Use of Simple Fat Analysis for Evaluating Quality Changes in Intermediate Moisture Fish Stored at Tropical Temperature

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Changes in the quality of intermediate moisture (IM) fish during storage at 38°C were monitored by assessing the moisture content, pH, acid value, peroxide value and thiobarbituric acid (TBA) value periodically. Results adequately portrayed the hydrolysis and peroxidation of fats and the concomitant protein degradation and crosslinking reactions that have been shown by more sophisticated methods to occur in intermediate moisture fish. Since these changes markedly affect the organoleptic quality, acceptability/shelf-life and nutritive value of IM flesh-foods their predictability by simple fat analytical techniques is of practical value where/when the more sophisticated monitoring techniques are not feasible.

Fish fat consists predominantly of triglyceryl esters that are mostly heterogeneous (Lovern, 1932) with minor proportions of free fatty acids, vitamins, colouring matters, hydrocarbons, sterols and phosphatides (Brody, 1965). Only about 16% of the fatty acids present in fish fat are saturated, mainly palmitic (C_{10}) acid with smaller amounts of myristic (C_{14}) and stearic (C_{18}) acids. The bulk of the fatty acids (about 84%) are unsaturated comprising large proportions of C_{18} and C_{20} acids, with less C_{16} and C_{22} acids and only traces of C_{14} acids (Lovern, 1932; Kinsella *et al.*, 1977). Oleic acid occurs as the major constituent of these unsaturated fatty acids followed by clupanodonic acid which has five double bonds (Brody, 1965). In general, while marine fish oils have a relatively complex composition with high proportions of C_{18}, C_{20} and C_{22} acids, fresh-water fish oils contain small amount of C_{22} but large amount of palmitic acid and C_{18} unsaturated acids (Kinsella *et al.*, 1977).

Fish fat, by virtue of its composition, renders fatty fish prone to deterioration (Allen & Foegeding, 1981) particularly through oxidative rancidity which for intermediate moisture (IM) foods has been shown to be four to five times faster with the cheap, simple desorption technology than with adsorption processing (Labuza *et al.*, 1972). A recent study (Obanu, 1983) showed that a fatty fish *Chrysicthys nigrodigitatus)* preserved by desorption in glycerol-salt solution deteriorated faster particularly in colour and odour during storage at 30°C than similarly preserved lean fish *(Citharinus citherus)*. It is well known that oxidative deterioration through free radical initiation and propagation produces hydroperoxides, aldehydes, acids, ketohydroxy acids, epoxy compounds, furans and semi-aldehydes (Lundberg, 1962) many of which can interact with proteins (Gardner, 1979; Obanu *et al.*, 1980) to form melanoidins (brown polymer) or with amino acids to form acyl esters (with bitter taste). Since fish muscle fat has such marked influence on the quality of IM fish products (Obanu, 1983), it will be plausible in quality control if these complex fat-induced changes can be monitored cheaply and readily by simple, easy-to-perform assays. The present study, with this in view, attempts to evaluate through simple fat analytical techniques the storage changes in desorption-processed IM fish flesh from tropical fatty fishes.

Materials and Methods

Three freshwater fatty fish species *Chrysicthys nigrodigitatus, Bagrus docamac* and

Eutropius niloticus - caught by the gill-net from the lower River Niger were bought alive from fishermen. These were killed in the laboratory, gutted, washed and stored in polythene bags in a freezer at -25°C until required (the next day).

For the experiment, the fishes were thawed in tap water and steamed for 5 min to inactivate enzymes and micro-organisms and to facilitate filleting. The fillets from each species were cut into roughly 1 cm^3 pieces which were mixed thoroughly. One sixth of each species was stored back at -25°C as frozen control while the remaining five-sixths were cooked for 15 min in 1.5 times their weight of an infusing solution containing 35.4% glycerol, 9.5% salt (NaCl) and 0.5% potassium sorbate as antimycotic and subsequently equilibrated in the infusing solution overnight (12 h) at room temperature (Obanu *et al.*, 1975 a). The resulting intermediate moisture (IM) product of each fish species was divided into five equal portions that were packaged separately in polythene bags. One package per species was removed for prestorage analysis while the rest were stored at 38°C in a thermostatically controlled hot-air oven. At 3-week intervals one package of each species was taken at random and analysed along with the frozen control cooked for test in 1.5 times by wegiht of distilled water for 15 min.

The ether-extractable fat of cooked/desorbed fish flesh pieces was determined from fish pieces ground after pre-drying at 80°C for 5 h and extracted with petroleum ether for at least 7 h in a soxhlet apparatus (AOAC, 1975). The acid value and peroxide value were determined as described by Pearson (1976). Moisture was determined by drying 5 g duplicates of each fish sample in a hot air oven at 80°C overnight (at least 10 h) while pH was determined on a suspension of 1 g minced fish samples in 10 ml water. Thiobarbituric acid (TBA) value was obtained from 10 g fish samples by the distillation method of Tarladgis *et al.* (1960). All analyses were done in duplicate and results expressed as mean of the duplicate determination.

Results

Fat content of the fatty fishes

Fresh (steamed) samples of *C. nigrodigitatus, B. docmac* and *E. niloticus* contained 12.69%, 12.96% and 14.42% fat respectively while the freshly processed IM samples had 13.07%, 13.58% and 16.38% fat respectively (Table 1).

Table 1. *Ether extractable fat content (% w/w) of the flesh of the fatty fishes studied*

Fish species	Frozen control (cooked for test)	Intermediate moisture fish (freshly processed)
C. nigrodigitatus	12.69	13.07
Bagrus docmac	12.96	13.58
Eutropius niloticus	14.42	16.38

Moisture content of the IM fatty fishes

Moisture contents of the *Chrysicthys nigrodigitatus, Bagrus docmac* and *Eutropius nilotics,* after the cook-soak-equilibration processing, dropped from 70.91%, 67.30% and 66.50% to 42.90%, 51.30% and 37.00% respectively. Further moisture losses occurred at varying rates in all three fish samples during storage (Fig. 1). *E. niloticus* appeared to have the best moisture retention especially within the first 6 weeks of storage while *C. nigrodigitatus* appeared to have the worst moisture retention. These differences in moisture retention were statistically significant ($P<0.05$). It is pertinent to observe that *E. niloticus* which had the lowest initial moisture content and best moisture retention during storage (Fig. 1) had the highest initial fat content (Table 1). Nevertheless, all three samples had fairly good moisture retention comparable with IM meats stored at the same temperature, 38°C (Obanu *et al.*, 1975 b, 1976).

pH of the IM fatty fishes

The pH valves of freshly processed IM samples were similar to those of conventionally cooked fish (Table 2). However, during storage of the IM samples at 38°C there were slight increases in acidity (ie. pH drop). The pH of *C. nigrodigitatus* and *E. niloticus* declined from prestorage values of 6.75 and 6.60

Table 2. *pH of intermediate moisture (IM) fish flesh stored at 38°C*

Fish species	Frozen control (cooked for test)	Weeks of storage of IM fish flesh 0	3	6	9	12
C. nigrodigitatus	6.80	6.75	6.40	6.15	6.20	6.35
Bagrus docmac	6.60	6.60	6.50	6.50	6.50	6.45
Eutropius niloticus	6.70	6.60	6.50	6.40	6.20	6.30

to minima of 6.15 at 6 weeks and 6.20 at 9 weeks respectively, while *B. docmac* had the least pH drop from an initial value of 6.60 to 6.45 at the termination of storage.

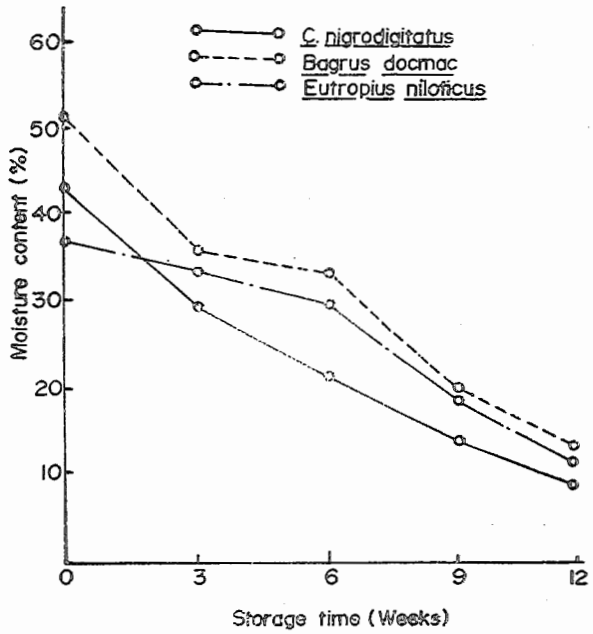

Fig. 1. Moisture retention of intermediate moisture fatty fish samples during storage at 38°C.

Acid values of the IM fish fats

Both the freshly processed IM samples and the cooked fishes had no detectable acid values. During storage, however, acid values increased sharply after a lag which was minimal for *B. docmac,* attaining peak levels at 6 weeks for *B. docmac* and 9 weeks for both *C. nigrodigitatus* and *E. niloticus* (Fig. 2). The increases in acid values for the latter two fishes appeared to have been detected by pH measurements (Table 2) while the increased acid values of *B. docmac* did not lead to corresponding pH drop.

It is pertinent to observe that *B. docmac* which had the highest moisture content especially early in storage (Fig. 1) also had the highest and fastest free acid production early in storage (Fig.2). Conversely *C. nigrodigitatus* with the poorest moisture retention and hence lowest moisture contents during storage (Fig. 1) had the least free acid production (Fig. 2).

Peroxide values of the IM fish fats

Freshly processed IM fish from all three species had no detectable peroxide values, as in the cooked fish controls. During storage, there was a lag of 3 weeks in peroxide formation in both *C. nigrodigitatus* and *E. niloticus* (Fig. 3); even in *B. docmac* peroxide formation was slow in the first 3 weeks. *Bagrus docmac* which had the fastest and highest peroxide formation also had the highest residual moisture (Fig. 1) and highest free acid generation (Fig. 2) without concomitant pH drop (Table 2). In all three fatty fishes, peroxide formation, once initiated, progressed all through the 12 weeks of storage (Fig. 3).

Thiobarbituric acid (TBA) values of the fatty fishes

Thiobarbituric acid values increased with storage for all three fatty fishes attaining peak levels at 9 weeks for *C. nigroditatus* and *E. niloticus* and at 6 weeks for *B. docmac;* thereafter all values declined fast (Fig. 4). Since TBA value is a measure of malonaldehyde-type compounds, this trend indicates generation in storage (within 6 or 9 weeks) of carbonyl compounds which are subsequently used up. This is also the trend observed for free acids as measured by acid value (Fig. 2).

Discussion

The moisture and fat contents of the freshly processed IM fishes (Fig. 1 and Table 1 respectively) confirm the inverse relationship between these components while the storage changes in Fig. 1 illustrate the moisture retention effect of muscle fat since water diffusion is slower when the fat content is higher (Jason, 1965). Moisture losses (Fig. 1) attributable to protein crosslinking reactions (Obanu *et al.*, 1975a, b, 1976, 1980)

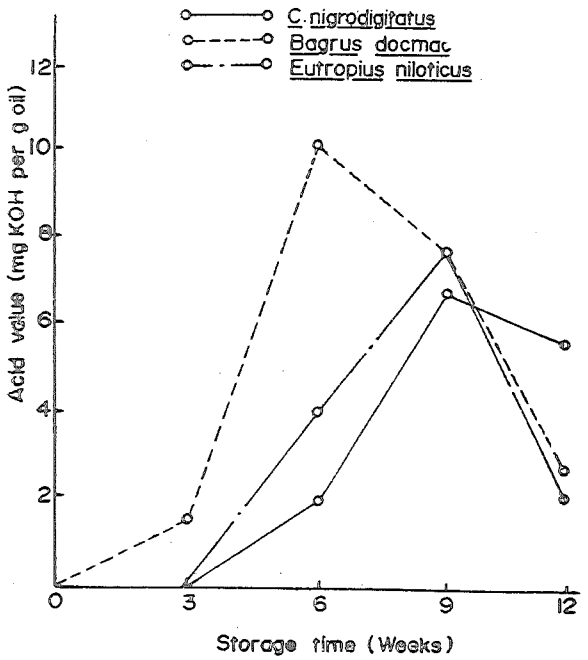

Fig. 2. Acid value of the muscle fat of intermediate moisture fatty fish samples stored at 38°C

are common in intermediate moisture (IM) meat (Obanu *et al.*, 1975 b, 1976) and fish (Obanu, 1983) stored at tropical temperature and affect product texture (Obanu *et al.*, 1975 b). This deteriorative crosslinking of proteins is invariably accompanied by a decrease in pH (Obanu *et al.*, 1975a, b, 1976; Labuza, 1975; Webster, 1980) as Table 2 confirms. It is interesting that the pH decrease is associated with increased release of free fatty acids resulting in increased acid value in the first 6 or 9 weeks of storage at 38°C (Fig. 2). Sharp (1953) observed similar increase in acidity of fats in dried raw meat, which although reduced by precooking was not eliminated and it is now accepted that the fat splitting action of lipase may persist, albeit slowly, in cooked dried meat (Acker, 1962). Fatty acid release was expectedly highest in the *Bagrus docmac* that contained the highest residual moisture (Fig. 1) after the cook-soak-equilibration processing.

The oxidation of unsaturated lipids, which is maximal at intermediate water activity (Labuza *et al.*, 1972) and faster in the higher-moisture cintaining desorption-processed IM foods than in adsorption-processed foods of same water activity (Chou *et al.*, 1973) is monitored in this study simply by peroxide value and thiobarbituric acid (TBA) value. The initiation of lipid oxidation is indicated by the onset of peroxide value rise at about 3 weeks storage at 38°C (Fig. 3). The initiation of lipid oxidation naturally leads to molecular degradation producing lower molecular weight acids that raise the acid value (Fig. 2). These scission products of lipid oxidation are known to be TBA active hydroperoxides (and charged radicals) that readily participate in lipid protein complexing interacitons. The peroxide value (Fig. 3) shows

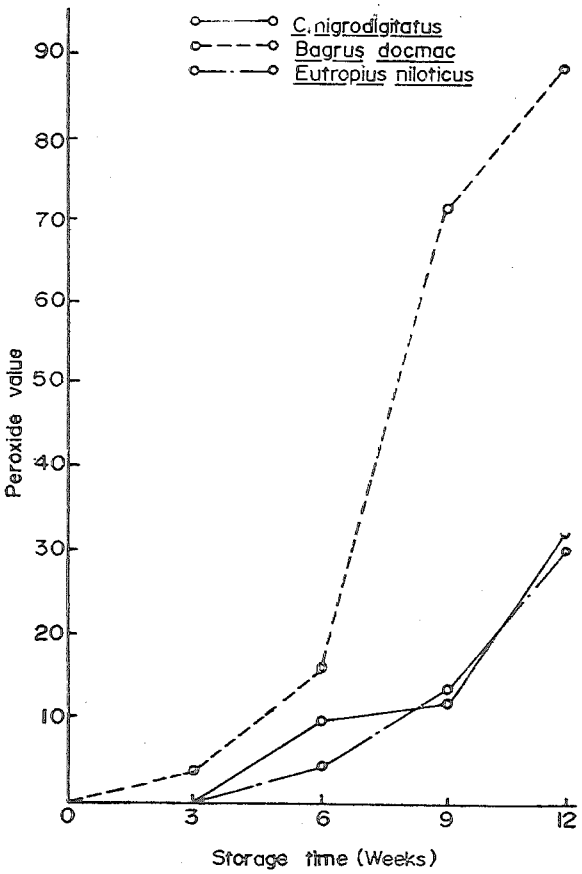

Fig. 3. Peroxide value of the muscle fat of intermediate moisture fatty fish samples stored at 38°C

the production of these hydroperoxides while the TBA value monitors their fate showing their production to be faster than their use-up early in storage (within the first 6 or 9 weeks) thereafter they are used up faster than their

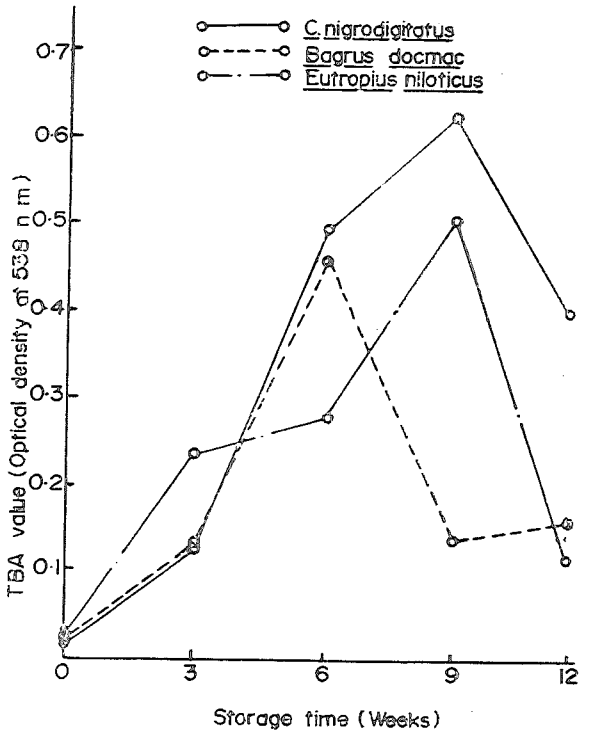

Fig. 4. Thiobarbituric acid (TBA) value of intermediate moisture fatty fish samples stored at 38°C

production rate (Fig. 4). The hydroperoxide interactions in muscle foods are very important because they readily affect organoleptic and nutritional quality (Gardner, 1979; Obanu *et al.*, 1980; Allen & Foegeding, 1981) and the present study uses easy fat assay techniques to demonstrate their occurrence in IM fish.

That these all important deteriorative reactions in IM fish and meat can be evaluated by such simple fat analytical techniques as peroxide and TBA values may be of great practical value in quality control, especially where/when the more orthodox sophisticated methods are not feasible owing to lack of facilities and/or expertise. These methods apart from being simple and easy-to-perform are also cheap and employ readily available laboratory facilities and know-how.

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