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## RESEARCH ARTICLE

# QUALITY OF ACID SILAGE FROM TILAPIA (*Oreochromis niloticus*) FILLETING WASTE DURING STORAGE

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**ABSTRACT**

The fish acid silage is an age old organic matter preservation technique, elaborated starting from whole fish or their parts, to which acids are added. The objective of this research was to produce and characterize acid silage from tilapia filleting residues elaborated with organic acids during 28 days of storage. For such, three acid silage types were elaborated from residues of tilapia filleting using 5% v/p of formic, acetic and propionic organic acids. The moisture and ethereal extract variables increased in relation to the initial ensilage time. In compensation, the protein and ash content underwent a decrease. The extracted oils of the silage remained stable during the experiment, peroxide formation not being detected. Due to the high preservation efficiency, the three acids (formic, acetic and propionic) are suitable in the elaboration of fish acid silage.

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## INTRODUCTION

Currently, humanity is concerned with quality of life, a concern that calls special attention to environmental conservation. Within this reality an activity which degrades the environment in any aspect becomes inadmissible. The use of residues (solids, liquids and gaseous) contributes to the minimization of environmental impacts, besides generating, most of the time, by-products with high aggregrate value. Included in the term 'fish residues'are: pre-canning flakes, dark meat, non-standard size fish for industrialization, heads and carcasses (Oetterer, 1993). An alternative process, economically viable and environmentally favorable, can be adopted based on the preservation of the organic matter through the ensilage technique. The ensilage of fish waste is an old organic matter preservation technology. The preservation of the material is activated by the reduction of the pH, which can be chemically obtained through direct acidification using organic acids, such as formic, acetic and propionic, among others, and/or mineral acids, such as hydrochloric and sulfuric (acid silage), by the addition of lactic acid producing microorganisms (biological silage) or by the combination of the two methods (Kompiang, 1981; Tatterson & Windsor, 1974; Borghesi, 2004). The investigation of the storage effects on the nutritional characteristics of the products is as important to know as the their nutritional value after preparation. The use of different types of acids, raw materials, temperatures or storage types leads to the obtaining of different final products, making the choice of variables that produce a good final product at the end of storage fundamental. Thus, the objective of this work was to produce and to characterize physiochemically, chemically and microbiologically, three types of acid silage from tilapia filleting residues, elaborated with acetic, propionic and formic acid during 28 days of storage.

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## MATERIALS AND METHODS

### Preparation of acid silage from tilapia filleting residue (*Oreochromis niloticus*)

The raw material used in the elaboration of the silage was composed of residues from the filleting of Nile tilapia

(*Oreochromis niloticus*) including bones, fins, tails, flakes (between bones) and few scales. Such residues were acquired from producers in Boa Esperança-MG- Brazil area. The residues were transported frozen to the Animal Nutrition Laboratory of the Animal Science Department at the Federal University of Lavras, where they were ground in an electric meat grinder. The total content after grinding was weighed using an analytical scale. The total weight of triturated residue was 29.1 kg. After manual homogenization, the biomass was divided in three equal parts of 9.70 kg used in the elaboration of silage with the following formulations:

- Silage from tilapia filleting residue using formic acid (FAS): 9.70 kg of ground tilapia filleting residues, 0.1% p/p butylhydroxytoluene (BHT) antioxidant and 5%v/p formic acid;
- Silage from tilapia filleting residue using acetic acid (AAS): 9.70 kg of ground tilapia filleting residues, 0.1% p/p butylhydroxytoluene (BHT) antioxidant and 5%v/p of acetic acid;
- Silage from tilapia filleting residue using acid propionic (PAS): 9.70 kg of ground tilapia filleting residues, 0.1% p/p butylhydroxytoluene (BHT) antioxidant and 5%v/p of acid propionic;

The silage were stored in 15kg polyethylene buckets. In the covers of those containers 3 holes were opened to permit the escape of gases. The silage was stored for 28 days at room temperature. During everyday of storage the systems were agitated in order to provoke the highest possible contact between the acid (preservation agent) and the biomass.

**Sample Collection**

Before each collection the systems were agitated. Samples were collected soon after the preparation of the silage and weekly, in other words, on the 0, 7th, 14th, 21st and 28th day of the ensilage process.

**Physicochemical determinations**

The room temperature in the silage storage local was monitored daily, using a maximum minimum thermometer. The temperature inside the biomass was measured by a mercury thermometer before each daily turnover. The hydrogen potential (pH) was determined in a digital potentiometer, with results carried to two decimal places.

**Proximate composition**

The proximate composition of the silage was determined using the following methods proposed by the Association of Official Analytical Chemists, AOAC (1990): moisture (gravimetric method by oven drying at 105ºC), etherial extract (Soxhlet method using ethyl ether as solvent), crude protein (Micro Kjeldahl method using the factor of 6.25 for transformation of the total nitrogen in raw protein) and ash (gravimetric method by incineration in a Muffle furnace at 550ºC).

**Non-protein nitrogen**

The level of Non-Protein Nitrogen (NPN) was determined using trichloroacetic acid to precipitate the nitrogen originating from the protein fraction, just measuring the non-protein nitrogen by the Micro Kjeldahl method. Both analyses were conducted following procedures described by AOAC (1990).

**Crude oil characterization analysis**

To characterize the oil originating from silage, the lipidic content was extracted by centrifugation of a 10g sample at 3000 xG (Arruda, 2004). The following analyses were made: peroxide index (using the methodology proposed by Lovern, 1965 and American Oil Chemists ' Society, AOCS, 1995), iodine index, saponification index and acidity index (the last three variables were determined following the methodologies proposed by Moretto & Alves, 1986).

**Fatty acids profile**

The fatty acids were extracted from the acid silage of the tilapia filleting residues according to the methodology proposed by Folch, Lees & Sloane-Stanley (1957), after 28 days of storage. The fatty acids were esterified and the resulting esters were submitted to gas chromatography analysis in a Shimadzu GC 2010 apparatus, with flame ionization detector (FID), using capillary column (100m x 0.25mm x 0.2μm). Thew following conditions were used: split injetor, using the helium as carrier gas, flow rate 1.09mL.min$^{-1}$ for a total time of 60 minutes; initial column temperature 140ºC, remaining for 5 minutes, rising 4ºC. min$^{-1}$ up to 240ºC. The stationary phase of the column was composed of bis-cyanopropyl polysiloxane. The identification and quantification of the fatty acids were made by comparison of ester retention times contained in the Supelco$^{TM}$ 37 FAME MIX standard with those of the sample. That determination was conducted in the Chemistry Department Chemical Analysis Center at the Federal University of Lavras.

**Determination of the minerals calcium and phosphorus**

The minerals calcium and phosphorus were determined by atomic absorption spectrophotometry, in the Chemistry Department Leaf Analysis Laboratory at the Federal University of Lavras, only in the samples of the last storage time. The results were expressed as a percentage and were not submitted to statistical tests (they were only descriptive).

**Microbiological determinations**

The analyses of total mesophilic microorganism count and the total mold and yeast count were carried out based on Silva *et al*. (1997). For the first analysis the culture medium used was SCA (Standard Count Agar) and for second the culture medium used was PDA-cloranphinicol (PDA (Potato-Dextrose Agar-Cloranphinicol). The microbiological assays were developed at the Laboratório de Microbiologia da Empresa de Pesquisa Agropecuária de Minas Gerais – Centro Tecnológico do Sul de Minas - EPAMIG/CTSM, located on the campus of UFLA.

**Statistical analysis**

The experiment followed a Completely Randomized Design (CRD), in a factorial outline, the factors being: type of acid

silage (Acetic Acid Silage, Propionic Acid Silage and Formic Acid Silage) and storage times (0, 7, 14, 21 and 28 days). In each storage period, 7 repetitions of each treatment were removed, totaling 105 portions (except for the silage oil extract characterization analyses, where 4 repetitions of each treatment were removed). The statistical model used was: $y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + e_{ijk}$ , where: $y_{ijk=}$ variable response; $\mu$ = constant associated to each response; $\alpha_i$ = effect of $i^{th}$ level of the silage type factor; $\beta_j$= effect of the $j^{th}$ level of the storage time factor; $\alpha\beta_{ij}$= effect of the interaction between the two factors; $e_{ijk}$ = error observed for each observation. The data obtained for each variable were stored in electronic spreadsheets using the Excel program, and later transformed in dBase (DBF IV) files and processed by Sisvar (Ferreira, 2000).

## RESULTS AND DISCUSSION

### Physicochemical determinations

### Monitoring of room temperature and that within the silage biomass

During the silage storage, the average observed for the maximum and minimum temperatures were 31°C and 15°C, repectively. The average temperature within the mass did not present differences among the treatments in any storage period, presenting an average from 16.4°C to the end of the 28 days of ensilage. That result shows that the three types of acids (formic, acetic and propionic) used in the elaboration of the silages self-solubilized in a similar way in the raw material and none stood out by causing endo or exothermic alterations in the biomasses.

### Hydrogen potential (pH)

Significant interaction was observed ($P < 0.01$) between the storage time and the acid type. The letters present in the Figure 1 represent the result of the test of averages (Scott knott) and same letters indicate that the averages do not differ amongst themselves at the level of 5% of probability, for the referred test.

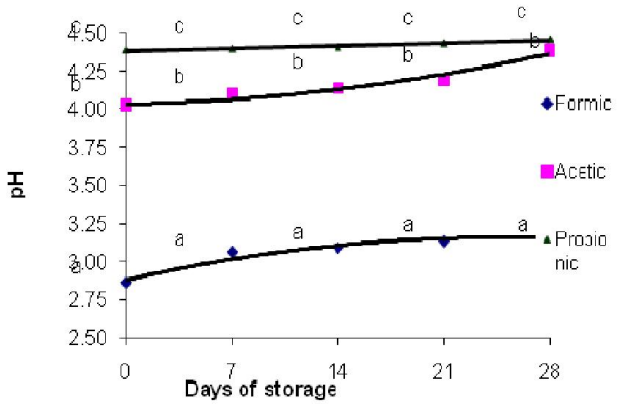


**Figure 1. Variation of pH in acid silage from fish residues throughout the days of storage**

The different averages pH in all of the storage times in each silage type are due to the different degrees of dissociation of the acids inunder study. Benites (2003) emphasizes that the pH limit to maintain the microbiological quality of the silage is 4.5. For all the 5 sampling times, that limit was not crossed by any of the silage under study, which contributes positively to the use of those acids under 5% v/p concentration in the production of fish chemical silage. The highest average pH was observed in the last storage time in the propionic acid silage (4.46) and to lowest average was observed in the formic acid silage (2.86), in the first sampling time. Through the behavior of the pH in function of the storage time of the three produced silages, which can be visualized in Figure 1, the elevation of the pH along the ensilage days is noticed, a fact that can be attributed to the formation of secondary products during proteolysis, such as amino acids, and biogenic amines, among others. Some of those substances can have alkaline characteristics, affecting the buffer capacity of the acids under study, provoking the pH increase of the biomass (Lindgren & Pleje, 1983; Dapkevicius, Nout, Roumbouts & Houben, 2000).

### Proximate composition and non-protein nitrogen

### Moisture, etherial extract and ash

For the moisture, etherial extract and ash level variables, a significant difference ($P < 0.01$) for the silage type and storage time was observed, however significant difference was not verified for the interaction between those two factors ($P>0.05$). The average values for those variables in each silage type are listed in Table 1.

**Table 1. Average moisture, ether extract and ash values in the fresh matter[1] (%) of FAS, AAS and PAS silage in each storage period**

| Type of silage | M (%) | EE (%) | A (%) |
|---|---|---|---|
| FAS | 40.57 a | 18.80 b | 13.64 b |
| AAS | 40.83 a | 18.17 a | 13.21 a |
| PAS | 39.55 b | 19.75 c | 13.76 b |
| CV (%) | 2.63 | 6.16 | 5.32 |

[1]Averages with the same letter on the line do not differ among themselves at 5% probability by the Scott-Knott test.

Symbols used: M= moisture, EE= ether extract, A= ash; FAS: Formic acid fish silage, AAS: Acetic acid fish silage, PAS: Propionic acid fish silage.

The formic acid and acetic acid silage presented statistically similar moisture averages (40.57 and 40.83%, respectively) and superior to that of propionic acid silage (39.55%). The smaller the carbon chain of the acid, the higher its polarity and, consequently, the higher its solubility in water. After solubilizing in water, the acetic and formic acids can be evaporated together with other polar substances when oven-dried at 105°C, being detected as water in the proximate composition analysis, contributing to the moisture increase in relation to the raw material *in natura*. The propionic acid, by being more apolar, has a smaller fraction associated to the water, being able to be associated to the lipids, not being lost in proportions similar to the other acids in question. The effect of time and storage on the moisture level in the acid silage can be observed in Figure 2a. The days of ensilage presented a increasing linear effect (positive angular coefficient) on the moisture level. During hydrolysis, secondary products are

formed, such as fatty acids, peptides and amino acids, which, when soluble, contribute to the moisture increase during the storage periods. Oliveira, Pimenta, Camargo, Fiorini, Pimenta & Logato (2006), using formic acid at the concentration of 3% v/p in the acid silage production of tilapia filleting residues observed, as in the present study, an increase in the moisture level, which went from 40.20% (first day of ensilage) to 42.09% (thirtieth day of ensilage). The high lipid levels can harm the sampling, hindering the homogenization of the material, mistakes possibly occurring in the determination of that variable (Espe & Lied, 1999). It is important to point out that before the collection of each ensilage period the maximum possible homogenization of the systems under analysis was undertaken, in order to minimize the experimental errors. The final composition of the silage primarily varies as to the lipid level, according to the type of residue employed, the sex of the animals and their capture time (Borghesi, 2004). In Figure 2b the growing increase of the ethereal extract level can be observed in function of the silage storage. A maximum ethereal extract level point occurrs around the 19th day of ensilage. Dapkevicius, Batista, Nout & Roumbouts (1998), observed an increase in the lipid level from 11.30 (at 0 days) to 14.90% (at 15 days) based on the dry matter. A decrease in the ethereal extract level during the days of ensilage was observed in the acid silage studied by Oliveira *et al*. (2006), however such authors did not use antioxidants as one of the ingredients for the elaboration of the silage, thus, lipid oxidation could have occurred, leading to the decrease in the ethereal extract level.

Regarding the ash content in the acid silage, acetic acid silage presented a different and lower average than the others (Figure 2). Acid solutions can solubilize the silage nutrients, which dislocates them to the supernatant portion of the silage, causing the minerals to concentrate on the solid part and resulting in a high concentration of minerals in the silage meal (Benites, 2003). The high level of minerals present in the silage can be due to the use of carcasses (without visceras and heads), as mentioned by Oliveira *et al*. (2006). Figure 2c, shows a linear decrease in the ash level during the days of storage. The decrease in the mineral content was also observed by Dapkevicius *et al*. (1998), who observed a reduction of 13.4% at 0 days to 11.8% of ash in the dry matter at 15 days. The highest ash concentrations observed in the first storage periods might have occurred because of the non-solubilization of bones and scales, which hinders the sampling. With passing of the days, such materials were becoming more soluble, since in the works of Oliveira *et al*. (2006) and Valério (1994) the ash fraction during the storage time was practically invariable.

**Crude protein and non-protein nitrogen**

Regarding the crude protein content and non-protein nitrogen content, significant interaction was observed between the storage time and the acid type ($P<0.01$). Figure 3 presents the protein averages (Figure 3a) and non-protein nitrogen averages (Figure 3b) in the fresh matter along the ensilage process for the treatments, as well as the result of the statistical analyses (Scott Knott test of averages).

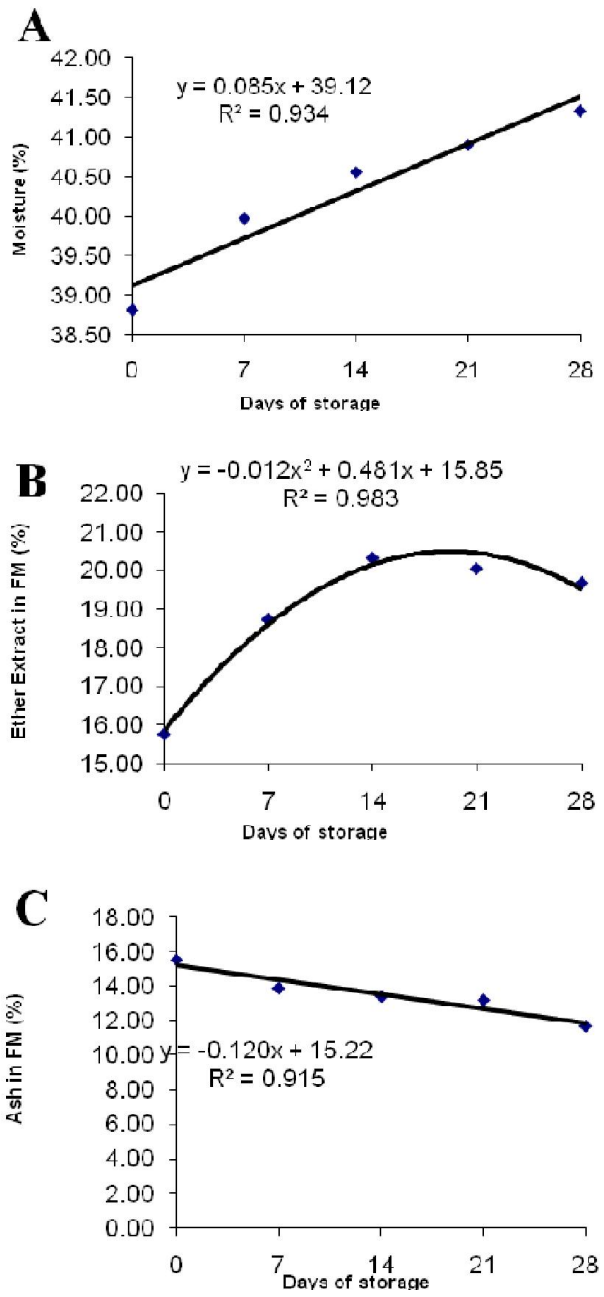


**Figure 2. Variation of the (A) moisture percentage (B) ether extract in fresh matter (C) ash level throughout the days of storage**

The choice of the acid is crucial for the protein quality of the silage. The acids can denature, break or precipitate proteins. The action of the acid will be determined by the acidic conditions. In mild acidity there is practically no protein degradation, loss of solubility being able to happen in the proteins whose isoelectric points are in the lightly acidic pH range. However, in highly acidic solutions, the proteins can be partially or totally hydrolyzed. Some amino acids can be totally destroyed, such as the tryptophan, or partially lost, as in the case of serine, threonine and cysteine (Sgarbiéri, 1996).

For the times 14 days and 21 days, the averages of the crude protein values in the fresh matter of all of the silages were statistically similar. At 0 days, the highest averages were found

in the acetic and propionic acid silages. However, at 7 days, the formic and acetic acid silages stood out in relation to of propionic acid silage by their possessing higher crude protein content. After 28 days of storage, to lowest average was observed in the acetic acid silage (23.48%), while the silages of formic and propionic acid presented higher levels of crude protein (25.45 and 24.65%, respectively). Arruda (2004) working with raw material from tilapia filleting, obtained 13.49 and 12.85% of crude protein. The proteolytic processes are quite intense during the first week of ensilage, when most of the proteins present in the biomass are converted into peptides and free amino acids. Those transformations can improve the digestibility of the silage (Morales-Ulloa & Oetterer, 1997). The resulting amino acids of the protein hydrolysis can be deaminated and decarboxylated, acting as precursors of biogenic amines, potentially toxic compounds to some animals (Baraquet & Lindo, 1985).

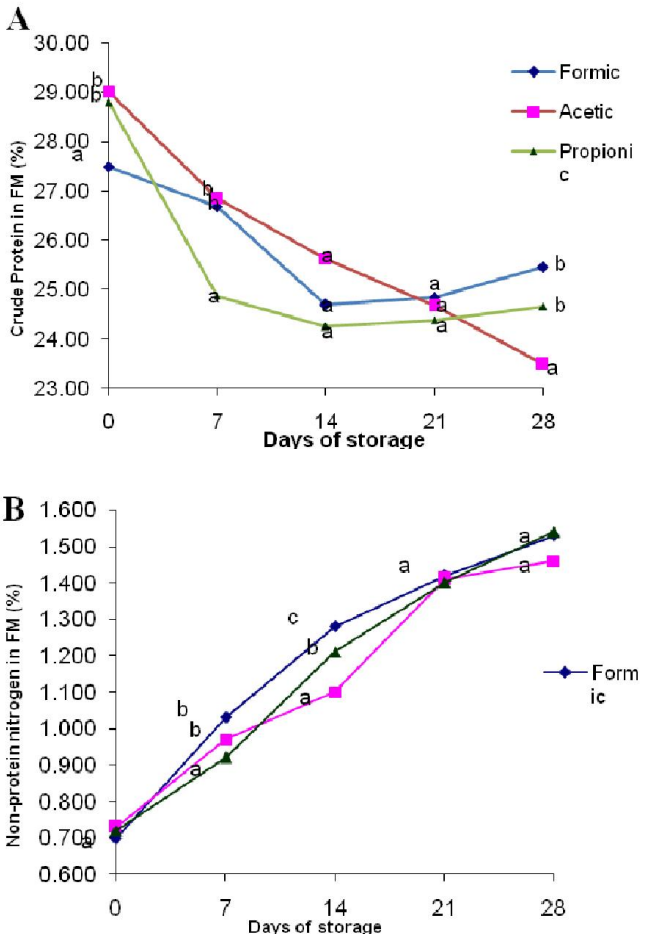


**Figure 3. Variations of the (A) crude protein content (B) level of non-protein nitrogen presents in acid silage from fish residues throughout the days of storage**

In all of the systems a decrease of the protein content was observed during the storage period (Figure 3a). For the silages FAS and PAS, a quadratic effect was observed during the days of ensilage, lower values of crude protein occurring close to 20 and 19 days, respectively; while, for AAS, a linear decrease effect was observed. The reduction in the protein content is due to protein hydrolysis, which converts the proteins into ammonium, that can volatilize during the silage processing and storage (Santana-Delgado, Avila & Stelo, 2008). Geron *et al*. (2007), observed a 9% fall of the crude protein level in acid silage from tilapia filleting residues, after 180 days of storage, in relation to the raw material. From the evaluation of Figure 3a, it can be noticed that the nutritional value, in terms of the crude protein level, is indirectly related with the storage time. The highest nutritional value is attributed to the period soon after the preparation, in other words, the fresh silage is nutritionally superior to the stored silage (Stone & Haardy, 1986; Valério, 1994). It is worth pointing out that even having a decrease of the protein level during the storage time, the remaining content is high after 28 days, and the possible benefits in the digestibility improvement can certainly make up for that small loss of nutritional value. The degree of protein hydrolysis is an important chemical criterion in the evaluation of the nutritional quality of fish silage. According to Valério (1994), the degree of hydrolysis can be measured by visual observation, by the nitrogen conversion rates (the higher NNP, the more hydrolyzate in the system) and by the digestibility after the days of ensilage. The autolytic activity that occurs during the storage period provokes the increase of nitrogenated substances, such as ammonium, amines, amino acids and peptides, which are quantified as non-protein nitrogen (Lindgren *et al*., 1983; Gonzáles & Marín, 2005). The nitrogen solubilization values, and consequently the protein solubilization expressed as NNP, can be visualized in Figure 3b. A rapid increase in the nitrogen solubilization during the first week in all of the silages is observed in Figure 3b. In general, this continued to proceed until the end of the experiment. Stone *et al*. (1986), affirm that in the first days of ensilage (from 3 to 7 days) accentuated proteolysis occurred and most of the proteins are transformed into amino acids and short chain peptides, increasing the digestibility of the silages. Gonzáles *et al*. (2005), studying biological silages of sardine residues stored for 60 days, verified a significant increase in the level of NNP to the 13th day of ensilage, continuing its ascension, however at a slower speed, until the end of the experiment.

**Determination of the minerals calcium and phosphorus**

The analyses of calcium and phosphorus were conducted in order to inform about the level of those minerals after 28 days of ensilage, just being descriptive (they were not submitted to statistical tests). The calcium and phosphorus levels in the dry matter of the acid silage after 28 days of storage were: 3.34 and 3.01% in FAS, 3.27 and 2.91% in AAS and 3.35 and 2.99% in PAS, respectively. The values are close to those obtained by Geron *et al*. (2007), who found 4.9% and 4.3% of calcium and phosphorus, respectively, in acid silage of tilapia filleting residues with based on the dry matter, and superior to those found by Maia Júnior (1998), who obtained 1.6% of calcium and 1.2% of phosphorus in meal of acid silage of from tilapia filleting residues.

**Physical characteristics of silage crude oils**

**Peroxide Index**

The peroxide index is a very sensitive indicator in the initial stage of oxidation, and its presence is an indication that flavor

and odor deterioration, in function of its instability, is about to occur (ARAÚJO, 2004). The oils extracted from the silages under study, at all storage times, did not present peroxide formation, proving the efficiency of protection of the BHT antioxidant, which, according to Araújo (2004), it is the most efficient in fats or animal oils. Seibel (2002), prepared acid silage using 15% of acetic acid for 2 elaborations, one using BHT (200mg/kg of residue) and the other without BHT addition, varying the amount of acetic acid. After 25 days of storage, the peroxide formation was not detected in either elaboration. According to the author, the acetic acid might have acted as an inhibitor in the peroxide formation.

**Iodine index**

The iodine index is a measure of the oil unsaturation (Araújo, 2004). The higher its content, the higher the number of unsaturated fatty acid constituents of the oils. For the iodine index significant differences were not detected between the silage type and storage time factors, nor for the interaction between them (P>0.05). The average values found for that variable were of 119.99; 120.27 and 120.12 gI/100g for formic acid, acetic acid and propionic acid silage, respectively. Those results indicate that changes did not exist in the number of unsaturated fatty acids during the storage, nor were differences observed for the silage type. The averages are close to those found by Benites (2003).

**Saponification index and acidity index**

The saponification index is an important attribute of oil quality, indicating the average molecular weight of the fatty acids esterified to glycerol. A low saponification index indicates the existence of fatty acids with high molecular weight, and a high index indicates the existence of fatty acids of low molecular weight (Araújo, 2004). However, the acidity index reveals the existence of substances that associate to sodium hydroxide, possessing acid characteristics. For those two variables, significant differences were observed among the silage types studied ($P < 0.01$). Differences were also detected among the storage times ($P < 0.01$), however the interaction between the silage type and the storage time was not significant ($P>0.05$). The results for saponification index showed that the formic acid silage presented the highest average (171.54 $mgKOH.g^{-1}$), followed by acetic acid silage (164.14 $mgKOH.g^{-1}$), the lowest average being verified in the propionic acid silage (153.97 $mgKOH.g^{-1}$), indicating that in the latter, there is a higher content of long chain fatty acids. Those results are close to the values obtained by Seibel (2002) and superior to the values observed by Maia Júnior *et al.* (1998), who observed the indexes 157.87 mg $KOH.g^{-1}$ and 126.78 $mgKOH.g^{-1}$, respectively. The increase in the saponification index as the days of storage passed, shown in Figure 4a, can be indicative of the short chain fatty acid increase, originating from of the hydrolysis of the long chain fatty acids. Regarding the acidity index, the highest level of that variable was observed for the formic acid silage (9.03 mg $NaOH.g^{-1}$) due to the fact that of the organic acid being stronger in relation to the others, which leads to lower silage pH and higher oil acidity. The averages of the acidity indexes for acetic and propionic acid silage were similar statistically (6.66 $mgNaOH.g^{-1}$ for both silages), which corroborates the close pH values of both silages. The acidity values are close to those found by Benites (2003). The decrease observed (Figure 4b) in the acidity index of the oils extracted from the silages along the storage periods is due to the protein hydrolysis increase, that liberates alkaline substances, provoking destabilization of the buffering capacity of the silages (Dapkevicius *et al.*, 2000), which can act neutralizing the excess acid, thus reducing the acidity index of the systems.

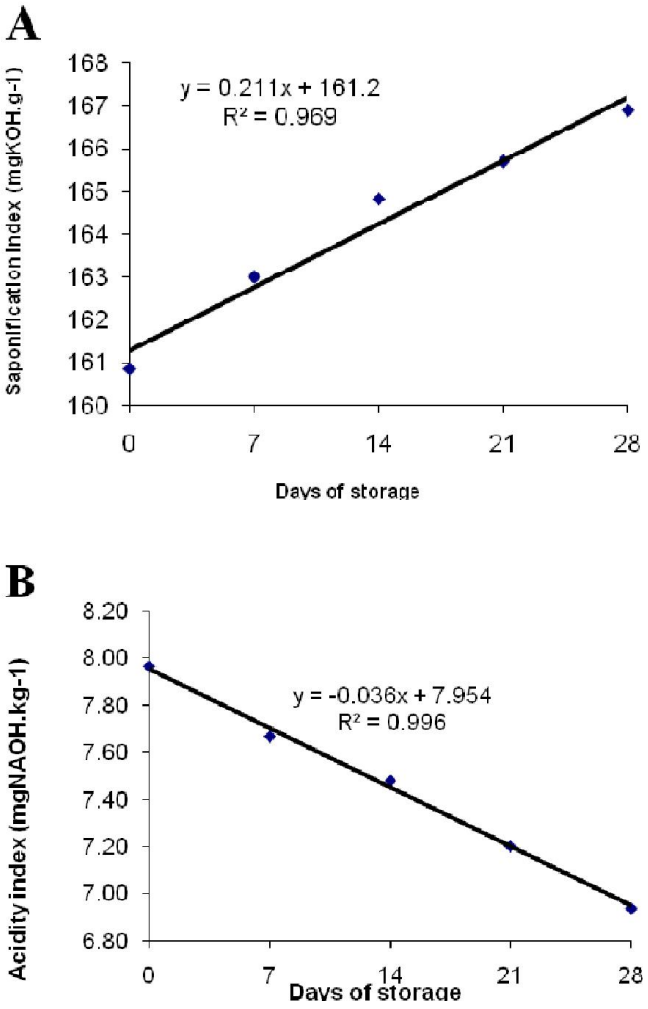


**Figure 4. Variation of the (A) saponification index (B) acidity index throughout the days of storage**

**Profile of acid silage fatty acids**

From the data contained in Table 2, one can notice the similarity among the oils of the different silages as to their fatty acid contents. The highest percentage in terms of area, among the saturated fatty acids, were observed for the palmitic acid. Among the unsaturated fatty acids, the highest percentage was observed for oleic acid.

The similarity among the types of silages is a strong indicator that the acids used did not affect the fatty acid profile of the silages.

**Table 2. Profiles of fatty acid in the silage after 28 days of storage (%) of acid silage from tilapia filleting residues[1]**

| Fatty Acids | Symbol | % in FAS | % in AAS | % in PAS |
|---|---|---|---|---|
| Myristic | C14:0 | 2.83 | 2.67 | 2.80 |
| Palmitic | C16:0 | 21.66 | 20.65 | 22.50 |
| Margaric | C17:0 | 0.29 | 0.25 | 0.27 |
| Stearic | C18:0 | 5.59 | 5.11 | 5.48 |
| Tricosanoic | C23:0 | 0.92 | 0.73 | 0.78 |
| Σ saturated | | 31.29 | 29.41 | 31.83 |
| Linolenic | C18:3n3 | 0.87 | 0.81 | 0.82 |
| Docosahexaenoic | C22:6n3 | 1.85 | 1.50 | 1.57 |
| Σ omega 3 | | 2.72 | 2.31 | 2.39 |
| Linolenic | C18:2n6c | 12.35 | 14.64 | 12.24 |
| γ- Linolenic | C18:3n6 | 0.73 | 0.66 | 0.72 |
| Eicosadienoic | C20:2n6 | 1.12 | 1.06 | 1.04 |
| Eicosadienoic | C20:3n6 | 0.66 | 0.63 | 0.64 |
| Σ omega 6 | | 14.86 | 16.99 | 14.64 |
| Palmitoleic | C16:1n7 | 4.75 | 4.70 | 4.67 |
| Σ omega 7 | | 4.75 | 4.70 | 4.67 |
| Elaidic | C18:1n9t | 0.46 | 0.28 | 0.64 |
| Oleic | C18:1n9c | 31.92 | 34.61 | 31.91 |
| Cis-11- Eicosenoic | C20:1n9 | 1.51 | 1.50 | 1.50 |
| Σ omega 9 | | 33.89 | 36.39 | 34.05 |
| Σ unsaturated | | 56.22 | 60.39 | 55.75 |

[1] % refers to the normalization of the total peak area (identified and unidentified).

Symbols use= FAS: Fish silage with formic acid, AAS: Fish silage with acetic acid, PAS: Fish silage with propionic acid.

Regarding the omega 3 fatty acids, the linolenic and docosahexaenoic acids were identified. The values of linolenic acid found in the present research are very close to those found by Santana-Delgado *et al*. (2008). For the docosahexaenoic acid, the values verified in the present research were superior to those found by Geron *et al*. (2007) and Arruda (2004), both working with acid silage from tilapia filleting residues (*Oreochromis niloticus*), who observed 0.1 and < 0.01% of docosahexaenoic acid, respectively. However, the values wrer far from those observed by Benites (2003), who when investigating the profile of acid silage fatty acid from Argentine croaker (*Umbrina canosai*) residue, obtained a percentage of 11.39% docosahexaenoic acid. The high percentage values in terms of area found for the unsaturated fatty acids, especially the poliunsaturated, indicate that the fish acid silage is a promising source of fish oil of high nutritional quality.

**Microbiological Analyses**

Aerobic mesophilic microorganisms nor molds and yeasts were detected during all of the sampling periods in the elaborated silages. This fact can be attributed to the low pH and the antimicrobiological effect of the organic acids used in the elaboration of the silagens. These results corroborate the works of Carmo, Pimenta, Pimenta, Oliveira, Logato & Ferreira (2008), who did not detect the presence of molds and yeasts in silages of tilapia filleting residue after 20 days of ensilage for the acetic, propionic and formic acid silages. Under the conditions in which the experiment was conducted, it could be noticed that the storage time interfered in the analyzed nutritional variables. In spite of small differences, all the tested acids were efficient in the maintenance of the microbiological and nutritional quality of the silages for 28 days. The choice of the acid type to be used in the elaboration of the silages will depend on the cost and availability of those reagents.

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