



RESEARCH ARTICLE

CHARACTERIZATION OF ACID SILAGE OBTAINED FROM TILAPIA FILLETING WASTES (*Oreochromis niloticus*) DURING STORAGE

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ABSTRACT

The present study aimed to obtain acidic silages of different types of waste from tilapia (*Oreochromis niloticus*) and its characterization through chemical, physical and microbiological analysis over the storage time. To obtain the silage different residues were used, which were divided into five treatments: T1 100% guts, T2 - 100% head, T3 - 100% carcass, T4 - 30% head + 70% carcass and T5 - 15% guts + 20% head + 47% carcass + 15% leather + 3% scales. Each treatment was performed by adding 5% acetic acid and 0.1% p/p of BHT / Kg. Analyses were performed at days 0, 7, 14, 21, 28, 60 and 90. It was found that the texture of the ensiled biomass showed no visible immediate changes, prevailing caramel coloring. The first hydrolysis signals were seen from the 4th day of storage. T1 and T5 treatments had lower temperatures in the biomasses. The pH of the silages were less than 4.5. T1 obtained the highest values of malondialdehyde / kg. The results of microbiological analysis have not detected the presence of aerobic mesophilic microorganisms, yeasts and molds for all treatments of the silages. The use of acetic acid and BHT acted efficiently to preserve silage quality. Thus, it can be said that the silages in the process are of high quality, with applicability in food and feed, as well as commercially valuing the fish byproduct and reducing to almost zero waste generation.

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INTRODUCTION

The waste generated during the industrialization of fish, when not properly treated, presents major environmental impacts. On the other hand, when its technological potential is used it becomes profitable, due to value added to a by-product and benefiting the environment (Aguar; Limberger; Silveira, 2014). In this sense, the use of edible leftovers from traditional fish filleting or cutting operations becomes very important, as it lowers costs of key inputs, minimizes the problems of production and the unit cost of raw materials compared to finished products (Oetterer, 2002). The high protein content found in fish makes the main destination for processing the waste fishmeal production, for animal feed, a product that has about 70% protein with the advantage of low cost (Arruda et al. 2006; Seibel; Souza-Soares, 2003). Fish silage is one more way to recover production, manufacture and sale waste, being a

liquid product which can be obtained from whole fish or parts, adding acids, enzymes or lactic acid producing bacteria, wherein the liquefying mass originates from the action of enzymes naturally present in the fish, producing acidic, enzymatic or biological silage, respectively (Sales, 1995). The fish silage has been a known process for a long time and it basically consists of acidifying the pH of the ground dough, leaving the tissue enzymes actions free, which end up liquefying the product. The conventional silage is acidified to a pH between 3.9 and 4.2, and for three days at an ambient temperature between 27 ° C and 30 ° C and it liquefies satisfactorily, restoring the lipid layer and maintaining the enzyme activity for many months (Vidotti; Goncalves, 2006). When compared to obtaining fishmeal, silage has the advantages of process simplicity and practicality that is independent from scale. However, one of the barriers in the production is that the product is bulky in a paste form, but it can go through drying process for use in animal feed, in the dehydrated form (Arruda; Borghesi; Oetterer, 2007). The silage also provides nutritional benefits opposed to fishmeal, allowing

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improvement of the nutritional value of the raw material related to the increase in protein digestibility and the presence of lysine and methionine, amongst other essential amino acids. In general, silages are deficient in tryptophan, an unstable amino acid under acidic conditions, when present on its free form (ARRUDA *et al.*, 2006; Morales-Ulloa; Oetterer, 1995). The investigation of the effects of storage on the nutritional characteristics of ensiled products is as important as knowing its nutritional value after preparation. The use of different types of acids, raw material, temperature or storage manner leads to obtaining different end products, making it essential to select variables that produce a good product at the end of storage (Carmo, 2009). There are several studies related to the applicability of fish waste silage for animal feed related to storage time (Vidotti *et al.*, 2004); body composition and efficiency of nutrient utilization in fingerlings of Nile tilapia (*Oreochromis niloticus*) with diets containing fish meal and fermented silage of tilapia waste (Honorato and Carneiro, 2003); fattening of juvenile Nile tilapia using fish waste silage and whole soybean to replace fishmeal, without undermining growth and carcass quality (Assano, 2004); the use of up to 40% of tilapia filleting waste acid silage may replace fish meal, without sacrificing performance and without causing mortality (Oliveira *et al.*, 2006; Pimenta *et al.*, 2008). In this context, the present study aimed to obtain acidic silages of different types of waste generated in the process of filleting tilapia (*Oreochromis niloticus*) and its characterization through chemical, physical and microbiological analysis throughout the storage time.

MATERIALS AND METHODS

Preparation of acid silage from tilapia filleting residue (*Oreochromis niloticus*). This study was conducted at the Federal University of Lavras (UFLA) located in the municipality of Lavras – Minas Gerais. The structures and equipment of the Central Analysis Laboratory and Fish Technology Laboratory were used, at the Department of Food Sciences (DCA). Microbiological tests were conducted at the Microbiology Laboratory of the University José do Rosário Vellano (Unifenas), located in the city of Alfenas, Minas Gerais. The raw material (waste) used was composed of waste from the filleting of Nile tilapia (*Oreochromis niloticus*) (guts, head, carcass, leather and scales), supplied by Cristalina fish farming, located in the city of Fartura, in the state of Sao Paulo. Residues were frozen separately, vacuum packed on site and transported by refrigerated truck to the Fish Technology Laboratory where they were kept in a freezer. At the beginning of the work, the residues were washed with running water (except the guts) and then ground by an electric meat grinder model C.A.F. 10I.

Preparation and characterization of acid silage made from different fractions of waste from the filleting of tilapia

For the preparation of acidic silages a total of 120kg of different waste which were divided into five treatments were used: T1 100% guts, T2 - 100% head, T3 - 100% carcass, T4 - 30% of head + 70% carcass and T5 - 15% guts + 20% head + 47% carcass + 15% leather + 3% scales. For each treatment, 24 kg of ground fish waste was used, adding 5% acetic acid

concentration and adding 0.1% p/p of BHT / kg. Later, each treatment was placed in 8 40 cm 100mm PVC tubes (3kg per tube), in a total of 40 tubes. These tubes were capped with a PVC cap, and each cap had a whole through which the gases left. The ensiled biomasses were stored for 90 days at room temperature. The analyses were performed at 0, 7, 14, 21, 28, 60 and 90.

Sensory Characteristics of acidic silages

The silages were evaluated according to Valério (1994), observing the attributes: texture, phase separation, staining the sedimented phase, and odor, during the storage period.

Microbiological analyses

All tests followed the methods shown by Silva *et al.* (2005), as follows: Total count of aerobic mesophilic microorganisms, filamentous fungi, and yeasts, coliforms at 35°C and 45°C through the multiple tube method with confirmatory tests for fecal coliforms and *E. coli*, positive coagulase *Staphylococcus*, *Salmonella*, *Bacillus cereus* and *Clostridium sulfite* reducers. After the production of silages, a product sample was withdrawn aseptically from each treatment in all storage periods (0, 7, 14, 21, 28, 60 and 90 days) to perform the analyses.

Room temperature

The room temperature of the silage storage location was monitored weekly until the 28th day and at the 60th and 90th days, using a thermometer of maximum and minimum.

Temperature within the biomass

The temperature within the biomass was measured using a mercury thermometer before each daily tilling.

Hydrogenionic potential - pH

It was determined in digital pH meter, with results with two decimal places.

Lipid Oxidation

The determination of TBARS (thiobarbituric acid reactive substances) was used according to the methodology described by Tarladgis, Watts and Younathan (1960) with some modifications.

Statistical analysis

The completely randomized design (CRD) was used, with split plot and factorial scheme 5X7, being: 5 types of treatment (T1, T2, T3, T4 and T5), 7 storage times (0, 7, 14, 21, 28, 60 and 90 days) and 3 replicates. Analyses were made so that when detected interaction between the factors, the split of the sum of squares of the factor "time" within each "treatment" was performed, and the time factor studied through polynomials because it is a quantitative factor. On the other hand, the "treatment" sum of squares of factors within each "Time" was

split, and the factor treatment evaluated by the Scott-Knott test at 5% probability, using the SISVAR program (FERREIRA, 2000). Statistical analyses for the sensory characteristics parameters, microbiological analyses, and room temperature were not performed.

RESULTS AND DISCUSSION

Characterization of acid silage

For the characterizations of silages, the following analyses were performed: sensorial characteristics, microbiological analyses, room temperature, biomass temperature, pH and lipid oxidation.

Sensory Characteristics

Regarding textures of acid silage made with different fractions of tilapia filleting waste, no immediate visible changes were observed in any of the ensiled biomass in the five treatments with the addition of acetic acid and the antioxidant BHT. The caramel coloring prevailed for all silages elaborated. The first signs of hydrolysis of biomass were viewed from the 4th day of storage, where we observed the separation of two phases, a pasty one and an oily other. The higher oil volume was observed for the T5 treatment, that is, the silage produced with 15% guts + 20% head + 47% carcass + 15% leather + 3% scales. Similar results were also observed by Carmo (2009), who observed the phase separation on the 5th day of ensiling and Oliveira *et al.* (2006) in their studies, with tilapia filleting waste silage using formic acid, report that the phase separation even in the first week of storage was observed. According to Haard *et al.* (1985), the material is continuously hydrolysed during storage, whose fish protein undergoes hydrolytic processes for at least three months. Regarding the sensory characteristics, through visual examination and silage aroma, a change in mass was observed, leading to a semi-pasty form which persisted until the end of the storage period, except for the T1 treatment which had a soft pasty consistency with characteristic odors from the beginning of putrefaction starting at 90 days. According to the authors Disney, Tatterson, and Ollen (1977), Espe, and Njaa Raa (1989) and Haard *et al.* (1985), sensory evaluation through visual examination and chemical evaluation of silages is critical and defines the lifetime of the product.

Microbiological analyses of acidic silages

On Table 1, the results of the total count of aerobic mesophilic microorganisms; filamentous fungi and yeast; coliforms at 35°C and 45°C; *Escherichia coli*; *Staphylococcus aureus*, *Salmonella sp.* and *Bacillus cereus* are shown. Microbiological testing for aerobic mesophilic microorganisms, molds, yeasts, coliforms (35 ° C and 45 ° C); *Escherichia coli*; *Staphylococcus aureus*, *Salmonella sp.* and *Bacillus cereus* of different silages did not present these in all analyzed treatments in all storage times (Table 1). According to the National Health Surveillance Agency (ANVISA), in Resolution - RDC number 12, of January 2nd, 2001, technical regulation on microbiological standards for fish-based products follows the standards: for coliforms at 45 ° C, maximum of 10³ NMP / g, coagulase positive staphylococci with maximum count of 10³ NMP / g and absence of *Salmonella* in 25 g of food (National Health Surveillance Agency - Anvisa, 2001).

The Brazilian legislation does not specify or quotes tolerance limits for total count of aerobic heterocyclic mesophilic bacteria, nor the detection and quantification of filamentous fungi and yeast, and *Bacillus cereus* count. A similar result was observed by Boscolo *et al.* (2010), in their experiments, which constituted assessing the acid silage of Nile tilapia waste, with the addition of 5% v / p of acetic acid and storage for a period of up to 201 days. The authors concluded that the tilapia filleting waste acid silage with addition of 5% acetic acid can be stored for 201 days without showing proliferation of microorganisms. Sales (1995) reports that in the silage obtained from tilapia waste a decrease in the rate of mesophilic bacteria during storage, thereby causing a significant reduction of micro-organism count to room temperature and the effect was more pronounced after the second week of the experiment, obtaining counts below 10³ UFC / g, ensuring therefore good product stability over a period of 160 days. Aguiar and Goulart (2014), analyzed the production of oil and flour using fish scales and leather waste from the Araguaia region. Through microbiological analyses, the hygienic conditions of the flour produced was verified and negative results for coliform and *Salmonella* were obtained. However, as to the molds and yeasts, a growth of 1,58x10⁵ ufc / g in the sample kept at 24 ° C for 5 days was observed. The absence of microorganisms confirm that safety and hygienic procedures were followed correctly from the capture of the fish to the preparation of the

Table 1. Microbiological analysis of acidic silages prepared with different fractions of waste from tilapia filleting at different storage times

TREAT**	Storage time/days						
	COL. 35°C NMP/g	COL. 45°C NMP/g	FU/ YEAST	<i>E. c.</i>	<i>S. aureus</i> NMP/g	SAL. <i>Salmonella</i>	BAC. <i>Bacillus cereus</i>
T1	<3	<3	-	-	<3	-	-
T2	<3	<3	-	-	<3	-	-
T3	<3	<3	-	-	<3	-	-
T4	<3	<3	-	-	<3	-	-
T5	<3	<3	-	-	<3	-	-

*Absence (-) **Treatments - T1- silage 100% guts, T2 – silage 100% head, T3 – silage 100% carcass, T4 – silage 30% head + 70% carcass and T5 – silage 15% guts + 20% head +47% carcass + 15% leather + 3% scales. ***COL. Coliforms at 35°C (NMP/g); COL - Coliforms at 45°C (NMP/g); FU/YEAST - Fungi e Yeasts; *E. c.* - *Escherichia coli*; *S. aureus* - *Staphylococcus aureus* (NMP/g); SAL. *Salmonella* e BAC. *Cereus* - *Bacillus cereus*

raw material. It further demonstrates the high preservation efficiency of acetic acid and BHT, which maintained the quality of silage. The short chain organic acids, due to its low solubility, flavor intensity and low toxicity to the human body, such as acetic, benzoic, citric, propionic, sorbic and lactic acid, are most commonly used in food (SOCCOL 2002). These acids are classified as conservative or acidifiers as seen in the Brazilian law, Decree No. 55871 from 6/23/1965 (BRAZIL, 1965).

Room temperature

For the average temperatures recorded of acid silage made with different fractions of tilapia filleting waste at different storage times, it was found that during the silage storage period (0, 7, 14, 21, 28, 60 and 90 days), from August to October 2012, maximum temperatures of 28 °C and minimum of 14 °C, were respectively reached. It was also observed that there was no significant variations in maximum temperature as much as in minimum throughout the period. According to Backhoff (1976 cited by VIDOTTI 2001), at room temperatures of 27° at 30 °C the hydrolysis of the ensiled biomass occurs, separating the lipid layer and retaining the enzyme activity for several months. Jackson, Kerr and Crowey (1984) in their studies, verified the influence of temperature on protein hydrolysis in silage sprats (*Sprattus sprattus*) using sulfuric acid and formic acid and observed that the silages submitted to storage at a temperature of 10 °C showed lower levels of proteins, lipids and ashes compared to silage stored at 20 °C. Santana-Delgado, Avila and Stelo (2008) tested the effect of two storage temperatures on the hydrolysis of acid silage made with 1.3% v / p sulfuric acid + 1% v / p propionic acid with room temperature of 37°C. The authors concluded that by storing the silage at a temperature of 37 °C there is a decrease in hydrolysis time, showing the effect of temperature on hydrolytic processes.

Temperature of different ensiled biomasses

On Table 2, the mean values of temperatures of the different biomasses ensiled are represented. It appears that at time zero there was significant difference ($P < 0.01$) compared to treatments, where T1 showed a lower temperature (16,40° C) than other treatments. The highest temperature was observed for T2 (19,60° C). On the 7th, 14th, 21st, and 28th days of storage, there were no significant differences among treatments ($P < 0.01$). At 60 days of storage, the temperature was higher ($P < 0.01$) for T4 and T5 in relation to others and at 90 days, it was observed that T1 and T5 presented lower temperatures (22.8 and 22.9°C), while T3 and T4 showed the highest temperatures (23.9°C).

Table 2. Average values of the temperature of ensiled biomass (acid silage) of different types of tilapia filleting waste at different storage times

Treatment*	Storage time/days						
	0	7	14	21	28	60	90
T1	16,4d	19,0a	19,60a	19,90a	21,1a	23,4b	22,8c
T2	19,60a	19,0a	19,7a	20,1a	21,3a	23,3b	23,4b
T3	17,5c	19,1a	19,9a	20,2a	21,1a	23,6b	23,9a
T4	18,0b	19,1a	19,8a	20,2a	21,3a	24,1a	23,9a
T5	17,4c	19,1a	20,2a	20,1a	21,3a	24,4a	22,9c
CV1 (%)	1,18						
CV2 (%)	1,47						
Standard error	0,18						

Averages followed by same letter in the column do not differ by the Scott-Knott test at the 5% level of probability ($P < 0,05$).*(T1- silage 100% guts, T2 – silage 100% head, T3 – silage 100% carcass, T4 – silage 30% head + 70% carcass and T5 – silage 15% guts + 20% head + 47% carcass + 15% leather + 3% scallies)

On Figure 1, the mean temperature values of biomasses for each treatment and at different times are shown. A quadratic effect ($P < 0.01$) for all treatments was observed. There also was a similar behavior among them, where there was a gradual increase in temperature to around 60 days and then there was some stabilization by the end of the period. According Santana-Delgado, Avila and Stelo (2008) the increase in temperature also increases the rate of reaction, reducing the hydrolysis time, which improves the digestibility of the ensiled material.

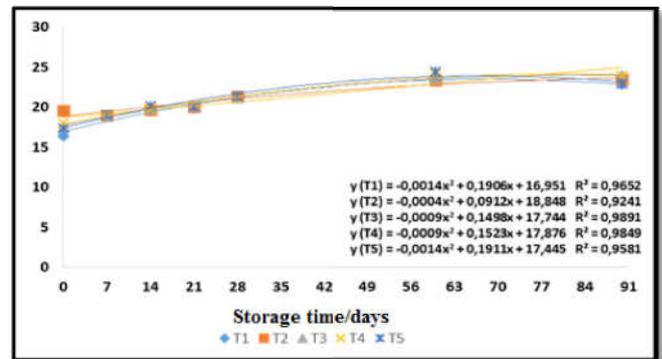


Figure 1. Regression equations for temperature of ensiled biomasses of acid silage made from different fractions of filleting tilapia waste according to different storage times

Hydrogenionic potential (pH) of silage

On Table 3, pH average values observed in each time are found, depending on the different treatments of acidic silages prepared with different fractions of waste from tilapia filleting. At the time when the biomass to be ensiled was acidified (zero time), low pH values ($P < 0.01$) ranging from 3.62 to 3.82 were observed. Lower mean values were observed at T1 and T4. The other treatments (T2, T3 and T5) had higher values, which did not differ from one another, with an average pH of 3.8. Seven days after the preparation of silage, values remained low for all treatments, with the lowest pH value at T3 ($P < 0.01$) with an average pH value of 3.60. The other treatments (T1, T2, T4, and T5) showed higher pH values ($P < 0.01$) and were statistically equal. At 14 days after ensiling, the pH of the silages were low, very acid, ranging from 3.72 to 3.97, noting that the lowest values ($P < 0.01$) were obtained for T1 and T3. The other treatments (T2, T4 and T5) did not differ and showed higher values than the others. At 21, 28, 60 and 90 days of preparation of silage, the lowest pH value ($P < 0.01$) was found for T1. The treatments T2, T3, T4 and T5 showed similar results and greater than 4. On Figure 2, the curves are related to the pH for each treatment at different times.

Table 3. Average pH values of acid silage made from different fractions of waste from tilapia filleting at different storage times

Treatment *	Storage time/days						
	0	7	14	21	28	60	90
T1	3,62b	3,60a	3,72b	3,82b	3,90b	3,95b	3,98b
T2	3,82a	3,74a	3,91a	4,07a	4,18a	4,20a	4,23a
T3	3,81a	3,32b	3,80b	4,10a	4,14a	4,21a	4,26a
T4	3,65b	3,67a	3,87a	4,17a	4,13a	4,18a	4,18a
T5	3,77a	3,78a	3,97a	4,17a	4,180a	4,24a	4,24a
CV1%	1,18						
CV2%	1,47						
Standard error	0,054						

Averages followed by same letter in the column do not differ by the Scott-Knott test at the 5% level of probability ($P < 0,05$).*(T1- silage 100% guts, T2 – silage 100% head, T3 – silage 100% carcass, T4 – silage 30% head + 70% carcass and T5 – silage 15% guts + 20% head +47% carcass + 15% leather + 3% scabies)

Quadratic effect was observed for all treatments, and T1 presented the best fit ($R^2 = 92.14$). It is noted that there was a general increase in pH in early times, reaching maximum values of pH of 4.01 at 72 days for T1; 4.33 after 76 days for T2; 4.17 after 58 days for T3; 4.20 after 53 days for T4 and T5 was 4.24 at 90 days. The presented results seem to be fairly consistent since they noticed a very low pH in the first 14 days, showing a higher acid hydrolysis during this period and a slight increase as the reaction ceased and silage stabilized. The maintenance of low pH throughout the process contributed to the microbiological stability of the silage.

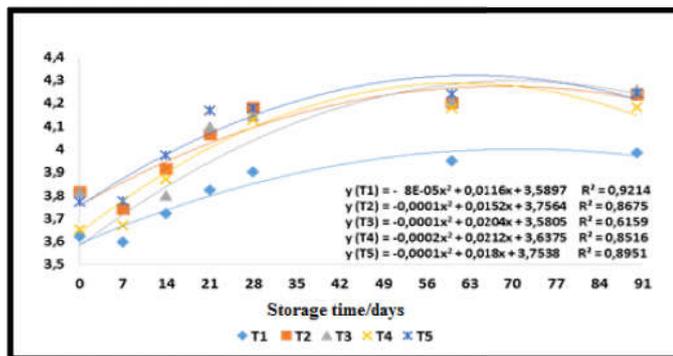


Figure 2. Regression equations for pH values of acid silage made from different fractions of filleting tilapia waste according to different storage times

The antimicrobial activity of short-chain organic acids is related to the fact they cause reduction of pH and dissociation capacity of its carboxyl groups. In undissociated state, low molecular weight organic acids have passive penetration ability in the microbial cell. After penetration, the release of protons and anions occur, which results in the lowering of the intracellular pH. Increasing the ionic strength increases the pressure inside the cell, causing the death of the microorganism (Rodríguez-Palenzuela, 2000; VIOLA; Vieira, 2007). According to Benites (2003), the pH limit for maintaining the microbiological quality of the silage is 4.5. In this sense, all treatments, regardless of storage time, had lower average. Similar results were found by Carmo *et al.* (2008), who studied acidic silage made with tilapia residues with 5% v / p of acetic acid, observed a pH around 4.37. However, Maia Jr. (1998) in his studies using the concentration of 17% v / p of acetic acid, in the preparation of acid silage of tilapia waste found pH ranging from 3.80 to 4.00 for 60 days of storage.

Also, Oliveira *et al.* (2006) evaluated the nutritional value of the tilapia filleting waste acid silage (carcass), which used the concentration 3% v / p of formic acid, in order to use the silage to replace fishmeal in feed of aquatic organism, observed average values of pH of 3.95 at the end of 30 days in the results. These values are intermediate to the present study. According Dapkevicius *et al.* (1998) the pH below 4.5 and physical characteristics of fish silage lead to lower oxygen concentration in the ensiled material and favor the action of the enzyme amino-acid-decarboxylase of endogenous origin, responsible for the formation of biogenic amines considered a risk to animal health.

Lipid Oxidation of acidic silages

It is noted from the results of Table 4 that there were significant variations in the results of malondialdehyde concentrations in acidic silage made with different fractions of tilapia filleting waste at different storage times, and at the beginning and end of the storage time, or is, at 0 and 90 days, the T1 (100% viscera) showed higher concentration. T4 treatment (30% + 70% carcasses heads) had lower mean values at time zero, 60 and 90 days. At 7 days, the treatments T2 (100% head), T3 (100% carcass) and T4 obtained the lowest values and did not differ. At 14 days, T3 treatment had lower average. Yet, at 21 and 28 days, T2 was the treatment that showed lower values than the others. By the results of the regression analysis regarding the TBA data of silages (Figure 3), there was a significant effect for all treatments, where the model that best fit was cubic. For the T1 treatment there was a marked reduction in the TBA concentration from time zero until it reaches a minimum value of 0.75 mg malonaldehyde / kg after 25 days of storage, with significant increases reaching a maximum value of oxidation of 3.70mg of malondialdehyde / kg at 84 days of storage. For other treatments the changes were small, the lowest and the highest value was reached for the treatment T2 (0.18 mg malonaldehyde / kg at 24 days, and at 83 days 0.67 mg malonaldehyde / kg). According to Araujo (2001), thiobarbituric acid content analysis (TBA - Thiobarbituric acid) is a widely used method to evaluate the oxidation of lipids, which is based on thiobarbituric acid condensation reaction with the products of decomposition of hydroperoxides. Stevanato *et al.* (2007) in their studies, found increasing amounts of TBA rates in tilapia head flour during storage, with a significant difference for the different months. The values ranged from 0.74 to 3,87mg of malondialdehyde /

kg of flour for time zero and 90 days, respectively, and these results higher than those found in this study for the treatment T2 (100% head), which varied during the same storage time (0 to 90 days) from 0.18 to 0.53 mg malonaldehyde / kg of flour.

causes foods to become unsuitable for consumption, causing yet others changes that can affect both the nutritional quality, the integrity and safety of food (Reische; Lillard; Eitenmiller, 2002).

Table 4. Malondialdehyde average values (mg / kg) obtained from samples of acid silage made from different fractions of tilapia filleting waste at different storage times

Treatment *	Storage time/days						
	0	7	14	21	28	60	90
T1	3,36a	1,17a	0,94a	1,10a	1,00a	2,36a	3,08a
T2	0,56b	0,22c	0,26b	0,18d	0,20d	0,43b	0,53b
T3	0,44c	0,23c	0,23c	0,25b	0,26b	0,36c	0,41c
T4	0,34d	0,21c	0,25b	0,22c	0,23c	0,28d	0,33d
T5	0,56b	0,38b	0,28b	0,23c	0,23c	0,44b	0,52b
CV1%	2,08						
CV2%	2,45						
Standard error	0,009						

Averages followed by same letter in the column do not differ by the Scott-Knott test at the 5% level of probability ($P < 0,05$). *(T1 - silage 100% guts, T2 - silage 100% head, T3 - silage 100% carcass, T4 - silage 30% head + 70% carcass and T5 - silage 15% guts + 20% head + 47% carcass + 15% leather + 3% scales)

Fabricio *et al.* (2013) using pirambema and tilapia waste in the preparation of compressed fish broth, found a significant increase in the concentration of the compressed malondialdehyde in broths when observed at different days after manufacturing. Bragadóttir, Pálmadóttir and Kritbergsson (2004), report that fishmeal stability depends on the conditions of processing and storage, but the habitat and seasonal variations are also very important. In this sense, a study with capelin fish meal during the four seasons, for four months of storage was conducted. In their results they found TBA values from 1 to 4 mg MA / kg. They also found that the flour produced with fish caught in the spring and summer showed no significant differences, but those produced in the fall, the value of TBA had reduced by 40%. Regarding the flour produced in winter the amount of TBA was almost doubled in two months, or changed from 2 to 4 mg MA / kg.

Conclusion

The textures of the ensiled biomass showed no immediate visible changes, prevailing caramel coloring. The first signs of hydrolysis of biomass were observed from the 4th day of storage. The treatments T1 (100% guts) and T5 (15% guts + 20% heads + 47% carcass + 15% leather + 3% scales), had lower temperatures inside the biomass, while T3 and T4 had the highest temperatures. The pH of the silages were less than 4.5. Treatment with 100% guts obtained the highest values of malondialdehyde / kg. The results of microbiological analysis have not detected the presence of aerobic mesophilic microorganisms, yeasts and molds for all treatments of the silages. The use of acetic acid and BHT acted efficiently to preserve silage quality. Thus, it can be said that the silages in the process are of high quality, with applicability in food and feed, as well as commercially valuing the fish byproduct and reducing to almost zero waste generation.

REFERENCES

- Agência Nacional De Vigilância Sanitária. Resolução RDC nº 12, de 02 de janeiro de 2001. Regulamento técnico sobre padrões microbiológicos para alimentos. Disponível em: <www.anvisa.gov.br>. Acesso em: 22 out. 2014.
- Aguiar, G. P. S., Goulart, G. A. S. 2014. Produção de óleo e farinha a partir de coprodutos de pescado provenientes da bacia Tocantins-Araguaia. Revista Eletrônica Interdisciplinar, Barra do Garça, v. 1, n. 11, p. 67-71.
- Aguiar, G. P. S., Limberger, G. M., Silveira, E. L. 2014. "Alternativas tecnológicas para o aproveitamento de resíduos provenientes da industrialização de pescados." Revista Eletrônica Interdisciplinar, Barra do Garça, v. 1, n. 11, p. 225-229.
- Araújo, J. M. A. 2001. Química de alimentos: teoria e prática. 2. ed. Viçosa, MG: UFV. 416 p.
- Arruda, L. F. *et al.* 2006. Nutritional aspects of Nile tilapia (*Oreochromis niloticus*) silage. Ciência e Tecnologia de Alimentos, Campinas, v. 26, n. 4, p. 749-756.
- Arruda, L. F.; Borghesi, R.; Otterer, M. 2007. Use of fish waste as silage: a review. Brazilian Archives of Biology and Technology, Curitiba, v. 50, p. 879-886.

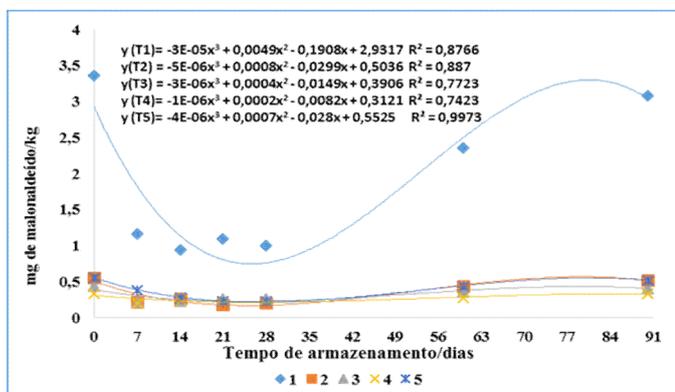


Figure 3. Regression equations for malondialdehyde values of acid silage made from different fractions of filleting tilapia waste according to different storage times

Lipid oxidation is responsible for producing unpleasant odors and flavors making the food unfit for consumption, even causing other changes that may affect both the nutritional quality due to degradation of fat-soluble vitamins and essential fatty acids, the integrity and food safety, through the formation of potentially toxic polymeric compounds (Brazil, 2001; Reische; Lillard; Eitenmiller, 2002). To reduce lipid oxidation, the Brazilian Compendium of Animal Nutrition (Brazil, 2005) suggests the addition of antioxidants, as oxidation, being the cause of the development of unpleasant odor and taste,

- Assano, M. 2004. Utilização de diferentes fontes e níveis de proteína no crescimento da tilápia do Nilo (*Oreochromis niloticus*). 2004. 34 p. Dissertação (Mestrado em Aqüicultura) - Universidade Estadual Paulista, Jaboticabal.
- Benites, C. I. 2003. Farinha de silagem de resíduo de pescado: elaboração, complementação com farelo de arroz e avaliação biológica em diferentes espécies. 2003. 159 p. Dissertação (Mestrado em Engenharia e Ciência dos Alimentos) - Fundação Universidade Federal do Rio Grande, Rio Grande.
- Boscolo, W. R. *et al.* 2010. Manual técnico: criação de pacu em tanque-rede para produção de carne mecanicamente separada (CMS). Toledo: Convênio de cooperação técnico-financeira para o desenvolvimento de pesquisas relacionadas à produção de peixes em sistema de tanques-rede no Reservatório de Itaipu -GEMAq/Unioeste/Itaipu Binacional. 50 p.
- Bragadóttir, M.; Pálmadóttir, H.; Kristbergsson, K. 2004. Composition and chemical changes during storage of fish meal from capelin (*Mallotus villosus*). *Journal of Agricultural and Food Chemistry*, Easton, v. 52, n. 6, p. 1572-1580.
- Brasil. Decreto nº 55.871, de 26 de março de 1965. Modifica o Decreto nº 50.040/61 referente às Normas Reguladoras de Emprego de Aditivos em Alimentos, alterado pelo Decreto nº 691/62. Diário Oficial [da] República Federativa do Brasil, Rio de Janeiro, 9 de abril de 1965, retificado pelo de 20 de abril de 1965.
- Brasil. Ministério da Agricultura Pecuária e Abastecimento. 2005. Compêndio brasileiro de alimentação animal. São Paulo.
- Brasil. Ministério da Agricultura Pecuária e Abastecimento. Instrução Normativa n.º 6, de 15 de fevereiro de 2001. Regulamentos técnicos de identidade e qualidade de paleta cozida, produtos cárneos salgados, empanados, presunto tipo serrano e prato elaborado pronto ou semi-pronto contendo produtos de origem animal. Diário Oficial [da] República Federativa do Brasil. Brasília, DF, 19 de fevereiro de 2001.
- Carmo, J. R. *et al.* 2008. Caracterização de silagens ácidas de resíduos de tilápia (*Oreochromis niloticus*). *Revista Eletrônica Nutritime*, Viçosa, MG, v. 5, n. 5, p. 664-672, set./out.
- Carmo, J. R. 2009. Qualidade de silagens ácidas de resíduos da filetagem de tilápia (*Oreochromis niloticus*) elaboradas com ácidos orgânicos. 2009. 157 p. Dissertação (Mestrado em Ciência dos Alimentos) – Universidade Federal de Lavras, Lavras, 2009.
- Dapkevicius, A. *et al.* 1998. Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. *Journal of the Science of Food and Agriculture*, London, v. 77, n. 1, p. 140-146.
- Disney, G. J., Tatterson, I. N., Ollen, J. 1977. Recent development in fish silage. In: CONFERENCE ON THE Handling Processing and Marketing of Tropical Fish, 1, London. Proceedings... London: Tropical Products Institute, 1977. p. 321-40.
- Espe, M.; Raa, J.; Njaa, L. R. 1989. Nutritional value of stored fish silage as a protein source for young rats. *Journal of Science Food and Agriculture*, London, v. 49, n. 3, p. 259-70.
- Fabricio, L. F. F. *et al.* 2013. Elaboração de caldo de peixe em cubos compactados utilizando pirambeba (*Serrasalmus brandtii*) e tilápia (*Oreochromis niloticus*). *Semina. Ciências Agrárias*, Terezina, v. 34, p. 2411-252.
- Ferreira, D. F. 2000. SISVAR: sistema de análises de variância para dados balanceados. Lavras: UFLA.
- Haard, N. F. *et al.* 1985. Stabilisation of protein and oil in fish silage for use as a ruminant feed supplement. *Journal of the Science Food and Agriculture*, London, v. 36, n. 4, p. 229-241, Apr.
- Honorato, C. A.; Carneiro, D. J. 2003. Corporal composition of Nile tilapia fingerling (*Oreochromis niloticus*) fed with diets containing both different souces and protein levels and proportions of animal protein. In: WORLD AQUACULTURE, 1., 2003, Salvador. Anais... Salvador: [s. n.]. p. 354.
- Jackson, A. J.; Kerr, A. K.; Crowey, C. B. 1984. Fish silage as a dietary ingredient for salmon: I. nutritional and storage characteristics. *Aquaculture*, Amsterdam, v. 38, n. 3, p. 211-220, May.
- Maia Júnior, W. M. 1998. Adequação do processo de silagens de resíduos de tilápias, caracterização dos lipídios e da fração seca em pó. João Pessoa, 1998. 116 p. Dissertação (Mestrado em Tecnologia de Alimentos) - Universidade Federal da Paraíba, João Pessoa.
- Morales-Ulloa, D. F., Oetterer, M. 1995. Bioconversão de resíduos da indústria pesqueira. *Ciência e Tecnologia de Alimentos*, Campinas, v. 15, n. 3, p. 206-214.
- Oetterer, M. 2002. Industrialização do pescado cultivado. Guaíba: Agropecuária.
- Oliveira, M. M. *et al.* 2006. Silagem de resíduos da filetagem de tilápia do Nilo (*Oreochromis niloticus*), com ácido fórmico: análise bromatológica, físico-química e microbiológica. *Ciência e Agrotecnologia*, Lavras, v. 30, n. 6, p. 1218-1223, nov./dez.
- Pimenta, M. E. S. G. *et al.* 2008. Desempenho produtivo e digestibilidade pela tilápia do Nilo (*Oreochromis niloticus* Linnaeus, alimentada com dietas suplementadas com níveis crescentes de silagem ácida de pescado. (prelo). *Ciência e Agrotecnologia*, Lavras, v. 32, n. 6, p. 1953-1959, nov./dez.
- reische, D. W.; Lillard, D. A.; Eitenmiller, R. R. 2002. Antioxidants. In: AKOH, C. C.; MIN, D. B. *Food lipids: chemistry, nutrition and biotechnology*. 2nd ed. New York: M. Dekker. p. 489-516.
- Rodriguez-Palenzuela, P. L. 2002. Os ácidos orgânicos como agentes antimicrobianos. In: *Avances in Nutrición y Alimentación animal*, 16., 2000, Barcelona. Proceedings... Barcelona: FEDNA, p. 155-167.
- Sales, R. O. 1995. Processamento, caracterização química e avaliação nutricional da despesca da tilápia do Nilo (*Oreochromis niloticus*, Linnaeus) em dietas experimentais com ratos. 1995. 174 p. Tese (Doutorado em Tecnologia de Alimentos) - Universidade Estadual de Campinas, Campinas.
- Santana-Delgado, H.; Avila, E.; Stelo, A. Preparation of silage from Spanish mackerel (*Scomberomorus maculatus*) and its evaluation in broiler diets. *Animal Feed Science and Technology*, Amsterdam, v. 141, n. 1/2, p. 129-140, Mar. 2008.
- Seibel, N.F., Souza-Soares, L. A. Produção de silagem química com resíduos de pescado marinho. *Brazilian Journal of*

- Food Technology, Campinas, v. 6, n. 2, p. 333-337, jul./dez. 2003.
- Silva, N. et al. Manual de métodos de análise microbiológica de alimentos. 3. ed. São Paulo: Varela, 2005.
- Socol, M. C. H. Otimização da vida útil da tilápia cultivada (*Oreochromis niloticus*), minimamente processada e armazenada sob refrigeração. 2002. 124 p. Dissertação (Mestrado em Ciências) - Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo,
- Stevanato, F. B. et al. (2007). Aproveitamento de resíduos, valor nutricional e avaliação da degradação de pescado. PUBVET, Londrina, v. 1, n. 7. Disponível em: <<http://www.pubvet.com.br/texto.php?id=74>>. Acesso em: 10 maio 2014.
- Tarladgis, B. G.; Watts, B. M.; Younathan, M. T. A. A 1960. distillation method for the quantitative determination of malonaldehyde in rancid foods. Journal American Oil Chemists Society, Madison, v. 37, p. 44-48.
- Valério, A. C. R. 1994. Elaboração de silagem enzimática de pescado como alternativa ao processo tradicional. 102 p. Dissertação (Mestrado em Ciência e Tecnologia de Alimentos) - Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, 1994.
- Vidotti, R. M. 2001. Produção e utilização de silagens de peixe na nutrição de Pacu (*Piaractus mesopotamicus*). 65 p. Tese (Doutorado em Aquicultura) – Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, 2001.
- Vidotti, R. M. et al. 2004. Caracterização das silagens ácidas e fermentadas e determinação do coeficiente de digestibilidade aparente da proteína para tilápia após 7 e 30 dias da produção. In: Congresso Da Sociedade Brasileira De Aquicultura E Biologia, 1., 2004, Vitória. Anais... Vitória: [s. n.], p. 84.
- Vidotti, R. M.; Gonçalves, G. S. 2006. Produção e caracterização de silagem, farinha e óleo de tilápia e sua utilização na alimentação animal. Disponível em: <ftp://ftp.sp.gov.br/ftppesca/producao_caracterizacao.pdf>. Acesso em: 10 maio 2013.
- Viola, E. S.; Vieira, S. L. 2007. Suplementação de acidificantes orgânicos e inorgânicos em dietas para frangos de corte: desempenho zootécnico e morfologia intestinal. Revista Brasileira de Zootecnia, Viçosa, MG, v. 36, n. 4, p. 1097-1104.
