9). Both the freeze-dried coffee pulp and the air-dried fermented product were hammer milled to 60 mesh before their inclusion in the diet formulation.

Statistical evaluation of the data was carried out through analysis of variance and covariance as described by Snedecor and Cochran (23).

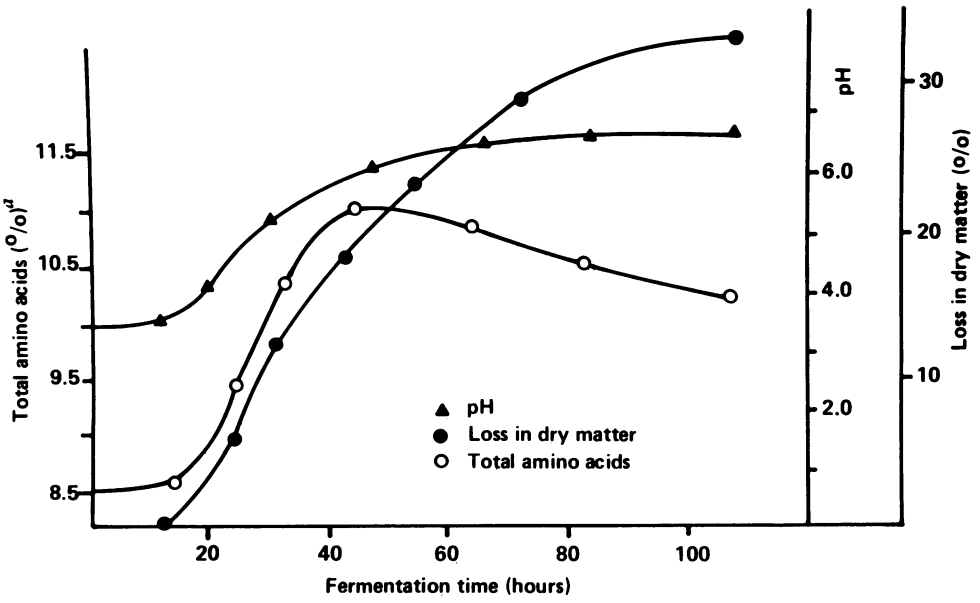
RESULTS AND DISCUSSION

The effects of the moisture content of the pulp used as substrate and of the fermentation temperature on the growth rate of *A. niger*, as determined by the total amino acid content of the product obtained after 24 and 67 h of fermentation, are presented in Table 1. The pH of the products and the dry matter recovery data after the two fermentation times studied are also included. The fermentation time and temperature, as well as the initial moisture content of the substrate, proved to exert a significant ($P < 0.01$) effect on the total amino acid content of the final product, indicative of the growth rate of *A. niger*. That coffee pulp required a much higher moisture content (80%) than that reported for starchy substrates (around 50%) to attain maximum growth of *A. niger* (19) indicates that the constituents of the pulp bound a larger amount of moisture than the starchy materials (such as cassava and the like) and, therefore, need more water to allow the level of available water required for the mold growth. Since coffee pulp has been reported to contain 6 to 8% mucilagenous constituents (mainly pectin), it is possible that these components are mainly responsible for the difference in binding water with the starchy materials (12).

The increase in total amino acid content showed a significant ($P < 0.01$) positive correlation ($r = 0.98$) with the increase in pH of the material during the fermentation process. Also, the total amino acid content of the products showed a significant ($P < 0.01$) negative correlation ($r = -0.98$) with the dry matter recovered after the process. This correlation was expected, since a higher rate of growth of *A. niger* would imply a higher utilization of the pulp as energy source and, therefore, a higher loss of dry matter during the process.

Since at a higher moisture content (85%) no further improvement in the total amino acid content of the product was obtained, it was decided to establish the coffee pulp solid-state fermentation kinetics by using a moisture content of 80%, a temperature of 35°C, and a pH of 3.5 as fermentation conditions. The results of the kinetic studies are presented in Fig. 1. The highest concentration of total amino acids in the fermentation product was obtained after 43 h of fermentation. The increment rate in total amino acid content of the material during the active growth (log) phase was determined to be 0.095 g/100 g (dry basis) of substrate per h; these data are in accordance with the findings reported by Raimbault (19) for molds studied with different fermentation systems. The increase in total amino acid content of the product proved to have a significant ($P < 0.01$) positive correlation both with the changes observed in pH ($r = 0.98$) and the loss in dry matter ($r = 0.94$). Preliminary findings indicate that an increase in total amino acid content of fermented coffee pulp always shows a positive correlation with an increase in pH, even when no external nutrients (e.g., nitrogen source) are added to the pulp. These findings suggest that alkalinizing compounds are being produced by *A. niger* during the fermentation of coffee pulp and that the rate at which they are produced could be taken as indicative of the growth rate of the mold.

The chemical characteristics of both the original freeze-dried coffee pulp and the product obtained after 43 h of fermentation are presented in Table 2. As expected, the fermented product showed a much higher total amino acid content (indicative of true protein content) than the original coffee pulp did. Also, the former showed a much lower cell



a o/o dry basis, based on the original weight of substrate.

FIG. 1. Effect of fermentation time on total amino acids, pH, and dry matter loss of coffee pulp subjected to solid-state fermentation, using *A. niger*. Fermentation conditions: initial moisture, 80%; temperature, 35°C; pH 3.5; aeration, 8 liters/min per kg.

wall component value than the latter, due primarily to a lower cellulose and hemicellulose content. The percentages of lignin, tannin, and caffeine remained similar for both materials. It should be noted, however, that to obtain similar percentages of these components in the raw material and in the final product the mold would have had to use or degrade them partially to compensate for the dry matter loss obtained during the fermentation process (Fig. 1). This would imply that the remaining fractions of these components (lignin, tannin, and caffeine) suffered partial modifications in their structures.

The reduction of the cellulose of coffee pulp through the fermentation process, using *A. niger*, is not surprising considering that this species has been reported to have cellulolytic activity (3). That the fermented product showed a much higher ash and potassium content than the original material can be explained by the addition of the nitrogen and phosphorus sources to the fermentation media [KH_2PO_4 and $(NH_4)_2SO_4$] as well as by the loss in organic matter obtained through the process (Fig. 1). That the percentages of tannin and caffeine of both the original coffee pulp and the fermented product were very similar was considered of relative significance, since both of the above-mentioned components have been thought to be possibly responsible for the antiphysiological effects observed when feeding coffee pulp in excess of 6 and 30% of the rations of growing chickens and ruminants, respectively (4, 8–10). However, as mentioned above, the tannin and caffeine remaining in the product could have been modified in their chemical structures through the fermentation process.

The amino acid profiles of both the original pulp and that fermented for 43 h are presented in Table 3. The fermentation process caused an increase in the concentrations of all amino acids when the results were expressed per 100 g of dry material; however, the concentration of all amino acids tended to remain constant when expressed per 100 g of total amino acids. This last observation suggests that the nutritive value of the protein of both products, as determined by chemical analysis, remains very similar, since the essential amino acid balance does not change appreciably. In fact, a very similar protein score was obtained for both the original and the fermented pulp (66.1 and 70.1, respectively) when calculated against the Food and Agriculture Organization protein reference pattern (13). Nevertheless, it should be noted that the methionine content of the product is proportionally higher than that of the starting material. This is of interest considering the methionine has been generally reported to be the most limiting essential amino acid of unicellular proteins (1, 19).

The formulation of the standard diet used in the chicken growth study is presented in Table 4. The original freeze-dried and milled coffee pulp and the air-dried and milled fermented product were added at a 10% level at the expense of yellow corn. The relatively high ash content of the fermented product was not considered for the formulation of the diets. The results obtained in the chicken growth exper-

TABLE 2. Composition of the original coffee pulp and the product obtained after 43 h of fermentation

Component	% Composition (dry basis)	
	Original coffee pulp	Fermented product
Ether extract	2.19	2.39
Crude fiber	23.69	13.14
Total nitrogen	1.74	5.76
Total amino acids	8.42	14.32
Ash	4.99	12.64
Potassium	1.39	3.65
Cell wall constituents	31.44	23.48
Hemicellulose	0.98	0.05
Acid detergent fiber	30.46	23.43
Cellulose	18.65	11.97
Lignin	12.20	11.72
Tannins	2.33	2.81
Caffeine	0.68	0.76

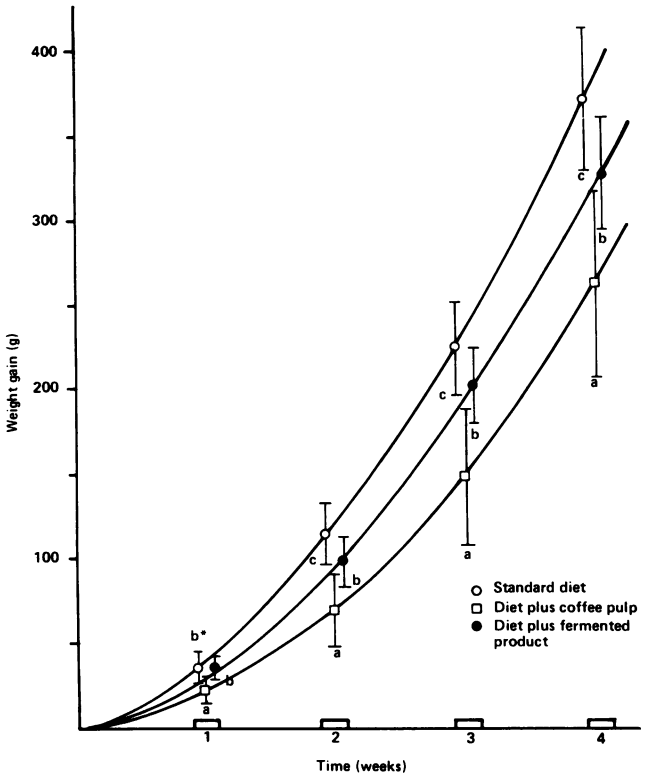
TABLE 3. Amino acid composition of the coffee pulp and of the product obtained after 43 h of fermentation

Amino acid	Composition (g/100 g of dry material)	
	Coffee pulp	Fermented product
Lysine	0.46 (5.5)[a]	0.82 (5.7)
Histidine	0.26 (3.1)	0.46 (3.2)
Arginine	0.38 (4.5)	0.69 (4.8)
Aspartic acid	0.84 (10.0)	1.38 (9.6)
Threonine	0.32 (3.8)	0.67 (4.7)
Serine	0.33 (3.9)	0.57 (4.0)
Glutamic acid	0.70 (8.3)	1.21 (8.4)
Proline	0.53 (6.3)	0.90 (6.3)
Glycine	0.42 (5.0)	0.70 (4.9)
Alanine	0.49 (5.8)	0.90 (6.3)
Valine	1.16 (13.8)	1.90 (13.3)
Methionine	0.32 (3.8)	0.58 (4.0)
Cystine	0.13 (1.5)	0.23 (1.6)
Isoleucine	0.51 (6.1)	0.89 (6.2)
Leucine	0.56 (6.6)	0.96 (6.7)
Tyrosine	0.20 (2.4)	0.44 (3.1)
Phenylalanine	0.28 (3.3)	0.47 (3.3)

[a] Numbers in parentheses represent grams of amino acid per 100 g of total amino acids as determined electrophoretically.

iments are presented in Fig. 2. The weight gain obtained with the standard diet was significantly ($P < 0.05$) better than that obtained with both the diet containing the original coffee pulp (from week 1 of the experiment) and that containing the fermented product (from week 2). On the other hand, the feed consumption recorded for the standard diet was significantly ($P < 0.05$) higher than that recorded for the diets containing the original and the fermented pulp, which in turn were not significantly different from each other (Table 5). In turn, the feed efficiency (feed consumption/weight gain) of the standard diet and that of the diet containing 10% of the fermented product turned out to be very similar and significantly ($P < 0.05$) lower than the value presented by the diet containing 10% of the original coffee pulp (Table 5). This indicates that the nutritive value of the former diet is very similar to and significantly ($P < 0.05$) better than that of the latter one.

Based on the chemical differences between the original pulp and the fermented product mentioned above (Tables 2 and 3), it is considered that the significantly ($P < 0.05$) better feed efficiency (or nutritive value) of the diet containing 10% of the fermented product, when compared with that containing an equal percentage of the original material (Table 5), could be explained by a reduction in the cell wall constituents and a possible modification of the structure of the



* Different letter at the same time period indicates significant difference ($P < 0.05$).

FIG. 2. Weight gain of growing chickens fed diets containing 10% of either coffee pulp or the fermented product.

antiphysiological factors of the pulp (tannin and caffeine) through the fermentation process, and an improved bioavailability of the nutrients in the diet containing the fermented product. This, considering that, as stated earlier, the protein chemical score of the original material was very similar to that of the fermented product, indicates that the protein quality of both materials was similar. It is of interest to note that an improvement in the nutritional characteristics of the pulp was obtained, even though the percentages of caffeine and tannins remained very similar in both the fermented and the original products. The possibility that the fermentation process is modifying the structure of caffeine and tannins in some way also as to lower or eliminate their antiphysiological activity should be studied further. It is our belief that the slightly higher protein concentration found in the diet con-

TABLE 4. Formulation of the standard diet used in the chicken growth study

Ingredient	Concn (%)
Soybean meal	35.00
Yellow corn flour[a]	55.35
Cottonseed oil	5.00
Bone meal	2.10
Calcium carbonate	1.50
Common salt (NaCl)	0.45
DL-Methionine	0.30
Vitamins, minerals, and antibiotics premix (Pfizer-100)	0.30

[a] In the case of the diets containing coffee pulp and the fermented product, these were added at a 10% level at the expense of the yellow corn flour.

TABLE 5. Average feed consumption and feed efficiency obtained for diets containing coffee pulp and the fermented product when fed to growing chickens for 4 weeks

Diet	Feed consumption (g/4 wk, ± SD)	Feed efficiency (feed consumed/wt gain, ± SD)
With 10% coffee pulp	663.73 ± 37.84[b]	2.53 ± 0.06[b]
With 10% fermented product	695.47 ± 32.98[b]	2.14 ± 0.11[a]
Standard	802.37 ± 12.34[a]	2.19 ± 0.21[a]

[a,b] Different letters in the same column indicate significant difference ($P < 0.05$).

taining 10% of the fermented product versus that of the one containing the same percentage of the original pulp (0.6% difference) had no effect on the biological findings.

That the standard diet presented a significantly ($P < 0.05$) higher feed consumption value than the diet containing 10% of the fermented product (Table 5) is considered to be due to possible differences in the palatability of both diets, since both presented a similar feed efficiency value. Further, considering that, as stated above, both diets presented a similar (statistically equal) feed efficiency value (Table 5), it is our contention that the difference observed in weight gain between the standard diet and that containing 10% of the fermented product (Fig. 2) is due to a difference in feed consumption and not to a difference in nutritive value (represented by feed efficiency) between the diets. Consequently, in future experiments planned along these lines, the aspects of palatability (including texture) of the diet containing the fermented product will be considered as highly important. The high ash content of the fermented product could be a factor affecting the palatability of the diet.

From the evidence presented herein, we may conclude that solid-state fermentation represents an alternative way to improve the nutritive value of coffee pulp for monogastric animal feeding. A lowering in the cellulose and hemicellulose content of coffee pulp and a partial utilization of the caffeine, lignin, and tannin components seem to be enough to warrant the inclusion of the material at a 10% level in a growing chicken's diet, ensuring a feed efficiency equal to that of the standard diet. Consequently, future fermentation trials are being planned with other cellulolytic and lignolytic molds, besides *A. niger*, to evaluate the possiblity of a further improvement of the pulp through this alternative. Also, the possibility of using the molds reported to use caffeine (21) and polyphenolic compounds (14), alone or as mixed inoculum, is being considered to improve the palatability of the fermented product. Also, alternatives to lower the high ash content of the final product are being considered, to improve palatability. Efforts are also being made to develop a simple, large-scale, solid fermentation system that would allow for the future implementation of this alternative at the coffee-processing site. Finally, experiments are being planned to determine the minimum amount of external nitrogen sources to add to the coffee pulp to assure the efficiency of the process. All of the above work will be carried out with the final intention of establishing the technical and economic viability of implementing this alternative at the coffee-processing site in the near future.

ACKNOWLEDGMENTS

This research was carried out with funds from the International Development Research Centre, Ottawa, Ont., Canada (grant-in-aid INCAP-841), and the U.S. Agency for International Development, Washington, D.C. (grant-in-aid INCAP 550).

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