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Nutritive Value of Ensiled Mangrove Leaves by *Lactobacillus plantarum* I. Fermentation Characteristics and Chemical Composition

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Abstract: The aim of this study was to obtain a qualitative and quantitative determination of chemical constituents of *Avicenna marina* leaves and its nutritive value during ensiling process. *Avicenna marina* leaves of Mangrove plant were fermented after inoculation with *Lactobacillus plantarum* using nylon bags technique. Samples were tested at zero time up to 75 days for fermentation characteristics and changes in chemical composition. Inoculation with lactic acid bacteria (LAB) plus addition of molasses and/or urea improved fermentative characteristics of mangrove silage. No great change in number of *L. plantarum* growth during the first week was occurred, after which the counts of lactic acid bacteria increased rapidly during the fermentation process, while the counts of other native microflora decreased periodically. A progressive increase in total sugars, crude protein, fat and lactic acid content reached5.8, 14.0, 9.3 and 6.5%, respectively, with molasses as an additive. On other hand, pH value was decreased to 4.2, which supports the ensiling process. Cellulose, hemicellulose and lignin contents decreased from 19.0, 15.8, 31.0 to 14.5, 10.4 and 14.2%, respectively. At the end of six weeks of ensiling period, the scanning electron microscope revealed that there was tissues degradation and laceration. Thus, during ensiling of fresh *A. marina* leaves, it was found that supplementation of lactic acid bacteria ($1x10^6$/g) with 4% molasses showed the best results concerning fermentation characteristics, nutritive value and chemical composition of silage. To the best of our knowledge, this is the first study reported the nutritive value of Mangrove leaves for silage production.

Key words: Animal feed · Fermentation · *Lactobacillus plantarum* · Leaves · Mangrove · Nutritive value · Silage

INTRODUCTION

Growing human population urges the immense need to exploit the existing livestock resources to meet the animal protein requirements. In future, it is expected that ruminants will be more dependent on forages because readily expanding human population will have direct competition with livestock for edible grains. Ensiling, a better way to preserve forage, has been known for hundreds of years and used in different countries since 18th century. Silage is defined as a semi-liquid or paste product obtained from the residues or fermented raw material. It is estimated that 200 million tons of dry matter are ensiled worldwide annually. During the ensiling process, from one day to three weeks, soluble carbohydrates are converted to lactic and acetic acids, ethanol, mannitol, acetaldehyde and carbon dioxide by anaerobic bacteria [1]. Silage microflora plays a key role in the successful outcome of the conservation process. Several workers have concluded that the addition of molasses increased the dry matter and lactic acid contents, reduced the pH and ammonia nitrogen contents in treated silages [2]. Moreover, urea can improve the digestibility, nitrogen retention and ruminal fermentation [3]. Lactic Acid Bacteria (LAB) are used to control the ensiling process, improve quality, inhibit undesirable microorganisms and ferment water-soluble carbohydrates (WSC) under anaerobic conditions. On other hand, some

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Fig. 1: Mangrove plants grow as a shrub or high trees that live along shores, rivers and estuaries (*left and middle images*) in the tropics and subtropics and characterized by survival in salt water by abundant roots (left image).

additives are used when making silage such as molasses which is a by-product of the sugar-cane and sugar-beet industries. Molasses has extensively been used for ensilage fermentation using *Lactobacilli* spp. [4] and in numerous silage experiments has been proven to be an effective additive for the ensiling process in terms of promoting lactic acid fermentation and generally decreasing organic matter losses [5].

To deal with salt, all Mangrove trees exclude some salt at the abundant root level (*left image*) and all can tolerate more salt in their tissues than normal plants, often in quantities that would kill other plants. Salt that gets through are believed to be concentrated and stored in old leaves which are later dropping down. Leaves with a waxy coating (*right image*) limits saltwater penetration and salt-secreting pores on the leaves allow the plant to get rid of excess salt [8]. When used in silage fermentation, *Lactobacillus* spp. reducing silage pH and discouraging *Clostridial* fermentation and proteolysis. Urea is also commonly used as a feed additive to increase crude protein content [6]. Urea as a source of ammonia is relatively safe and convenient in handling method of chemically treating forage. Many renewable raw materials have extensively been used for preparation of silage [7]. Mangroves are plants grow along shores and are usually found only in the tropic climates, as they need consistently warm conditions for development and survival. Medicinal extracts of Mangrove have been extensively reported worldwide [8]. Different plant materials have low crude protein content [9] and high structural carbohydrate contents, which usually lead to low nutritive value of silage [10, 11]. *Avicenna marina*, a kind of Mangrove plants commonly known as gray mangrove, belongs to the family *Aviceniaceae*. It grows as a shrub or tree to a height of three to ten meters, or up to 14 meters in tropical regions, growing in the saline [12]. They live in water up to 100 times saltier than most other plants can tolerate, growing where land and water meet and can thrive despite twice-daily flooding by ocean tides. They occur in approximately 112 countries and territories [13]. Mangrove litter of low nutritive value is decomposed and converted into a rich nutrient which serves as food for fishes [13]. Studies on Mangroves have been mainly focused on ecology, ecological physiology, plant taxonomy, ecological community, bioactive compounds and anti-inflammatory effect [14-18]. Until quite recently, Mangrove plants have not yet been exploited for making silage. However, there are only a few scattered studies on chemical composition and biological activities of a certain Mangrove plants. No researches were found to use Mangrove leaves in the food area. To the best of our knowledge, this is the first report deals with the ensilage of Mangrove leaves by *Lactobacillus plantarum*. Effects of adding molasses and/or urea on the fermentation process were also investigated. Chemical changes and nutritive value during fermentation of Mangrove leaves are also reported herein.

MATERIALS AND METHODS

Plant Material: Mangrove plants were collected from mangrove forests at Kilo 8 of Red Sea in BortSudan City, Sudan was identified as *Avicenna marina* at Marine Biology Department, Faculty of Marine and Science Fisheries, Red Sea University. The plant leaves were washed with tap water shade dried.

Culture and Inoculum Size: *Lactobacillus plantarum*, used in the present study for silage fermentation, was purchased from the cultural collection center (Mersin/Ain Shams University). Preparation of inoculum size (1 x 10^6per gram) of wet leaves for ensiling experiment was performed according to Paviz *et al*. [19].

Additives for Ensiling Process: Sugarcane molasses and urea were used, individually or in combination, as additives during ensilage process to achieve higher nutritive value. The rate was as follows: molasses, 4%, urea 0.3%, an inoculum of *L. plantarum* at a rate of $1X10^6$ g^{-1}.

Ensilage Preparation: *Avicenna marina* leaves were ensiled using nylon bags [20, 21] after being thoroughly mixed with molasses and/or urea as additives and inoculated with *L. plantarum*. Up to 200g of Mangrove leaves per one nylon bag were used, as whole leaves, where 64 bags were used for the whole period of ensiling process. The amount of water required to raise the leaves moisture to 60% water holding capacity was calculated before mixing with the leaves. Before ensiling, leaves were compacted until air between particle size get off and then the nylon bags were closed tightly. Experiments were done in duplicate and carried out by taking two bags at zero time up to 15,30,45,60 and 75 days, respectively. During ensiling, all silage bags were stored in dark place [22].Fermented leaves were analyzed for estimation of dry weight loss, total sugars content, nitrogen, lipids and protein. In addition, total lignin, cellulose, hemicellulose, ash, dry matter digestibility, pH and lactic acid content were also determined. Dry matter (DM) loss was determined by the difference between dry weight before and after fermentation and calculated as percentage of initial weight. Temperature was regularly measured every 24 h. during the ensiling period. The pH of each sample was determined in duplicate using, approximately, 25 g ensilage added to 100 ml of distilled water. After hydration for 10 min using a blender, the pH was measured using a digital pH meter [22].

Chemical Analysis: All chemical analysis experiments were achieved in Animal Production Department, National Research Center, Dokki, Giza, Egypt by Dr. Abeedo. Ensiled samples were dried in a forced air oven at 50°C [23]. Residual ash was assayed at 600°C for 4h [23]. Crude protein and fat were determined by micro- Kjeldahl method according to A.O.A.C [24]. Soluble sugars were determined by the phenol method [25]. Lactic acid content was determined according to the method of Friedemann and Graeser [26]. Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) contents were determined as described by van Soest *et al*. [27]. Hemicellulose contents were estimated as the difference between NDF and ADF, while cellulose content was the difference between ADF and ADL [27]. Acid detergent lignin (ADL) was determined according to A.O.A.C [28].

Moisture Content: A 50 g of silage were dried at 105°C to constant weight. Difference in weight indicates the moisture content:

Moisture content% = (Wet weight - Dry weight) / Wet weigh x 100.

Scanning Electron Microscopy: Structural degradation of fermented *A. marina* leaves was observed using scanning electron microscope (SEM). Specimens were prepared for the SEM by a modified method of Akin and Amos [29]. Samples were described for Scanning Electron Microscope (SEM) by the Central Lab for Services (NRC).

RESULTS ANDDISCUSSION

The present study was conducted to investigate the effects of adding 4% molasses and/or 0.3% urea on fermentation dynamics, crude protein, crude fat, lactic acid and structural carbohydrate degradation of *A. marina* leaves. The major goal for ensilage of mangrove leaves is to retain its original nutritional value at the highest value possible during fermentation process.

Bacterial Populations During Ensiling Process: The microbial count of solid-state fermented *A. marina* leaves during ensiling processes was estimated. At the beginning of the fermentation process, there was a wide competition between the native microflora and inoculated *L. plantarum*. It was estimated that there is no great change in *L. plantarum* growth during the first week, after which the counts of lactic acid bacteria increased rapidly during the fermentation process, while the counts of other native microflora decreased periodically [30]. Count of yeast sharply decreased, whereas coliforms and fungi showed little increase during the first seven days of fermentation and maintained an elevated level until 15 day, thereafter the levels dropped back. *Bacilli* showed little increase during the first 7 days of incubation but dropped after 30 days. In a fermentation study, it was reported that, controlled fermentation typically involves lactic acid bacteria, molds, yeasts and bacillus as inoculum because they can achieve more predictable results under optimal conditions. When a combined liquid culture of *Lactobacillus fermentum*, *Saccharomyces cerevisae* and *Bacillus subtilis*, was used, it was found that a successful fermentation process was occurred, where *Saccharomyces cerevisae* was added to consume the oxygen inside the fermenting nylon bag to promote the growth of anaerobes including lactic acid bacteria and Bacillus. On the other hand, *Bacillus subtilis* has been shown to be a potential alternative to antibiotics for use in animal feeds because it inhibits the effect of *E. coli* [30]. Bacterial populations and enzymes produced during ensiling process will be further amplifying studied.

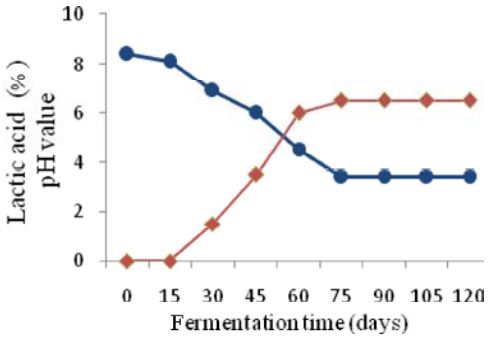

Fig. 2: Enhancement of lactic acid (♦) content and reduction in pH (•) values during ensilage process by *lactobacillus plantarum* grown with molasses and urea up for 120 days.

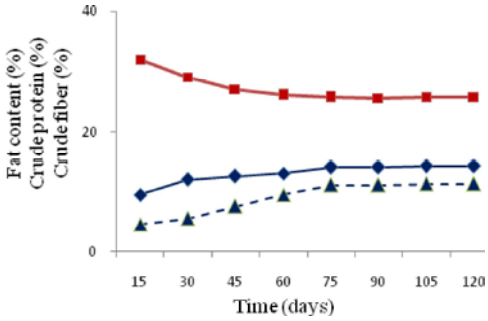

Fig. 3: Nutritive value (%) of silage treated with molasses and urea after fermentation for 75 days by *lactobacillus plantarum*; (▲) fat content, (♦) crude protein and (■) crude fiber.

Nutritive Value of Silage During Fermentation: Lactic acid, in molasses-treated silage, increased periodically during ensiling process due to the accelerated growth of lactic acid bacteria, which fermented sugars to lactic acid (Fig. 2). Since the majority of the microflora in the original Mangrove leaves is aerobes, they would be superseded by the facultative anaerobes (*L. planetarum*), which established anaerobic conditions. Gram-negative microorganisms (*coliforms*) would be replaced by Gram-positive bacteria (*lactobacilli*). So, cessation of coliform growth is due to the accumulation of lactic acid produced by *L. plantarum*. It was found also that the number of *L. plantarum*, which is well known to be antagonistic to other silage microorganisms, increases as silage matures. During ensiling, increasing organic acids such as lactic and acetic acids concentration can inhibit the growth of undesirable microorganisms [31]. Increment in lactic acid in silage was found to be stable after 75 days up to 120 days of fermentation (Fig. 2). Nutritive value of silage increased during fermentation, where crude protein and fat content increased gradually, with an obvious decrease in the crude fiber (Fig. 3). Decreases in fiber content may be due to partial hydrolysis of hemicelluloses and lignin [32]. It is well known that the initial lactic acid content of Mangrove leaves is nil. Therefore, the existence and increase of this acid during the ensiling process was due to the accelerated growth of *L. plantarum* which ferments sugars to lactic acid. On other hand, average temperatures were 17, 23, 24 and 26°C in March, April, May and June, respectively. Low temperatures are optimum during ensiling. Higher temperatures (> 49°C/120°F) often result in poorer-quality silage and could reduce the fermentation quality, enhance protein degradation and reduce the rapid pH decline that are necessary for an efficient fermentation. Excessively heated or heat-damaged silages have a brown to dark brown color with a tobacco-type smell [33]. Part of the protein in heat-damaged silages is complexed with carbohydrates and become less digestible. The concentration of heat-damaged protein depends on both the temperature and the length of time during which the temperature is elevated. Heat-damaged silage may be palatable, but part of the protein and some of the energy it contains will be unavailable to livestock [33]. When treated with molasses, silage showed the best nutritive value compared with other treatments (Table 1). Maximum values of lactic acid, crude protein and fat content reached 6.5, 14.0 and 9.3%, respectively. On other hand, no comparable values were observed between silage treated with urea and silage treated with molasses and urea, irrespective of the fat content (Table 1). On other hand, no great difference was observed in dry matter digestibility between silage treatments. Use appropriate additives can increase nutritional value and have positive effect on silage quality [34].

Table 1: Nutritive value after fermentation of silage treated with molasses and urea after 75 days by *lactobacillus plantarum*.*

Treatment*	pH	Dry matter digestibility	Lactic acid	Crude fiber	Crude protein	Fat content
Control	8.3	0.0	0.0	29.5	11.5	4.5
LAB without additives	5.1	5.8	4.6	26.3	12.8	4.7
LAB + Molasses	4.2	7.2	6.5	25.5	14.0	9.3
LAB + Urea	4.9	7.0	6.0	25.3	13.0	7.5
LAB+ Molasses+ Urea	4.5	7.5	5.5	25.6	13.2	9.6

*All chemical composition values were expressed as percentage (%).

Table 2: Chemical composition after fermentation of silage treated with molasses and urea after 75 days by *lactobacillus plantarum*.*

Treatment	Total sugars	Ash	Cellulose	Hemicellulose	Lignin	Organic matter
Control	4.6	14.7	19.0	15.8	31.0	85.5
LAB without additives	5.1	19.6	15.2	10.8	16.5	83.0
LAB + Molasses	5.8	20.4	14.5	10.4	14.2	81.3
LAB + Urea	4.7	19.2	14.8	10.6	15.2	81.4
LAB+ Molasses+ Urea	6.5	21.3	14.2	10.0	15.8	81.2

*All chemical composition values were expressed as percentage (%).

Table 3: Nutritive value and chemical composition of silage during fermentation by *lactobacillus plantarum*

Period (days)	pH	Lactic acid	DMD*	Crud protein	Fat content	Cellulose	Hemicellulose	Lignin	Total sugars	Ash
0	8.4	0.0	0.0	6.5	4.5	19.0	15.8	25.6	0.0	14.2
7	8.4	0.0	0.0	6.5	4.5	19.0	15.8	25.6	0.0	14.2
15	7.9	0.0	0.6	6.8	4.8	18.9	15.6	25.1	0.9	14.6
21	7.6	0.8	1.0	8.0	5.1	18.2	15.2	24.4	1.4	15.4
30	6.5	1.6	2.2	9.5	5.9	17.4	14.8	22.6	3.2	16.2
45	6.0	2.3	3.1	10.8	6.2	16.2	13.9	19.2	4.0	17.1
60	5.4	3.5	4.6	11.2	6.8	15.8	12.2	17.4	4.7	18.8
75	5.1	4.6	5.8	12.8	7.4	15.2	10.8	16.5	5.1	19.6

*DMD = Dry matter digestibility

Degradation of Lingo-Cellulosic Carbohydrates: As shown in Table 2, results obtained indicated that there was a pronounced decrease in cellulose, hemicellulose and lignin content during fermentation of all treatments (indicated below). It may be related to the effect of enzymes such as cellulase and hemicellulase produced during fermentation process. It could also be attributed to acidic conditions as a result of the organic acids developed in silage and subsequently, the prevalence of anaerobic bacteria. Lignin content was decreased (Table 2) during fermentation period, probably due to the microbial degradation of the bonds between lignin and carbohydrate polymers, i.e. cellulose and hemicellulose. Decrease in carbohydrate polymers was associated with tissues laceration and destructed structure which appears through electron microscope. These results are in accordance with previously reported study for millet silage [32].The crude fiber digested difficultly was gradually easily digested and crude protein increased at the same time [35]. During fermentation process, silage tissues changed and degraded [32]. Hemicelluloses can act as reservoir of fermentable sugars in silage [36]. High degradation of carbohydrate polymers such as cellulose, hemicelluloses and lignin could be attributed to the prevalence of anaerobic conditions, which were suitable for the development of some microorganisms having the ability to aid in breakdown of these polymers by carbohydrate-active enzymes [36].

Ensiling Process

Silage Fermentation by Lactic Acid Bacteria: Inoculation with LAB, essentially lowered the pH value, improved lactic acid production and improved dry matter digestibility [37]. In silage treated by *L. plantarum*, homo-fermentation is stimulated and lactic acid produced [31]. When used for silage fermentation, *L. plantarum* has proved to be an effective strain; decreasing the pH value from 8.4 to 5.1, increasing lactic acid content up to 4.6 and an overall increment in fat content, crude protein, dry matter digestibility as well as total sugars and ash (Table 3). On other hand, cellulose, hemicellulose and lignin contents are decreased from 19.0, 15.8 and 25.6% to 15.2, 10.8 and 16.5%, respectively (Table 3). Many different additives can be used to improve silage fermentation or provide supplementary nutrients. However, addition of molasses at ensiling can increase the fermentation rate by increasing organic acid production and lowering the pH value [33]. On other hand, it was found that addition of molasses and/or urea to *L. plantarum* during the ensiling process obviously increased silage quality as will be discussed below.

Silage Fermentation by Lactic acid Bacteria and Molasses: There are different objectives of using silage additives. The main target is to prevent secondary fermentation and to decrease butyric acid production. The effectiveness of additives depends on the degree of preventing such fermentation in silages [5]. Molasses contains nutrition sources such as mono- and oligo-sugars, some nitrogenous compounds such as amino acids as well as a minor amount of fatty acids [38]. Therefore, molasses is considered a rich energy source for growth of microorganisms. During the second stage, from one day to three weeks, soluble carbohydrates are

Table 4: Nutritive value and chemical composition of silage during fermentation by *lactobacillus plantarum* and molasses.

Period (days)	pH	Lactic acid	DMD*	Crud protein	Fat content	Cellulose	Hemicellulose	Lignin	Total sugars	Ash
0	8.3	0.0	0.0	6.5	4.5	19.0	15.8	25.6	0.0	14.2
7	8.3	0.0	0.0	6.6	4.5	19.0	15.8	25.6	0.0	14.2
15	7.6	0.0	0.9	7.8	5.2	18.3	15.4	25.2	1.2	14.8
21	6.4	0.0	1.2	9.2	6.8	17.6	14.8	24.4	2.3	16.6
30	5.4	2.0	3.4	10.5	7.2	16.3	13.7	21.8	4.5	17.2
45	5.1	4.0	5.3	11.0	7.8	15.4	12.0	18.7	4.7	17.9
60	4.6	6.2	6.8	11.5	8.6	14.8	11.8	15.8	4.9	19.0
75	4.2	6.5	7.2	14.0	9.3	14.5	10.4	14.2	5.8	20.4

*DMD= Dry matter digestibility

Table 5: Nutritive value and chemical composition of silage during fermentation by *lactobacillus plantarum* and urea.

Period (days)	pH	Lactic acid	DMD*	Crud protein	Fat content	Cellulose	Hemicellulose	Lignin	Total sugars	Ash
0	8.4	0.0	0.0	6.5	4.5	19.0	15.8	25.6	0.0	14.2
7	8.4	0.0	0.0	6.5	4.5	19.0	15.8	25.6	0.0	14.2
15	7.8	1.2	0.8	6.8	4.8	18.2	15.5	25.2	1.0	14.5
21	7.4	1.8	1.2	8.2	5.3	17.8	15.0	24.6	1.6	16.0
30	6.3	2.4	2.9	9.8	6.2	16.4	14.5	22.2	2.0	17.2
45	5.6	4.3	4.2	11.4	6.8	15.7	13.6	18.4	2.8	18.3
60	5.1	5.8	5.8	12.6	7.2	15.2	12.1	16.6	3.5	19.0
75	4.9	6.0	7.0	14.0	7.5	14.8	10.6	15.2	4.7	19.7

*DMD= Dry matter digestibility

converted to lactic and acetic acid, ethanol, mannitol, acetaldehyde and carbon dioxide by anaerobic bacteria [1].

Therefore, qualitative changes occurring within the lactic microflora could be explained. Therefore, qualitative changes occurring within the lactic microflora could be explained on the basis of antibiosis antagonistic activities, the changes in conditions within ensilage bags from aerobic to anaerobic conditions and acid tolerance of lactobacilli to the pH (3.8-4.2). The antagonistic activity of *lactobacilli* against other silage microflora and the antibiosis activity among the *lactobacilli* are more plausible explanations for the dominance of lactic acid bacteria. Successful production of silage from mangrove leaves by fermentation depends on their adequate amount of C-source, N-source, minerals and the establishment of anaerobic conditions. Because mangroves leaves are naturally low in protein and sugars, accordingly, nutrient additives such as energy source (molasses) was added to improve both nutritive value and fermentation quality. Obviously, when molasses was added to LAB during fermentation, all of lactic acid, crude protein, fat content and DMD were increased by 6.5, 14.0, 9.3 and 7.2%, respectively, associated with a decrease in pH to 4.2 (Table 4). On other hand, lignocellulosic material decreased to 14.5, 10.4 and 14.2% for cellulose, hemicellulose and lignin, respectively (Table 4). A pronounced decrease in fiber content may be due to partial hydrolysis of carbohydrate polymers [32]. The high content of minerals in silage treaded with molasses resulted in higher ash content from 14.2 to 20.4% (Table 4). These results are in accordance with other reported studies [3, 22, 39, 40].

Silage Fermentation by Lactic Acid Bacteria and Urea: Addition of urea to LAB for silage fermentation was tested. Urea is apt to be hydrolyzed to ammonia due to the activity of urease which is a common enzyme existing in plants and microbes. Urea as a source of ammonia is relatively safe and convenient in handling method of chemically treating forage and is commonly used as a feed additive to increase crude protein content [3]. Silage treated with urea is characterized with high protein content (Table 5).

Fermentation of Silage by Addition of Lactic Acid Bacteria, Molasses, Urea: Fat content of *A. marina* leaves increased during fermentation period in all treatment samples compared with control; it reached a maximum in molasses + urea treated sample (Table 6). Fermentation of silage with molasses + urea was characterized with increment in DMD, fat content, total sugars and ash; where it reached 7.5, 9.6, 6.5 and 21.3%, respectively, in comparing with other treatments. These results might be due to the capability of different microorganisms to utilize urea in presence of molasses,

Table 6: Nutritive value and chemical composition of silage during fermentation by *L. plantarum*, molasses and urea.

Period (days)	pH	Lactic acid	DMD*	Crud protein	Fat content	Cellulose	Hemicellulose	Lignin	Total sugars	Ash
0	8.4	0.0	0.0	6.5	4.5	19.0	15.8	25.6	0.0	14.2
7	8.4	0.0	0.0	6.5	4.5	19.0	15.8	25.6	0.0	14.2
15	8.1	1.0	0.9	6.9	5.1	18.2	15.4	25.1	0.9	14.6
21	7.5	1.6	2.6	8.5	6.4	17.4	14.8	23.2	1.7	16.2
30	6.2	2.2	3.8	10.2	7.0	16.1	13.6	21.4	4.2	17.0
45	5.8	4.0	5.2	11.3	7.8	15.2	11.9	19.2	4.8	19.5
60	5.2	5.2	6.8	11.8	8.6	14.6	11.2	17.5	5.2	20.2
75	4.5	5.5	7.5	13.2	9.6	14.2	10.0	15.8	6.5	21.3

*DMD = Dry matter digestibility

where molasses contains nutrition sources such as oligo- and mono-sugars, some nitrogenous compounds such as amino acids in addition to a minor amount of fatty acids [41].The degradation of carbohydrate polymers in all treatments took place during the first three weeks in appreciable amounts. This might be due to the effect of some enzymes produced by microorganisms during the fermentation process and/or the acid conditions developing in silage as a result of the organic acids produced. Moreover, it could be concluded that, presence of ammonia liberated by the action of urease of microbial origin, enhanced the microbial degradation of such polymers. During ensiling of *A. marina* leaves, it was observed that lactic acid was increased; it reached about 6.5% at the end of 60 days. At the same time there was a decrease in pH value which reached 4.2 whereby lactic acid bacteria convert WSC into organic acids, mainly to lactic acid [11].

At beginning of silage process, lactic acid bacteria grow very fast where lactic acid declined the pH values. Rapid drop in pH during ensiling and increasing organic acid concentration can inhibit the growth of undesirable microorganisms such as *Clostridia*. Silage treated with molasses maintained high lactic acid which indicating stable lactic acid fermentation. This could be attributed for molasses addition which supply sufficient substrate and played an important role in promoting efficient fermentation. From these results it could be concluded that, supplementation of lactic acid bacteria with 4% molasses and 0.3% urea during ensiling process is recommended. Molasses and molasses/urea treatment increased the DM and WSC contents compared with control. This might be attributed to high DM and soluble sugar of molasses [42]. During the second stage, from one day to three weeks, soluble carbohydrates were converted to lactic [1]. Lactic acid bacteria are classified to homo-fermentative or hetero-fermentative according to the end products of fermentation [33]. The antagonistic and antibiosis activities of *L. plantarum* stimulate homo-fermentation and increase lactic acid concentration, subsequently the palatability increased. In the present study, *L. plantarum* raise homo-fermentation and decline the pH value, where such acidic environment can preserve WSC and residual WSC provides an additional advantage to the aerobic stability of silage [35]. On other hand, *Clostridial* fermentation (hetero-fermentation) could be enhanced when conditions are unfavorable for silage due to the changes in conditions within the bags from aerobic to anaerobic. However, acid tolerance of *Lactobacilli*, the pH value (3.8-4.2), antagonistic and antibiosis activities of *Lactobacilli* against other silage microflora, are more plausible explanations for the dominance of lactic acid bacteria and inhibiting other native microflora.

Scanning Electron Microscopy: Our study was also designed to assess the degradation and hydrolysis features of Mangrove leaves during the fermentation process. Leaf sections observed with SEM was recorded with degraded tissues (Fig. 2). Intact leaf section incubated with *L. platarum* and observed with the SEM revealed differential tissues destruction (Fig. 4). Mesophyll and cell walls were obviously degraded and most tissues had lost structural integrity, whereas sclerenchyma and rigid vascular tissues resisted microbial digestion [29].The tissues digested after longer incubation times (45 days) [32].The zones of degradation were sharply defined in contrast to the diffuse zones inside cell tissues. Applying ammonia not only increased crude protein content but also promoted degradation of structural carbohydrate due to the hydrolytic action of ammonia on linkages between lignin and structural polysaccharides [43, 44].

Long Shelf-life of Silage: Silage is high moisture forage, stored in the absence of oxygen and preserved by acids produced during fermentation process. The silage remains preserved as long as air is kept out because spoilage-causing yeasts in silages remain dormant in the absence of oxygen. Entry of oxygen into the silo revives the yeasts and may cause spoilage. The present study

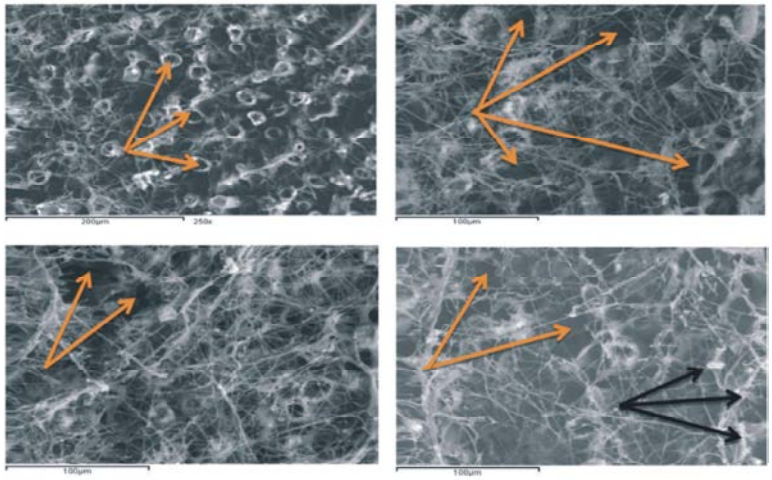

Fig. 4: Structural degradation of fermented *A. marina* leaves using scanning electron microscope revealed that, the rigid vascular tissues, epidermis, mesophyll and sclerenchyma are intact (7 days, *Above/left image*).Areas of degraded zones are seen (21 days, *Above/right*).Cells do not have a uniform shape (30 days, *Down/left images*). Obvious progressive degradation is observed indicated by destructive structural integrity tissues (45 days *Down/right image*) and attachment of bacterial cells to the tissues (Black arrows).

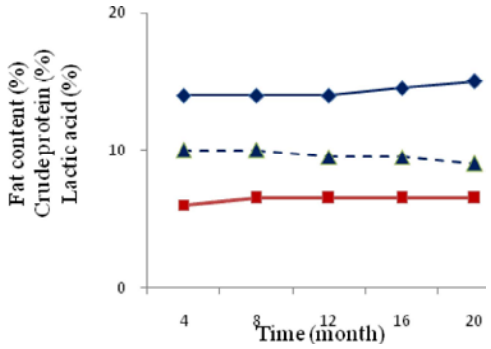

Fig. 5: Stability of silage constitutes: (▲) fat content, (♦) crude protein and (■) lactic acid,during a long period of storage.

produced a mangrove silage possesses several advantages characterized with long-shelf life and maintain its high quality and odor, kept its color, smell and stability against spoilage. In addition, keeping the quality of silage for long period may support this assumption. Moreover, Mangrove silage is characterized with high levels of minerals and amino acids, among which seven essential amino acids [37, 45, 46]. Mangrove Silage has high concentration of fermentable carbohydrates, it is, thus, rich in nutritive value and is, economically, inexpensive. Generally, properly made silage has several advantages includes: 1) Stable composition of silage for a longer period (up to 5 years); 2) Plants can be efficiently used by livestock; 3) More economical use of plants with high yield of green mass; 4) Allows by-products (such as sugar-cane and sugar-beet processing, etc.) to be optimally used; and 5) Requires 10 times less storage space compared to hay; 6) Greater flexibility and fit for many livestock feeding programs [47]. Disadvantages, however, include: 1) Higher moisture content results in heavier forage that is less economic to haul; 2) Requires specialized equipment for harvesting, storing and feeding operations; 3) High loss potential if silage is not made well; and 4) Shorter shelf life after the silo is opened [47].

CONCLUSION

In the present study, the major goal of ensilage of *A. marina* leaves is to retain its original nutritional value at the highest possible value. It is based on lactic acid fermentation under anaerobic conditions. The successful formation of silage from mangrove leaves by fermentation depends on the dominance of lactic acid bacteria. Nutrient additives as energy source (molasses) and nitrogen source (urea) were tested to improve both nutritive value and fermentation quality. It is suggested that, improvement of nutritive value of silage materials treated with molasses was associated with the increase of bacterial digestion of fibrous material which caused structural changes of cell wall and bacterial degradation of cell constituents. Growth of lactic acid bacteria decreases the pH value. Attainment of low pH is one of the important determinants for final silage fermentation quality. Bacteria maintained in silage are considered naturally proteins and contain more than 75% true protein.

Inoculation of lactic acid bacteria at $1x10^6$ per gram and addition of 4%molasses, during ensiling of *A. marina* leaves, showed the best results concerning fermentation characteristics, nutritive value and chemical composition. Supplementation with 0.3% urea, during ensiling of *A. marina* leaves, is also recommended.

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