

FATTY ACID COMPOSITION OF ACID, BIOLOGICAL AND ENZYMATIC FISH SILAGE

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The use of the fish silage as an ingredient in feed for aquatic organisms is an alternative to solve sanitary and environmental problems caused by the lack of an adequate destination for the residues generated by the fishing industry. It would also lower the costs with feed, and consequently the fish production costs, since the expenses with the feed account for approximately 60% of the total cost. The objective of this study was to evaluate the fatty acid composition of the acid silage (AS), biological silage (BS) and enzymatic silage (ES) produced from discardings of the culture and from processing residues of the Nile tilapia (*Oreochromis niloticus*). The values found for lipids (dry matter basis) were: 12.45; 12.25 and 12.17 g 100 g⁻¹ for BS, AS, and ES, respectively. The fatty acids present in the lipid fraction of the silages are predominantly unsaturated. Oleic acid was present in larger amounts (30.49, 28.60 and 30.60 g 100 g⁻¹ of lipids for BS, AS and ES, respectively). Among saturated fatty acids, palmitic and stearic acids were present in larger amounts. Only traces of eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids were found. The silages produced from discardings of the culture and processing residues of the Nile tilapia are not a good source of EPA and DHA for fish feeds.

KEY-WORDS: FISHING INDUSTRY - WASTE; NILE TILAPIA; SILAGE; OIL.

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1 INTRODUCTION

Mainly in tropical countries, large quantities of fish are wasted during capture, commercialization, and industrialization processes (VIDOTTI, VIEGAS e CARNEIRO, 2003). Due to a lack of adequate use, fisheries wastes and rejects may accumulated and cause environmental, health, and economical problems; but when processed and turned in by-products, they can become alternative, potentially good feedstuff for aquafeeds. That has been considered to be the case of fish silage, which according to BEERLI, BEERLI & LOGATO (2004), CARVALHO et al. (2006), VIDOTTI, CARNEIRO e VIEGAS (2002), and VIDOTTI, VIEGAS & CARNEIRO (2003), is a product of high biological value.

Fish silage is a liquid or semi-liquid product resulting from the preservation (ensilage) of whole fish or parts by the addition of acids (acid silage) or carbohydrate-induced microbial fermentation (biological silage). In the ensilage process, proteins are hydrolyzed by naturally present and (or) added enzymes (enzymatic silage); the process is favored by the adjustment of pH. The final result is a paste-like product, rich in protein, short-chain peptides and free amino acids (GODDARD & PERRET, 2005; SHIRAI et al., 2001; STONE & HARDY, 1986).

The composition of silage is very similar to the raw material, though its nutritional value may be negatively affected with the increase in the storage time due to the formation of a large quantity of free amino acids and lipid oxidation (ESPE & LIED, 1999; KOMPIANG, 1981; VIANA, GUSMAN & ESCOBAR, 1999).

Several authors have demonstrated the nutritional and economical feasibility of using fish silage in diets of several fish species, not to mention environmental benefits of this practice (FAGBENRO & JAUNCEY, 1995; GODDARD & PERRET, 2005; VIDOTTI, CARNEIRO & VIEGAS, 2002).

The objective of this work was to evaluate the fatty acid composition of acid, biological and enzymatic silages of Nile tilapia (*Oreochromis niloticus*).

2 MATERIAL AND METHODS

2.1 RAW MATERIAL

The raw material was 20% of Nile tilapia processing (filleting) wastes – heads, viscera, scales, fins, vertebral column, skin, adhered tissues – plus 80% whole Nile tilapias (trash fish), grinded in an electrical mincer (ML-4.0 WEG-µline) to yield 60 kg of a homogenous mass.

2.2 INOCULUM SOLUTION

Lactobacillus plantarum, a lactic acid producer, was used as inoculum. Cell multiplication was carried out from an aliquot of 0.1 mL of the previously activated culture, inoculated in a test tube with 5 mL of Mann Rugosa Sharp (MRS) Broth Lactobacilli produced by DIFCO, and incubated at 35°C for 24 hours. After 24 hours, 5 mL aliquots of the test tubes were inoculated in Erlenmeyers flasks containing 500 mL of the MRS medium. After that they were incubated at 35°C for 48 hours in order to obtain concentrations of at least 10^8 cells mL⁻¹ (inoculum medium).

The material containing microorganisms was centrifuged in a BHG- Hermle/Z320 (4000 x G), at 25°C, for 15 min to separate the cell mass. The centrifuged products were suspended in a saline solution, 0.85% up to 40 mL (inoculum solution) (MORALLES-ULLOA, 1994).

2.3 SILAGE PRODUCTION

The homogenous mass obtained was split into six plastic containers (10 kg/container) and added of 200 ppm of butyl-hydroxy-toluene (BHT) dissolved in ethylic alcohol plus 3% of a 1:1 mixture

(volume/weight) of 88% formic acid and 100% propionic acid. After homogenous acidification (hand mixing) of the mass, the containers were covered with plastic lids and set to rest at room temperature.

Acid silage became a pre-treatment for other silages. Two containers were reserved as acid silage (AS), and incubated for four weeks; the others were used to produce the biological and enzymatic silages.

Biological silage was prepared from the acid silage three days after incubation, when the supernatant lipid layer was removed. To obtain the biological silage (BS), 14,000 ppm of inoculum solution of *Lactobacillus plantarum* plus 18% of sugarcane molasses (volume/weight) were added and homogenized to acid silage containers, which were covered with plastic lids and kept at room temperature for 3 weeks.

Enzymatic silage (ES) was obtained by the addition of 10 g of protease type II from *Aspergillus oryzae* (0.13 unit mg⁻¹ solids; Sigma Chemical Corporation) diluted in 100 mL of distilled water to AS containers, also three days after incubation. After hand homogenization, the containers were covered with plastic lids and kept at room temperature for one week.

2.4 CRUDE LIPID EXTRACTION AND PREPARATION OF METHYL ESTERS

The analyses of the silages were carried out on a dry matter basis, according to the AOAC (1984) under the standardization of the *Compêndio Brasileiro de Nutrição Animal* (BRASIL, 1998). The crude lipid was determined after extraction with chloroform-methanol (FOLCH, LEES & SLOANE-STANLEY 1957).

Methyl esters were obtained according methodology described by HARTMAN & LAGO (1973). Approximately 200 mg of sample fat material (crude lipid) was added to a 50 mL volumetric flask. Five milliliters of 0.5N methanoic sodium hydroxide was added to the sample which was heated on a 65-70°C water bath for 15 minutes. Ten milliliters of ester reagent (solution of ammonium chloride and sulphuric acid in methanol) was added to the heated sample. This mixture was boiled for ten minutes. At room temperature, two milliliters of petroleum ether was added and the volume was completed with a saturated sodium chloride solution. The solvent was then evaporated on a 60°C water bath. The esters are now ready for high resolution gas chromatography (HRGC) analysis.

2.5 FATTY ACIDS ANALYSIS

Fatty acids were analyzed, after esterification, by HRGC on a Konic/HRCG 4000A, equipped with a flame ionization detection and fitted with a fused silica capillary column (CP Sil 88 Tailor Made FAME-Chrompak). The gas chromatography analyses were carried out according to AOCS (1998). Injector and detector temperature were programmed to 300°C. Column temperature was programmed to 205°C. Hydrogen was used as a carrier gas with a flow rate of 0.5 mL min⁻¹. The reference standard used was the 1-decanoic methyl ester. The fatty acids identification was obtained by the comparison of the retention time and the fatty acids pick areas with the reference standard.

3 RESULTS AND DISCUSSION

The decrease in the lipid contents of the silages in comparison to the raw material was due to the removal of the supernatant lipid portion three days after the development of the silages. Some authors mention that this fraction interferes in the quality of the silage, for the fatty acids that constitute the lipid fraction of fish are predominantly unsaturated and can easily oxidize resulting in changes in flavor, color, texture, nutritional value, besides being considered potential producers of toxic components. The oxidation process can be sped up if the fish silage is in contact with the light and the air (RAA & GILBERG, 1982; SALES, 1995).

According to NELSON & COX (2000), lipid oxidation can cause the formation of peroxides which can make proteins more complex through physical and covalent bonds. These covalent bonds among oxidized products and proteins can destroy amino acids such as tryptophan, oxidize methionine and bond lysine to other compounds making these amino acids unavailable (SALES, 1995). Therefore, due to all these factors there are authors (ARRUDA, 2004; DISNEY, TATTERSON & OLLEY, 1977; KOMPIANG, 1981; RAA & GILBERG, 1982; TATTERSON & WINDSOR, 1974) who defend the removal of the lipid fraction during the elaboration of silage for the production of an uniform and more stable product.

The high contents of lipids found in the silages produced in this research are due to the presence of viscera and body cavity fat in the raw material used in their elaboration.

The results obtained for the lipid fraction by FAGBENRO (1996) and FAGBENRO & JAUNCEY (1995) for biological silage was 10.86 and 10.63 g 100 g⁻¹, respectively. RISTIC, FILIPOVIC & SAKAC (2002), DISNEY, TATTERSON & OLLEY (1977) and SALES (1995) analyzed acid silages and found values of 14.84; 12.20 and 14.29 g 100 g⁻¹, respectively. DAPKEVICIUS et al. (1998) studied acid and biological silages and verified lipid values of 14.90 and 10.30 g 100 g⁻¹, respectively. All these results are close to the ones found in this research.

The final composition of silage varies considerably with the type of raw material employed, particularly as to the contents of lipids, which varies according to the harvest season, species and type of waste used (viscera) and gender (BROWN & SUMNER, 1985; DISNEY, TATTERSON & OLLEY, 1977; HAARD et al., 1985).

To avoid off-flavor problems, when the silage is offered for swine or poultry, the fish oil cannot be over 1% of the total lipid fraction of the ration (RAA & GILBERG, 1982).

In general, the proximate composition is extremely variable from one species of fish to the other and within the same species depending on the time of the year, type of diet and stage of gonadal maturation and, in the same fish, it will vary depending on the part analyzed (SALES, 1995). As the composition of the silage is very similar to the composition of the raw material, the nutritional value of silage will also vary according to the parameters mentioned above.

As observed in Table 1, fatty acids present in the lipid fraction of the silages are predominantly unsaturated. Oleic acid was present in larger amounts. Among saturated fatty acids, palmitic and stearic acids were present in larger amounts. These results are in accordance with those presented by MAIA et al. (1998), SALES (1995), VALÉRIO (1994) and GERON et al. (2007).

The fatty acids composition of fish silage is highly variable, depending directly on the raw material used in its production. ESPÍNDOLA FILHO (1999) evaluated acid silage produced from different species of fish and found larger amounts of saturated than unsaturated fatty acids, which was associated to the presence of pink shrimp (*Farfantopenaeus brasiliensis*) and sea-bob shrimp (*Xiphopenaeus kroyeri*) as components of the raw material used in the elaboration of acid silage.

In this research, only traces of Eicosapentaenoic (EPA) and Docosahexaenoic (DHA) fatty acids were found, diverging from results obtained by MAIA et al. (1998), who found values of 2.3 and 9.7 g 100 g⁻¹ of lipids, respectively, in the oil of acid silages made from tilapia waste. Also in the oil of acid and biological silage, GERON et al. (2007) found DHA values of 0.1 and 0.5 g 100 g⁻¹ of lipids, respectively. On the other hand, the results found in this research agree with those reported to acid fish silage made from tilapia's waste (ARRUDA, 2004).

The fatty acids composition of tilapia's waste (heads) also was evaluated by VISENTAINER et al. (2000). In this material, the authors found linolenic acid (4.94 mg g⁻¹ of lipids) and DHA (3.93 mg g⁻¹ of lipids). VISENTAINER et al. (2003), evaluating tilapia's heads fatty acids compositions also found linolenic acids (4.88 g 100 g⁻¹ of lipids) and DHA (0.29 g 100 g⁻¹ of lipids) but, as in the present search, the authors didn't found EPA fatty acid.

MOREIRA et al. (2003) evaluating the fatty acids composition of matrinxã's (*Brycon cephalus*) heads found values of 0.10 and 0.53 g 100 g⁻¹ of EPA and DHA, respectively.

The results obtained by MAIA et al. (1998) and VISENTAINER et al. (2000) can be explained

by the large amount of tissues that are part of the nervous system (brain, eyes) in the raw material, which according to TOCHER, MOURENTE & SARGENT (1997), are rich in EPA and DHA.

TABLE 1 - FATTY ACIDS IN ACID, BIOLOGICAL, AND ENZYMATIC SILAGES

Fatty acids		Biological Silage	Acid Silage	Enzymatic Silage
		-----g 100 g ⁻¹ of lipids-----		
Dodecanoic	C12	<0.01 ¹	0.11	0.23
Myristic	C14	3.80	4.74	5.36
Pentadecanoic	C15	0.70	0.81	0.60
Palmitic	C16	28.50	33.19	35.19
Palmitoleic	C16:1	8.60	9.94	10.70
Margaric	C17	0.90	0.56	1.00
Stearic	C18	6.08	5.60	5.70
Oleic	C18:1	30.49	28.60	30.60
Linoleic	C18:2	17.38	16.30	14.10
Linolenic	C18:3	3.10	3.10	3.20
Octadecatetraenoic	C18:4	1.52	1.50	1.20
Arachidic	C20	0.80	1.30	1.20
cis-11-Eicosenoic	C20:1	2.79	1.60	1.20
Eicosapentaenoic	C20:5	<0.01	<0.01	<0.01
Docosahexaenoic	C22:6	<0.01	<0.01	<0.01
Crude lipids ²		12.25 ± 0.45	12.45 ± 0.45	12.17 ± 0.18

¹ The values are averages of two replicates.

² The values are averages of three replicates ± standard deviation.

Evaluating the fatty acids composition of acid silage produced from different species of marine fish, considered an important source of EPA and DHA fatty acids, ESPÍNDOLA FILHO (1999) found values of 1.1 and 1.1 g 100 g⁻¹ of lipids of EPA and DHA, respectively.

High contents of EPA (between 7.01 and 7.92 g 100 g⁻¹ of lipids) and DHA (between 17.99 and 18.21 g 100 g⁻¹ of lipids) were found in fish silages by VALÉRIO (1994) and MORALLES-ULLOA (1994). It can be explained, because both authors used sardine (*Sardinella brasiliensis*), a fish specie rich in EPA and DHA fatty acids, as raw material.

Unsaturated fatty acids are greatly accountable for oxidative alterations in foods, producing undesirable effects such as rancidity, color fading and loss of nutrients. For this reason, the adequate use of an antioxidant is important (SALES, 1995).

According to SARGENT et al. (1999), eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and, probably, arachidonic acid (AA, 20:4n-6) are considered essential in diets for marine aquatic organisms for normal growth and survival, but are not essential for tropical freshwater fishes (SARGENT, TOCHER & BELL, 2002). This is primarily due to a capacity that most freshwater fish species have to convert the 18:3n-3 fatty acid to 20:5n-3 (EPA) e 22:6n-3 (DHA) fatty acids (MARTINO & PORTZ, 2006).

4 CONCLUSION

As the silages prepared in this research showed unexpressive values of these fatty acids, they can not be used in fish diets as a source of EPA, DHA, and arachidonic acid. However, the lipid fraction of the silages could be used as a feed stimulant or as a source of energy in freshwater fish feed.

Resumo

COMPOSIÇÃO EM ÁCIDOS GRAXOS DAS SILAGENS ÁCIDA, BIOLÓGICA E ENZIMÁTICA DE PESCADO

A utilização da silagem de pescado como ingrediente em rações para organismos aquáticos constitui alternativa para solucionar os problemas de ordem sanitária e ambiental, causados pela falta de destino adequado dos resíduos gerados pela indústria do pescado. Além disso, podem diminuir os custos com alimentação e, conseqüentemente, os custos de produção do pescado, já que os gastos com a alimentação correspondem a aproximadamente 60% do custo total. O objetivo deste estudo foi avaliar a composição em ácidos graxos da silagem ácida (SA), silagem biológica (SB) e silagem enzimática (SE), produzidas a partir de descartes e resíduos do processamento da tilápia do Nilo (*Oreochromis niloticus*). Os valores encontrados de lipídios (base na matéria seca) foram: 12,45; 12,25 e 12,17 g.100 g⁻¹ para SB, SA e SE, respectivamente. Os ácidos graxos predominantes na fração lipídica das silagens são os insaturados. O ácido oléico foi encontrado em maiores quantidades (30,49; 28,60 e 30,60 g 100 g⁻¹ de lipídios para SB, SA e SE, respectivamente). Entre os ácidos graxos saturados, o palmítico e o esteárico estavam presentes em maiores quantidades. Apenas traços dos ácidos graxos poliinsaturados eicosapentaenóico (EPA) e docosahexaenóico (DHA) foram encontrados. As silagens produzidas a partir de descartes da piscicultura e resíduos do processamento da tilápia do Nilo não são boas fontes de EPA e DHA em dietas para peixes.

PALAVRAS-CHAVE: INDÚSTRIA DA PESCA - RESÍDUOS; TILÁPIA DO NILO; SILAGEM.

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AKNOWLEDGEMENTS

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES.
Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP.