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Chemical and nutritional quality of fermented fish silage containing potato extracts, formalin or ginger extracts

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Fermented fish silages were prepared from whole tilapia (*Oreochromis niloticus*), 15% molasses and 5% *Lactobacillus plantarum* starter culture. After fermentation for 72 h, potato extracts (PE), formalin or ginger extracts (GE) were added at 5 ml/kg silage as proteolytic inhibitor or lipid antioxidant, and incubated at 30°C for 30 days. The effects of presence or absence of these additives on various protein and lipid quality parameters were periodically examined. PE had a slight and insignificant effect on protein solubilization and proteolytic activity; hence it was not effective as a proteolytic inhibitor. Formalin prevented further hydrolysis of protein and reduced ammonia production. GE proved effective as an antioxidant in fermented tilapia silage, as the thiobarbituric acid value remained low after 30 days' incubation. The tilapia silages were incorporated into dry tilapia diets and the digestibility of nutrients determined. The formalin-treated tilapia silage diet gave significantly reduced ($P < 0.5$) digestibility of dry matter, nitrogen or lipid compared to the control, PE-treated or GE-treated silage diets.

INTRODUCTION

The nutritional quality of unpreserved fish products varies markedly with the extent to which the protein and lipids have been hydrolysed. During ensilage and storage of fish, liquefaction occurs mainly by endogenous proteolytic enzyme activity and yields high contents of soluble peptides, free amino acids and ammonia (Batista *et al.*, 1989; Dong *et al.*, 1993). Perfect preservation could therefore be achieved by inhibiting enzymes or by limiting the degree of proteolysis. Proteolytic inhibitors from plants have successfully suppressed proteolysis in minced fish (Lanier *et al.*, 1981; Gowda & Karunasagar,1985), and in particular, potatoes (*Solanum tuberosum*) contain inhibitors for trypsin, chymotrypsin, carboxypeptidases and cathepsins (Ryan *et al.*, 1974; Busse & Belitz, 1976; Pearce *et al.*, 1982). Formaldehyde has also inhibited proteolysis and lipid rancidity in acid fish silage (Haard *et al.*, 1985; Husain & Offer, 1987), but proved toxic to livestock.

High levels of unsaturated lipids in fish silage make it susceptible to oxidation and consequent formation of toxic products which caused the reduced growth performance reported in some fish-silage nutritional trials. Raa and Gildberg (1982) suggested that lipid oxidation in fish silage could be checked by adding antioxidants such as ethoxyquin, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). These synthetic antioxidants are expensive; furthermore, they are slowly metabolized in fish muscle (Lovaas,1989); hence they are prohibited in many meat and fish products. A variety of natural antioxidants from vegetable extracts or spices may offer cheap alternatives (Chipault *et al.*, 1952; Pratt & Watts, 1964; Bishov & Henick, 1978; Larson, 1988). Ginger (*Zingiber officinale*) has strong antioxidative properties (Lee *et al.*, 1986; Jitoe *et al.*, 1992) and has proved effective in minimizing lipid oxidation in fish oils (Byun *et al.*, 1986). Comparatively, the total saturated fatty acids are slightly higher in freshwater fish than marine fish. Generally, marine species contain more longer-chain polyunsaturated fatty acids (PUFA). Tilapias are low-fat freshwater fish and thelr body lipids comprise about 16·5–21% PUFA, 39·2–44·1% monounsaturated fatty acids and 31·6–32·6% saturated fatty acids (Stickney & McGeachin, 1983; Viola *et al.*,1988).

In earlier studies, degradation of proteins in fermented tilapia silage was minimised by preheating minced tilapia to 90°C for 30 min or addition of 5% salt (Fagbenro & Jauncey, 1993). However, limitations remain, in that salt is a prooxidant (Stanton & Yeoh, 1977; Gildberg *et al.*, 1984) and will only suppress the presence of heterofermentative lactic acid bacteria (Batista, 1987) while heating may not be cost- or energy-effective if a large volume of fish silage is produced (Lindgren & Pleje, 1983). Therefore, this study was designed to determine the effectiveness of:

(a) extracts of potato tubers as proteolytic inhibitors in preserving tilapia silage;
(b) formalin as an agent to inhibit autolysis of fermented tilapia silage;
(c) extracts of ginger rhizomes as lipid antioxidants during fermentation and storage of tilapia silage.

MATERIALS AND METHODS

Silage preparation

Juvenile tilapias, culled as a result of routine husbandry techniques, were collected and kept frozen (−20°C), and later thawed at room temperature before use. Molasses (International Molasses Ltd, Grangemouth) was added as a carbohydrate source. A prefermented starter, prepared with freeze-dried *L. plantarum* culture (NCIMB 11974, NCIMB Ltd, Aberdeen) inoculated into molasses for large scale production and incubated until it gave 10^7 bacteria/g molasses, was added as inoculum. Ginger rhizomes and potato tubers (TESCO Stores, Stirling) were separately peeled, sliced, crushed and soaked in water (2 litres/kg) and homogenized. The homogenate of ginger was used as antioxidant without further purification, while the water-soluble fraction of potato was used as an extract for proteolytic inhibition. Formalin (40% formaldehyde, FISONS Ltd, Loughborough) was used as an additive to wet silage. Corn flour (TESCO Stores, Stirling) was used as a filler for drying the fish silage.

Ungutted thawed tilapias were minced and passed through a die with 3-mm diameter holes. Minced tilapia was divided into four 1-kg batches (A, B, C, D), representing the experiments, each of which was thoroughly mixed with 15% molasses and 5% inoculum (w/w) inside 5-litre plastic buckets and sealed air-tight. After fermentation (72 h later), PE was added to Batch A, formalin was added to Batch B, GE was added to Batch C, each at 5 ml/kg silage, while Batch D received no additives and served as the control. The silages were incubated at 30°C for 30 days, after which they were heated to 90°C and maintained for 30 min to halt autolysis. During incubation, they were stirred daily for the first 15 days and later, when samples were taken. The surface of the silages and inner walls of the containers were sprayed with 1% potassium sorbate solution after each sampling to prevent mould growth.

Characterization of silage product

Triplicate 30-g samples of silages were taken for analysis on days 0, 2, 4, 7, 15 and 30. 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.

The pH was determined with a digital pH meter. Protein autolysis was estimated as non-protein nitrogen (NPN) and determined by the trichloroacetic acid (TCA) precipitation method (Backhoff, 1976). Ammonia nitrogen (NH_3-N) was determined by the microdiffusion method (Haaland & Njaa,1988). Free fatty acid (FFA) content was determined by Pearson's (1971) procedure after extraction with a methanol/chloroform mixture. Peroxide (PV) and thiobarbituric acid (TBA) values were determined by a distillation method (Pearson, 1971). Proteolytic activity (PA) was estimated as the release of TCA-soluble Folin positive material from haemoglobin at pH 4·4 (pepsin activity) using tyrosine as a standard (Gildberg & Raa, 1983). PA was expressed as μmol tyrosine equivalent/h at 25°C.

Nutrient digestibility trial

After 30 days, each fermented fish silage was blended with corn flour (3:2) and co-dried in an oven at 45°C for 48 h. Four diets were formulated, each containing 75% dry weight of one of the co-dried fish silages. Other ingredients were present in the following proportions (g/100 g dry weight): soybean meal, 10%; cellulose flour (non-nutritive filler), 5%; vegetable oil, 3%; fish oil, 2%; mineral mix, 2% and vitamin mix, 1% (Jauncey & Ross, 1982); carboxymethyl cellulose (binder), 1%; and chromic III oxide, 1%, used as an inert marker for the determination of apparent digestibility coefficient (ADC). 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 analysed by the acid digestion method (Furukawa & Tsukahara, 1966). ADC for dry matter, protein and lipid were calculated according to Austreng and Refstie (1979) as follows:

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

where

a = nutrient in feed/chromic III oxide in feed,
b = nutrient in faeces/chromic 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

Proximate composition and pH of tilapia silages

The proximate composition of the tilapia silages after incubation for 30 days is presented in Table 1; it shows slight differences which were not significant ($P > 0{\cdot}05$). The pH of the silages (Table 2) dropped rapidly below 4·5 within 48–72 h of fermentation (prior to the addition of PE, formalin or GE). The pH declined further in the raw silage, though at a slower rate, reaching 3·9

Table 1. Proximate composition (g/100 g dry matter) of the Tilapia silages after 30 days

	Tilapia silage			
	Raw	PE-treated	Formalin-treated	GE-treated
Dry matter	$30{\cdot}55 \pm 1{\cdot}14^a$	$26{\cdot}26 \pm 1{\cdot}06^b$	$25{\cdot}48 \pm 1{\cdot}42^b$	$26{\cdot}15 \pm 1{\cdot}51^b$
Crude protein	$42{\cdot}4 \pm 2{\cdot}50^a$	$44{\cdot}4 \pm 2{\cdot}08^a$	$43{\cdot}5 \pm 2{\cdot}77^a$	$42{\cdot}6 \pm 2{\cdot}06^a$
Crude lipid	$10{\cdot}6 \pm 1{\cdot}53^a$	$11{\cdot}09 \pm 1{\cdot}50^a$	$10{\cdot}28 \pm 1{\cdot}32^a$	$10{\cdot}54 \pm 1{\cdot}16^a$
Ash	$15{\cdot}55 \pm 1{\cdot}67^a$	$17{\cdot}57 \pm 1{\cdot}72^a$	$16{\cdot}54 \pm 1{\cdot}38^a$	$16{\cdot}28 \pm 1{\cdot}29^a$

^{a,b,} Values in the same row with similar letters are not significantly different ($p = 0{\cdot}05$).

after 30 days' incubation. The pH decline in both PE-treated and GE-treated tilapia silages followed a similar trend, which suggests that they were well preserved. In the formalin-treated silage, however, the pH remained constant at 4·3 over the 30-day incubation (Table 2). Thus, the prevention of further pH decline shows that lactic acid bacteria activity may have been restricted, thus minimising lactic acid production.

Effect of potato extract on proteolytic activity in tilapia silage

The NPN content of the silages is presented in Table 2; it shows little difference ($P > 0{\cdot}05$) between the raw and PE-treated silages. This suggests that the solubilization of protein was not affected by PE. This observation agrees with Makinodan *et al.* (1985), who also found no effect of PE on protein hydrolysis in white croaker. However, Aksnes (1989) reported that protease inhibitors from PE- inhibited protein hydrolysis in minced capelin by decreasing the access to free amino acids (arginine, tyrosine and lysine), thus suppressing the medium for microbial (spoilage bacteria) growth. This may not apply to fermented fish silages because fermentation involves lactic acid bacteria, which utilize carbohydrates (preferably, rather than amino acids), as nutrients for growth (Raa, 1980; Van Wyk & Heydenrych, 1985). Moreover, because of the acidic medium of fish silages, pepsin is presumably the main enzyme involved in fermentation (Orejana & Liston, 1982); it seems, therefore, that protease inhibitors from PE do not affect peptic activity, as reported by Aksnes (1989) for stored capelin.

Effect of formalin on protein hydrolysis in tilapia silage

The protein content of the raw tilapia silage became increasingly soluble as reflected by a high percentage of NPN (Table 2). This indicates a high degree of protein hydrolysis, presumably to peptides and free amino acids during storage. The addition of formalin resulted in a significant ($P < 0{\cdot}05$) lowering of the soluble nitrogen content to 22·5% NPN over the same incubation period (Table 2). This signifies that proteolysis was inhibited, possibly by affecting proteolytic activity as suggested by Husain and Offer (1987), who observed a similar occurrence with formalin-treated formic acid whiting silage kept for 10 days. When formalin was added after liquefaction (48 h) of formic acid cod silage, protein hydrolysis was halted, leaving only 20–30% of the crude protein as NPN after 36 days (Haard *et al.*, 1985).

When autolysis is allowed to continue, the resultant free amino acids are further degraded to ammonia, its production being higher in fermented fish silage than in an acid fish silage (Batista, 1987). Despite reduced protein solubilization in formalin-treated silage, NH_3-N production increased markedly ($P < 0{\cdot}05$) up to 15 days of storage and declined slightly afterwards (Table 2). This does not refute the inhibition of protein hydrolysis by formalin as it would be expected that degraded products would affect the buffering capacity of the

Table 2. Changes in pH and characteristics of fermented Tilapia silages at 30°C

Storage period (days)	Raw							PE-treated			Formalin-treated			GE-treated			
	pH	NPN	PA	NH_3N	TBA	PV	FFA	pH	NPN	PA	pH	NPN	NH_3N	pH	TBA	PV	FFA
0	6·7	16·0	1·17	7·8	22·2	143	1·21	6·6	16·3	1·20	6·7	16·2	7·6	6·7	22·5	143	1·20
2	4·5	19·6	ND	8·6	19·4	106	ND	4·5	20·0	ND	4·5	19·8	8·4	4·4	19·7	106	ND
4	4·3	21·7	ND	10·8	17·7	93·1	ND	4·3	21·5	ND	4·3	20·5	12·3	4·2	16·2	129	ND
7	4·1	28·5	1·91	12·3	16·1	82·5	1·37	4·1	28·9	1·91	4·3	20·8	18·8	4·0	14·8	156	1·29
15	3·9	36·8	4·95	13·7	15·6	63·2	1·81	4·0	37·4	1·43	4·3	21·7	28·5	3·9	11·9	163	1·61
30	3·9	45·8	6·58	20·8	13·0	39·0	2·36	3·9	46·2	1·40	4·3	22·5	24·2	4·0	9·1	138	2·57

NPN = non-protein nitrogen (g/100 g N).
PA = proteolytic activity (μmol tyrosine equivalent per hour at 25°C).
NH_3N = ammonia nitrogen (g/100 g N).
TBA = thiobarbituric acid (milliequivalent/kg lipid).
PV = peroxide value (milliequivalent/kg lipid).
FFA = free fatty acid (as oleic acid, %).
ND = not determined.

silage and increase pH (Sinell, 1980), but this was not the case with pH in this treatment.

Although the evidence of decarboxylation or deamination of amino acids by *L. plantarum* is conflicting (Meyer, 1965; Jonsson *et al.*, 1983), it is unlikely that the NH_3-N was derived from amino acids because previous studies showed no differences in the total amino acids of fermented fish silages (Kompiang *et al.*, 1980). It seems that the NH_3-N was formed by hydrolysis of amide nitrogen, degradation of nucleic bases or oxidation of amines by bacterial aminooxidases as suggested by Hassan and Heath (1987).

The general effect of formalin on protein has been reviewed by Barry *et al.* (1973). Formalin forms methylene cross linkages between proteins, at the E-amino group of lysine. The linkages in the resultant methyol compounds are hydrolysed under the acid-pepsin conditions and the proteins become liberated. The level of formalin added should be closely related to the protein content of the ensiled material (Mackie, 1971). If the level is too high, irreversible bonding occurs and lysine is destroyed, and If the level is too low, it would be possible for a clostridial fermentation to occur. The latter is undesirable in lactic acid-fermented fish silages. However, the level used in this study was within the safe range recommended for fish silages (Haard *et al.*, 1985: Husain & Offer, 1987).

Effect of ginger extract on lipid oxidation in tilapia silage

The changes in FFA, PV and TBA values of tilapia silages during the 30 days of incubation are also shown in Table 2. The increase in FFA content of both raw and GE-treated tilapia silages were marginal and identical, suggesting that GE addition did not affect lipid hydrolysis (production of FFA). initially, there was a decrease in PV values of raw and GE-treated silages and, as expected, the decrease in PV value was further sustained in raw silage because fermentation under anaerobic conditions limits oxygen availability. With the addition of ginger extracts after fermentation for 72 h, there was a rapid development of hydroperoxides in the lipid of the GE-treated silage, which reached a PV value of 163 by day 15, after which it dropped to 138 by day 30. This probably reflected the degradation of part of the hydroperoxides to form secondary breakdown products such as aldehydes as suggested by Jackson *et al.* (1984) and, according to Labuza (1971), the addition of an antioxidant to an actively oxidizing system cannot destroy peroxides or their breakdown products or ameliorate their destructive effects, but may prevent further build up of these reactive species.

TBA value decreased in the raw silage, and the decrease was faster ($P < 0.05$) with addition of GE (Table 2). As such, there being no oxidation changes during incubation, GE proved to be effective as an antioxidant. The potency of GE is dependent on pH, with the maximum values at pH 5 (Lee & Ahn, 1985), The pH 3·9 attained in GE-treated silage (Table 2) is considered optimum and was therefore ideal for its antioxidative effectiveness. Thus, silage stability determined by TBA values was improved by GE inclusion. Although there are no established values associated with rancid/lipid quality in fish silages, it is safe to say that GE was very effective in preventing lipid oxidation when tilapia silage was incubated for 30 days. Since tilapia cannot be considered as a fatty fish (TRS, 1989), the extent of lipid protection that GE can provide for fish silages is unknown. Further studies need to be conducted using fatty fish such as mackerel in order to assess the full potential of GE as an antioxidant.

Table 3. Digestibility of fermented silages in Tilapia (O. niloticus)

	Coefficient of digestibility		
	Dry matter	Nitrogen	Lipid
Raw	80.5^a	90.4^a	87.5^a
PE-treated	78.6^a	87.1^b	86.0^a
Formalin-treated	70.8^b	76.0^c	71.2^b
GE-treated	79.6^a	84.7^b	85.1^a

^{a,b,c} Values in the same column with similar letters are not significantly different ($p = 0.05$).

Nutrient digestibility studies

The data on nutrient digestibility are presented in Table 3. Comparatively, the formalin-treated tilapia silage diet gave reduced apparent digestibilities of dry matter, nitrogen and lipid, which were significantly different ($P < 0.05$) from nutrient digestibility coefficients of the other diets. The level of formalin added to tilapia silage after fermentation in this study is equivalent to 0·5% by weight. Ferguson *et al.* (1967) and Offer *et al.* (1971) used similar levels in ruminant livestock rations without creating undesirable side effects. Barry *et al.* (1973) fed formalin-treated silages (containing 1·7% formalin (w/w) to sheep and obtained increased dry matter intakes and live-weight gains compared with those given untreated silages. Similarly, Haard *et al.* (1985) and Husain and Offer (1987) used 0·25% or 0·29% and 0·1–0·5% formalin-treated fish silage, respectively, in sheep diets without any adverse effects on growth or nutrient utilization. However, the lower level of 0·4% used by Vanbelle *et al.* (1978) caused reduced feed intake and poor utilization of dry matter, energy, fibre and nitrogen by sheep.

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