In Vitro Digestibility of Selected Agricultural Wastes at Various Moisture Levels Treated with Microwave Energy[1]

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Individually dried and milled cotton stalks (*Gossypium hirsutum* L.), kudzu (*Pueraria lobata* Willd.), pine needles (*Pinus eliotitti* Engelm.), wheat straw (*Triticum aestivm* L.), peanut hulls and skins (*Arachis hypogaea* L.), poultry waste and separated dairy cattle waste were hydrated at the rate of 0, 5, 10, 15, 20, and 25% (dry basis) and subjected to batch microwave treatment for 0, 1, 2, 3, 4, and 5 min. General linear model analyses and generation of predicted surface response curves showed differences in in vitro dry-matter digestibility (IVDMD) estimates for waste resource, microwave treatment time, and moisture content. In general, IVDMD values decreased with increasing moisture content or microwave treatment time.

INTRODUCTION

Agricultural byproducts represent potential ruminant feed resources, or microbiological substrates, if economically viable means are developed to convert these materials into highly digestible substrates. Various approaches to improve digestibility have been evaluated using biological, chemical, thermal, and γ irradiation treatments (Han and Anderson, 1975; Hartley, 1983; Holzer et al., 1978; Ibrahim and Pearce, 1980; Ibrahim and Pearce 1983a; Ibrahim and Pearce, 1983b; Jayasuriya, 1979; Reade and McQueen, 1983). Although these treatments have demonstrated increased digestibility, economic and other restraints have limited their commercialization. Alternative efficient and economically viable means of enhancing the digestibility of lignocellulosic wastes are needed.

Microwave energy use is becoming more prevalent and utilizes frequencies in the range from about 1 to 30 GHz (Albrecht and Landau, 1978; Nelson, 1985). Microwave energy is used in the food industry for determining moisture content, heating, cooking, and drying various products (Nelson, 1979; Nelson et al., 1981; Pour–El at al., 1981; Mudgett and Schwartzberg, 1982; Nelson et al., 1985). Radiofrequency and microwave energy are also used in the wood industry (Peyskens et al., 1984) and in other industrial applications (Allan et al., 1980; Chan and Krieger, 1981) and have been investigated for insect control (Nelson and Stetson, 1974; Nelson and Whitney, 1960). Although human health concerns over microwave usage have been raised, the process usage has increased overall.

Microwave heating of lignocellulosic wastes was demonstrated to enhance the enzymatic susceptibility of highly lignified and hydrated wastes above 160 °C. The moderately low intensity microwave treatment (<8 min) resulted in no substantial change in cellulose crystallinity; however, hemicellulose and lignin underwent acid-catalized autohydrolysis (Azuma et al., 1984). High-intensity microwave energy however, has been shown to degrade cellulose (Allan et al., 1980). Starch polymers also undergo degradation when treated with microwave energy (Khan et al., 1979).

The objective of this study was to assess the effects of moisture content and microwave treatment time on the in vitro dry-matter digestibility (IVDMD) of selected agricultural wastes.

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[1]Mention of commercial or proprietary products in this paper does not constitute recommendation or endorsement of these products by the U.S. Department of Agriculture.

METHODS

Harvested cotton (*Gossypium hirsutum* L.) stalks were mechanically pulled (Sumner et al., 1984) and collected on a cotton farm in South Georgia. Growing kudzu (*Pueraria lobata* Willd.) stalks and leaves, mature fallen pine needles (*Pinus eliotitti*), and harvested wheat (*Triticum aestivm* L.) straw were collected at the Coastal Plain Experiment Station. Peanut (*Arachis hypogaea* L.) hulls and skins were recovered from commercially produced mature harvested peanuts in a laboratory sheller (Davidson and McIntosh, 1973). Fresh (<1 day old) poultry waste (guano, feathers, and wasted feed) was collected at a commercial grower farm. Screen-separated dairy cattle solids were collected from a dairy by a water flush waste disposal system (Erdman, 1985).

All forages and wastes were individually processed. Samples were dried in a forced-air oven at 50 °C for 24 h and passed through a hammer mill (No. 10 Modern, C. S. Bell Co., Hillsboro, OH) using a screen size diameter of 0.64 cm. Proximate analyses were performed in duplicate by AOAC procedures (1980). Triplicate subsamples at each moisture and microwave treatment level were distributed into 3.8-L resealable plastic bags (Ziploc freezer bags, Down Chemical Co., Indianapolis, IN). Water was added to the samples at the rate of 0, 5, 10, 15, 20, and 25%; the bags were resealed, mixed, and incubated at 16 °C for 7 days. During the incubation all samples were remixed in the sealed plastic bags on days 2 and 5 to facilitate uniform distribution of the water. Total weight of the sample and water was 100 g. Following incubation the sample contents were remixed and the bags partially opened to allow for vapor pressure release during microwave treatment. The bag and contents were batch treated in a household microwave oven (Model 1009.000, Litton Systems, Inc., Minneapolis, MN) at 2450 MHz (700–W rated output) for 0, 1, 2, 3, 4, or 5 min. Some samples (especially the pine needles) were partially treated and allowed to cool prior to receiving the remaining total dosage to prevent sample combustion. Total calculated energy (joules = watts × seconds) exposure was estimated as 0, 4.2×10^4, 8.4×10^4, 1.26×10^5, 1.68×10^5, and 2.1×10^5 J at 0, 1, 2, 3, 4, and 5 min, respectively. Following microwave treatment the samples were dried (50 °C for 24 h), and in vitro dry-matter digestibility was determined in duplicate by the procedure of Tilley and Terry (1963). The in vitro procedure utilized 0.4 g of forage for a 48-h rumen fluid digestion, followed by a 48-h acid pepsin digestion. Rumen fluid inoculum for the in vitro digestions was taken from fistulated steers fed a constant diet of Coastal Bermuda grass hay.

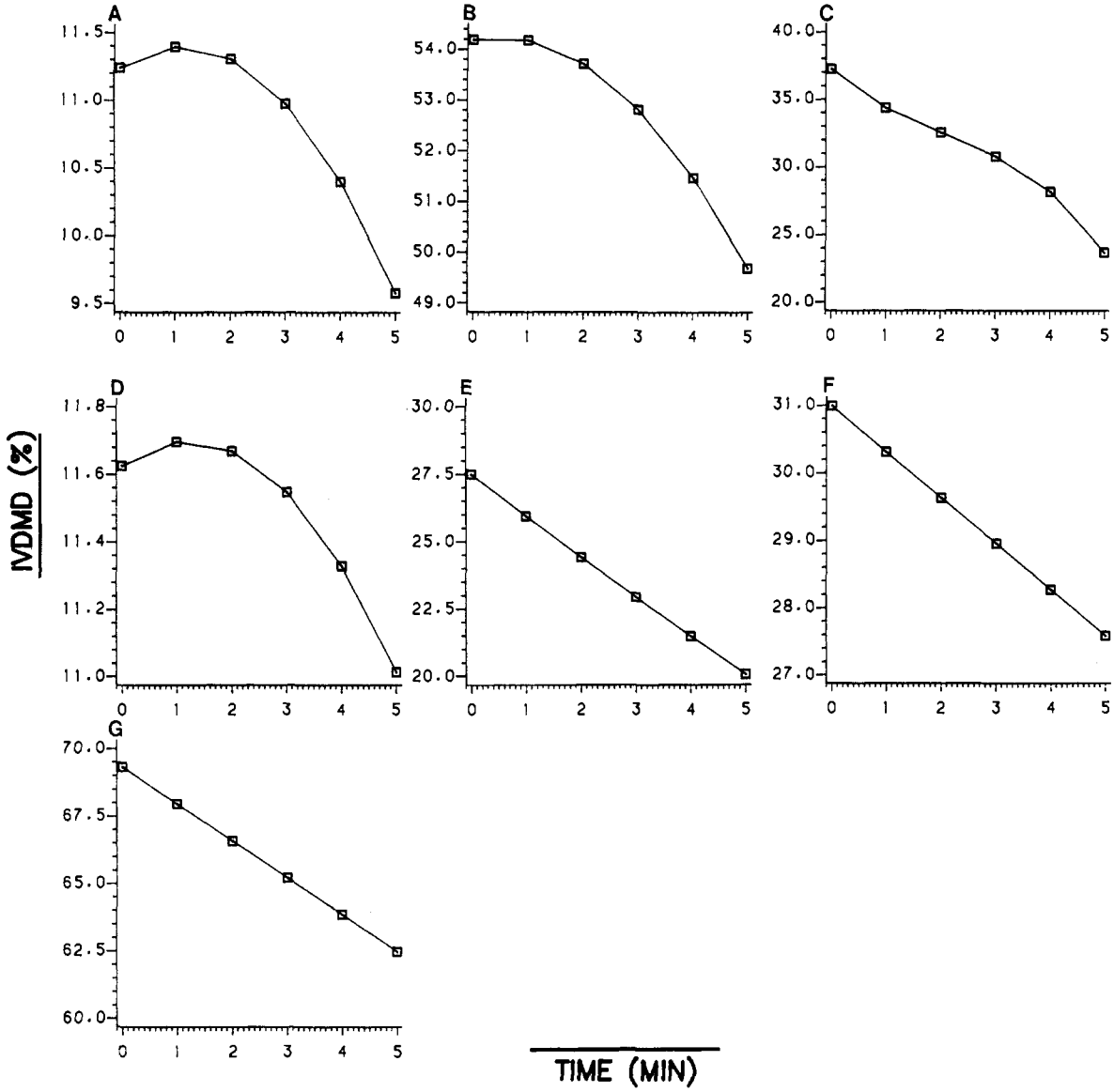

Figure 1. Effect of microwave treatment time (min) on in vitro dry-matter digestibility of dry cotton stalks (a), kudzu (b), peanut waste (c), pine tree needles (d), wheat straw (e), screen-separated dairy cattle waste (f), and poultry waste (g).

Data were analyzed by the general linear models procedure. Linear, quadratic, cubic, and all possible interactions were originally used (SAS Institute, Inc., 1981). The equations presented contain all values and interactions where $P < 0.05$. Response surface curves were generated from the equations developed for predicted IVDMD (%) vs. moisture content (%) and microwave treatment time (SAS Institute, Inc., 1982).

RESULTS AND DISCUSSION

Waste Characteristics. A diverse group of agricultural wastes was selected to address the wide regional waste resources common to agriculture. The in vitro nutrient characteristics of these agricultural wastes are presented in Table I. Crude protein concentrations of the wastes ranged from low values in cotton stalks, pine needles, and wheat straw to moderately high levels in kudzu, peanut, dairy, and poultry wastes. Ether extract values were 2.6% or less except for pine needles. The ether extract value for the pine needles was high because of solvent extractables in the waste. Ash content ranged from 2.6 to 8.8%, except for peanut waste which had a 12.4% ash content. Crude fiber content ranged from a low value of 15.4% in poultry waste to a high value of 55.5% in cotton stalks. Nitrogen-free extract, neutral detergent fiber, and acid detergent fiber ranged from 20.3 to 83.4, 20.9 to 71.2, and 4.3 to 35%, respectively. Gross energy content ranged from 2980 to 5251 cal/g.

Predicted IVDMD. The overall regression analyses equations for predicted IVDMD at 0–25% moisture content and 0–5-min microwave treatment times are presented in Table II. The equations contain all values and interactions where $P < 0.05$. The coefficients of determination for predicted IVDMD ranged from 23.5 to 68.0% while the standard error ranged from 0.7 to 3.1% when compared to actual IVDMD values. The predicted values were used for the generation of response surface curves.

A series of dry samples were subjected to microwave treatment (Figure 1). Predicted IVDMD decreased with increasing microwave treatment time except for small increases in cotton stalks and pine needles treated for 1 min. Predicted reductions in IVDMD ranged from 5.8 to 57.2% and were greatest in peanut hulls and smallest in pine needles. Microwave treatment of dry cotton and pine wastes for 1 min increased IVDMD 1.4 and 0.6%, respectively, when compared to untreated controls.

Response surfaces for predicted IVDMD at various moisture and treatment times are presented in Figure 2. The response surfaces differed for each waste evaluated, and IVDMD values generally decreased with increasing

Table I. Nutrient Characteristics of Evaluated Biomass[a]

source	crude protein, %	ether extr, %	ash, %	crude fiber, %	N-free extr, %	neutr deterg fiber, %	acid deterg fiber, %	acid deterg lignin, %	gross energy, cal/g
cotton stalks	3.85 (0.15)	1.02 (0.03)	2.55 (0.05)	55.45 (1.05)	37.13 (1.28)	83.35 (0.55)	71.20 (0.70)	27.15 (0.35)	4607.37 (4.16)
kudzu	15.60 (0.20)	2.64 (0.05)	8.00 (0.00)	39.10 (0.70)	34.67 (0.86)	51.20 (0.60)	41.20 (0.30)	7.65 (0.05)	4494.59 (20.00)
peanut waste	14.55 (0.05)	2.31 (0.02)	12.35 (0.05)	50.50 (0.00)	20.30 (0.12)	70.45 (1.15)	61.80 (0.50)	20.85 (0.05)	4507.97 (19.69)
pine needles	3.40 (0.00)	7.93 (0.01)	2.50 (0.00)	35.40 (0.80)	50.77 (0.79)	60.05 (0.05)	61.90 (0.20)	35.00 (0.20)	5251.34 (1.34)
wheat straw	4.05 (0.05)	1.11 (0.07)	6.20 (0.10)	47.85 (0.15)	40.80 (0.37)	87.70 (0.20)	58.25 (0.45)	7.20 (0.10)	4352.73 (17.64)
dairy waste	10.45 (0.15)	1.13 (0.05)	8.85 (0.05)	30.65 (0.35)	48.93 (0.20)	72.05 (0.35)	44.00 (0.50)	11.90 (0.20)	4295.36 (23.70)
poultry waste	22.50 (0.20)	0.21 (0.00)	3.60 (0.00)	15.35 (0.25)	58.34 (0.05)	36.50 (0.80)	20.85 (0.05)	4.30 (0.10)	2979.66 (24.63)

[a] Mean (SEM). IVDMD defined as in vitro dry-matter digestibility.

Table II. Regression Analysis Table for Predicted Digestibility[a]

parameter	cotton stalks	kudzu	peanut waste	pine needles	wheat straw	dairy waste	poultry waste
N	193	207	177	207	185	188	205
intercept	11.239 (0.290)	54.073 (0.557)	37.227 (0.807)	11.625 (0.155)	27.508 (1.052)	31.002 (0.351)	69.315 (0.452)
moisture, %							
L	−0.433 (0.063)	−0.043 (0.025)	−0.040 (0.099)	0.026 (0.020)	−0.815 (0.254)	−0.120 (0.023)	−0.803 (0.084)
Q	0.031 (0.006)		−0.010 (0.004)	−0.004 (0.001)	0.043 (0.022)		0.029 (0.003)
C	−0.001 (0.0001)				−0.001 (0.001)		
time, min							
L	0.279 (0.248)	0.017 (0.436)	−3.692 (1.049)	0.118 (0.097)	1.577 (0.886)	−0.677 (0.117)	−1.367 (0.156)
Q	0.122 (0.048)	0.181 (0.084)	0.991 (0.520)	−0.048 (0.020)	0.018 (0.174)		
C			−0.159 (0.069)				
interactions							
$M_L \times T_L$	−0.030 (0.016)		0.083 (0.016)		0.003 (0.079)	0.019 (0.008)	0.204 (0.029)
$M_Q \times T_L$					0.006 (0.002)		0.007 (0.001)
$M_L \times T_Q$	0.008 (0.003)			−0.002 (0.001)	0.026 (0.011)		
$M_Q \times T_Q$							
RSD	0.892	3.049	3.014	0.669	3.098	1.516	1.691
R^2, %	53.25	23.53	51.03	67.97	60.54	30.91	41.04

[a] Mean (SEM). C, L, M, Q, and T defined as cubic, linear, moisture, quadratic, and time, respectively.

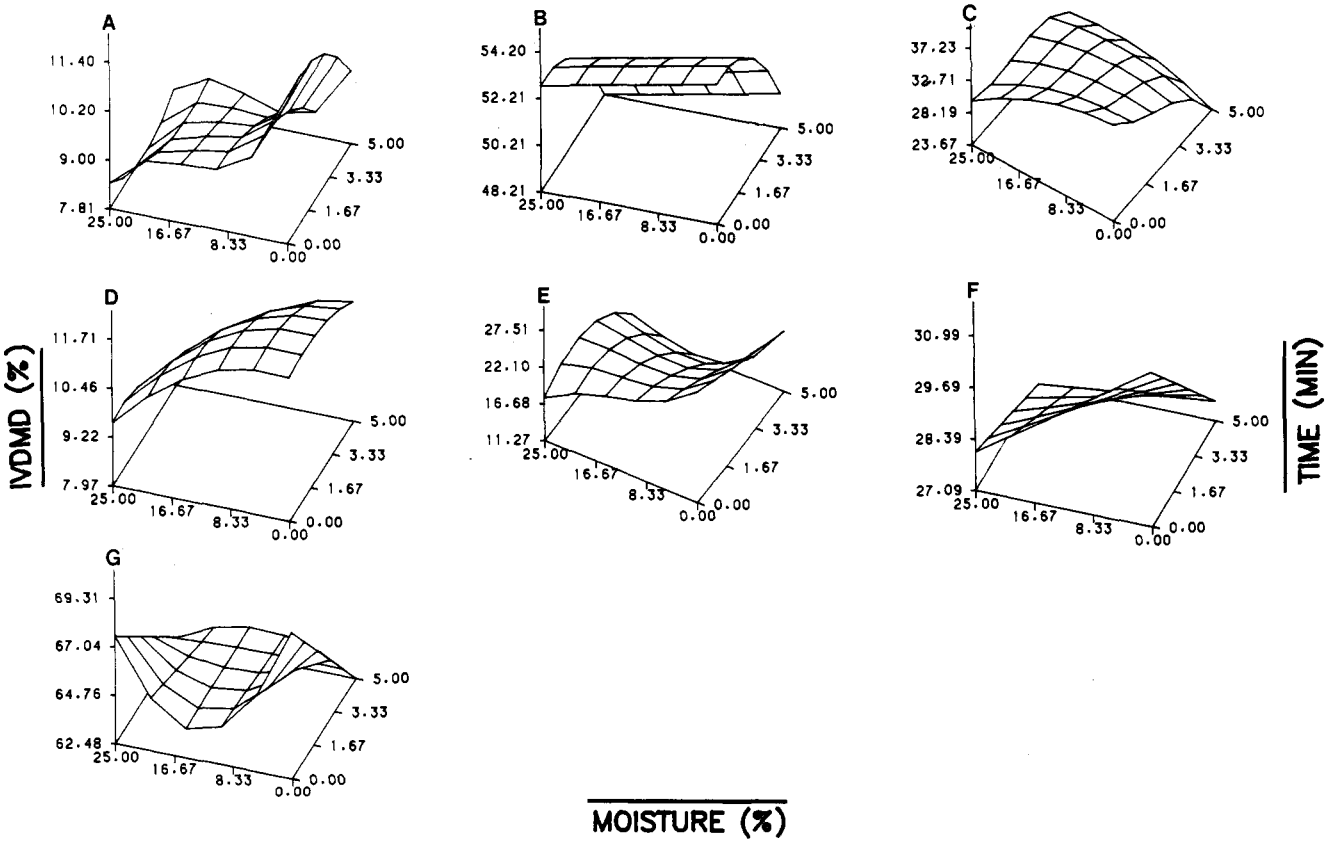

Figure 2. Effect of microwave treatment time (min) and moisture content (%) on in vitro dry-matter digestibility of cotton stalks (a), kudzu (b), peanut waste (c), pine tree needles (d), wheat straw (e), screen-separated dairy cattle waste (f), and poultry waste (g).

moisture content and treatment time. Low-level microwave treatment following mechanical milling resulted in no apparent improvement in the IVDMD of the wastes evaluated, and presumably no enhanced feed value for ruminants with the conditions tested. Azuma et al. (1984), however, have been successful in enhancing the enzymatic susceptibility of lignocellulosic wastes at temperatures above 160 °C and showed a maximum at 223–228 °C,

independent of lignocellulosic waste source. In their process the irradiated samples were treated with Meicelase (Meiji Seika Industry, Co., Ltd.) at a 2% substrate concentration, 0.2% enzyme concentration, followed by incubation for 48 h. Differences existed between enzymatic treatments in the two processes and may account for the differences observed (Faithful, 1984).

In the study reported here, temperature was not measured; however, the typical dark brownish color was apparent, indicating caramelization of the sugars released during microwave treatment. Samples had a characteristic burned smell. The preliminary results presented indicate that microwave treatment reduced the in vitro digestibility of the agricultural wastes evaluated. The lack of an observed enhancement in IVDMD may be due to relatively low microwave power settings since there is a poor coupling of microwave energy to lignin (Chan and Krieger, 1981) although less treatment time is required for lignocellulosic wastes than for woody plants (Azuma et al., 1984).

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Nitrite–Tryptophan Reaction: Evidence for an Equilibrium between Tryptophan and Its Nitrosated Form

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Nitrite reacts with *N*-acetyltryptophan (NacTRP) in aqueous solution to yield *N*-acetyl-N^1-nitrosotryptophan (NacNOTRP), which was found to be unstable. The reaction was followed by means of UV spectrometry using different combinations of the operating parameters (relative concentrations, pH, temperature). It was concluded from the collected data that an equilibrated reaction takes place between NacTRP, nitrite, and NacNOTRP. The reacting species of nitrite was found to be HNO_2. The value of the equilibrium constant equals 455.6 ± 0.13 M^{-1}. The temperature effect study yielded ΔH_0 = −54 000 J·mol^{-1} and ΔS_0 = −140 J·mol^{-1}·K^{-1}. These results provide an insight on the release of nitrite observed on cured meat and myofibrillar proteins and the "transnitrosation" reactions that occur from protein tryptophan residues toward myoglobin heme groups.

Nitrite in cured meat products is responsible for color stabilization and bacteriostatic protection and induces a characteristic flavor. The color mechanism is known as the fixation of one or two NO on the heme group of the myoglobin (Tarladgis, 1962), and some explanations have been given for the bacteriostatic effect (Yarbrough et al., 1980; Reddy et al., 1983). A better knowledge of the reaction mechanisms of nitrite with meat constituents is required for a more precise understanding of the role of nitrite in the development of the characteristic flavor of treated meat products and for preventing the formation of nitrosamine in cured meat. Studies have been made on the distribution of nitrite in the different meat fractions, showing that, during the curing process, the loss of the added nitrite is considerable. Cassens et al. (1977) reported a 5–20% recovery of the initial $Na^{15}NO_2$ in meat systems as free nitrite, but 20–30% of the ^{15}N was bound to protein fraction. Woolford et al. (1976) reported that 30% of the nitrite added to a bovine serum albumin solution could bind to the protein. Noël et al. (1981) observed a release of nitrite from nitrosated meat and myofibrillar proteins after washing with water. The nitrite recovery between first and last washing increased when the incubation pH decreased. The authors concluded an equilibrium exists between a part of bound nitrite and free nitrite. Reactivity of the side chains of protein has been studied for a long

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