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Evolution of Fermentation Parameters of Ensiled Sugar Beet Pulp During Storage

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Abstract

This study was carried out to evaluate the silage quality of sugar beet pulp throughout the storage period and so obtain useful information for farmers and livestock feed industry. All silages were prepared in bags of 50 kg in anaerobic conditions. Silages were sampled on months 0, 3 and 6.the results indicate a net decrease of the pH to around 3.8 after 3months silage and remained stable, an important increase of the lactic acid and a slight increase in the acetic acid. But there is a total absence of propionic acid and butyric acid and that after 3 months and 6 months silage. These results have allowed classifying silage 3 months and 6 months between a good and excellent. the results indicated that there is a positive and significant correlation between lactic and acetic acid (r = 0.955 **) and between DM and pH (r = 0 .630**), but there is a negative and significant correlation between pH and lactic acid (r = -0.896**), between DM and lactic acid (r = -0.731**), between acetic acid and pH (r= -0.921**) and between acetic acid and DM (r= -0.678**).

Keywords: Sugar beet pulp; silage; VFAs; pH; Storage period.

1. Introduction

Silage is a conservation process of moist crops by anaerobic fermentation. The lactic acid bacteria convert the soluble sugars to organic acids, mainly lactic acid; accordingly, the pH decreases and the silage is preserved as long as possible so it is not exposed to air.

The Silage method is characterized by two main phases which differ in length and intensity (Weinberg & Muck, 1996; Pahlow & al., 2003): The Initial aerobic phase when air is still trapped between the forage particles and the pH value is still neutral, which enable aerobic microbial and enzyme activities. This phase lasts for several hours until the complete anaerobic. The second phase begins in this anaerobic medium, with the death of plant cells and releases by plasmolysis their cell contents. The soluble carbohydrates released by the cell are then used by the anaerobic bacterial flora; almost all of these molecules disappear and allow the production of lactic acid and volatile fatty acids (VFA): it is the lactic acid fermentation which takes 1 to 2 weeks (Demarquilly, 1994). This fermentation results in a significant and rapid pH drop ($\leqslant$4) which generates predominant lactic acid bacteria (more resistant to low pH) and thereby inhibits the adverse butyric fermentations. (Demarquilly, 1994).

The quality diagnostic of silage depends on various chemical analysis aimed at whether the techniques used to preserve fodder have been mastered for better acidification of the medium, it is essentially the determination of the main fermentation parameters such as pH and organic acids (acetic, propionic, butyric and lactic acids).

Monitoring these fermentative parameters and inspecting the conservation status is an essential step to ensure the condition of the food provided to animals and subsequently ensure the qualitative and quantitative profitability in terms of milk production or meat.

Good conservation of silage minimizes the loss of dry matter and preserves the nutritional value of feed and at same time can improve its nutritional quality. On the other hand when conditions are unfavorable and silage is not controlled, there is a large deterioration in the quality of food which constitutes a real risk to the health of animals.

According to (Leduc & Fournier,1998) the pH provides an indication of the quality of preservation of the silage, because when the pH is sufficiently low it can stop microbial activity.

However the pH which ensures the stability of anaerobic silage and which stops the activity of clostridial bacteria, depend on the variation of to dry matter. PH should therefore be interpreted taking into account the dry matter content of silage.

In accordance with (AFSSA, 2004), lactic acid is the limiting factor for lactic fermentation; it is mainly responsible for the lowering of the pH and quality of silage. Its content in silage mainly depends to the dry matter content and to soluble sugar. Lactic acid is derived to the anaerobic degradation of glucose.

For acetic acid its produced early in the silage during the aerobic and heterolactic phase, it should not exceed 2 % DM silage. If the quantity is higher, she signed a delay of lactic fermentation and competitive sugar consumption at the expense of lactobacilli. Consequently the production of lactic acid is penalized, acidifying the forage is slowed (AFSSA, 20004).

The researches of (Dulphy and Demarquilly,1981; Demarquilly Andrieu,1988 ; Leduc & Fournier,1998) showed that butyric acid is an indicator of poor conservation and instability silage. An excellent quality silage contains less than 0.1% and can be considered average to good silage that contains less than 0.5%.

The study of (Macdonald & Clark, 1990) showed that the presence of propionic acid is both an index of the degradation of silage and the most effective mold inhibitor among the various organic acids. According to Amyot 2003 the propionic acid is a very good inhibitor of yeast and mold since it is mainly these organisms that grow in the presence of oxygen during recovery or when there is air infiltration into the silo, its existence significantly improves aerobic stability of silage, that is to say its ability to withstand heating and mold growth when exposed to the air.

Recently, some authors were interested in the study of the evolution of chemical constituents depending on the storage life as silage. Some consider it stable after 6 weeks (Pahlow & al. (2003), others consider that the silage is not stable and show that there is a great loss in DM as juice or gas if the storage life is long (Hermann & al, 2011; Der Bedrosian & al, 2012). The study of (Weinberg & Chen, 2013) shows that there is instability from the 3rd month of storage by conversion of lactic acid to acetic acid, improves in passing aerobic stability of silage, since the acetic acid is an inhibitor of yeast and mold. The main goal in silage making is to maintain the original quality of the preserved crop as much as possible. (Wilkinson & Davies, 2012).

In recent years the integration of by-products of the food industry in animals alimentation is increasing and that thanks to their high-energy or high protein content, so they can establish a competitive alternative to traditional energy sources used in animal feed (Westendorf, 2000;Vasta et al, 2008; Jaramillo & al ., 2010). Sugar beet is one of the most important crops for the production of sugar. However, it can generate a by-product such as beet pulp, which occupies an important place in the field of animal feed whatever its nature: fresh or preserved by silage.

The current study has been undertaken to obtain data on the quality of sugar beet pulp silage used after prolonged storage and to monitor the quality of conservation and fermentation parameters pH, lactic acid and volatile fatty acids: acetic, butyric and propionic acid during the storage period.

2. Materials and Methods

2.1 Sugar beet pulp

Samples of beet pulp were collected from Cosumar company (SUTA) in Beni Mellal zone (Morocco) during the sugar companion 2014. The samples have not undergone any treatment based bacterial inoculant, enzyme or by adding organic acids, salts or ammonia..

2.2 Sampling

30 bags of 50 Kg of beet pulp were analysed, these sample bags were divided into 3 periods and for each period were taken 10 sample. The T0 time present a period when the pulp is at the outlet of the press, T 3 time after 3 months silage and T 6 time after 6 months silage. Once collected, each sample was divided into 2 sub-samples and each was treated differently, one was subjected to drying for obtain the dry matter and the other was stored at -20 ° C until the analysis.

2.4 Statistical Analysis:

The data were analysed through one-way analysis of variance (ANOVA) to determine the effect of storage time, and Tukey test were performed to determine the statistical significance of the differences between means using SPSS statistical software (SPSS for Windows, Release 19).

2.3 Analysis

Dry matter (DM) was determined by oven drying for 48 h at 60◦C. To determine the fermentation parameters of the silages, a sub-sample of silages 50 g was macerated with 150 ml of distilled water and stored in a refrigerator at 4◦C for 12 h (Bureenoket al., 2006). The pH was measured with a glass electrode pH meter (HI2211 pH/ORP Meter, Hanna Instruments). Lactic acid (LA) was determined by the HPLC methods. The extracts was centrifuged for 10 min at 10,000 × g, then supernatant was reserved for volatile fatty acids (VFAs) analysis: acetic acid, propionic acid and butyric acid , which were determined using gas chromatography (GC) HP 6890 brand with automatic HP 6890 passer and a controller for automatic injection. The GC was equipped with a flame ionization detection (FID) system. The column used is a polar column, reference CP-SIL 19 CB 60m * 0.25mm * 0.15 µm. Program column: initial temperature: 40 ° C / 5 min, final temperature: 260 ° C / 5min. Detector type is FID (flame ionization detection): temperature 250 ° C. Gaz detector: Air at a flow rate of 400 ml/ min and hydrogen with a flow rate of 40ml / min. the vector gas is nitrogen with a flow rate of 2ml / min The injection is in split mode and the injection volume is of the order of 1µl.

3. Results and Discussion:

It is well-documented that one of the most important factors affecting silage quality is the rate of decrease in pH of plant material being preserved (McDonald & al., 2010). A pH range of 3.7– 4.2 is generally considered beneficial for the preservation of forages (Kung & Shaver, 2001). According to the standards set by (Dulphy & Demarquilly ,1981; Demarquilly & Andrieu ,1988) the silage is considered as an excellent conservation quality when its pH is less than 4.

Figure 1 shows a decrease in pH since 5.96 at T 0 periods to the stability at 3.8 corresponding to the T 3 period and after this period of storage the pH stay stable until T6 ensuring excellent lactic fermentation. . It's show also a decrease in dry matter since bagging with a value of 27% DM until T6 time where the value is 24.5%.

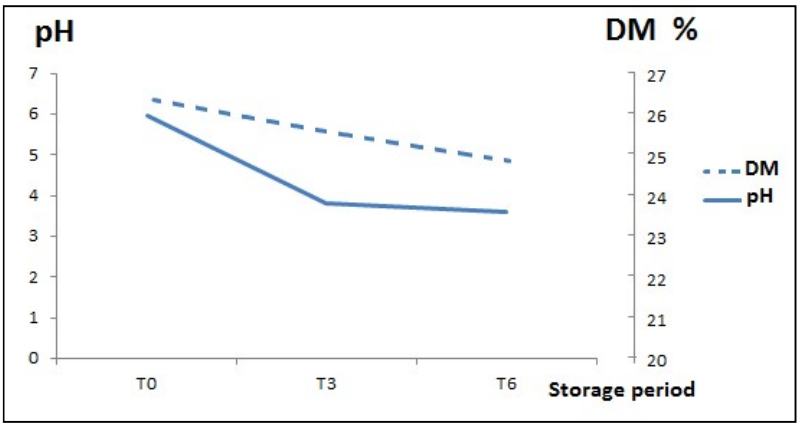

Figure 1: Evolution of the pH and DM of sugar beet pulp during silage period.

text According to (AFSSA,2004), when DM is lower than 30%, the pH must not exceed 4 and with higher levels in DM, pH can increase proportionally but should not exceed 4.4 (proliferation risk of Clostridia and / or Listeria). A low pH is unfavorable to the growth of lactobacilli, and depending on the strain, their prolif-eration is limited to a value between 3.2 and 3.8 (AFSSA, 2004). In silage rich in sugar, too acid pH can promote the growth of yeasts (poor aerobic stability). Finally it can decrease the rumen buffering capacity and reduce the amount of silage consumed.

Figure 2 reveal a decrease of pH accompanied by an increase of the lactic acid passing a value of 2.97% DM

when the pH is 5.6 at T0 time to a value of 6.83% DM when the pH is equal to 3.8 at T6 period. This can be explained by the fact that plant cells dying in an anaerobic environment by lack of oxygen and the intracellular contained is released by plasmolysis it is then used for the growth of anaerobic bacterial flora present initially on plants. While the pH decreases, the lactic flora resistant to the acidity of the medium expands, inhibits the growth of other microorganisms and transforms the soluble carbohydrates into lactic acid until pH reduced in the vicinity of 4 (R. Baumont &al.,2011). Lactic acid (LA) is the strongest of all silage acids and its presence will drop pH more effectively than VFAs (McDonald & al., 2010).

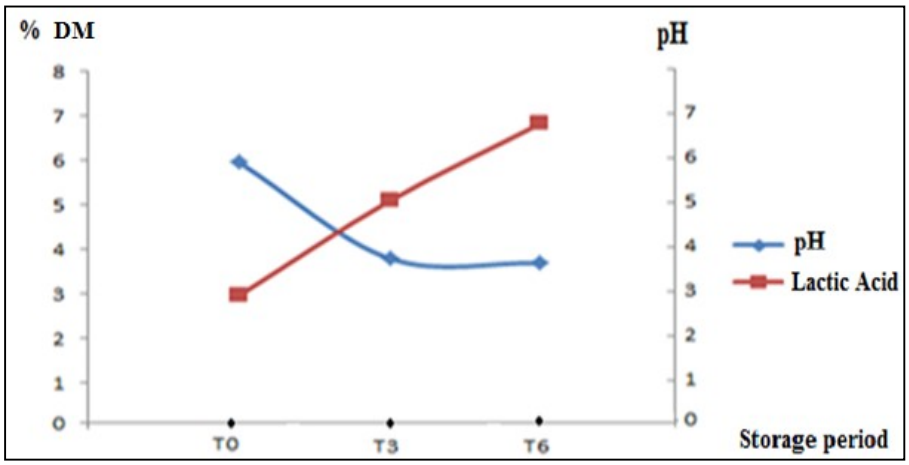

Figure 2: Evolution of de pH and Lactic acid during storage period

The results presented in the diagram (Figure 3) show that for all samples in periods T0, T3 and T6 are exempt to butyric acid and therefore absence of clostridia and butyric spores that pose a risk to animal health and risk of contamination of milk, we can conclude that the silage has an excellent quality since it contains less than 0.1% (Leduc & Fournier,1998).

The absence of propionic acid during storage period is an index of the non-degradation of silage and excellent fermentation quality.

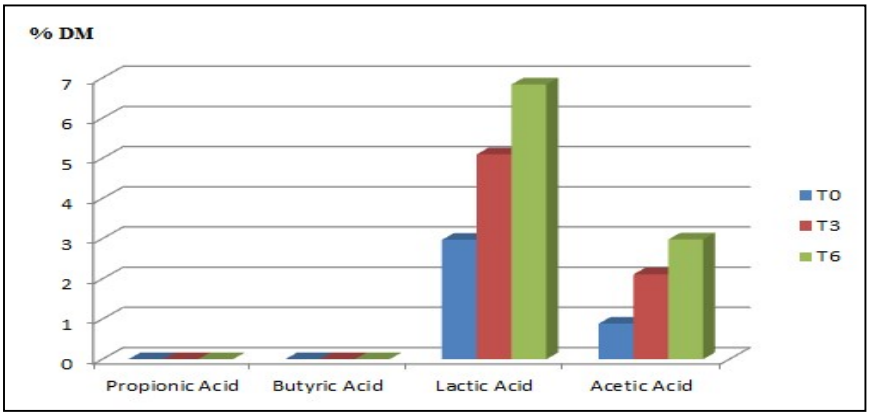

Figure 3: Diagram of contents of lactic, acetic, butyric and propionic acids during silage period

However, the lactic acid content increased going from the value 2.97 % DM at T0 time to 6.83% DM at T6 time. According to AFSSA, with less than 4% DM lactic fermentation is insufficient and below 2.5 % of DM silage, the palatability is poor. With more than 8% DM, silage is too acid, ingestibility decreases and this can promote the development of chronic ruminal acidosis. In our study the values found inform that we have high-quality silage.

The acetic acid also has grown during storage time, it increased from the value 0.8% DM at T0 time to the value 2.98 %DM at T6 time, the values shows that silage is good following Mhanna, 1994. The presence of acetic acid

in excessive amounts (greater than 4% of DM silage) gives a vinegar flavor few appreciated by the animals and can reduce their level of ingestion [AFSSA, 2004].

In Figure 4 we remark that the increase in lactic acid content is accompanied by an increase in acetic acid content with dominance of lactic acid and this is absolutely normal because the heterofermentative lactic acid bacteria present in the silage produce both acetic acid and lactic acid and the homofermentative lactic acid bacteria produce lactic acid alone.

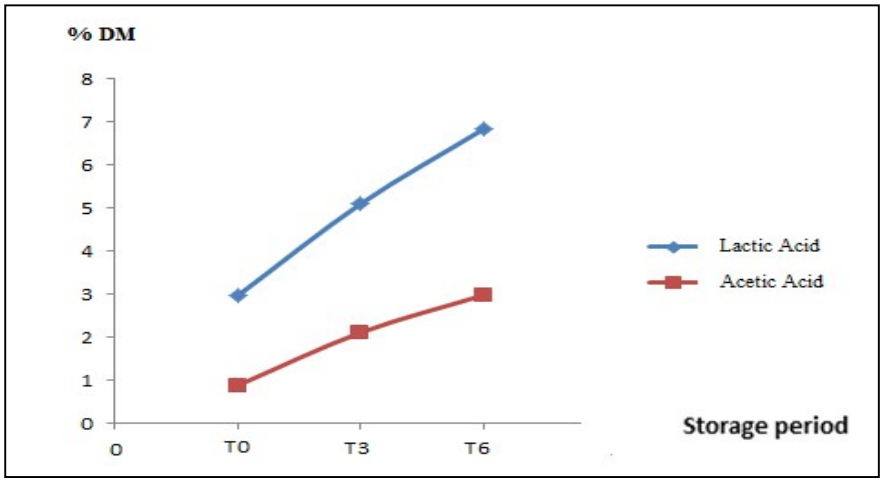

Figure 4: Evolution of the lactic and acetic acids during silage period

The proportions of organic acids relative to total acids are presented in the table 1, they provide information on the effectiveness of the silage and fermentation quality.

Table 1: Organic acids content and proportion of acid in pulp silage.

Organics Acid :	After 3 months silage	After 6 months silage
Propionic Acid (%DM)	0	0
Butyric Acid(%DM)	0	0
Lactic Acid(%DM)	5.1	6.838
Acetic Acid(%DM)	2.111	2.98
Total Acid(%DM)	7.211	9.818
The proportion of Acid		
Lactic Acid /Total acid %	70.7253	69.6476
Acetic Acid / Total acid %	29.2747	30.3524
Butyric Acid / Total acid %	0	0
Propionic Acid / Total acid %	0	0

The ratio (lactic acid / total acid) after 3 months and 6 months silage is of the order of 70%, this value is used to classify beet pulp silage performed in 50 kg bags between good and excellent quality of fermentation following WOOLFORD, 1984. Also the ratio Acetic Acid / Total acid are around 30% and according to the same author this value let's classify silage is good. The ratio (Butyric Acid / Total acid) and (Propionic Acid / Total acid) is equal to 0 (less than 1.5%) which signify that the silage is excellent.

For statistical analysis, ANOVA revealed a very highly significant effect of period for all variables considered that are: pH, DM, lactic acid and acetic acid.

For propionic acid and butyric acid the values stay stable in 0% during storage period then we can not signal significant difference (see Table 2):

Table 2: ANOVA results data of 6 parameters fermentation analyzed:

ANOVA				
	df	MS	F	Signification
Lactic Acid	2	112587	3195.789	0
Acetic Acid	2	43.615	1234.784	0
Propionic Acid	2	0	.	.
Butyric Acid	2	0	.	.
pH	2	47.001	6742.889	0
DM	2	34.114	47.335	0

Pearson correlations presented in Table 3 between different fermentation parameters: lactic acid, acetic acid, pH and DM, show that there is a positive and significant correlation between lactic and acetic acid (r = 0.955 **) and between DM and pH (r = 0 .630**), but there is a negative and significant correlation between pH and lactic acid (r = -0,896**) , between DM and lactic acid (r = -,731**), between acetic acid and pH (r= -0,921**) and between acetic acid and DM (r=-,678**).

Table 3: correlations between lactic and acetic acid, pH and DM

Correlations					
		Lactic acid	Acetic acid	pH	DM
Lactic acid	Pearson correlation	1	,955**	-,896**	-,731**
	Sig. (bilateral)		0	0	0
Acetic acid	Pearson correlation	,955**	1	-,921**	-,678**
	Sig. (bilateral)	0		0	0
pH	Pearson correlation	-,896**	-,921**	1	,630**
	Sig. (bilateral)	0	0		0
DM	Pearson correlation	-,731**	-,678**	,630**	1
	Sig. (bilateral)	0	0	0	

** The correlation is significant at the 0.01 level (bilateral).

The Pearson correlation of the propionic acid and butyric acid with other variables could not be established because the values found in these two acids are constant and they not vary during storage period (see Table 1).

5. Conclusion

Sugar beet pulp silage performed in 50kg bags has undergone a good lactic fermentation, the pH stability has been reached, a slight increase of acetic acid relative to the standard that is 2%, however there is a total absence of propionic acid and butyric and this after 3 months and 6 months of silage which could place it between a good to excellent silage. These data are important for the livestock feeding industry and for farmers who need to use silage at different times of storage.

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