ISSN:
Print - 2277 - 0755
Online - 2315 - 7453

Journal of Agricultural Science and Environment

DETERMINATION OF VOLATILE FATTY ACIDS WITH LENGTH OF FERMENTATION DAYS OF WHEAT STRAW SILAGE and ADDITIVES

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ABSTRACT

Volatile fatty acids of acetic acid, propionic acid and butyric acid are products of silage fermentation. The concentrations of volatile fatty acids in wheat straw ensiled with urea, poultry litter, watermelon peels and pineapple peels were determined in 0, 6, 12, 18, 24, and 30 fermentation days. The experiment was laid in a factorial randomized complete block design consisting 5 treatments; SWS (sole wheat straw), UWS (urea 2.5% + wheat straw), PLWS (poultry litter 25% + wheat straw), WPWS (watermelon peels 25% + wheat straw), and PPWS (pineapple peels 25% + wheat straw) each treatment were placed in triplicates. Samples from each treatment and fermentation day were collected and analyzed for concentration of volatile fatty acids (acetic, propionic, and butyric acids). The results showed a significant difference (P<0.05) in the concentrations of the acids in all treatments and the days of fermentation. The control (SWS) showed the highest levels of acetic (1.27 at 18 FDs), propionic (0.086 at FDs), and butyric acids (0.011 at 18 FDs), then PLWS but WPWS, PPWS, and UWS showed ranging similar values in acetic acid. In contrast, WPWS and PPWS show similar values in propionic acid and decreased butyric acid. In all the treatments, the concentration of the acids increased with an increase in fermentation days. It was concluded that wheat straw can be ensiled solely or with poultry litter for higher concentrations of volatile fatty acids.

Keywords: Wheat straw, Silage, Acetic acid, Propionic acid, Butyric acid, Fermentation days

INTRODUCTION

Ensiling is the most active and economical procedure to conserve forages (Tao *et al.,* 2020). It can improve palatability and prolong storage duration through anaerobic fermentation (Wang *et al.,* 2018). Anaerobic fermentation during the silage process yields volatile fatty acids through the action of a series of desirable microorganisms such as acetobacter, giving rise to acetic acid, propionic acid and butyric acid. Acetic acid is among the highest acids in concentration produced in a well - fermentative silage, when consumed by ruminants. It can be absorbed from the rumen and used for energy or be incorporated into milk or body fat. *Pro-*

pionibacteria converts glucose and lactic acid to propionic acid during a fermentation process. The use of additives containing propionic acid to improve the aerobic stability increases its content in the final product. (Krooneman *et al.,* 2002). Similarly, consumed propionic acids are absorbed by the rumen and converted to glucose in the liver, but butyric acid should not be detected in a well-fermented silage. The presence of butyric acid indicates metabolic activity from clostridial organisms which leads to deterioration of the final product (Pahlow *et al.,* 2003).

Generally, use of silage additives improves the rate and concentration of volatile fatty acids. Use of fruit peels has been reported as an inhibitor for volatile fatty acid (VFA) production in silage (Rani 2004). Conserving low-quality forages and crop residues through ensiling with different additives have been effective in the tropics but Morais *et al.,* (2017) recorded lower concentrations of volatile fatty acids in straw silage with poultry litter as an additive. Adli and Sjofjan (2018) observed a sufficient quantity of acetic acid with 20% and improved quality in poultry litter added silage.

Crop residues like wheat straws have the potency of being used as animal feed (Abdurrahaman *et al.,* 2021). With adequate processing techniques like ensiling with additives, wheat straw was reported to be a good source of ruminant feed (Bhandari, 2019). The objective of this study is to determine the range of fermentation days required for the production of volatile fatty acids in wheat straw silage.

MATERIALS AND METHODS

Study area

The research was conducted at the laboratory. Department of Animal Science, Faculty of Agriculture, Federal University, Dutse, Jigawa State. Located at latitude: 11.69174° N and 9.34525⁰ E longitude, with an average temperature ranging between 20°C and 39.76°C. The dry season lasts for about 7 months and the rainy season for about 4 months (NIMET, 2022), which makes animal feed scarce within the year.

Collection and preparation of experimental materials

Wheat Straw was obtained from a farm in Kiyawa Local Government Area, Jigawa State after mechanical threshing of wheat grains. This was screened for other impurities and foreign particles to prevent contamination; and was transported to the study area. The screened wheat straw was weighed and mixed with silage additives adequately in the recommended quantities. The additives were added as follows; urea was used as 2.5% of the wheat straw as reported by (Morais *et al*., 2017). 25% of poultry litter was used plus 75% of wheat straw ensiled, watermelon peels were used at rate of 25%, and Pineapple peel at the rate of 25%.

Experimental design

The experiment was laid in a factorial Randomized Complete Block Design (RCBD) (2x5x6) consisting of five different (5) treatments with 3 replications each, (Table 1)

Table 1: Treatment combinations

Treatments	Combinations
T1 Control (SWS)	Sole wheat straw
T2 UWS	Wheat straw + urea
T3 PLWS	Wheat straw + poultry litters
T4 WPWS	Wheat straw + watermelon peels
T5 PPWS	Wheat straw + pineapple peels

Ensiling procedure

Wheat straw including additives was thoroughly mixed, homogenized, and ensiled in an open mouthed Kilner jars (Cope BS 910-8, 1000 ml). Treatments were varied in 0, 6, 12, 18, 24, and 30 fermentation days in triplicates, a total of 90 bottles were ensiled. The mouth was sealed tightly to prevent air from entering into the jar and was stored in the laboratory.

Analytical methods

Samples were collected according to the days of fermentation for each treatment (days 0, 6, 12, 18, 24, and 30). The jar was opened; the upper layer of the material was scrubbed off and samples were taken from the middle of the jar to prevent possible contamination

Determination of volatile fatty acids (VFA)

The concentrations of VFA content were determined in silage extract by high- performance liquid chromatography (HPLC) according to earlier procedures of Kostulak-Zielińska and Potkański (2001), Gąsior (2002). The fresh silage samples were homogenized in a manual blender (Bosch) in an ice bath (for 2min), pouring in five times more water than the weight of the given sample. The homogenate was filtered by straining through miller gauze; the filtrate was passed through a soft filter (Filtrak No. 388), deproteinized with 24% (w/v) metaphosphoric acid (FLUKA) and centrifuged (7min., 10000x g at 4°C) in an MPW-350R centrifuge.

The supernatant was filtered and analyzed by HPLC system, RP, column: METACARB 67H (Organic Acids Column, Varian), mobile phase: 0.002M (v/v) sulphuric acid solution (95%, Sigma-Aldrich) in deionized water, flow rate 1cm3/min., loop 20 l, detector SDP-20A UV/Vis - 210nm). The external-standard method was employed using the SUPELCO standards of acetic, propionic and butyric acids. A mixture of standards was prepared as acetic acid 0.5mg/cm^3, propionic acid 0.495mg/cm^3, and butyric acid 0.482mg/cm^3. The peak areas from the sample were compared with the peak areas from the standards.

Data analysis

All data generated were subjected to analysis of variance (ANOVA) according to standard procedure of the Generalized Linear Model (GLM) procedures of GenStat version 17.5 means were separated using fishers LSD.

Results and discussion

The interaction of silage additives and fermentation days on acetic, propionic, and butyric acids of the resultant silage show a significant difference ($P<0.05$) among treatment groups. The values of acetic acid (Table 2) were within the range <1.00 in 18 FD in UWS, PLWS, WPWS and PPWS compared to the control SWS as described by Hou and Nishino (2021). Propionic acid (Table 3) was also within the range described by Hou and Nishino, (2021), Chen Lei *et al* (2016) and Coskuntuna *et al,* (2010) indicating better fermentation qualities, while butyric acid as (Table 4) and recommended by Steinbrenner *et al*, (2021) and Kung *et al,* (2018) should be <0.1 in a better fermentative silage product which is also obtained in this research. As observed in the above A x FD tables, Acetic acid production peaked at 24 FD for most of the additives (SWS, UWS, and PPWS) and 30 FD for PLWS and WPWS, though the increase in Acetic acid in the latter was very trivial. Propionic acid was observed to be highest at 24 FD in all treatments, while butyric acid displays the lowest values at 24 FD. Then, it can be thought that the production of volatile fatty acids silages was optimal at 24 FDs.

Table 2 : Effect of silage additives and fermentation days on acetic acid (%) of the resultant silage

Treatments	Fermentation days						P-value
	0	6	12	18	24	30	
SWS	$0.16^{p}\pm0.01$	$1.13^{c}\pm0.01$	$1.01^{d}\pm0.01$	$1.27^{b}\pm0.01$	$1.13^{c}\pm0.01$	$0.99^{e}\pm0.01$	<0.001
UWS	$0.75^{fg}\pm0.01$	$0.73^{gh}\pm0.01$	$0.55^{o}\pm0.01$	$0.68^{j}\pm0.01$	$0.69^{ij}\pm0.01$	$0.62^{lm}\pm0.01$	<0.001
PLWS	$1.32^{a}\pm0.01$	$0.65^{k}\pm0.01$	$0.62^{lm}\pm0.01$	$0.72^{hi}\pm0.01$	$0.62^{lm}\pm0.01$	$0.65^{k}\pm0.01$	<0.001
WPWS	$0.62^{lm}\pm0.01$	$0.64^{kl}\pm0.01$	$0.76^{f}\pm0.01$	$0.73^{gh}\pm0.01$	$0.64^{kl}\pm0.01$	$0.69^{ij}\pm0.01$	<0.001
PPWS	$0.60^{mn}\pm0.01$	$0.73^{fgh}\pm0.01$	$0.69^{ij}\pm0.01$	$0.75^{fg}\pm0.01$	$0.59^{n}\pm0.01$	$0.54^{o}\pm0.01$	<0.001

Means within rows and columns with different superscripts are significantly different ($P<0.05$) SWS = sole wheat straw, UWS = urea + wheat straw, PLWS = poultry litter + wheat straw, WPWS = watermelon peels + wheat straw and PPWS = pineapple peel + wheat straw.

Table 3: Effect of silage additives and fermentation days on propionic acid (%) of the resultant silage

Treatments	Fermentation Days						P-value
	0	6	12	18	24	30	
SWS	$0.01^{o}\pm0.0007$	$0.076^{c}\pm0.0007$	$0.073^{d}\pm0.0007$	$0.086^{b}\pm0.0007$	0.076 ± 0.0007	$0.067^{e}\pm0.0007$	<0.001
UWS	$0.051^{f}\pm0.0007$	$0.041^{ghi}\pm0.0007$	$0.036^{n}\pm0.0007$	$0.046^{j}\pm0.0007$	$0.047^{j}\pm0.0007$	$0.042^{kl}\pm0.0007$	<0.001
PLWS	$0.092^{a}\pm0.0007$	$0.044^{k}\pm0.0007$	$0.043^{k}\pm0.0007$	$0.047^{i}\pm0.0007$	$0.043^{k}\pm0.0007$	$0.043^{k}\pm0.0007$	<0.001
WPWS	$0.042^{kl}\pm0.0007$	$0.043^{k}\pm0.0007$	$0.051^{fg}\pm0.0007$	$0.041^{ghi}\pm0.0007$	$0.042^{kl}\pm0.0007$	$0.046^{j}\pm0.0007$	<0.001
PPWS	$0.041^{lm}\pm0.0007$	$0.050^{fgh}\pm0.0007$	$0.046^{j}\pm0.0007$	$0.050^{fgh}\pm0.0007$	$0.039^{m}\pm0.0007$	$0.036^{n}\pm0.0007$	<0.001

SWS = sole wheat straw, UWS = urea + wheat straw, PLWS = poultry litter + wheat straw, WPWS = watermelon peels + wheat straw and PPWS = pineapple peel + wheat straw.

Table 4: Effect of silage additives and fermentation days on butyric acid (%) of the resultant silage

Treatments	Fermentation days						P-value
	0	6	12	18	24	30	
SWS	$0.003^{i}\pm0.0002$	$0.009^{c}\pm0.0002$	$0.008^{d}\pm0.0002$	$0.011^{a}\pm0.0002$	$0.008^{c}\pm0.0002$	$0.007^{e}\pm0.0002$	<0.001
UWS	$0.006^{f}\pm0.0002$	$0.005^{g}\pm0.0002$	$0.004^{h}\pm0.0002$	$0.005^{g}\pm0.0002$	$0.005^{g}\pm0.0002$	$0.005^{g}\pm0.0002$	<0.001
PLWS	$0.010^{b}\pm0.0002$	$0.005^{g}\pm0.0002$	$0.005^{g}\pm0.0002$	$0.005^{g}\pm0.0002$	$0.005^{g}\pm0.0002$	$0.005^{g}\pm0.0002$	<0.001
WPWS	$0.005^{g}\pm0.0002$	$0.005^{g}\pm0.0002$	$0.006^{f}\pm0.0002$	$0.005^{g}\pm0.0002$	$0.005^{g}\pm0.0002$	$0.005^{g}\pm0.0002$	<0.001
PPWS	$0.005^{g}\pm0.0002$	$0.006^{f}\pm0.0002$	$0.005^{g}\pm0.0002$	$0.006^{f}\pm0.0002$	$0.004^{h}\pm0.0002$	$0.004^{h}\pm0.0002$	<0.001

Means within rows and columns with different superscripts are significantly different (P<0.05) SWS = sole wheat straw, UWS = urea + wheat straw, PLWS = poultry litter + wheat straw, WPWS = watermelon peels + wheat straw and PPWS = pineapple peel + wheat straw.

Acetic Acid

Acetic acid is another acid that indicates a well fermented silage. The standard values of acetic acid are 2%-3% DM which were about 0.90, 0.89, 0.88, 0.85 and 0.79 in SWS, UWS, PLWS, WPWS and PPWS, respectively (Table 2). The highest values of acetic acid were obtained in the control (SWS). This it may be because of lack of additive similar to Danner *et al.* (2003) who reported that in a similar silage with additives, acetic acid was higher in thin control group. Relatively, higher values of acetic acid were determined in PLWS, lower in UWS and PPWS. All values obtained were within the recommended range of acetic acid for ruminant nutrition. The recommended range of acetic acid in silages is desirable in order to minimize growth of yeasts and molds during aerobic exposure at feeding periods (Gerlach *et al,* 2021). Acetic acid above the recommended range were associated with lower feed intake in ruminants feeding (Krizsan and Randby, 2007).

Propionic Acid

Propionic acid and butyric acid were the lowest acid produced in silage (Table 3). Propionic acid is usually undetectable (especially in drier silages) or in very low concentrations (<0.1%) in good silages (Chen *et al,* 2016). *Propionibacteria* that convert glucose and lactic acid to propionic and acetic acid have been found in silages, but it is doubtful that natural populations can flourish in most silages. High concentrations of propionic acid (>0.3 –0.5%) are more commonly found in clostridial fermentations, likely a result of *Clostridium propionicum* (Kung *et al,* 2018), such is contrary to the findings of this study. The highest propionic acid was recorded on SWS (0.065) and from 0.052 to 0.044 in PLWS and PPWS respectively (Table 3) which were within the range of well -fermented silage.

Butyric acid

Butyric acid is the most unwanted acid in silage, its safety value is <0.1 because the presence of butyric acid indicates the action of clostridial bacteria which leads to a lost in DM and poor recovery energy. (Pahlow et al. 2003). The level of butyric acid obtained in SWS was higher (0.0079) because no additive was used in the treatment and no any fermentation enhancer or inhibitor was used, (Table 4) then it led to the growth of clostridial or undesirable microorganisms in the resultant silage. Stefani et al. (2000) explained that silages with higher butyric acids have some growth of undesirable bacteria. UWS, WPWS and PPWS have the lowest, but the range from this study was 0.0050, 0.0052, and 0.0050, respectively (Table 4). This is because the production of other acids hinders the activity of undesirable microbes responsible for the production of butyric acid in these treatments

Table 5: Effect of silage additives and effect of fermentation days on biological acids

Treatments	**Parameters (%)**		
Additives (A)	**ACT**	**PRP**	**BUT**
SWS	0.96^{a}±0.004	0.065^{a}±0.0003	0.0079^{a}±0.00009
UWS	0.67^{d}±0.004	0.045^{c}±0.0003	0.0050^{c}±0.00009
PLWS	0.76^{b}±0.004	0.052^{b}±0.0003	0.0058^{b}±0.00009
WPWS	0.68^{c}±0.004	0.045^{c}±0.0003	0.0052^{c}±0.00009
PPWS	0.65^{e}±0.004	0.044^{d}±0.0003	0.0050^{c}±0.00009
P-value	**<0.001**	**<0.001**	**<0.001**
Fermentation days (FD)			
0	0.69^{e}±0.005	0.048^{e}±0.0003	0.0059^{b}±0.0001
6	0.77^{b}±0.005	0.052^{b}±0.0003	0.0060^{b}±0.0001
12	0.74^{c}±0.005	0.050^{c}±0.0003	0.0056^{c}±0.0001
18	0.83^{a}±0.005	0.056^{a}±0.0003	0.0065^{a}±0.0001
24	0.73^{c}±0.005	0.049^{d}±0.0003	0.0056^{c}±0.0001
30	0.70^{d}±0.005	0.047^{f}±0.0003	0.0052^{d}±0.0001
P-value	**<0.001**	**<0.001**	**<0.001**
Interaction	*	*	*

Means within rows and columns with different superscripts are significantly different (P<0.05) SWS = sole wheat straw, UWS = urea + wheat straw, PLWS = poultry litter + wheat straw, WPWS = watermelon peels + wheat straw and PPWS = pineapple peel + wheat straw.

CONCLUSION

From this research, it was concluded that ensiling wheat straw with poultry litter or without additive for 30 FDs yields better production of volatile fatty acids for livestock feeding.

Acknowledgement

We will like to acknowledge Tetfund Institutional Based Research Ggrant for funding this research (FUD/VC/RU/ADM.054) and Federal University, Dutse for the support provided during the research work.

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(Manuscript received: 3rd March, 2024; accepted: 27th November, 2024).