

PUFA content of silages prepared from tuna cannery wastes

S. Tamadoni Jahromi^{1*} ; A. Ghoroghi² ; K. Rouhani³ and
K. Ejlali⁴

Stamadoni @yahoo.com

1,3 & 4- Persian Gulf and Oman Sea Ecological Research Centre,
P.O.Box: 1597 Bandar Abbass, Iran

2- Iranian Fisheries Research Organization, P.O.Box: 14155-6116
Tehran, Iran

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Abstract: Ensiling as one of the best methods for utilization of tuna wastes was investigated. docosahexaenoic acid [DHA C22:6(n-3)]- rich products were obtained from the wastes (viscera and dark meat) of four tuna species, namely longtail, skipjack, yellowfin and kawakawa by a procedure involving ensiling in organic acids followed by neutralization. Identification of fatty acids in the samples was performed by comparison with chromatograms of fatty acids standard.

Total lipid content of silages varied from 10.41% in skipjack dark meat silage to 22.01% in kawakawa viscera silage, but all lipids contained high percentages of DHA and EPA [eicosapanteonic acid C20:5(n-3)]. The highest DHA ratio (15% of total lipids) was found in the lipid of skipjack viscera silage and the highest ratio of EPA (11% of total lipids) belonged to the lipids of kawakawa dark meat silage. ANOVA test results indicated that DHA ratio was significantly high before silage preparation and linolenic acid [C18:3(n-3)] content significantly differed between the species ($P < 0.05$).

Keywords: Silage, PUFA, Tuna wastes, DHA, EPA, Persian Gulf

* Corresponding author

Introduction

Artificial diets based on less expensive protein sources are becoming increasingly important as alternatives to live feeds in the aquaculture industry (Coutteau & Sorgeloos, 1992). Currently fish meal and fish oil are the main ingredients in finfish and marine shrimp feeds. Together they provide a good balance of high quality protein (amino acid composition) and lipids (containing long chain n-3 polyunsaturated fatty acids) in a highly dense form of digestible energy. Studies have shown that diets containing fish-based ingredients have generally performed better in terms of growth and feed efficiency than diets containing alternative plant-based sources (Moyano *et al.*, 1992). However, the aquaculture industry is now actively investigating alternative nutrient sources (Naylor *et al.*, 2000).

Ensiling is one of the methods chosen for processing the fish wastes. Silages prepared from fishery by-products are used in the formulation of diets for aquaculture and have been the subject of several investigations. (Hardy *et al.*, 1984; Jakson *et al.*, 1984; Heras *et al.*, 1994). Fish silage, preserved by adding a combination of organic or inorganic acids, an acceptable dietary ingredient for salmonid feed, has a nutritional value as good as that of the fresh raw material (Viena *et al.*, 1999). It can contain from 27 percent to 35 percent dry matter (Machin *et al.*, 1982). The lowered pH prevents bacterial putrefaction, which allows the silage to be stored for several months. Acids that can be used in this process are the organic acids propionic and formic (Rattagool *et al.*, 1980; Green *et al.*, 1983; Machin *et al.*, 1982), and certain mineral acids, either sulphuric or hydrochloric (Alvarez, 1972). The fish wastes silages have been prepared specifically for use in larval stage of marine fishes and other animal, which may have limited digestive capabilities. The fact that ensiling results in considerable hydrolysis of proteins to smaller peptides and amino acids may have specific benefits (Raa & Gildberg, 1976).

Marine lipids have well documented beneficial health effects. High amounts of long chain polyunsaturated fatty acids (PUFA) like eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6n-3) make marine lipids unique

compared to other lipid sources. The omega-3 fatty acids have been found to reduce the risk of cardiovascular diseases, hypertension, autoimmune and inflammatory diseases. It has also been found that fish oils in the diet may help protect against various cancers. Dietetic research has shown that most people do not have enough n-3 fatty acids in their diet (Garcia, 1998).

It is well known that dietary n-3 polyunsaturated fatty acids (PUFA) are essential for the optimal growth and development of fishes. Marine fish larvae have a requirement for large quantities of n-3 PUFA, particularly DHA and dietary deficiencies of these products induce a range of abnormalities in fish including behavioral (Henderson & Tocher, 1987).

The scientists confirm that fish silages are rich sources of protein and lipids especially DHA that can be compared with other products like fish meal. Moreover, there is a growing demand for natural lipids highly rich in DHA as nutritional supplements during larval development.

This study is focused on silage preparation, determination and extraction of their PUFAs contents from wastes (viscera and dark meat) of four tuna species, namely skipjack (*Katsuwonus pelamis*), kawakawa (*Euhtynnus affinis*), longtail (*Thunnus tonggol*) and yellowfin (*Thunnus albacares*). The major objectives of the present study are to get access to the available resources of PUFAs in commercial tuna fishes in the Persian Gulf and Oman Sea and develop ways in utilization of tuna wastes in processing industry.

Material and Methods

Material:

Random samples of tuna wastes (dark meat and viscera) were collected from Bandar Abbass tuna cannery. Ensiling fluid was an ammoniated solution of 75% formic acid (Merck, Darmstadt, Germany); Gas-Chromatograph: varian model vista 6000 Germany; column type: fused silica (30 m, BPX), pepsin, butylated hydroxyanisol (BHA), propyl gallate, citric acid, propylene glycol and sodium hydroxide were purchased from Merck Chemical Co. Ltd Darmstadt Germany.

Method of silage preparation

Fish wastes (viscera or dark meat) were removed from the fish body and weighed. The weighed samples were minced prior to the addition of ensiling fluid. Ensiling fluid up to 3.5% by weight was added and the resultant mixture blended using a mechanical stirrer (Eurostar RS232).

Crude pepsin was added to the mixture at 0.1% (1 gm per kg) followed by 0.1% of an antioxidant mixture (containing 60mg ml⁻¹ butylated hydroxyanisole, 60mg ml⁻¹ propyl gallate and 40mg ml⁻¹ citric acid) dissolved in propylene glycol.

The reaction vessel was placed in water bath at 25°C and slowly mixed. The reaction was continued for 16 hours by which time the ensiling process was complete. Liquid silages were neutralized by the addition of solid sodium hydroxide pellets. The amount of sodium hydroxide was determined by calculation based on the known amount and concentration of acid (ensiling fluid) added. (Tocher *et al.*, 1997).

Fatty acid analysis

Fatty acid methyl esterase was prepared from total lipid and purified as described by AOAC (1990). Methyl esterase was analyzed with a varian GC vista 6000 gas chromatograph equipped with a fused silica capillary column (30 m × 0.32mm, BPX type) and FID detector. The carrier gas was nitrogen. The samples was run through a temperature gradient from 125 up to 220°C with an increase rate of 6.0° C/min and total run time of 40 min. The detector was operated at 260°C. All solvents contained 0.01 BHT (butylated hydroxytoluene).

Identification of fatty acids in the samples was performed by comparison with chromatograms of fatty acids standard (C4-C24 fatty acids) from Sigma Chemicals. Fatty acids composition was calculated from the total identified fatty acids area and the values were always the average of at least two to three injection of each duplicate extract.

Data analysis:

ANOVA was used to compare the amount of PUFAs between different tissues and species or before and after silage production. SPSS software and MS Excel were applied for statistical analysis and graph drawing respectively.

Results

Total lipid contents of the silage samples (dry weight) from viscera and dark meat ranged from 10.41% to 22% (Table 1). The highest (22%) and the lowest (10.95%) lipid contents of viscera silages were recorded from kawakawa and yellow fin tuna, respectively; the highest (15.8%) and the lowest (10.41%) lipid contents of dark meat silages were found in longtail and skipjack tuna, respectively.

Table 1: Total ratio of lipid content in viscera and dark meat of tuna wastes (gr/100 gr/ dry weight)

Species	Tissue (raw material)		Silage	
	Dark meat	Viscera	Dark meat	Viscera
Longtail	16.84	18.09	15.8	15.65
Skipjack	12.77	15.79	10.41	18.51
Yellowfin	12.83	15.8	14.61	10.95
Kawakawa	16.69	21.91	15.4	22.01

ANOVA test results indicated that linolenic acid [C18:3(n-3)] contents significantly varied among species, and that DHA ratio was significantly different before and after silage preparation (Table 2).

Table 2: Critical point of P value in tuna species, waste tissues and silages

	Between species		Between Tissues		Before & after silage preparation	
	df	Sig.	df	Sig.	df	Sig.
Total PUFA	3	0.279	1	0.333	1	0.757
C18:2(n-6)	3	0.439	1	0.635	1	0.497
C18:3(n-3)	3	*0.010	1	0.570	1	0.506
C20:4(n-3)	3	0.600	1	0.467	1	0.846
C20:5(n-3)	3	0.269	1	0.354	1	0.124
C21:5(n-3)	3	0.346	1	0.598	1	0.232
C22:6(n-3)	3	0.946	1	0.655	1	*0.040

* significant

The DHA ratio of the lipids was significantly high in both dark meat and viscera tissues. The highest DHA content (15.43%) in viscera silage was found in

skipjack tuna sample, while the highest DHA content (13.88%) in dark meat silage was found in kawakawa sample (Figs.1 & 2 and Table 3).

Table 3: Total ratio of C22:6(n-3) content in different tissues of tuna wastes (gr/100gr of total lipids)

Species	Tissue (Raw material)		Silage	
	Dark meat	Viscera	Dark meat	Viscera
Longtail	13.71	16.25	11.99	12.05
Skipjack	11.69	16.43	8.51	15.43
Yellowfin	13.76	13.14	12.59	11.25
Kawakawa	14.92	12.96	13.88	8.02

Discussion

In this study ensiling was chosen as a method for processing fish wastes. The silages were generally produced from whole fish or fish offal and dried for use primarily as a fish meal replacement in normal diets for growing fish such as rainbow trout (Hardy *et al.*, 1984) and Atlantic salmon (Jackson *et al.*, 1984; Espe *et al.*, 1992). The result showed that level of PUFA type C18:2 (n-6) (linoleic acid) in kawakawa raw dark meat was higher than its silaged form (Fig. 1A & 1B). ANOVA test results also indicated that DHA ratio was significantly higher than before silage preparation (Table 2).

The different results observed before and after preparation of silages may be due to different physico-chemical characteristics that could affect the processing of silage, e.g. light (photolysis), suitable temperature during silage preparation, pH and enzymes effects. These agents may break down the chains of fatty acids and decrease or increase the value of different types of fatty acids in silage.

The main factor limiting the application of these PUFAs in food products is their susceptibility to lipid oxidation, which may affect the flavour and decrease the nutritional value. Oxidation is influenced by factors like oxygen, temperature, light and chemical composition of the material (Amarowicz & Shahidi, 1997; Kim *et al.*, 2001).

The highest level of EPA was found in kawakawa viscera/dark meat silage (9.46% and 10.62% of total lipids, respectively) (Fig. 1B & 2B). The result of this study showed that kawakawa viscera silage had the highest (22% of dry weight)

total lipid content followed by skipjack tuna (18.51%), longtail tuna (15.65%), and yellowfin tuna (10.95%). The result also revealed high total lipid content (18.51%) in skipjack viscera silage. The highest levels of DHA [C22: 6(n-3)] were recorded in the skipjack viscera (15.43%) and kawakawa dark meat silages (13.88%) (Fig. 1B & 2B). It means that these products contain a high percentage of DHA, which potentially deliver the greatest amount of DHA per unit weight of silage, suggested to be one of the best types of silages as an enriching material for live prey and promoting growth of larval and juvenile fish (Kanazawa, 1985; Hung *et al.*, 1987; Olsen *et al.*, 1991).

Studies have shown that tuna contain a high concentration of DHA (20-25%) and EPA (5-8%) in body and viscera oils. Phospholipids of fishes typically have a total of 35-40% of these two fatty acids (Polvi & Ackman, 1992). These high proportions of EPA and DHA are the result of the tuna species natural diet and many scientists observed that is hard to match in fish farming operation (Morioka *et al.*, 1999)

In marine environment, the prey of marine fish larvae can satisfy their requirements for DHA, but the situations in mariculture are more complicated where the supply of this fatty acid to larvae is a regular problem because the most common cultivated live preys, artemia and rotifers are naturally lack of DHA. The result of the present study has demonstrated that silages prepared from tuna wastes can be either rich in phospholipid or PUFAs depending on the species and tissue. It has been claimed that silages prepared from tuna wastes by hydrolysis of proteins and amino acid does not expire before 1.5-2 years (Raa & Gildberg, 1976). The techniques for preparation of the tuna wastes silage are relatively simple and do not need complicate instruments and high investment.

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