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UTILIZATION OF FISH AND MANGO WASTES ON BIOLOGICAL SILAGE PRODUCTION

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ABSTRACT

The aim of this study was to demonstrate the utilization of mango and fish wastes in biological silage production. Six treatments containing different levels of fish and mango wastes, sugar cane molasses and corn stover were inoculated with 4 % of Lactobacillus sp. B2 strain and incubated 15 days at 30 °C. The pH values of silages varied from 4.39 to 4.70 and the lactic acid content between 2.96 to 4.42 %. Silages showed the best acidifying at 7 day of fermentation; also they further increased the lactic acid bacteria counts and decreased the coliforms bacteria. Crude protein, ethereal extract and minerals values were higher in treatments with the upper level of fish wastes. The silage with the higher corn stover showed the upper crude fiber value and the lower in vitro dry matter digestibility. Contrary, the high inclusion level of fish wastes resulted in the highest dry matter digestibility. In conclusion, the combination of fish and mango wastes is a good alternative to produce biological fish silage as an ingredient for ruminants feed. The process of production is simple and environmental friendly.

Contribution/Originality: The present study contributes to knowledge in the use of mango and fish wastes to produce silage. The findings show that silage can be evaluated in ruminants feeds for increase the added value and avoid the environmental impact of waste landfill sites.

1. INTRODUCTION

The status of aquaculture production in 2014 reported 73.8 million tons without production of plants included [1]. Furthermore the estimated world production of fish wastes 17.9 and 39.5 million tons per year, representing an important source of nutrients, however these wastes are often eliminated causing lesser or greater environmental impacts [2, 3].

Recently studies conducted with fish wastes has been determined that more than 50% of fish tissues including fins, heads, skin and viscera should be valued nutritionally because world's fisheries and aquaculture discards exceed 130 million tons per annum [4]. In spite of the low value traditionally assigned to fishery wastes, from this huge mass of unused/under-utilized resources a significant amount of bioactive compounds with wide pharmaceutical and biotechnological applications could be produced, such as proteins (enzymes, collagen), protein hydrolysates, lipids,

astaxanthin, chitin and oils rich in Polyunsaturated Fatty Acids (PUFAs), especially Eicosapentaenoic Acid (EPA) and Docosaesaenoic Acid (DHA) are particularly interesting for their high commercial value, as well as for their possible use as animal feed components [5]. Silage is an alternative to preservation and use of fish wastes [6, 7]. During the silage manufacturing process proteins can be hydrolyzed into peptides chains and a good profile of essential amino acids of high nutritive value for animal feed is obtained [8, 9].

According to FAO's 2012 data [10] worldwide mango cultivation area was approximately 5,170,000 hectares with an annual production of 42,140,000 tons. Major production countries are India (2,300,000 tons), China (460,000 tons), Thailand (320,000 tons), Indonesia (230,000 tons) and the Philippines (190,000 tons) [11]. In Mexico the fruit cultivated area in 2011 was about 174,969 ha with a total production of 1,632,649 tons [12]. Mango wastes, especially seeds and peels, which represent approximately 35 % to 60 % of the fruit are considered to be cheap sources of valuable food and nutraceutical ingredients [13, 14] these biologically active components include the major polyphenols: mangiferin, catechin, quercetin, kaempferol, cinnamic acids, tanins, vanillin, coumarin, rhammetin, antocyanins, gallic and ellagic acids, propyl and methyl gallate, benzoic acid, and protocatechuic acid [15, 16] besides, vitamin E and vitamin C, minerals, β-carotenes, rhamnetin glycosides, flavonols, xanthones, anthocyanins and pectins among others [13, 14, 17-21].

In México and many parts of the world mango wastes represent an environmental problem because they do not have adequate management and large volumes of these resources are regularly thrown into landfills [22, 23]. Therefore, the utilization of fish and mango wastes is relevant from an economical and environmental perspective [24]. The feed cost can be as high as 70 % of total cost of animal product formed through the livestock operation and the use of food wastes is also likely to reduce the cost of feeding resulting in higher profit to livestock rearers [25]. There are not reports on the utilization of fish and mango wastes together; therefore, the aim of this study was to demonstrate the use of these industry wastes for biological silages production.

2. MATERIALS AND METHODS

2.1. Material Preparation

2.1.1. Fish and Mango Wastes

Fish wastes of commercial marine species such as *Bagre panamensis*, *Peprilus snyderi*, *Sphyraena ensis*, *Trachynotus ovatus*, *Argyrosomus regius*, *Diplodus vulgaris* and *Bagre panamensis* (estuarine fish) were obtained from San Blas, Nayarit, Mexico. Fish wastes were minced, extruded and the mixture was stored at -20 ºC until used according to the methodology reported previously [9]. Mango (*Mangifera indica* L.) wastes variety Tommy that included shell, remains pulp and seed were provided by Mexifrutas Company, Nayarit, Mexico. The handling process of mango wastes included milling with a hammer mill (Honda, model GX390), sieving material (mesh size of 5 mm) and frozen at -20 ºC until further use.

2.1.2. Sugar Cane Molasses and Corn Stover

Sugar cane molasses supplied by the sugar mill "El Molino" Nayarit, Mexico, was used like main carbohydrates source and presented a water content of 25.1 %, an ashes content of 10.4 % and a soluble carbohydrates content of 55.7 %. Corn stover was ground through a blade mill using a 0.5 cm screen (Willey, model 4, Philadelphia, USA).

2.1.3. *Lactobacillus* Strain

Starter culture of *Lactobacillus* sp. strain B2 was cultivated in MRS broth (de Man Rogosa and Sharpe, MRS, Merck Darmstadt) at 30 °C for 24 h until to reach a final concentration of 1 X 10^9 CFU/mL [26].

2.2. Silage Production

An experiment of six treatments was designed for silage evaluation (Table 1). Subsamples of well mixed fish

and mango wastes also cane molasses and corn stover were supplemented with 4 % of *Lactobacillus* spp. B2 as starter culture and homogenized.

Table-1. Composition (%) of biological silage added with different levels of fish and mango wastes supplemented with sugar cane molasses and corn stover.

Ingredient	Ingredient Treatments					
	1	**2**	**3**	**4**	**5**	**6**
Fish wastes [a]	40	60	30	41	60	40
Mango wastes	47	24	57	41	14	24
Sugar cane molasses	9	12	9	9	12	12
Corn stover	0	0	0	5	10	20
Inoculum [b]	4	4	4	4	4	4

[a] Mixture of fish wastes obtained from several fish species.
[b] *Lactobacillus* spp. B2 cultivated in MRS broth [7, 26].

The mixed material of each treatment was pressed firmly into small plastic bags (total weight 100 g), subsequently air was extracted for one minute using a vacuum pump (Power Electric, model FDE1446RB1A, MABE, Mexico) and incubated at 30 ºC, moreover during the first week air inside was extracted by gently pressing and bags remained closed.

A standard protocol was followed to evaluate the kinetics of acidification continuously for 7 days and after the 15th day of fermentation, it is consisting of pH measurements using a potentiometer UB10 UltraBasic (Denver Instrument, USA) and lactic acid percentage determination with the procedure reported [7] briefly, the silage samples in triplicate were diluted in distilled water (1:10) and the lactic acid concentration was determined by titration with 0.1N NaOH until a final pH of 7.5. The milliliters of used NaOH were recorded to calculate the percentage of lactic acid using the following formula: $\%\ \text{lactic acid} = \frac{(\text{ml of NaOH})(N)(0.090)}{\text{weight of sample}} X\ 100;$ where: N = normality of NaOH, and 0.090 = milli equivalent weight of lactic acid.

2.3. Chemical and Microbiological Analysis

Fish and mango wastes were analyzed on its chemical composition of dry matter, crude protein (N × 6.25), crude fat (ethereal extract), crude fibre and ash using standard methods [27]. Once fermentation time which achieved the best silage acidification, the silages were analyzed for their chemical composition [27] neutral detergent fiber content (NDF) and acid detergent fiber content (ADF) [28]. The microbiological quality of fish silage was evaluated by colony enumeration of lactic acid bacteria and total coliforms cultivated in MRS broth and Eosin Metilen Blue agar, respectively [7, 26].

2.4. Digestibility

The silages prepared were analyzed to determine *in vitro* digestibility of dry matter using the two-step technique with rumen fluid and pepsin [29].

2.5. Statistical Analysis

The data obtained were statistically analyzed by ANOVA and when significant differences existed among treatments Tukey test for means comparison was used at the 0.05 level of significance [30]. The analyses were processed statistically using NCSS 2007 program (NCSS Inc. USA) [31].

3. RESULTS AND DISCUSSION

3.1. Chemical Composition of Raw Material

The chemical composition of raw material in this study is shown in Table 2. The results of mango wastes were better than reported by Fontes-Vieira, et al. [32] in crude protein (3.87 %) and crude fiber (14.60 %), however they reported higher values of ethereal extract (4.36 %) and NFE as carbohydrates (81.92 %).

Table-2. Proximate composition in dry basis (%) of fish and mango wastes.

Component	Fish	Mango
Dry matter	29.70 ± 0.4	23.65 ± 0.19
Crude protein	52.4 ± 0.9	4.75 ± 0.11
Ethereal extract	24.5 ± 0.6	2.76 ± 0.10
Crude fiber	–	9.89 ± 0.79
Ash	19.0 ± 0.5	4.56 ± 0.49
NFE [a]	4.1	78.04

Data are means ± standard deviation of three replicate determination (n=3).
– Not determined.
[a] NFE: Nitrogen free extract, 100 – (% crude protein + % ethereal extract + % ash + % crude fiber).

The values reported for protein (4.68–4.19 %), ethereal extract (1.21–2.72 %) and nitrogen free extract (76.13–47.79 %), respectively, peel and seed of *Mangifera indica* wastes [33] highlight in evidence compared to the values of this research.

Generally mango wastes have low nitrogen content, high fiber content and low lipid concentration, however they have a high carbohydrate content, therefore their use may improve fermentation mainly to be used for making silage in combination with fish wastes.

3.2. Silage acidity conditions

The six treatments based on mango and fish wastes showed adequate acidification during fermentation from one day to the fifteenth day, with pH values between 4.39 and 4.70 (Fig. 1), and lactic acid values from 2.96 to 4.42 % ($p \leq 0.05$), (Table 3).

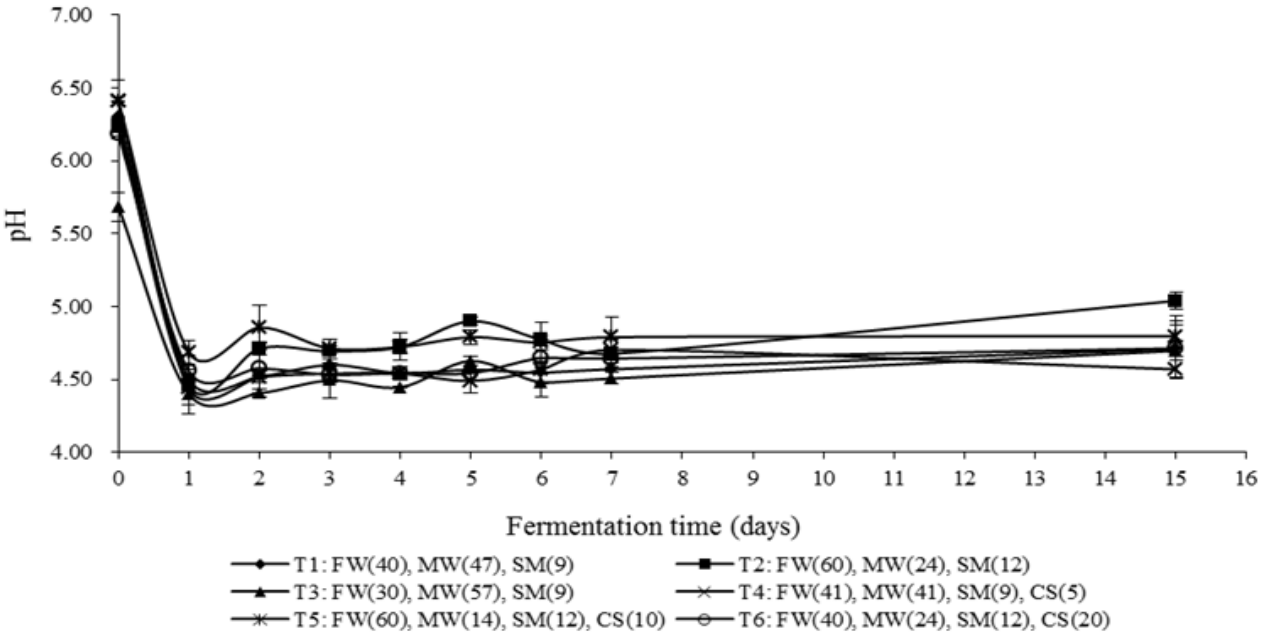

Figure-1. pH values of silages with different levels (%) of fish wastes (FW), mango wastes (MW), sugar cane molasses (SM) and corn stover (CS).

Table-3. Lactic acid concentration (%) of silage added with different levels fish and mango wastes, in combination with sugar cane molasses and corn stover.

Fermentation time (days)	Treatments					
	1	2	3	4	5	6
0	0.33±0.02 b	0.24±0.02 a	0.31±0.05 b	0.24±0.03 a	0.21±0.03 a	0.42±0.07 c
1	2.03±0.40 a	3.53±0.37 c	2.75±0.18 b	2.31±0.26 a	3.26±0.79 c	3.38±0.66 c
2	3.41±0.98 b	3.82±0.61 c	3.23±0.55 b	3.75±0.25 c	2.02±0.16 a	2.36±0.33 a
3	3.32±0.31 b	2.63±0.12 a	2.98±0.31 b	2.86±0.37 b	2.83±0.22 b	2.48±0.14 a
4	3.25±0.16 a	3.06±0.04 a	3.21±0.22 a	3.22±0.22 a	3.15±0.24 a	3.28±0.33 a
5	3.10±0.32 a	3.19±0.12 a	3.21±0.96 a	3.55±0.25 a	3.43±0.27 a	3.46±0.19 a
6	3.38±0.38 a	3.64±0.28 a	3.19±0.21 a	3.86±0.25 a	3.77±0.28 a	3.22±0.23 a
7	3.34±0.30 b	3.80±0.35 c	3.39±0.23 b	3.64±0.29 b	2.96±0.40 a	3.78±0.19 c
15	3.49±0.67 a	3.77±0.41 a	4.04±0.66 a	4.42±0.30 a	4.32±0.14 a	4.33±0.57 a

Values with different letters in a row indicate significant differences among treatments ($p<0.05$).

The values of acidity in the silages obtained in this study are consistent with those reported for a silage made from trout and white perch inoculated with *Lactobacillus plantarum* in silage stored when the silage was stored at both ambient temperature and 37 °C for 35 days [33] also with those reported for a silage based on tilapia filleting wastes with anaerobic fermentation (50 g/kg *L. plantarum*) [8] using fresh sardines wastes (viscera, heads and frames) without addition of inoculum [34] as well as a mixture of wastes from various marine species and *Lactobacillus* spp. B2 as starter culture [7]. It has been reported pH values of 3.99 (lower than our results) in biological silage of hake wastes using sucrose (7 %) as carbon source and three different strains of lactic acid bacteria [35]. Furthermore our results are better than those reported by Zakaria, et al. [36] who obtained values of pH equal to 5 and 1 % of lactic acid when evaluating a silage made from scampi (*Nephrops norvegicus*) waste supplemented with 10 % of glucose as carbon source [36]. Likewise, it was reported that fermented shrimp wastes with glucose [37] and sucrose [38] both in concentration of 10 % were able to reach pH values of 4.6 using 10 % of *Lactobacillus* spp. B2 as inoculum. However, the carbon sources used by these authors are much more expensive than mango wastes and sugar cane molasses used in this study, which could significantly reduce production costs of silage. An advantage of the mango wastes and sugar cane molasses is the high content of fermentable carbohydrates; therefore, acidification of the silage was improved. Moreover, the strain *Lactobacillus* spp. B2 used in this study is homofermentative and has high acidifying capacity, which has been reported in other research projects conducted producing silages [7, 37].

As shown in Fig. 1, the pH values tended to increase at 15 days fermentation being more remarkable in the treatments 2 and 5, which had in its composition more fish wastes than mango wastes (Table 1) and therefore higher percentages of crude protein and minerals (Table 2). The increase in pH in these silages can be attributed to the buffer effect of amino acids, small peptides, proteins and mineral salts present in fish wastes as well as the partial neutralization of lactic acid by the calcium [39]. The relative lower pH values in all treatments were presented at 7 days of fermentation (Fig. 1), therefore this time was selected to evaluate their chemical composition, dry matter digestibility *in vitro* and microbiological quality.

During the first three days of fermentation the lactic acid production was different between treatments resulting higher in silages with more molasses content (Table 3; $p\leq0.05$). However, the evaluation period of lactic acid fermentation on days 4, 5 and 6 showed no significant difference between treatments ($p\geq0.05$), indicating a possible degradation of mango wastes becoming more available sugars for fermentation of lactic acid bacteria.

The treatments with the highest levels of fish wastes (60 %) and corn stover (20 %) inclusion were significantly higher in lactic acid content at 7 days of fermentation ($p\leq0.05$; Table 3). This is relevant because the high levels of addition of these ingredients in the silages 2 and 6 not adversely affect the acidification, besides, the addition of 20 % corn stover improved consistency of silage, which could facilitate handling in diets preparation for ruminants. As shown in Table 3, the lactic acid production of the most treatments increased between 7 and 15 days of

fermentation, which is probably due to the availability of soluble carbohydrates in the silage and acid tolerance of *Lactobacillus* spp. B2.

3.3. Proximate Composition and Digestibility

In Table 4 are presented the proximal composition, values of NDF, ADF and dry matter digestibility *in vitro* of treatments at 7 days of fermentation. In this period of evaluation the higher values of dry matter in silages occurred in the treatments 1 and 5 compared with the treatments 2, 3, 4 and 6 ($p \leq 0.05$), moreover both groups had similar dry matter content between treatments ($p \geq 0.05$).

Table-4. Chemical composition and digestibility (%) of silages added with fish and mango wastes at 7 days of fermentation.

Analysis	Treatments					
	1	**2**	**3**	**4**	**5**	**6**
Dry matter	32.32±0.68 b	30.36±0.74 a	30.58±0.82 a	30.24±1.01 a	36.48±1.13 b	29.46±3.0 a
Ash	13.15±0.81 b	17.68±2.5 c	10.08±0.70 a	9.3±1.05 a	15.4±0.81 c	13.5±0.65 b
Crude protein [a]	28.09±0.71 d	36.92±1.5 e	22.60±0.52 c	17.65±0.39 b	35.46±0.66 e	14.05±0.42 a
Ethereal extract	9.34±0.50 a	14.42±0.9 c	8.15±0.47 a	11.6±0.43 b	8.94±0.6 a	7.5±1.2 a
Crude fiber	8.34±0.61 b	4.7±0.59 a	8.5±0.43 b	7.35±0.01 b	6.41±0.09 b	13.2±1.9 c
NDF [b]	24.27±3.95 a	21.50±3.55 a	30.02±4.34 b	23.09±2.43 a	21.36±2.92 a	32.07±1.28 b
ADF [c]	11.11±1.95 a	12.81±0.25 a	18.70±3.17 b	18.59±1.74 b	10.98±0.59 a	22.22±1.28 c
NFE [d]	41.07	26.28	50.65	54.15	33.75	51.7
DMD [e]	78.40±1.58 b	81.89±2.02 c	77.34±4.17 b	81.70±1.58 c	79.48±1.53 b	75.44±0.77 a

Values with different letters in a row indicate significant differences among treatments ($p<0.05$)
[a] Total nitrogen x 6.25
[b] NDF: Neutral detergent fiber
[c] ADF: Acid detergent fiber
[d] NFE: Nitrogen free extract, 100 – (% crude protein + % ethereal extract + % ash + % crude fiber)
[e] DMD: Dry matter digestibility

The higher moisture content in treatments 2, 3, 4 and 6 was mainly due to the contribution of the fish and mango wastes to the silage (Table 1). The protein content ranged from 14.1 to 36.9 %, for lipids from 7.5 to 14.4 % and for minerals from 9.3 to 17.7 %, with higher values in silages with higher content of fish wastes ($p \leq 0.05$), treatments 2 and 5 respectively (Tables 1 and 4). The latter is because the fish wastes have a higher content of these nutrients compared to other ingredients used in the production of silages [34, 40]. Crude fiber values were different between the silages ($p \leq 0.05$) (Table 4) registering data between 4.7 and 13.2 %. The treatments 2 and 5 with low content of mango wastes and high content of fish wastes (Table 1) showed the lowest crude fiber content. On the other part, the treatment with higher content of corn stover (6) had the highest content of crude fiber. The value of neutral detergent fiber was higher in silages with higher content of mango wastes (57 %) and corn stover (20 %), treatments 3 and 6 respectively; this trend was similar to the content of acid detergent fiber which it was higher in the same treatments ($p \leq 0.05$). Nitrogen-free extract values were different between silages (Table 4), resulting lower in treatments 2 and 5 with higher content of fish wastes and the highest content showed the silages with higher content of mango wastes and sugar cane molasses, treatments 1, 3, 4 and 6 respectively.

Dry matter digestibility *in vitro* ranged from 75 to 82 % and there were differences between silages ($p \leq 0.05$), presenting the lowest digestibility treatments inclusion levels 10 and 20 % of corn stover which is most likely due to the high fiber content of corn stover (Table 4). Contrary treatments 2 and 4 had the highest dry matter digestibility, which is attributed to the higher content of fish wastes in its composition.

The latter is because the fish wastes used contain viscera, being an important source of proteolytic enzymes, plus meat scraps containing highly digestible protein [41]. Although treatment with the higher content of corn stover (T6) had the lowest dry matter digestibility, it improved its paste-like consistency relative compared to other silages, which could facilitate handling when being included in animal diets, such aspects that should be analyzed in ruminant bioassays for estimating *ad libitum* feed intake and its effect on growth.

3.4. Microbiological Quality

Regarding microbiological load, all silages showed a significant increase average of 3 log units in lactic acid bacteria with values of 7.5 and 10.3 log CFU/g at 0 and 7 days of fermentation, respectively ($p \leq 0.05$). The high population of LAB found in silage pointed out the suitable conditions for growth of the culture and also the efficiency of the strain used. In contrast, total coliforms decreased significantly from 5 to 2.6 log CFU/g being better silage with greater acidification ($p \leq 0.05$). The diminution of coliforms and silage preservation has been attributed to lactic acid, low pH and possibility to antibiotic produced by BAL [41] which likely favors the preservation of silage since none of the six treatments showed signs of decomposition. The microbiological quality of silages in this study showed a similar tendency compared with the results of other studies with fish silages [7, 26].

4. CONCLUSION

The inclusion of fish and mango wastes in combination with the ingredients sugar cane molasses and corn stover affected in different proportions the parameters acidification, nutritional composition, digestibility of dry matter and microbial load of silage. However, all silages were stables and had good nutritional quality, therefore their evaluation in ruminants feed is suggested. Silage production based on fish and mango wastes is feasible, economic and environmentally sustainable from a view of the use of these agroindustrial resources.

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