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Microbial kinetics, fermentative and chemical characteristics in solid state fermentation of apple bagasse

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ABSTRACT

The objective of this study was to evaluate the microbial kinetics, fermentative and chemical characteristics during solid state fermentation (SSF) of apple bagasse (AB), which was determined over the course of 4 incubation times: 0, 24, 48, 72 h, in a completely randomized design with 4 repetitions. pH values, lactic acid concentration, numbers of total aerobic bacteria, yeast and lactobacilli, dry matter digestibility and neutral detergent fiber digestibility were determined. True and crude protein, neutral and acid detergent fiber were also measured. Results revealed that pH decreased over the 4 sampling times. The lactic acid concentration increased over time. There was a reduction in numbers of total aerobic bacteria. Numbers of lactobacilli also reduced. Yeast populations (CFU/ml) were stable at 24 h, but decreased thereafter. *In vitro* dry matter digestibility (IVDMD) increased during incubation. *In vitro* neutral detergent fiber digestibility (IVNDFD) similarly increased, with a maximum value observed at 72 h. True protein (TP) increased during fermentation, achieving a high value at 24 h; however, crude protein (CP) showed no change during incubation. Neutral detergent fiber (NDF) content did not change during fermentation however acid detergent fiber (ADF) reduced. It is concluded that the increased content of lactic acid and the accompanying decrease in pH during SSF of AB negatively affected the yeast and total bacteria populations whereas true protein content increased likely because of formation of unicellular protein during the process.

Key words: Apple waste, Bacteria, Chemical composition**,** Fermentation, Yeast

Currently, biotechnological methods such as solid state fermentation (SSF) of agro-industrial byproducts to enrich protein contents for animal feeding are important because of the low cost and easy operation. Apple bagasse (AB) is the most abundant byproduct produced during juice extraction. This residue is rich in soluble carbohydrates and pectin (Vicente *et al.* 2005), and microorganisms like yeasts (Martorell 2006) which are low in protein but of high biological value (Rodríguez *et al.* 2008). During SSF the microorganisms in the substrate use nitrogen, carbohydrates, minerals and vitamins for their growth and reproduction. Consequently, the final product is enriched in high quality protein and low fiber contents (Rodríguez *et al.* 2006). For instance Rodríguez *et al.* (2001) reported an increase in true protein in SSF of sugarcane (*Saccharum officinarum*) and sweet potato (*Ipomea batata*) due to propagation of its epiphyte flora. Nevertheless, the research using AB as substrate for SSF is limited. The present research objective was to determine fermentation and chemical characteristics and assess microbial work during SSF of apple bagasse under microaerobic condition.

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MATERIALS AND METHODS

Location: The present research was performed in the Animal Nutrition laboratory of the Faculty of Animal

Husbandry and Ecology at the Autonomous University of Chihuahua located in Chihuahua, México.

Experimental procedure: Apple bagasse (AB) obtained, ground and mixed (Elias *et al.* 1990) with 1.5% urea, 0.2% ammonium sulphate, and 0.5% a mineral salts mixture containing macro and micro elements. These ingredients were mixed and then 342 g portions were distributed to separate sterile 500 ml Erlenmeyer flasks for solid state fermentation (SSF). Each flask was plugged with cotton and incubated under static conditions at 32°C for 0, 24, 48, and 72 h. At each sampling time, 4 flasks were withdrawn from the incubator and the total contents of each flask were collected and individually homogenized. A 5 g sample of each resultant homogenate was combined with 45 ml sterile distilled water in a 100 ml Erlenmeyer flask and shaken for 30 min with shaker equipment and then filtered through sterile gauzes and refrigerated at 4° C for further analyses.

Laboratory analysis: The pH of the filtrate obtained as described in the experimental procedure, was immediately recorded with a table potentiometer. For quantification of lactic acid, 0.25 ml from the sample filtrated was diluted in 9.75 ml of distilled water. From this solution, 0.5 ml was used for lactic acid determination according to the calorimetric method described by Madrid *et al.* (1999). The microorganism extractions were made with the same procedure used for aqueous extractions for pH measurement, but in aseptic conditions. An aliquot of 1 ml of filtered suspension was serially diluted to 10^{-11} in peptone-water (0.01%). Diluted samples were spread to plate count agar, malt extract agar supplemented with 0.01 g chloramphenicol/ L, and to MRS agar, for enumeration of total aerobic bacteria, yeast and lactobacilli, respectively. All incubations used 4 dilutions; for total viable bacteria were 10^3 to 10^6, for yeast 10^4 to 10^7 and lactobacilli 10^5 to 10^8. Inoculated media were incubated at 37°C aerobically for 24 h to enumerate total aerobes and yeast and anaerobically for 48 h to enumerate lactobacilli. These were carried out in a count-colonies chamber. The chosen dilutions that had 30 to 300 colonies were counted. Dry matter (DM), organic matter (OM), ashes (AS), and crude protein were analyzed according to AOAC (1990). For true protein (TP) the method of Berstein (1970) was used as per Meir (1986). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured according to Van Soest (1994). These analyses were sequentially determined in the fiber analyzer , using filter bags F57 with a porosity of 30 microns. *In vitro* dry matter digestibility (IVDMD) and *in vitro* neutral detergent fiber digestibility (IVNDFD) values were obtained with incubator (ANKOM Technology 1998).

Statistics: A completely randomized design was used with 4 repetitions by each sampling time. The statistical analysis of the data was made using the GLM procedure.

RESULTS AND DISCUSSION

Fermentation, chemical characteristics and microbial growth: The results are shown in Table 1.

The pH decreased significantly (P< 0.007) at 24 h and then remained almost unchanged. The results on lactic acid concentration through the 4 fermentation times indicated increase until 72 h which may be related to the growth of lactic acid bacteria. During the fermentation process ammonia nitrogen concentration increased (P< 0.0009) as

Table 1. Fermentative, chemical characteristics and microbial growth of the apple bagasse under solid state fermentation

Item (%)	Fermentation time (h)				SEM	P=
	0	24	48	72		
pH	4.13^{a}	3.85^{b}	3.86^{b}	3.81^{b}	0.05	0.007
N-NH_3 (mmol/g of DM)	1.92^{a}	1.95^{a}	1.99^{b}	2.00^{b}	0.01	0.0009
Lactic acid (µg/g of DM)	22.24^{a}	22.50^{a}	22.87^{b}	23.75^{c}	0.13	0.0001
Total bacteria[1] (CFU/ ml)	$7.14^{a}(1.4x10^{6})$	$5.96^{b}(9.2x10^{4})$	$4.73^{c}(5.4x10^{3})$	$4.55^{d}(3.5x10^{3})$	0.03	0.0001
Lactobacilli[1] (CFU/ ml)	$8.60^{a}(3.9x10^{7})$	$8.14^{b}(1.3x10^{7})$	$8.73^{c}(5.4x10^{7})$	$8.53^{a}(3.4x10^{7})$	0.02	0.0001
Yeast[1] (CFU/ ml)	$7.42^{a}(2.6x10^{6})$	$7.35^{a}(2.2x10^{6})$	$6.0^{b}(1x10^{5})$	$4.85^{c}(7.1x10^{3})$	0.05	0.0001
IVDMD %	89.0^{a}	89.21^{a}	92.10^{b}	94.28^{c}	0.67	0.0005
IVNDFD %	81.1^{a}	83.41^{a}	87.11^{b}	88.63^{b}	1.29	0.004
Dry matter	18.0^{a}	18.92^{b}	18.97^{b}	19.12^{b}	0.16	0.001
Organic matter	95.86^{a}	96.14^{b}	96.13^{b}	96.01^{b}	0.07	0.05
Ashes	4.14^{a}	3.86^{b}	3.87^{b}	3.99^{b}	0.07	0.05
True protein	16.89^{a}	21.15^{b}	19.50^{ab}	18.12^{ab}	0.91	0.03
Crude protein	36.19	36.02	35.57	35.47	0.30	N.S
Neutral detergent fiber	52.65	53.14	53.22	53.76	0.45	N.S
Acid detergent fiber	41.57^{a}	41.45^{a}	38.80^{b}	39.42^{b}	0.30	0.0001

$^{a\,b\,c\,d}$ Different superscripts in the same row differ statistically;[1] the counts of microorganisms were according to logarithmic function. CFU,colony-forming unit; IVDMD, *in vitro* dry matter digestibility;IVNDFD, *in vitro* neutral detergent fiber digestibility.

the incubation time progressed (Table 1). This could be related to the ureolytic action of urease-positive bacteria that normally occur in SSF processes (Valiño *et al.* 1994). Moreover, protein synthesis takes place more slowly than urea hydrolysis (Rodriguez *et al.* 2001). The air present in the SSF mixtures limited the growth of lactic bacteria at 24 h; but at 48 h their growth was favored by oxygen consumption and pH reduction (P< 0.0001). Acid tolerant lactobacilli are able to grow at pH of 3 or below (Hyden 2001, Pandey *et al.* 2001). The decrease of lactobacilli at 72 h was attributable to substrate decline, which also caused a reduction in total bacteria and yeast counts. The results on total counts of aerobic bacteria showed a decrease (P< 0.0001) as the fermentation time passed. This depressive effect on total bacteria population could be related to the high lactic-acid concentration, which influences pH reduction causing a bactericidal effect (Hyden 2001). In the first 24 h the yeast population was constant, during the maintenance phase, and then it came into mortal phase, which could be attributed to low pH of the ecosystem, which is lethal for yeast (Elías and Lezcano 1994). Although in present study acetic acid content was not determined, the lethal phase also could be related to greater acetic acid production, which under certain conditions inhibits microbial growth and induces to the cellular death of the yeast (Ludovico *et al.* 2001). Accumulation of ammonia nitrogen also could influence yeast death by diminishing activity of the dehydrogenase L-glutamate enzyme, which is necessary to incorporate nitrogen ammonia into the cell protoplasm of the microorganisms (Metges and Loh 2003) and through that way make unicellular protein. Results for IVDMD and IVNDFD (Table 1) both increased significantly (P<0.0005 and P<0.004 respectively) at 48 and 72 h of incubation. DM and OM increased (P<0.001 and P<0.05 respectively) up to 24 h, and then remained constant. This increase could be due to the loss of water by evaporation. CP content showed no differences with the change in fermentation time. However, TP increased in the first 24 h (P<0.03) and could be related to the growth of microorganisms, which is in agreement with Pandey *et al.* (2001) stating that TP could be an indirect way to measure microbial growth in SSF processes. NDF composition was not affected by time of fermentation, but ADF decreased (P<0.0001) as the fermentation time passed, probably due to the degradation of the fibrous fraction by cellulolytic microorganisms; Valiño *et al.* (2002) utilized sugarcane as a substrate in SSF and reported reduction in the level of cellulose and NDF.

In conclusion, the SSF of apple bagasse enhanced its nutritive value through the growth of microorganisms that increased true protein and *in vitro* digestibility of dry matter and fiber.

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