Evaluation of the Nutritive Value of Citrus pulp Degraded with *Penicillium notatum* and *Penicillium citrinum*

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Abstract

This study was conducted out to assess changes in nutritive value of citrus pulp (CTP) fermented with *Penicillium notatum* and *Penicillium citrinum* using *in vitro* gas production technique. After the fungal biodegradation of CTP in a solid state fermentation, the chemical composition and *in vitro* digestibility of the undegraded and the degraded CTP were determined. The results showed a significant ($P<0.05$) increase in crude protein (CP) contents from 16.05% for the control, undegraded citrus pulp (UCTP) to 18.31 % for *Penicillium notatum* (PNT) and 20.11% for *Penicillium citrinum* (PCT) . The ash contents also increased significantly ($P<0.05$); it increased from 7.05% for UCTP to 8.02 % for PCT and 10.00% for PNT. The crude fibre (CF) significantly ($P<0.05$) decreased from 18.00% for UCTP to 15.50 for PCT and 16.26 % for PNT. Moreover, observable significant ($P<0.05$) differences were noted in acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (NDF). Organic matter digestibility (OMD) ranged from 28.46 to 33.09%; metabolizable energy (ME) improved from 6.42 to 8.38 MJ/kg DM and short chain fatty acid (SCFA) values ranged from 0.12 to 0.85µmol/g. There were significant ($P<0.05$) differences in the values obtained for potentially degradable fractions (b) ML. Gas production rate (C) was faster in the degraded CTP compared with the undegraded CTP. The obtained results revealed the possibility of using fungal treatment for the improvement of nutritional quality of corn offal for ruminant nutrition.

Key words: citrus pulp, *Penicillium notatum*, *Penicillium citrinum*, *in vitro* digestibility, chemical composition

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Introduction

The consistent reduction in the nutritive value of food consumed in the Sub saharan Africa arising from inadequate availability of animal protein has remained unabated. Owing to this, pre-school children, expectant and nursing mothers are subjected to malnutrition which results in diseases such as kwashiorkor, beriberi, marasmus, and intellectual depravity. To put an end to these nutritional deficiencies, proactive steps must be taken to increase the quantity and the quality of animal protein in the diets. The productivity of livestock is limited by the availability of feed in the right quality and quantity. This is due to the fact that majority of the feeding stuffs serve as staple food for humans and this brings about competition between animals and humans. In view of the above facts, future hope of feeding the teeming population and protecting their food security will depend on the better utilization of non-conventional feed resources, which cannot be used as food for humans. The main components of agro industrial residues (cellulose, hemicellulose and lignin) are complex and its biodegradability is low because of their resistance to degradation by ruminal microorganisms (Schettini *et al.*, 2013).Biotechnology, through the instrumentality of solid state fermentation (SSF), presents privileges to change the chemical structure of these substrates and therefore improves their digestibility. The advantage of using SSF to achieve both goals is low-tech fermentation system required plus the possibility of having it carried out on farm.SSF can be said to be a fermentation process in which micro-organisms grow on solid materials without the presence of free liquid (Pandey, 2003). The *in vivo* evaluation of feed quality is expensive and laborious. Hence, the need to use simple and economical techniques (Boluda Aguilar and Lopez-Gomes,*2013*), like in vitro gas production (Wiliams, 2000), which is used to determine the fermentation kinetics of cereal straws and grains (Rashid et al *et al.*, 2013) and other feed (Getachew *et al.*, 2004). Besides, the gas produced during in vitro incubation is related to production of short chain VFA (Getachew et al., 1999), which are the main energy source for ruminants. Citrus pulp is now mainly discarded as waste once the juice has been extracted from the orange. Industries producing citrus pulp have in time past incurred expenses for their proper disposal. It is a highly fermentable energy source with sweet taste and aroma. The purpose of this study is to evaluate the effect of *Penicillium notatum* and *Penicillium citrinum* on the nutritional quality of citrus pulp through chemical analysis and *in vitro* gas production technique.

Materials and Methods

Fungi

P. notatum and *P. citrinum* were the fungi used for this study. Slants of the microbes were obtained from the Department of Biological Science (Microbiology Unit), Bowen University, Nigeria. A portion of mycelia of each of the fungi was then subcultured on potato dextrose agar (PDA) in Petri dishes and incubated at 30^{o}C for 4 days (Iyayi and Aderolu, 2004).

Citrus pulp as the substrate

CTP was obtained from Funman Juice Industry in Ibadan, Nigeria. The collected CTP were screened to get rid of all foreign materials and then milled to pass through 1mm mesh screens. Enough quantity was bought at the commencement of the experiment to prevent fluctuation in quality with different batches because of the inconsistent processing techniques as observed by Onilude and Oso (1999). 50g of milled sample of CTP was placed in 250ml Erlenmeyer flask. The mouth of the flasks were clogged with cotton wool and then covered with aluminum foil. The flasks containing the substrates were autoclaved at 12^{o}C for 15 minutes. After autoclaving, it was moistened with 20 ml of basal medium containing :1.4g NH_4Cl, 0.3g urea , 2.0g KH_2PO_4,

1.4g $(NH4)_2SO_4$, 0.3g $MgSO_4.7H_2O$, 0.4g $CaCl_2.2H_2O$, 0.5mg Nicotinic acid, 0.5mg Riboflavin, 0.05mgThiamine and Biotin per litre of distilled water to obtain humidity of 38%. Flasks were inoculated with 1.0ml of aqueous spore suspensions of the respective fungus. Three set of flasks each containing CTP was aseptically inoculated with each of the 2 fungi. Another set of 3 flasks containing the autoclaved CTP was uninnoculated. This was done in order to ensure that each treatment was replicated. The flasks were covered back with sterilized cotton wool and placed in incubator set at 35^oC. Samples were withdrawn on the 7^{th} day and the action of the microbes was terminated by drying at 60^oC. The samples were dried to constant weight, thoroughly mixed and stored in sterilized containers for chemical analysis and *in vitro* digestibility studies.

***In vitro* gas production**

The rumen fluid was collected from six rams before morning feed was administered. The fluid was collected through the suction method (by means of the suction tube or hose) from six rams under the same feeding regime. The animals were fed according to their body weights; they were fed with 60% guinea grass and 40% concentrate feed (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soya bean meal, 10% dried brewer grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal). The collected rumen fluid was filtered through a four-layered cheese cloth into a flask flushed with carbon dioxide (CO_2) gas and stirred using an automatic stirrer. Incubation was carried out using the method of Menke and Steingass (1988).The buffer solution prepared was the McDougall's buffer containing: $NaHCO_3$+Na_2HPO_4+KCl+NaCl+$MgSO_4.7H_2O$+$CaCl_2.2H_2O$ in a ratio (1:4 v/v). 30ml of the innoculum was introduced into each of the pre-warmed syringes through the silicon tubes. Air bubbles trapped in the syringes were removed by shaking the syringe and then pushing the piston upward after which the steel clip on the tubes were screwed tightly. Blanks were also prepared with 30ml of innoculum, without the feed sample. The blanks were prepared as control. The incubation period lasted for 72 hours and the gas production was observed and recorded at an interval of 3 hours, including the blanks. Gas production was terminated at the end of the first 24hours, 48 hours and finally 72 hours. After each termination, 10 molar solution of NaOH was introduced through the silicon tube. After opening the steel clip, the mixture was thoroughly shaken; NaOH absorbed the CO_2 gas present in the syringe, leaving only the methane gas. The volume of methane gas was recorded and the volume of the CO_2 was determined by subtracting the volume of methane from total gas produced. Data of the *in vitro* gas production were analyzed using the equation $Y=a+b\ (1-e^{-ct})$ described by Ørskov and McDonald (1979) where Y=volume of gas produced at time t, a=intercept (gas produced from the soluble fraction), b=gas produced from the potentially degradable fraction, (a+b) = final gas produced, c=rate of degradation of b, t=incubation time. Metabolizable energy (ME, MJ/Kg DM) and organic matter digestibility (OMD %) were estimated as established (Menke and Steingass 1998) and short chain fatty acids (SCFA) was calculated as reported by Getachew *et al.*, (1999).

$ME = 2.20 + 0.136GV + 0.057CP + 0.0029CF$

$OMD = 14.88 + 0.88GV + 0.45CP + 0.651XA$

$SCFA=0.0239GV+0.0601$

Where GV, CP, CF and XA are: net gas production (ml/200mg, DM) crude protein, crude fibre and ash of the incubated sample respectively.

Chemical composition

The proximate analysis was carried out according to method of AOAC (1995).To determine the dry matter, 50g of the samples were weighed after drying at the termination of fermentation. The

weighed samples were wrapped in weighed foil papers and dried at 105^{o}C to a constant weight for 24 hours. Nitrogen (N) content of the milled dried samples was determined by the standard Kjeldhal method (AOAC 1995) and the crude protein (CP) was calculated (N x 6.25).Ash content was determined using muffle furnace. Neutral detergent fibre (NDF), Acid detergent fibre (ADF) and Acid detergent lignin (ADL) was determined using the method described by Van Soest *et al*., (1991).Hemicellulose was estimated as the difference between NDF and ADF, and cellulose estimated as the difference between ADF and AD.The nitrogen free extract (NFE) was then derived by calculation :100-(CP+CF+ash +ether extract).

Statistical analysis

The obtained were subjected to analysis of variance (ANOVA) according to the procedure of Steel and Torrie (1980) and means were separated by Duncan's multiple range test where there were significant differences using Statistical Analysis System (SAS) 1999 package.

Result and Discussion

Changes in chemical composition

Table 1: Chemical composition (g/100gDM) of *Penicillium notatum* and *Penicillium citrinum* degraded citrus pulp and undegraded citrus pulp

PARAMETERS	PNT	PCT	UCTP	SEM
Dry matter	94.90^{a}	94.28^{a}	86.71^{b}	0.31
Ash	10.00^{a}	8.02^{b}	7.05^{c}	0.36
Crude fiber	16.26^{b}	15.50^{b}	18.00^{a}	0.22
Crude protein	18.31^{b}	20.11^{a}	16.05^{c}	0.17
Acid detergent fiber	26.50^{b}	26.50^{b}	28.00^{a}	0.34
Acid detergent lignin	3.83^{b}	3.50^{b}	5.35^{a}	0.22
Neutral detergent fiber	32.65^{b}	33.00^{b}	36.00^{a}	0.59
Cellulose	33.21^{b}	30.31^{c}	36.72^{a}	0.23
Hemicellulose	22.41^{c}	24.32^{b}	27.01^{a}	0.41

a ,b ,c, means on the same column with different superscripts are significantly varied (P < 0.05) PNT = Penicillium notatum degraded citrus pulp, PCT = Penicillium citrinum degraded citrus pulp, UCTP=Control; it is the undegraded CTP. SEM= standard error of the mean.

The chemical composition of the treated and untreated citrus pulp is presented in Table 1. The crude protein changed from 16.05 to 20.11% when degraded with *Penicillium citrinum*, the ash (minerals) also improved from 7.05 to 10.00g/100DM after biodegradation with *Penicillium notatum.* The increase in the protein content of CTP after fungal biodegradation could be attributed to the possible release of some extracellular enzymes traceable to the fungal growth in an attempt to make use of the CTP as a source of carbon (Oboh and Akindahunsi, 2003; Boluda Aguilar and Lopez-Gomes,2013). Furthermore, the increase in the growth of the fungi in the form of single cell proteins could account for the increase in protein content of CTP (Oboh et al., 2002).

There were significant (P<0.05) differences observed in the CF, neutral detergent fibre (NDF), acid detergent lignin (ADL) and acid detergent fibre (ADF). The highest value of CF (18.00g/100DM) was obtained in the UCTP. The decrease in the crude fibre and the detergent fibre after biodegradation could be attributed to the ability of the fungi to hydrolyse fibre into glucose and ultimately the glucose will be used by the organisms to synthesize fungi biomass rich in protein. The proportionate increase in the protein content in the fungi fermented CTP could also account for the decrease in the fibre content (Njombolwana *et al. 2013*). Akinfemi *et al.*, (2009) opined that lignifications of structural polysaccharides not only inhibit ruminal microbial digestion of polysaccharides by forming 3-D matrix, but also that the presence of highly lignified tissues forms a physical barrier preventing accessibility of the otherwise highly digestible tissue to the action of hydrolytic enzymes of the rumen micro-organisms. Fungal biodegradation of citrus pulp therefore has the potential of breaking the structural carbohydrates and thereby increase its digestibility by the ruminant animals.

Organic matter digestibility (OMD), short chain fatty acid (SCFA), metabolizable energy (ME) and methane (CH_4) production

Table 2: Organic matter digestibility (%), short chain fatty acid (µmol) and metabolizable energy (MJ/Kg DM) of degraded and undegraded citrus pulp

PARAMETERS	PNT	PCT	UCTP	SEM
Organic matter digestibility (%)	33.09^{a}	31.82^{b}	28.46^{c}	0.17
Short chain fatty acid (µmol)	0.37^{b}	0.85^{a}	0.12^{c}	0.03
Metabolizable energy (MJ/Kg DM)	8.38^{a}	8.16^{b}	6.42^{c}	0.14

a, b, c, means on the same column with different superscripts are significantly varied (P < 0.05) PNT = Penicillium notatum degraded citrus pulp , PCT = Penicillium citrinum degraded citrus pulp, UCTP=Control; it is the undegraded citrus pulp. SEM= standard error of the mean.

Table 2 show the Organic matter digestibility (OMD), short chain fatty acid (SCFA), metabolizable energy (ME) and methane (CH_4) production. There were significant ($P<0.05$) differences in all these parameters. Organic matter digestibility was highest in *Penicillium notatum* degraded citrus pulp (33.09%). Metabolizable energy was highest in *Penicillium notatum degraded* citrus pulp (8.38 MJ/Kg DM). It is evident that the fungi degraded citrus pulp had higher percentage of organic matter digestibility .According to Getachew *et al.*, (2004). The higher OMD may be due to the microorganisms in the animals which improved the nutrient availability. Akinfemi *et al.*, (2009) used *Pleurotus sajor* and *Pleurotus pulmonarius* to biodegrade maize cob. The team observed that degraded maize cob had higher values and the highest value (42.09%) was found in Pleurotus *pulmonarius* degraded maize cob. The results showed that the ME ranged from 6.42 to 8.38MJ/Kg. Enzymes released by the fungi in the course of biodegrading the substrate (Citrus pulp) have the potential of increasing the ME as it can lead to an increase in the digestibility of cell wall components and this can enhance starch digestibility. Breakdown of β-glucan via fungi can result in more efficient starch utilization and hence increase metabolizable energy. The SCFA estimated from gas production were 0.37, 0.85 and 0.12 µmol for PNT, PCT and UCTP respectively. There were significant ($P<0.05$) differences expressed among the feedstuffs. Short chain fatty acids level indicates the energy available to the animal. It contributes up to 80% of animal daily energy requirement and it is directly proportional to the metabolizable energy (Akinyele and Akinyosoye, 2005). Since the degraded citrus pulp had higher SCFA compared with the undegraded citrus pulp, it suggests a potential to make energy available to the ruminants. Methane gas production is shown in figure 1. It is evident that the highest methane gas was produced by the UCTP. The degradation of dry matter in rumen by the microorganisms leads to production of hydrogen, carbon dioxide and methane. Methane gas is an important gas among gases produced by ruminants at fermentation and has been reported by Babayemi and Bamikole (2006) to be an energy loss to the animals and when emitted, it contributes to the depletion of ozone layer.

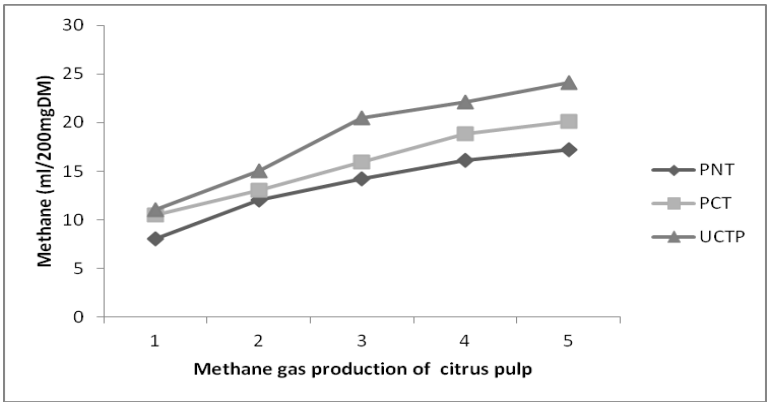

Figure 1: Methane gas production.

Gas production characteristics

Table 3: In vitro gas production characteristics of degraded and undegraded citrus pulp

PARAMETERS	PNT	PCT	UCTP	SEM
a	2.50^{c}	3.83^{b}	4.67^{a}	0.52
b	44.66^{b}	42.33^{c}	45.66^{a}	1.61
a+b	47.16^{b}	46.16^{b}	50.33^{a}	1.00
c(h^{-1})	0.075^{b}	0.078^{b}	0.085^{a}	0.00
t	14.50^{b}	14.45^{b}	16.78^{a}	0.44
y	6.27^{b}	9.66^{a}	8.33^{a}	1.01

a, b, c, means on the same row with different superscripts are significantly varied (P< 0.05), PNC = Penicillium notatum degraded corn offal, PCC = Penicillium citrinum degraded corn offal, UCO=Control; it is the undegraded CO. SEM= standard error of the mean, (a+b) = Potential extent of degradation, b= fermentation of the insoluble but degradable fraction, y= volume of gas produced, c= Rates of gas production

Table 3 shows the gas production characteristics. The results showed that there were significant (P<0.05) differences in the values of gas produced from the soluble fractions (a), gas produced from the insoluble fractions (b), a+b, gas production rate constant for the insoluble fraction (c), incubation time (t), and volume of gas produced at time t (y). Gas production is a reflection of carbohydrates degradation (Sommart *et al.*, 2000). Gas volume is a good yardstick that can be used to predict digestibility, fermentation end product and microbial protein synthesis of the substrate by rumen microbes in the in vitro system (Adenipekun and Fasidi, 2005; Sallam *et al.*, 2005) and Akinfemi *et al.*, (2009). Besides, gas production is essentially a product of fermentation of carbohydrates to acetate; propionate and butyrate .It has an indirect relationship with metabolizable energy in feedstuffs. In comparison, gas production from carbohydrates fermentation is higher that gas production from protein fermentation (Akinfemi *et al.*, 2009). The rate of gas production (c) from the table ranged between 0.075 and 0.085h^{-1}. The fastest rate of gas production was noted in UCTP. This may be due to the soluble carbohydrate fraction readily available to microbial population. Babayemi (2006) and Agbagla-Dohnani et al (2000) opined that gas production is not useful nutritionally, but, it strengthens the predictability of ME, OMD and SCFA.

Gas volume

Table 4 shows the results of the in vitro gas production over a period of 24 hours.

Table 4: In vitro gas production from degraded and undegraded corn offal for 24 hours

Incubation period (Hours)	3	6	9	12	15	18	21	24
PNC	2.20	5.30[a]	10.10[a]	15.25[a]	22.45[a]	28.15[a]	30.12[a]	34.15[a]
PCC	2.41	6.44[a]	10.01[a]	14.41[a]	19.11[b]	22.40[b]	25.41[b]	25.42[b]
UCO	2.01	2.46[b]	4.10[b]	6.12[b]	6.22[c]	8.52[c]	9.22[c]	9.23[c]
SEM	0.00	0.66	0.51	0.44	0.24	0.36	0.41	0.37

a, b, c, means on the same column with different superscripts are significantly varied (P< 0.05), PNC = Penicillium notatum degraded corn offal, PCC = Penicillium citrinum degraded corn offal, UCO=Control; it is the undegraded CO. SEM= standard error of the mean

Significant (P<0.05) were observed in the gas volume at different hours except for the 3rd hour. The highest was in PNC (34.15%) at the 24th hour followed by PCC (25.42%) also at 24th hour while the least (2.45%) was observed at the 3rd hour with the UCO. Gas volume is very important because the quality of gas produced during fermentation shows the amount of substrate digested and the microbial pathway. Gas production assists in determination of soluble and insoluble fractions of feed stuff (Akinfemi, 2009). Besides, France *et al*., (2000) opined that gas produced is directly proportional to the rate at which agro industrial by products are degraded. The higher the gas produced, the higher the short chain fatty acids and there is a direct relationship between OMD and gas production (Rodriguez *et al.*, 2002 and Schettini *et al.*, 2013).

Conclusion

1. The results showed that fungal biodegradation of citrus pulp with *Penicillium notatum* and *Penicillium citrinum* enhanced the crude protein content and reduced the detergent fibres and the crude fibre of the degraded citrus pulp.
2. There was an improvement in the in-vitro digestibility of the citrus pulp.

3. Inclusion of biodegraded citrus pulp in the diet would be of benefit to ruminant livestock.

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