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Chemical and nutritional quality of raw, cooked and salted fish silages

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Fermented fish silages were prepared from whole tilapia (*Oreochromis niloticus*), molasses and *Lactobacillus plantarum* starter culture. The effects of cooking and addition of 5% salt on the proximate composition, various protein and lipid quality parameters were measured over a 30-day period of incubation at 30°C. The silages were stable during fermentation and storage, and no appreciable loss of nutrient contents was noted. The changes observed were increased degree of autolysis and ammonia production. Addition of salt or cooking of substrate before fermentation prevented continued protein hydrolysis by inhibiting the activity of endogenous autolyzing enzymes and decreased the formation of total volatile bases. The apparent digestibility of dry matter, of nitrogen and of the energy content of silage-based diets for tilapia was higher in both the cooked and salted fish silages than in the raw fish silage.

INTRODUCTION

Ensilage of fish and fish by-products has been used as an alternative to fish meal in animal feeds with reports that its nutritional value is comparable with that of fish meal (Lopez, 1990). Ensilage involves lowering the pH either by adding mineral and/or organic acids (acid-preserved silage) or by lactic acid fermentation (fermented silage). Fermented silage is preferred in developing countries as it precludes the purchase of expensive acids and avoids risk of handling acids which may be corrosive to silage-making equipment (James *et al.*, 1977).

Feeding experiments have generally indicated that fish silage has food replacement value when it is partly substituted for fish meal in rations for livestock and fish (Lall, 1991). However, standardized methods of fish silage preparation do not exist and the nutritional value depends on the fish species used as substrate, proper methods of preparation, and knowledge of its quality and properties during fermentation and storage. Such information is limited, particularly for silage prepared from warm-water fish species, which may form an important substrate for silage manufacture in tropical developing countries where such technology is affordable and appropriate (Dhatemwa, 1989). This study was therefore conducted to determine the chemical changes that occur during the fermentation and storage of tilapia silage.

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The nutrient quality of fish silage can be improved by limiting the extent to which proteins are hydrolysed to polypeptides and free amino acids. Termination of the ensiling process after 3–7 days resulted in improved weight gain, protein efficiency ratio (PER), biological value (BV) and net protein utilization (NPU) when these products were fed to mink (Skrede, 1981), calves (Offer & Husain, 1987), salmonids (Lall, 1991) and rats (Espe *et al.*, 1992). Formaldehyde inhibits protein hydrolysis in fish silage (Haard *et al.*, 1985; Husain & Offer, 1987) but may prove toxic to some animals. As an alternative, the present study was also conducted to assess whether inhibiting autolysis of fish silage by cooking before fermentation or the addition of 5% salt (sodium chloride, NaCl) to the silage mixture would give a stable product during storage; the digestibility of these silages for feeding fish (*Oreochromis niloticus* Trewavas) was also assessed.

MATERIALS AND METHODS

Raw materials

Juvenile tilapias culled as a result of routine husbandry techniques were collected and kept deep-frozen (−20°C) and thawed in running water at room temperature before use. Molasses (International Molasses Ltd, Grangemouth, Scotland, UK) was added as carbohydrate substrate. A prefermented starter culture prepared with freeze-dried cultures of *Lactobacillus plantarum* (NCIMB 11974, NCIMB Ltd, Aberdeen, Scotland,

UK) was inoculated into molasses for large-quantity production and incubated until it gave 10^7 bacteria per g molasses. Cornflour (Tesco Stores, Stirling, Scotland, UK) was used as filler.

Silage preparation

Ungutted thawed tilapias were minced and passed through a die with 3-mm diameter holes. The minced fish was distributed into three batches (A, B, C) representing the experiments, each of which comprised triplicated treatments. Before fermentation, batch B was steamed for 30 min at 90°C and used as the preheated treatment while 5% salt (by weight) was added to Batch C and used as the salted treatment. For each replicate, 300 g of minced tilapia was thoroughly mixed with 15% (w/w) molasses and 5% (w/w) inoculum inside 500-ml storage glass bottles and sealed air-tight. For comparative purposes, an acid-preserved silage was also prepared by acidifying minced tilapia with 3% lactic acid.

The mixtures were incubated at 30°C for 30 days, after which the products were heated to 90°C and maintained for 30 min in order to halt autolysis. During incubation, silages were stirred daily for the first 15 days and later only when samples were taken. No antioxidant was added to the substrates during or after ensiling because of the low fat content of tilapias (Torry Research Station, 1989) but, to prevent mould growth, the surface of the silages and inner walls of the containers were sprayed with 1% potassium sorbate solution after each sampling.

Characterization of silage product

Triplicate 30 g samples of each fish silage were taken for analysis on days 0, 2, 4, 7, 15 and 30 after incubation. Similar analyses were made on minced tilapia before fermentation. Moisture was determined after oven-drying at 105°C to constant weight, and ash by incinerating the dried residue for 24 h at 550°C in a muffle furnace. Total nitrogen (N) was determined by the micro-Kjeldahl procedure and crude protein was estimated as N × 6·25. Crude lipid was determined after Soxhlet extraction of dried samples with petroleum ether. Fish silage was oven-dried at 80°C for 12 h, milled and used for gross energy assay by combustion in an adiabatic bomb calorimeter.

The pH was determined with a digital pH meter. Protein autolysis was estimated as non-protein nitrogen (NPN) and determined by trichloroacetic acid (TCA) precipitation according to Backhoff (1976). Total volatile nitrogen (TVN) and ammonia nitrogen (NH_3–N) were determined by the micro-diffusion method (Haaland & Njaa, 1988). Free fatty acid content was determined following the procedure of Pearson (1971) after extraction with a methanol/chloroform mixture. Peroxide and thiobarbituric acid (TBA) values were determined by the distillation method (Pearson, 1971).

Table 1. Ingredient composition (% dry matter) of silage diets

Ingredient	Raw silage diet	Cooked silage diet	Salted silage diet
Soybean meal	10	10	10
Cornflour			
Raw silage	75	0	0
Boiled silage	0	75	0
Salted silage	0	0	75
Cellulose flour[a]	5	5	5
Vegetable oil	3	3	3
Fish oil	2	2	2
Mineral mix[b]	2	2	2
Vitamin premix[b]	1	1	1
Carboxymethylcellulose[c]	1	1	1
Chromium(III) oxide[d]	1	1	1

[a] Non-nutritive filler.
[b] According to Jauncey & Ross (1982).
[c] Binder.
[d] Marker.

Protein digestibility trial

After 30 days, each fermented fish silage was mixed with cornflour (3:2) and co-dried in an oven at 45°C for 48 h. The co-dried fish silages were mixed with the basal diet (Table 1) containing 1% chromium(III) oxide as inert marker for the determination of the digestibility coefficient. All diets were formulated to have equal gross energy and crude protein contents and fed to tilapia (*O. niloticus*) fingerlings for 15 days. Faeces from 20 fish fed each diet were collected by anal extrusion, pooled on seven separate days, and lyophilized before analyses following the acid digestion method (Furukawa & Tsukahara, 1966). The apparent digestibility coefficient for dry matter, protein and gross energy were calculated according to Austreng & Refstie (1979) as follows

$$ADC = 10^2 \times (a - b)/a$$

where a = nutrient in feed/chromium(III) oxide in feed;
b = nutrient in faeces/chromium(III) oxide in faeces.

Statistical analysis

Data were analysed by analysis of variance (ANOVA). Duncan's multiple range test was used to compare differences among individual means.

RESULTS AND DISCUSSION

Fermentation characteristics

Raw silage

All the silages stored well and there were no signs of either bacterial or fungal spoilage at any time. The proximate composition and mineral content of the minced tilapia, fermented and lactic acid-treated tilapia silages are presented in Table 2. Generally the proximate composition of the silages and minced tilapia

Table 2. Proximate composition (g/100 g dry matter), gross energy (kcal/g dry matter) and mineral contents of minced tilapia and tilapia silages after 30 days

	Minced tilapia	Tilapia silage	
		Fermented	Lactic acid treated
Dry matter	26·3 ± 1·08	30·6 ± 1·14	26·7± 1·06
Crude protein	62·9 ± 3·40	42·4 ± 2·50	54·4 ± 2·08
Crude lipid	7·1 ± 1·45	10·6 ± 1·53	14·7 ± 1·50
Ash	18·0 ± 1·88	15·6 ± 1·67	17·6 ± 1·72
Gross energy	4·69 ± 0·21	5·28 ± 0·30	4·55 ± 0·24
Calcium	1·03 ± 0·05	0·95 ± 0·04	0·98 ± 0·04
Phosphorus	0·63 ± 0·04	0·54 ± 0·06	0·57 ± 0·05

varied little after 30 days of storage. This agrees with other reports that chemical analyses did not show any obvious differences between fish used as substrates and their respective silages (Hassan, 1982; Ajayl, 1985; Brown & Sumner, 1985; Espe, 1987).

Moisture content in both lactic acid-treated tilapia silage and minced tilapia were similar, and the corresponding dry matter content of fermented tilapia silage was higher than those in the minced tilapia and lactic acid-treated tilapia silage (Table 2). A slight increase in dry matter was similarly reported by Jackson *et al.* (1984) and Espe *et al.* (1989). Van Wyk & Heydenrych (1985) attributed this to the loss of carbon dioxide and ethanol (by evaporation) as a result of yeast fermentation whilst Espe *et al.* (1989) suggested that binding of water during proteolysis might be responsible.

Fermented tilapia silage contained 42·4 ± 2·5% protein whilst lactic acid-treated tilapia silage and minced tilapia contained 54·4 ± 2·0% and 62·9 ± 3·4% protein, respectively. The lower protein content in the fermented silage was due to the addition of carbohydrate (molasses) and slight dilution effect by the acid produced, whilst dilution by the added lactic acid was the reason in the acid-treated silage, as suggested by Batista (1987).

Lipid contents in both tilapia silages were higher than in minced tilapia (Table 2) and could be related to the extraction of lactic acid along with ether during lipid content determination. Stecher *et al.* (1968) reported that lactic acid is soluble in ether. The fact that tilapia is a low-fat fish is reflected in the low lipid content (<4%) of its silages. The lipid content is considered too low to pose a potential problem of rancidity during storage, compared with 6–18% obtained from sprat silage (Hall & Ledward, 1986). Tatterson & Windsor (1974) noted that it was desirable in a large-scale operation to remove oil from fish silage if it exceeded 4%.

There was a decline in pH accompanied by a corresponding increase in titratable acidity and a decrease in total carbohydrates (Table 3). The desired pH (4·5) was achieved within 72 h, by which time liquefaction had started, and pH remained at 3·9 up to 30 days of incubation. The change in consistency of the silage closely reflected the changes in trichloroacetic acid (TCA)-soluble nitrogen content. Storage of fish above freezing point without denaturation of proteolytic enzymes causes rapid liquefaction of the protein which is evident in the changes in TCA-soluble substances (Backhoff, 1976).

Initially, about 16% of total nitrogen (N) was TCA-soluble but, with time, there was a gradual increase in protein solubilization (Table 3) and NPN attained a maximum of 46·5% after 30 days. This value is lower than that obtained in acid-preserved silages, and is attributable to the adsorption of enzymes by the carbohydrates, thereby preventing their interaction, as suggested by Raa *et al.* (1983).

As a chemical quality criterion for fish silage, non-protein nitrogen (NPN) and/or ninhydrin-positive materials seem to be useful (Espe *et al.*, 1989) since the most obvious changes that occur during ensilage are autolysis of the tissues and release of ammonia (Backhoff, 1976; Haaland & Njaa, 1988). Before fermentation, minced tilapia had 13·2 mg TVN and 7·8 mg NH_3–N/g N, both of which increased slightly during storage (Table 3). The low total volatile nitrogen (TVN) value indicated that a fresh raw fish (substrate) was used.

The changes in free fatty acid (FFA), thiobarbituric acid (TBA) and peroxide values are also shown in Table 3. The initial low TBA values changed little with time; they might have arisen from the residual lipid. The reduction of the initially high peroxide value may have been due to the destruction of hydroperoxides.

Cooked silage

Within 48 h of fermentation, the cooked silage showed a slower pH decline compared with the raw silage (Table 3) and did not give a lower pH even after incubation for 30 days. While raw silage liquefied between

Table 3. Changes in pH and characteristics of fermented tilapia silages at 30°C

Fermentation period (days)	Raw									Cooked		Salted	
	pH	NPN	%TA	CHO	NH_3–N	TVN	TBA	PV	FFA	pH	NPN	pH	NPN
0	6·7	16·0	0·25	50·2	7·8	13·2	22·2	143	1·21	6·7	16·6	6·7	16·6
2	4·5	19·6	0·29	35·7	8·6	14·5	19·5	105	ND	5·4	16·8	5·5	17·5
4	4·3	21·7	0·40	24·9	10·8	15·1	17·7	93·1	ND	4·6	17·2	4·8	18·6
7	4·1	28·5	0·78	15·3	12·3	17·3	16·1	82·5	1·37	4·4	17·6	4·5	19·8
15	3·9	36·8	1·04	7·7	13·7	18·9	15·6	63·2	1·81	4·2	17·4	4·3	20·4
30	3·9	45·8	1·38	4·1	20·8	26·6	13·0	39·0	2·36	4·2	17·1	4·3	20·7

Abbreviations: %TA, titratable acidity; CHO, total carbohydrate (μg/g dry weight); NPN, non-protein nitrogen (%N); TVN, total volatile nitrogen (mg/g N); NH_3N, ammonia nitrogen (mg/g N); TBA, thiobarbituric acid (milliequivalents/kg lipid); PV, peroxide value (milliequivalents/kg lipid); FFA, free fatty acid (as oleic acid, %); ND, not determined.

48 and 72 h, cooked silage was 'porridge-like' up to 30 days of storage. This agrees with earlier reports that the prevention of liquefication is a normal phenomenon when the substrate is cooked before ensiling (Wood *et al.*, 1985; Batista, 1987), because, at 60°C and above, enzyme activity would have been destroyed (Strasdine *et al.*, 1988). Djajasewaka & Djajadiredja (1980) and Kompiang *et al.* (1980) reported that the nutritional value of boiled fish silage was superior to raw fish silage and attributed this partly to the denaturation of thiaminase during boiling.

In the raw silage, NPN increased from 16% to 45·8% within the 30 days of incubation while, in the cooked silage, it increased to 17·6% after 7 days and later decreased to 17·1% after 30 days (Table 3). Batista (1987) and Espe *et al.* (1992) reported a similar trend in fermented whiting and acid-preserved capelin silages, respectively. In acid-preserved silages, NPN may increase to 70–90% depending on the incubation/storage temperature (Tatterson, 1982; Batista, 1987) and such an increase in NPN is associated with the change in consistency. In the present study, cooked silage retained a dense consistency (less liquefication) for a longer period, which indicated that protein solubilization is essentially an enzymic process. Although heating accelerates the rate of proteolysis, temperatures below 60°C are required to maintain enzyme activity.

Salted silage

Addition of 5% NaCl did not improve the pH decline compared with the raw silage (Table 3). Visual inspection of the consistency of the salted silage indicated that an inhibition of autolysis occurred as very little liquefaction was noticeable after seven days. Such a delay in the pH decline within 48 h of incubation may be attributed to the partial inhibitory effect of salt on the growth of *L. plantarum* and proteolytic activity as suggested by Gildberg *et al.* (1984).

Pederson (1979) reported that 3·5% NaCl or more was detrimental to the growth of all bacteria in sauerkraut because it reduces the availability of soluble nutrients such as amino acids. Some lactobacillus strains degrade amino acids (Jonsson *et al.*, 1983) and develop ammonia. Apart from suppressing these ammonia-producing microbes (Subasinghe *et al.*, 1990), salt has also been used to inhibit hydrolysis in fish silage (Stanton & Yeoh, 1977) and fish sauce (Orejana & Liston, 1982; Gildberg *et al.*, 1984). Specifically, salt concentrations above 5% inhibited the activity of digestive enzymes, particularly pepsins, which are active under acid conditions.

Digestibility studies

When fed to tilapias, all the silage diets gave high digestibility values for dry matter (>80%), protein (>90%) and gross energy (>87%) the highest values being those of the cooked silage diet (Table 4). However, no significant difference ($P < 0.05$) occurred between digestibility values of the cooked and salted silage diets. In terms of protein digestibility, the lower values of the raw silage diet indicated that a significant proportion of the dietary nitrogen was not absorbed as observed previously in salmonids (Hardy *et al.*, 1983, 1984). This could be attributed to high levels of non-protein nitrogen (free amino acids, peptides) which might interfere with the mechanisms for peptide and amino acid absorption.

Table 4. Digestibility of fermented silages in tilapia (*O. niloticus*)

	Coefficient of digestibility[a]		
	Dry matter	Nitrogen	Gross energy
Raw	80·51a	90·37a	87·54a
Cooked	85·76b	96·22b	88·15a
Salted	84·20b	95·85b	88·10a

[a] a, b: values in the same column with similar letters are not significantly different ($P = 0.05$).

The loss of dietary nitrogen in feeds is detrimental to efficient protein utilization. This was evident from the effects shown by cooking and salt addition on the nutrient digestibility, particularly of nitrogen, and similar observations were noted in other fish (Djajasewaka & Djajadiredja, 1980; Kompiang *et al.*, 1980; Wood *et al.*, 1985). The high digestibility of protein in fermented tilapia silage is consistent with the results obtained by Wee *et al.* (1986) in experiments with the catfish, *Clarias batrachus*.

CONCLUSION

Tilapia silage products were stable during fermentation and storage. The pH did not vary appreciably within the desired range (4·0–4·5) and the rate of autolysis was influenced by cooking and addition of salt by inhibiting the activity of endogenous autolytic enzymes. This study has provided basic information for further work using pelleted fish feeds containing a high percentage of fermented fish silage as protein feedstuff. Thus it establishes an adequate alternative (to fish meal) which will result in lower cost for aquaculture production.

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