Revista Brasileira de Higiene e Sanidade Animal
Print version ISSN 1981 – 2965
Revista Brasileira de Higiene e Sanidade Animal, v. 07, n. 2, p. 16-30, jul-dez, 2013
http://dx.doi.org/10.5935/1981-2965.20130008

Artigo Cientifico

Biotecnologia

Evaluation nutritional of acid silage of Nile tilapia (*Oreochromis niloticus*), whole fish discarded, farmed in Indaiatuba – SP [1]

Ronaldo de Oliveira Sales *[2], Admar Costa de Oliveira [3]

ABSTRACT: The continental aquaculture with a production of 210,644,500 thousand tonnes produces a volume of solid waste causing a serious environmental problem, where 20% of the fish caught gets lost for lack of storage. The ideal would be to use raw materials and to recover byproducts as ingredients for animal feed. Aiming to increase the income and the capacity of production and, thus, to minimize environmental and health problems, deriving from fish waste, it was prepared the acid silage of the whole fish discarded from Nile tilapia (Oreocrhromis niloticus) after acidification and homogenisation of the biomass with 3% of formic acid and maintaining the pH around 3.7. Analyses were conducted to determine the humidity, protein, lipids and ash. Amino acids were examined in an auto-analyzer after acid hydrolysis, except for tryptophan determined by colorimetry. The new silage of Nile tilapia showed values higher than FAO standards for all essential amino acids, except for tryptophan. The highest values were found for glutamic acid, aspartic acid, lysine, leucine and glycine. The results indicate the potential use of the acid silage, prepared from the whole fish discarded from Nile tilapia, as a protein source in the formulation of animal feed, turning it into an excellent alternative for adding value to biowaste deriving from solid fish waste.

Key words: proximate composition, amino acid profile, acid silage, whole fish discarded

Avaliação nutricional da silagem ácida de tilápia do Nilo (*Oreochromis niloticus*), peixe inteiro descartado, cultivadas em Indaiatuba – SP [1]

RESUMO: A aqüicultura continental com uma produção de 210.644,5 mil toneladas produz um volume de resíduos sólidos causando um sério problema ambiental, onde 20% do pescado capturado chega a ser perdido por falta de armazenamento. O ideal seria o aproveitamento da matéria prima e recuperação dos subprodutos, como ingredientes para ração animal. Com os objetivos de aumentar a receita e a eficiência de produção e, consequentemente, minimizar os problemas ambientais e de sanidade, provenientes dos resíduos de pescado, procedeu-se à elaboração da silagem ácida do pescado inteiro descartado de tilápia-do-Nilo (Oreocrhromis niloticus) após homogeneização e acidificação da biomassa com 3% de ácido fórmico e manutenção do pH ao redor de 3,7. Foram realizadas análises para determinação da umidade, proteína, lipídios e cinza. Os aminoácidos foram examinados em auto-analisador após hidrólise ácida, à exceção do triptofano determinado por colorimetria. A silagem nova de tilápia-do-Nilo apresentou valores superiores ao padrão da FAO para todos os aminoácidos essenciais, com exceção do triptofano. Os valores mais elevados encontrados foram para o ácido glutâmico, aspártico, lisina leucina e glicina. Os resultados indicam a utilização potencial da silagem ácida, preparada a partir do pescado inteiro descartado de tilápia-do-Nilo, como fonte protéica na formulação de ração para animais domésticos, tornando-a excelente alternativa, por agregar valor ao biolixo proveniente dos resíduos sólidos do pescado.

Palavras claves: composição centesimal, perfil de aminoácidos, silagem ácida, despesca de pescado

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Part of the Doctoral Thesis of the first author.

[2] Federal University of Ceará - DZ / CCA / UFC. E-mail: ronaldo.sales@ufc.br

[3] State University of Campinas/UNICAMP

* To whom correspondence should be sent- E-mail: ronaldo.sales@ufc.br

Introduction

Environmental problems caused by large amounts of fish waste can be minimized by transforming such wastes into a product that can be incorporated as an ingredient for animal feed (ARRUDA et al., 2006). An alternative with great potential is the use of catch losses and wastes from fish processing (which can reach 60% of what is produced and / or captured) for the preparation of fish silage, and noble product with high biological value, making it a great alternative for adding value to fish waste (OLIVEIRA et al., 2012).

The nutritional value of fish silage is in the high protein digestibility due to the fact that protein is already well hydrolyzed and of the presence of lysine and tryptophan in high concentrations as well as of other essential amino acids. After bioconversion, the product is a high quality autolysed source of protein which can be used in animal feed and in the development of new foods, thus becoming a source of free amino acids and peptides of high quality, hardly obtained by other technological processes (BORGHESE et al., 2008). It may also be useful as organic fertilizer for the wealth of nutrients resulting from the presence of scales, fur, bones, collagen, blood, fat, swim bladder, gonads, eyes, brains, livers, digestive enzymes and carotenoids (ESPINDOLA FILHO, 1999; BEERLI et al., 2004).

The aim of this study was to evaluate the mineral proximate composition, the profile of amino acid of acid silage of Nile tilapia (*Oreochromis niloticus*), grown in Indaiatuba – S.P., as alternative ingredient in feed rations.

Materials and Methods

The raw material for obtaining the fish chemical silage, consisted of five samples of whole fish discarded of Nile tilapia (*Oreochromis niloticus*) totaling 5 kg in each sample, from the commercial cultivation in the region of Indaiatuba, S.P., fragmented in crusher, electrical equipment model ML-4, 0/Weg-uline. Then, it was homogenized, weighed and evenly distributed in hard plastic containers by adding formic acid in the ratio of 3% (w/w) of the volume of acid solution to the mass of

the residue (BERAQUET & GALACHO, 1983). The agitation of the material was often held to spread the enzymes and thus accelerate the rate of liquefaction (MACH DIEP & NORTVEDT, 2009) with daily pH control obtaining the acid silage of tilapia. Then the material was subjected to a drying process at room temperature for 15 to 20 days to obtain the dried silage with storage for 90 days at 27°C ± 3 °C and then it was subjected to the analysis of proximal chemical composition, minerals and amino acid profile as shown in Figure 1.

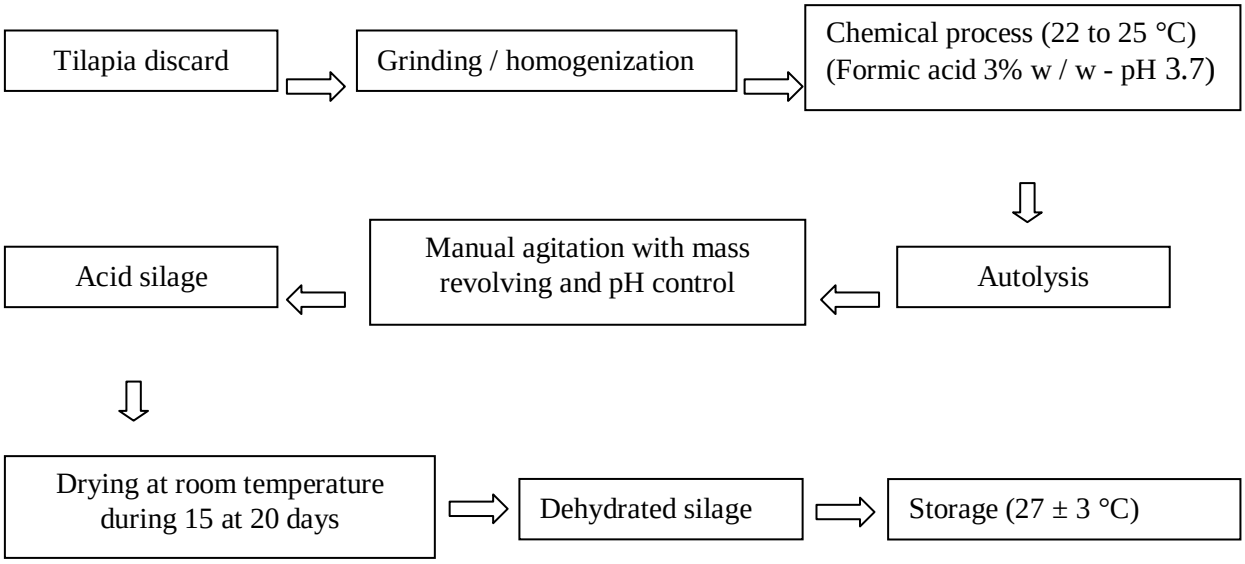

Figure 1. Flowchart processing for preparation of Nile tilapia acid silage

Analyses were conducted to determine the composition of the chemical silage of tilapia. All the tests were performed in triplicate and according to the (AOAC, 2000). Moisture was determined by gravimetric method, in a greenhouse at 105 °C until constant weight. The protein was calculated by the micro-Kjeldahl method with digestion of organic material in a digester block followed by distillation and titration with 0.01M sulfuric acid. The lipid fraction was obtained by Soxhlet extractor. It was used petroleum ether as solvent, followed by heating in an rotative evaporator until total removing of the solvent. The ash content was measured gravimetrically treating the sample in a muffle furnace at 550 °C for incineration of organic matter, and the levels of minerals calciumm, magnesium, iron and

phosforous were determined by atomic absorption, spectrophotometry. For quantification of amino acids, samples were hydrolyzed with 6N HCL for 22 hours at 110 ^{o}C (MOORE & STEIN, 1963). Samples were analyzed by ionic exchange chromatography in accordance with SPACKMANN et al. (1958), using an automatic analyzer. Tryptophan was determined after enzymatic hydrolysis with Pronase at 40 °C for 24 hours, followed by colorimetric reaction with 4 - dimethyl - amino-benzaldehyde (ABD) in 21.2 N sulfuric acid reading at 590 nm and is calculation from a standard curve, according to SPIES (1967). The chemical score was determined according to SGARBIERI (1987), through the relationship between the content of each essential amino acid content with the corresponding amino acid of a standard protein NATIONAL ACEDEMY OF SCIENCE (1980). The data were analyzed according to completely randomized design (BARBOSA & MALDONADO, 2010). For the analysis of variance and means comparison, Tukey test was used at 5% probability.

Results and Discussion

There was no significant difference (P>0.05) in proximate composition between the analyzed batches of Nile tilapia used for preparation of acid silage of fish wastes, with average value 76.62%, 17.07%, 3.57% and 2.33% for moisture, protein, fat and ash, respectively (Table 1).

These data are consistent with those reported by ESPINDOLA FILHO (1999) who, analyzing the composition of cruzamento da *Tilapia nilotica* (fêmea) com *Tilapia hornorum* (macho), found out that the moisture was presented into the range of 74.32% to 75.63%, protein from 17.52 to 17.62% ash, 1.73 to 2.32% and lipids 3.75 to 7.48%.

The largest coefficient of variation (CV%) occurred in the levels of ash with 26.29%, and then to a lesser extent by lipids, with 9.20% protein, with 2.24% and finally moisture,with 1.30%. Similar results were obtained by SALES (1995) that working with different species of Nile tilapia, observed no difference (P> 0.05) among the samples analyzed, with more pronounced variations in ash content (0.7 to 4.2 %), being the lowest in

moisture and protein, in which almost all species have similar values and the tilapia can be classified as lean fish (3.25 to 6.26%) of high protein content.

Table 1. Proximate composition of Nile tilapia (*Oreochromis niloticus*), (whole fish with viscera, skin and scales) from Indaiatuba, Sao Paulo, during the discard period.

Sample	Moisture %	Protein %	Lipids %	Ashes %
1	76.64 [a] ± 1.50	17.07 [a] ± 0.53	3.56 [a] ± 0.00	2.33 [a] ± 1.09
2	76.63 [a] ± 0.71	17.08 [a] ± 0.45	3.59 [a] ± 0.14	2.34 [a] ± 0.50
3	76.62 [a] ± 0.48	17.07 [a] ± 0.31	3.58 [a] ± 0.05	2.35 [a] ± 0.19
4	76.61 [a] ± 1.01	17.08 [a] ± 0.09	3.55 [a] ± 0.64	2.33 [a] ± 0.19
F	0.00[NS]	0.00[NS]	0.01[NS]	0.00[NS]
CV (%)	1.30	2.24	9.20	26.29
SMD	2.6162	1.0022	0.8590	1.6067
Averarge	76.62	17.07	3.57	2.33

Mean values and standard deviations of three determinations. Mean values in the same column marked with the same letters are not different by Tukey test at 5% probability.

* SMD Significant minimum difference.

According to ESPE & LIED (1999), the composition of Nile tilapia varies from one species to another and even between the same species, depending on season, type of food, the degree of maturation and sex. It may also vary in the same fish, depending on the analyzed part.

The four batches of the acid silage of Nile tilapia (*Oreochomis niloticus*), (whole fish with visceras, skin and scales) from Indaiatuba, Sao Paulo, from the *harvest* showed no difference (P> 0.05) among the samples analyzed (Table 2). The greatest change occurred in the lipid content with a coefficient of variation (CV) of 14.19%, followed, in lesser degree, by the ash content 1.91%, protein with 0.79% and finally moisture with 0.42%. Similar results were reported by ABIMORAD et al.,

(2009), which working with acid silage of tilapia waste, found in the proximate composition the same of the raw material that had originated it, demonstrating the homogeneity of the final product, which is proven by other authors (CARVALHO et al., 2006).

Table 2. Proximate composition of the full acid silage of Nile tilapia (*Oreochomis niloticus*), (whole fish with viscera, skin and scales) from Indaiatuba, São Paulo, during the discard period.

Sample	Moisture %	Protein %	Lipids %	Ashes %
1	77.24 [a] ± 0.11	16.30 [a] ± 0.07	3.28 [a] ± 0.50	3.19 [a] ± 0.01
2	77.25 [a] ± 0.24	16.31 [a] ± 0.21	3.27 [a] ± 0.36	3.18 [a] ± 0.01
3	77.26 [a] ± 0.29	16.32 [a] ± 0.05	3.26 [a] ± 0.05	3.17 [a] ± 0.08
4	77.27 [a] ± 0.52	16.31 [a] ± 0.12	3.25 [a] ± 0.69	3.16 [a] ± 0.09
F	0.00[NS]	0.01[NS]	0.00[NS]	0.14[NS]
CV (%)	0.42	0.79	14.19	1.91
SMD	0.8515	0.3356	1.2111	0.1585
Averarge	77.25	16.31	3.26	3.17

Mean values and standard deviations of three determinations. Mean values in the same column marked with the same letters are not different by Tukey test at 5% probability.

* SMD Significant minimum difference.

Thus, the solution of 3% formic acid (2%) in relation to ground fish fish (98%), as usually recommended in the literature (ARRUDA et al., 2006), was judged satisfactory, since it does not change the proximate composition data, being kept constant throughout the storage period of 90 days at a temperature of 27 ± 3 °C. ABIMORAD et al., (2009) working with fish waste, recommends a mixture of formic acid and propionic acid in a 1:1 ratio and addition of 5 to 3.0% (v/w) on the mass in order to obtain silage which is stable and free of pathogenic microorganisms. BORGHESE et al. (2008) evaluating the chemical composition and nutritional quality of acid silage (AS), biological silage (BS) and

enzymatic silage (ES), elaborated in the discard of Nile tilapia (*Oreochromis niloticus*), found values (dry matter basis) of: 54.25, 53.00 and 54.50 g/100 g for crude protein, 12.45; 12.25 and 12.17 g/100g for lipid, 8.03, 7.33 and 8.58 g/100 g for calcium and 4.71, 2.86 and 4.85 g/100 g for phosphorus, respectively, for AS, BS and ES.

It was observed in Table 3 that lipid content decreased from 3.26% to 0.48% after the process of oil removal by decanting, which lasted 15 to 20 days during storage. According to some authors OLIVEIRA et al. (2012), the lipids are immediate sources of energy and essential fatty acids for most fish species, and are present in large amounts in planktonic organisms. ABIMORAD et al., (2009) correlated the highest concentration of lipid body with the food type, degree of maturity and sex.

Table 3. Proximate composition of defatted acid silage of Nile tilapia (*Oreochromis niloticus*), (Whole fish with viscera, skin and scales) from Indaiatuba, SP, during the discard period.

Sample	Moisture %	Protein %	Lipids %	Ashes %
1	77.64 ^{a} ± 0.11	17.25 ^{a} ± 0.07	0.48 ^{a} ± 0.20	3.63 ^{a} ± 0.01
2	77.69 ^{a} ± 0.24	17.24 ^{a} ± 0.21	0.49 ^{a} ± 0.19	3.64 ^{a} ± 0.01
3	77.66 ^{a} ± 0.29	17.25 ^{a} ± 0.05	0.48 ^{a} ± 0.17	3.63 ^{a} ± 0.08
4	77.43 ^{a} ± 0.52	17.21 ^{a} ± 0.12	0.48 ^{a} ± 0.17	3.62 ^{a} ± 0.09
F	0.40^{NS}	0.07^{NS}	0.00^{NS}	0.05^{NS}
CV (%)	0.42	0.74	37.92	1.67
SMD	0.8515	0.3356	0.4784	0.1585
Averarge	77.66	17.23	0.48	3.63

Mean values and standard deviations of three determinations. Mean values in the same column marked with the same letters are not different by Tukey test at 5% probability.

* SMD Significant minimum difference

Table 4 illustrates the composition results in Ca, P, Mg and Fe in the acid silage of Nile tilapia. It is observed that there was no difference (P> 0.05) between the four samples, with a mean of 1.42 mg/100g, 0.97 mg/100 g, 0.61 mg/100g and 0.07 mg/100g respectively, calcium, phosphorus, magnesium and iron. The greatest variation occurred in iron, with a coefficient of variation (CV) of 24.74%, followed by the content of magnesium, with 1.63%, phosphorus, with a 1.03% and finally to a lesser extent by calcium, with 0.70%. Similar results were obtained by BUENO (2006), who studying the acid fish silage reports that calcium, ranges from 0.7 to 1.65 g mg/l00 silage, phosphorus, values between 0.4 to 2.0 mg/100 g of silage, magnesium, 0.5 to 1.05 mg/100 iron, 0.05 to 0.09 mg / kg of silage, no difference is shown (P> 0.05) among the samples.

Table 4. Composition in Ca, P, Mg and Fe in of acidic silage of Nile tilapia (*Oreochromis niloticus*) (whole fish with viscera, skin and scales) from Indaiatuba, Sao Paulo, during the discard period.

Sample	Calcium mg/100g	Phosphorus mg/100g	Magnesium mg/100g	Iron mg/100g
1	1.41 ^{a} ± 0.01	0.96 ^{a} ± 0.01	0.60 ^{a} ± 0.01	0.08 ^{a} ± 5.77
2	1.42 ^{a} ± 0.01	0.97 ^{a} ± 0.01	0.61 ^{a} ± 0.01	0.05 ^{a} ± 0.01
3	1.42 ^{a} ± 0.01	0.98 ^{a} ± 0.01	0.62 ^{a} ± 0.01	0.07 ^{a} ± 0.01
4	1.43 a ± 0.01	0.97 ^{a} ± 0.01	0.62 ^{a} ± 0.01	0.08 ^{a} ± 0.01
F	2.00^{NS}	2.00^{NS}	2.75^{NS}	2.00^{NS}
CV (%)	0.70	1.03	1.63	24.74
SMD	0.0261	0.0261	0.0261	0.0453
Averarge	1.42	0.97	0.61	0.07

Mean values and standard deviations of three determinations. Mean values in the same column marked with the same letters are not different by Tukey test at 5% probability.

* SMD Significant minimum difference.

In Table 5 we can compare the profile in essential amino acids (g/16 g N) of the new and old silages, soybean meal, casein and corn, as well as the values of chemical score to obtain the quality of those protein sources, obtained for the theoretical reference (NATIONAL ACADEMY OF SCIENCES, 1980). It was noticed, by comparing the acid silage of tilapia new and old (with 30 and 90 days of storage) with soybean meal, casein and corn that the latter has low amounts for each amino acid particularly in relation to sulfur as methionine. There are also very low levels of aspartic and glutamic acid. They are very similar to fish meal, when they are made from the same raw material, however, it must be remembered that such products are not obtained from the same raw material, which demonstrates that fish silages provide an optimal response in terms of amino acid profiles, growth rate and feed efficiency (SALES, 1995).

The chemical score (CS) indicated the relationship between the reference protein or default, the order of amino acids in the protein being studied, and the value found for the most limiting amino acid an estimate of biological or nutritive value of protein in the study (SGARBIERI, 1987). The standard protein was defined by the NATIONAL ACADEMY OF SCINCES (1980) as having the following concentrations for EAAs (g/16 g N) Isoleucine 4.2, Leucine 7.0, Lysina 5.1, Total súlfur 2.6, Total Aromatics 7.3, Treonine 3.5, Valine 4.8, Tryptophan 1.1, Histidine 1.7 e Aspartic Acid 7.8 (Table 5).

ESPE & LIED (1999) reported that the handmade silage has high amounts of essential amino acids, such as lysine (5.54 g / kg), histidine (5.33 g / kg) and glutamic acid (6.04 g / kg), making it an excellent alternative for adding value to biowate from fish solid waste.

It was observed that the decomposition of amino acids was higher in silage stored for 90 days than in the samples taken during the first 30 days, avoiding loss of aminoacids, during which it was found a smaller number of decomposed products, particularly essential amino acid as leucine, isoleucine, lysine and tryptophan, affecting significantly the

weight gain of animals in relation to silage stored for long periods of time (SALES, 1995).

Table 5. Profile of amino acids (g/16 g N) and chemical score of protein sources used for the preparation of diets, whose determination was made by ion exchange.

Amino acid	New Silage	Old silage	Soybean meal	Casein	Corn	Standard [1]
Isoleucine	5.64	3.80	1.81	5.4	2.5	4.2
Leucine	9.27	6.00	3.69	10.2	10.3	7.0
Lysine	9.90	6.80	2.65	7.8	2.2	5.1
Methionine	3.05	2.70	0.64	2.8	1.9	-
½ Cystine	1.22	0.98	-	0.2	-	-
Total súlfur	4.27	3.90	0.64	3.0	1.9	2.6
Tyrosine	3.20	3.12	-	6.1	-	-
Phenylalanine	4.36	4.10	2.11	5.6	3.6	-
Total Aromatics	7.56	7.22	2.11	11.7	3.6	7.3
Threonine	4.35	3.90	1.80	4.9	2.9	3.5
Valine	5.25	5.12	2.05	6.9	3.9	4.8
Serina	3.80	3.60	2.42	6.8	3.7	-
Alanine	8.10	4.23	2.12	3.2	6.3	-
Tryptophan	1.06	0.60	-	-	-	1.1
Histidine	2.20	2.10	-	2.9	-	1.7
Arginine	8.35	7.10	3.17	4.1	3.2	-
Glutamic Acid	18.27	12.30	8.82	27.4	17.2	-
Aspartic Acid	12.30	7.00	5.13	8.6	5.4	7.8
Glycine	8.12	6.05	1.98	1.9	3.0	-
Proline	4.25	3.90	2.33	10.0	7.2	
Chemical Score	96.30	54.5	24.6	110.25	43.1	

[1] Theoretial Standard (NATIONAL ACADEMY OF SCIENCES, 1980).

Covalent bonds between proteins and oxidized products can destroy the amino acids such as tryptophan, oxidize methionine and bind lysine to other compounds making them unavailable amino acids (NELSON & COX, 2000). Probable explanation for this

reduction of nutritional value may be the fact that free amino acids are quickly diverted from protein synthesis and enter the catabolic pathway, getting more available to be used as an energy source than the intact proteins (VIDOTTO, 2001; VIDOTTO et al., 2003). Free amino acids in the presence of hydroxyl from the sugar present Maillard reaction resulting in decreased nutritional value of silage (FAGBENRO & JAUNCEY, 1998).

Conclusion

The results demonstrate the possibility of the use of the acid silage prepared from the whole fish discard of Nile tilapia (*Oreochromis niloticus*) as a protein ingredient in balanced rations for animal feed, as a partial substitute for fishmeal, and that silage shoulder be consumed until 30 first days of preparation to avoid amino acids decomposition.

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Acesso em: 28 abr. 2012. doi: 10.1016/S0377-8401(03)00056-7.