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Use of fish waste and pineapple peel to produce biological silage

Aprovechamiento de desechos de pescado y cáscara de piña para producir ensilado biológico

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ABSTRACT

Six treatments were formulated to make silages with fish wastes, corn stubble, molasses, pineapple peel (PP) [15, 30 and 45%] and inoculum *Lactobacillus* sp. or *Lactobacillus* B2. The silages of each treatment were made in triplicate and incubated at 30 °C for 0, 2, 4, 7 and 14 days in order to evaluate the acidification under a 3 x 2 x 5 factorial design. The chemical composition and *in vitro* dry matter digestibility (IVDMD) were determined to the silages at end of fermentation. The highest acidification ($p<0.05$) was presented in the treatments with PP 15 and 30% and *Lactobacillus* B2 for 7 days. The highest dry matter content (39.3%) ($p<0.05$) was obtained with 15% of PP and the crude protein was from 26.5 to 31% without significant difference. The highest concentration of lipids (9.85%) was present in the treatments with PP 30 and 45% and *Lactobacillus* B2. Detergent fiber fractions decreased with increasing PP level and the highest IVDMD (82.9%) occurred in silages when using *Lactobacillus* B2, regardless of PP level. The silages obtained are an alternative in ruminant feeding.
Keywords: Fish waste, pineapple peel, biological silage, ruminant feeding.

RESUMEN

Se formularon seis tratamientos para elaborar ensilado biológico con desechos de pescado, rastrojo de maíz, melaza, cáscara de piña (CP) [15, 30 y 45%] e inóculo *Lactobacillus* sp. o *Lactobacillus* B2. Los ensilados de cada tratamiento se hicieron por triplicado y se incubaron a 30°C durante 0, 2, 4, 7 y 14 días con el propósito de evaluar la acidificación bajo un diseño factorial 3 x 2 x 5. A los ensilados se les determinó la composición química y la digestibilidad *in vitro* de la materia seca (DIVMS) al terminar la fermentación. La acidificación más alta ($p<0.05$) la presentaron los tratamientos con CP 15 y 30% y *Lactobacillus* B2 a los 7 días. Con 15% de CP se obtuvo el mayor contenido de materia seca (39.3%) ($p<0.05$) y la proteína cruda (26.5 a 31%, rango) fue igual ($p>0.05$). La concentración mayor de lípidos (9.85%) se presentó en los tratamientos con CP 30 y 45% y *Lactobacillus* B2. Las fracciones de fibra detergente disminuyeron al aumentar el nivel de CP y la DIVMS más alta (82.9%) se presentó en los ensilados al utilizar *Lactobacillus* B2, independientemente del nivel de CP ($p<0.05$). Los ensilados obtenidos son una alternativa para alimentación de rumiantes.
Palabras clave: Desechos de pescado, cáscara de piña, ensilado biológico, alimentación de rumiantes.

INTRODUCTION

World fisheries production in 2016 reached approximately 171 million tons, of which aquaculture accounted for 47% of the total and fishing for 53%, not including what was destined for the production of fishmeal and fish oil (FAO, 2018). More than 60% of residues made up of fins, scales, heads, viscera, skeleton, skin, roe and remains of meat derive from the industrial processing of fish (Ghosh *et al*., 2016; Renuka *et al*., 2016). These wastes in many parts of the world are discarded, which causes a great loss of nutrients such as proteins, lipids and minerals, in addition to polluting the environment (Olsen and Toppe, 2017). With fish waste, fertilizers, concentrates and protein hydrolysates, as well as fish meal and oil can be produced (Renuka *et al*., 2016; Ozyurt *et al*., 2017). Biotechnologies have also been developed for the fish waste conversion into high-value products such as polyunsaturated fatty acids, physiologically important peptides, carbohydrates and other bioactive compounds (Ghaly *et al*., 2013; Smichi *et al*., 2016). However, most of these technologies are not economically attractive because they require high investment (Ozyurt *et al*., 2017). The most important input in animal production is fishmeal due to its high protein content, but due to its high cost, alternative sources of protein are demanded in the market (Castillo *et al*., 2019). Fish silage is a product resulting from the preservation of whole fish or parts by the addition of organic or inorganic acids (chemical silage) or by bacterial fermentation (biological silage) (Ghaly *et al*., 2013; Olsen and Toppe, 2017). The fish silage produced by the biological method is a viable technological alternative from the economic and environmental point of view, which consists of mixing the ground fish waste with molasses or another carbohydrate source and a starter culture of lactic acid bacteria (LAB) (Ramírez-Ramírez *et al*., 2018; Castillo *et al*., 2019). During fermentation, LABs increase the production of acids, mainly lactic, so the pH decreases and microbial deterioration slows down. Furthermore, fish proteases are activated, accelerating proteolysis and consequently the digestibility of the product increases (Ghaly *et al*., 2013). Likewise, LABs generate compounds such as bacteriocins and hydrogen peroxide that help preservation and diacetyl, an aroma and flavor enhancing substance (Jini *et al*., 2011). In this sense, to achieve control of fermentation, the selection of LAB strains is important. According to various studies, fish silage is an excellent source of proteins, lipids and minerals with great biological properties for animal feed (Geron *et al*., 2007; Ghaly *et al*., 2013; Ramírez-Ramírez *et al*., 2016; Land *et al*., 2017), in addition, biological fish silage has antibacterial and antioxidant benefits and it is a possible source of probiotics (Jini *et al*., 2011; Ozyurt *et al*., 2017). On the other hand, pineapple (*Annanas comosus* Merr.) Ranks third as the most popular and most economically important fruit in the world, with a production of 24'785,762 tons (FAOSTAT, 2018). From pineapple industrialization, approximately 75% of the weight of the fruit is obtained as waste, which is a valuable fiber source, soluble sugars, protein, ascorbic acid, vitamins, minerals, water and bioactive compounds such as bromelain from multiple applications (Damasceno *et al*., 2016; Ketnawa *et al*., 2012). Pineapple waste can be used as a good quality substrate for microorganisms in fermentation processes; however, they are often thrown away causing serious contamination problems (Ketnawa *et al*., 2012). For all the above, the use of fish and pineapple waste could be a viable and relevant alternative from an economic and environmental point of view. To our knowledge there are no reports on the use of fish waste and pineapple peel together. Therefore, the objective of this research was to evaluate the use of these industrial wastes in the silage production by lactic fermentation.

MATERIAL AND METHODS

Raw material preparation

Fish waste and pineapple peel

Fish waste was obtained from commercial marine species such as *Bagre panamensis*, *Peprilus snyderi*, *Sphyraena ensis*, *Trachynotus ovatus*, *Argyrosomus regius*, *Diplodus vulgaris* and *Bagre panamensis* (estuarine fish) from San Blas port, Nayarit, Mexico. The waste was processed in a meat mill (Torrey Brand, model 32-3, Mexico) using the 0.5 cm diameter sieve and stored at -20 °C until use. The peel of pineapple (*Annanas comosus* Merr.), Cayenne smooth variety, was obtained manually with a knife and homogeneously ground in a food processor. The following table shows the chemical composition of these ingredients.

Table 1. Chemical composition (% DM) of fish waste and pineapple peel.

Component	Fish waste	Pineapple peel
Dry matter	29.72 ± 0.4	23.22 ± 0.37
Ash	18.94 ± 0.52	4.11 ± 0.12
Crude protein (Nt x 6.25)	52.43 ± 0.92	4.31 ± 0.13
Ethereal extract	24.50 ± 0.67	3.38 ± 0.46
Crude fiber	---	13.95 ± 0.55
Neutral detergent fiber	---	41.60 ± 1.10
Detergent acid fiber	---	22.71 ± 0.73
ELN	4.13	74.25

Mean ± standard deviation, n = 3.

ELN = 100 -% ash -% crude protein -% lipids -% crude fiber.

Cane molasses and corn stubble

Cane molasses with a moisture content of 25.15%, 10.37% ash and 55.73% total soluble carbohydrates was used. To improve the consistency of the silages, corn stubble processed in a knife mill was added using the 2 mm sieve (Willey, model 4, Philadelphia, USA).

Inoculums

Lactobacillus B2 and *Lactobacillus* sp. strains were evaluated, the latter being isolated from mango waste in our laboratory. The initiators were cultured in MRS broth (from

Man Rogosa and Sharpe, MRS, Merck Darmstadt) at 30 °C for 24 h until a final concentration of 1 X 10^9 cfu/mL was recorded.

Silage production

Six treatments were prepared with different amounts in percentage proportion of fish waste, PP (15, 30 and 45%, w/w), corn stubble, and sugar cane molasses (9%, w/w). As inoculum (I) *Lactobacillus* sp. or *Lactobacillus* B2 at 4% (v/p) (Table 2). The mixtures obtained were used to make 100 g mini silos in black plastic bags. Each treatment was prepared in triplicate and the silos were vacuum sealed and incubated at 30 ° C for 14 days.

Table 2.- Percentage composition of ingredients used in the production of silage by lactic acid fermentation.

Ingredient	Percentage					
Fish waste	57	47	37	57	47	37
Pineapple peel	15	30	45	15	30	45
Molasses	9	9	9	9	9	9
Corn stubble	15	10	5	15	10	5
Inoculum	4 A	4 A	4 A	4 B	4 B	4 B

A = *Lactobacillus* sp isolated from mango waste.
B = *Lactobacillus* B2.

Chemical analysis

The silages were analyzed every 0, 2, 4, 7 and 14 days (T) to determine the pH with a potentiometer model UB10 Ultra Basic (Denver Instrument, USA) and the content of lactic acid by titration. The silages obtained at the end of fermentation were analyzed to determine their proximal chemical composition (AOAC, 2005) and the contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) by the method of Van Soest *et al*. (1991).

In Vitro Digestibility of Dry Matter (IVDMD)

IVDMD was determined following the two-step technique of Tilley and Terry (1963). The silage samples were dried at 70 °C for 24 h in a forced air oven and processed in a Wiley mill to a particle size of 1 mm. Two uncastrated Blackbelly sheep weighing 35 kg of body weight and equipped with a cannula in the rumen were used to collect the ruminal fluid. The sheep were fed a diet based on 25% corn silage, 25% alfalfa and 50% concentrate. In the second step of the IVDMD technique, pepsin (Sigma P-7012, Sigma) was used.

Statistical analysis

The data of the fermentation parameters were treated by analysis of variance (ANDEVA) for a factorial design 3 x 2 x 5 (level of pineapple peel x inoculum x fermentation time). The data obtained from the chemical composition and IVDMD were analyzed by ANDEVA for a 3 x 2 factorial design (level of pineapple peel x inoculum).

When a significant difference was found, the means of the treatments were compared with the Tukey test ($p < 0.05$). The analysis was done with the Statistica 7 program (Statistica, versión 7.1).

RESULTS AND DISCUSSION

Fermentative parameters

The silage lactic acid content showed significant differences between treatments due to the inoculum factors (I) and fermentation time (T) (Table 3). The unfermented mixtures presented the lowest lactic acid content (average 0.55 ± 0.15%) with no difference between these treatments ($p > 0.05$). However, between days 2 and 7 of the process, most of the treatments with *Lactobacillus* B2 presented the maximum concentration of lactic acid (average 3.42 ± 0.17%). The high acidifying power of *Lactobacillus* B2 has been previously reported (Ramírez-Ramírez *et al*., 2008; 2018) and in this work it is reaffirmed. The results obtained agree with values of 2.96 to 4.42% of lactic acid recently reported for biological silage added with mixtures of fish and mango waste (Ramírez-Ramírez *et al*., 2018), as well as with the values of 2.96 and 3.08% of lactic acid for fermented shrimp head silage and fish waste; respectively (Castillo *et al*., 2019).

The pH was significantly affected by the main factors, pineapple peel level (PP), inoculum (I) and fermentation time (T); confirming the PP x I and PP x T interactions (Table 3). The unfermented mixtures presented the highest pH values and when the PP level increased there was a decrease in this parameter ($p < 0.05$). In silages, it was observed that when the lactic acid production increased, the pH decreased significantly, obtaining the best pH values between days 2 and 7 of fermentation (Table 3), which is due to the fact that LAB reaches the growth curve top. However, the PP x I interaction showed that at 7 days of the process the pH of the treatments with 15 and 30% of PP and inoculated with *Lactobacillus* B2 was better than with *Lactobacillus* sp, which was also observed in a similar way to the 14 days in silage with 15% PP. The silage pH was affected by the PP x T interaction during the 0, 7 and 15 days of the process ($p < 0.05$). However, between 2 and 4 days of fermentation, the pH values of the silages did not show significant differences, regardless of PP level and inoculum type ($p < 0.05$). The treatments with 30 and 45% PP at 14 days of fermentation showed a small increase in pH, which was related to a decrease of 0.72 percentage units in the lactic acid production. The increase in pH was due to the buffering effect of fish proteins and peptides derived from their hydrolysis (Ramírez-Ramírez *et al*., 2008; Ghaly *et al*., 2013), as well as the high amount of ashes provided by the bones from fish waste (Tables 2 and 4), since calcium salts act as neutralizers of lactic acid in silage during storage (Land *et al*., 2017).

Table 3. Effect of pineapple peel level, inoculum and fermentation time on the lactic acid content and pH of the silage.

Pineapple peel level (PP) (%)	Inoculum (I)	Fermentation time (T) (days)	Lactic acid (%)	pH
15	A	0	0.49 ± 0.08c	6.09 ± 0.05a
15	B	0	0.43 ± 0.01c	6.05 ± 0.14a
30	A	0	0.55 ± 0.02c	5.83 ± 0.01b
30	B	0	0.73 ± 0.03c	5.60 ± 0.10c
45	A	0	0.37 ± 0.14c	5.44 ± 0.25c
45	B	0	0.72 ± 0.07c	5.32 ± 0.28c
15	A	2	2.97 ± 0.45b	4.62 ± 0.03e
15	B	2	3.37 ± 0.05^{a}	4.65 ± 0.07^{e}
30	A	2	2.71 ± 0.25^{b}	4.64 ± 0.05^{e}
30	B	2	3.32 ± 0.21^{a}	4.52 ± 0.05^{e}
45	A	2	2.98 ± 0.41^{b}	4.36 ± 0.09^{e}
45	B	2	3.14 ± 0.15^{ab}	4.51 ± 0.05^{e}
15	A	4	3.09 ± 0.61^{ab}	4.69 ± 0.15^{de}
15	B	4	3.57 ± 0.06^{a}	4.63 ± 0.04^{e}
30	A	4	2.78 ± 0.14^{b}	4.65 ± 0.03^{e}
30	B	4	3.79 ± 0.34^{a}	4.49 ± 0.01^{e}
45	A	4	2.98 ± 0.41^{b}	4.44 ± 0.05^{e}
45	B	4	3.49 ± 0.25^{a}	4.56 ± 0.04^{e}
15	A	7	2.77 ± 0.28^{b}	4.77 ± 0.04^{d}
15	B	7	3.32 ± 0.47^{a}	4.68 ± 0.16^{e}
30	A	7	2.49 ± 0.14^{b}	4.82 ± 0.03^{d}
30	B	7	3.31 ± 0.33^{a}	4.50 ± 0.11^{e}
45	A	7	3.44 ± 0.12^{a}	4.43 ± 0.01^{e}
45	B	7	3.47 ± 0.13^{a}	4.50 ± 0.15^{e}
15	A	14	2.34 ± 0.74^{b}	4.89 ± 0.06^{d}
15	B	14	2.76 ± 0.04^{b}	4.68 ± 0.21^{e}
30	A	14	2.61 ± 0.26^{b}	4.77 ± 0.06^{d}
30	B	14	2.83 ± 0.55^{b}	4.83 ± 0.03^{d}
45	A	14	2.82 ± 0.31^{b}	4.82 ± 0.06^{d}
45	B	14	2.93 ± 0.44^{b}	4.77 ± 0.05^{d}
Effect			P Value	
PP			0.24	< 0.01
I			< 0.01	< 0.01
T			< 0.01	< 0.01
PP x I			0.12	< 0.01
PP x T			0.17	< 0.01
I x T			0.15	0.24
PP x I x T			0.57	0.09

A = *Lactobacillus* sp, isolated from mango waste.
B = *Lactobacillus* B2.
$^{a, b, c, d, e:}$ Means in the same column with a different superscript show a significant difference (p <0.05).

However, the silage obtained in this study presented acceptable sensory characteristics and did not show signs of decomposition. The pH values of this study silages agree with those of other reports (Castillo *et al*., 2019; Ramírez-Ramírez *et al*., 2018), although they were higher than the 4.2 obtained for biological silage from residues of tilapia filleting (Gerón *et al*., 2007).

Chemical composition

Table 4 shows the results of the chemical composition and IVDMD of the silages obtained after 14 days of fermentation. The dry matter content (DM) decreased significantly when the PP level increased, therefore the silages with 45% PP presented the lowest DM content, regardless of the inoculating strain (p <0.05). The silages with 15% PP presented the highest DM values (39.3%), despite having a high content of fish waste (57%) in their formulation, whose moisture percentage is high (Tables 1, 2 and 4). This was probably due to the addition of 15% corn stubble, which also improved the silage consistency. The DM results obtained agree with those of other reports (Geron *et al*., 2007; Castillo *et al*., 2019; Ramírez-Ramírez *et al*., 2018). The PP level and the PP x I interaction were significant in the ash content. The silages with 15% PP and inoculated with Lactobacillus sp. presented the highest ash content (14.5%) (p <0.05), however, the other treatments presented a good concentration of minerals, because fish waste is an important source of these nutrients Tables (1 and 4). The ash content obtained is similar to other investigation results (Castillo *et al*., 2019; Ramírez-Ramírez *et al*., 2018), although they were lower than 18.7% reported by Geron *et al*. (2007).

Table 4. Effect of pineapple peel level and inoculum on the proximal chemical composition, neutral detergent fiber (NDF), acid detergent fiber (ADF) and *in vitro* dry matter digestibility (IVDMD) of the silage

Pineapple peel (PP)	Inoculum (I)	Dry matter (%)	Ashes (%)	Crude protein (%)	Ethereal extract (%)	NDF (%)	ADF (%)	IVDMD (%)
15	A	39.6±0.7^{a}	14.5±0.6^{a}	27.5±1.5^{ab}	6.0±0.7^{c}	39.7±3.3^{a}	22.1±0.9^{a}	76.8±1.7^{b}
30	A	35.1±0.5^{b}	11.7± 0.3^{b}	26.5±1.9^{b}	5.2±0.6^{c}	41.9±2.4^{a}	22.7±1.7^{a}	76.2 ±1.1^{b}
45	A	28.5±1.3^{c}	12.5± 0.5^{b}	26.7±0.9^{b}	5.7±0.5^{c}	32.1±0.7^{b}	17.9±1.6^{b}	82.6±0.5^{a}
15	B	39.0±1.4^{a}	12.8±0.2^{b}	29.7±0.8^{ab}	8.0±1.0^{b}	37.5±2.4^{a}	21.6±2.0^{a}	80.6±0.3^{a}
30	B	33.5±0.5^{b}	12.3± 1.4^{b}	27.8±3.0^{ab}	9.7±0.8^{a}	35.7±1.6^{b}	24.6±1.5^{a}	84.4±3^{a}
45	B	28.9±1.5^{c}	12.9±0.7^{b}	31.0±3.6^{a}	10.0±0.8^{a}	28.0±0.9^{c}	18.4±0.8^{b}	83.8±2.1^{a}
Effectt, p value								
PP		<0.01	0.01	0.369	0.021	<0.01	<0.01	0.03
Inoculum		0.26	0.51	.028	<0.01	<0.01	0.39	<0.01
PP x Inoculum		0.33	0.04	0.52	0.30	0.97	0.42	0.08

A = *Lactobacillus* sp., Isolated from mango waste.
B = *Lactobacillus* B2.
$^{a, b, c}$: Values with different letters in each column differ statistically (p <0.05).

The silages showed a significant difference in the crude protein content due to the type of inoculum used. The silages with 45% PP and inoculated with *Lactobacillus* B2 presented numerically the highest crude protein content (31%), but statistically the PP level and the inoculum did not produce relevant changes in silage crude protein (Table 4). The crude protein content of silages obtained with 45% PP and *Lactobacillus* B2 coincides with 31.6% of biological silage from tilapia waste (Geron *et al*., 2007), although it was higher than 28.08% of crude protein from biological silage of fish and mango waste (Ramírez-Ramírez *et al*., 2018); however, it was lower than the 35.42% reported by Castillo *et al*. (2019) for biological silage of fish waste, molasses and yogurt.

Silage ethereal extract showed a significant difference due to the main effects. In this regard, treatments with 30 and 45% PP and inoculated with *Lactobacillus* B2 showed the highest lipid content (9.85%), which is very important from a nutritional point of view, since essential fatty acids are found in them for animal feed (Vidotti *et al*., 2011). The ethereal extract results of this study are lower than those reported by Geron *et al*. (2007) y Castillo *et al*. (2019); however, they coincide with the findings of another study (Ramírez-Ramírez *et al*., 2018). The NDF and ADF contents decreased with increasing PP level. Although PP is a good source of fiber (Table 1), the trend in the results was due to the addition of corn stubble in the formula, since the silages with a higher PP content at the same time contained a lower amount of corn stubble and therefore less NDF and ADF content (Tables 2 and 4). In this sense, the results are very important, since the contents of NDF and ADF in forages are negatively correlated with consumption and digestibility. The percentages of NDF and ADF obtained in the silages of the present study were higher than those reported by Ramírez-Ramírez *et al*. (2018).

In Vitro Digestibility of Dry Matter (IVDMD)

The digestibility values of food components are important parameters to evaluate the formulation of diets and to determine the use of a food component (Ozyurt *et al*., 2017). The IVDMD values of the silages presented a range of 76.2 to 84.4% with significant differences between treatments due to the main effects ($p < 0.05$). By increasing the PP level to 45% and with the addition of *Lactobacillus* sp., the silages presented an increase of 6.1 percentage units in the IVDMD to reach 82.6% ($p < 0.05$). However, this result was statistically the same when using *Lactobacillus* B2, regardless of PP level (Table 4). In fish silage production, the proteases present in the acidic medium hydrolyze the proteins into smaller fragments, peptides and amino acids, which affects the total digestibility (Ghaly *et al*., 2013; Geron *et al*., 2007; Ramírez-Ramírez *et al*., 2016; Olsen and Toppe, 2017). The PP effect on the increase in IVDMD was probably due to an increase in nutrient availability, which was related to the decrease in fiber fractions and consequently increased ruminal microbial activity. Likewise, the addition of PP probably increased the activity of the pepsin used in the second step of the digestibility test, which simulates stomach digestion and therefore the IVDMD of the silages increased. LABs are better known as starter cultures due to their versatile metabolic characteristics such as acidifying activity, proteolytic activity, and bacteriocin synthesis (Jini *et al*., 2011). In general, IVDMD of the silages was higher with *Lactobacillus* B2 than with *Lactobacillus* sp., due to the better acidifying power of *Lactobacillus* B2 and probably to a high production capacity of digestive enzymes. The IVDMD results obtained in this work agree with those of other reports (Ozyurt *et al*., 2017; Ramírez-Ramírez *et al*., 2018).

CONCLUSIONS

The inclusion of pineapple peel in 15 and 30% and *Lactobacillus* B2 caused the best acidification of silages at 7 days of fermentation. However, at 14 days all silages were stable and presented high nutrient content. Furthermore, the use of *Lactobacillus* B2, regardless of pineapple peel level produced the highest IVDMD of the silages. Silage production with fish waste and pineapple peel in combination with molasses and corn stubble is a simple, economical and environmentally friendly technological alternative. It is recommended to scale up the production process and evaluate the silage in ruminant feeding.

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