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# Effect of pre-icing duration on quality deterioration of iced Nile perch (*Lates niloticus*)

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#### Abstract

Quality deterioration of Nile perch iced 0, 3 and 6 h after landing was monitored to determine the effect of delayed icing on stability during storage on ice. Free fatty acids, volatile nitrogen bases and mesophilic microbial counts increased during the preicing duration. Delayed icing also increased the rate of lipolysis, degradation of nitrogenous compounds and growth of mesophilic microbes during subsequent storage on ice and reduced the time required for development of off odours. Psychrotrophic plate counts were, however, not affected by pre-icing duration. It was concluded that spoilage of Nile perch stored on ice is hastened by delay to ice and that mesophiles play a more important role in the spoilage of Nile perch, even when stored on ice. © 2003 Elsevier Ltd. All rights reserved.

Keywords: Nile perch; Icing; Fish deterioration; Fish preservation

#### 1. Introduction

Nile perch (*Lates niloticus*) is the most important fish species, commercially, for the East African countries of Uganda, Tanzania and Kenya. It is mainly derived from Lake Victoria, which is shared by the three countries. Much of the Nile perch caught in this region is exported either chilled or frozen. Artisan fishermen dominate fishing in this region. These fishermen in some cases do not have a supply of ice and the fish may spend several hours, at ambient temperature, before icing.

Nile perch is a fatty fish species (Ssali, 1989) and quality deterioration of fatty fish species is primarily caused by micro-organisms and lipid oxidation (Gram, Wedell-Neergaard, & Huss, 1990). Tropical (warm water) fish species, such as Nile perch, store longer on ice or under refrigeration than cold water fish species. It is estimated that Nile perch stored at ambient temperature has a shelf life of 13 h while storage on ice would extend the shelf life up to 28 days (Gram, Oundo, & Bon, 1989). Cold storage is, therefore, an efficient way of extending the shelf life of warm water fish species. This is because the dominant micro-flora on these species are mesophiles which do not proliferate readily under low temperature conditions.

High ambient temperatures and long pre-icing periods, however, may accelerate the deterioration of fish quality. Currently, little information is available regarding the quality changes that occur in Nile perch as a result of delays in icing and how these affect storage stability of the fish when it is eventually chilled and stored under refrigeration. The objective of this work was to determine the effect of delayed icing on the chemical, microbiological and sensory quality deterioration of Nile perch during subsequent storage on ice.

#### 2. Materials and methods

#### 2.1. Sample preparation

Nile perch weighing 3–5 kg were obtained from Lake Victoria immediately after landing. The fish were gutted, washed and iced immediately or after holding at ambient (23–26 °C) for 3 or 6 h. The fish were then packaged in polyethylene bags and stored on ice in insulated chests. Portions were drawn at five days intervals,

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over a storage period of 20 days and analysed to determine the extent of deterioration.

#### 3. Chemical analyses

# 3.1. Free fatty acids determination

Percent free fatty acids were determined by the method described by Kirk and Sawyer (1991). Diethyl ether (25 ml) and ethanol (25 ml) were mixed and neutralised with 0.1 M sodium hydroxide solution. A 2 g portion of the sample was dissolved in the diethyl ether and ethanol solvent mixture and titrated against 0.1 M sodium hydroxide with 1% phenolphthalein as indicator. The acid value was expressed as percent oleic acid equivalent.

# 3.2. Thiobarbituric acid (TBA) value

The thiobarbituric acid (TBA) value was determined colorimetrically by the method of Porkony and Dieffenbancher as described by Kirk and Sawyer (1991). A 200 mg portion of the sample was weighed into a 25 ml volumetric flask. An aliquot (1 ml) of 1-butanol was added to dissolve the sample. The mixture was made to volume and mixed. A 5 ml portion of the mixture was measured into a test tube and 5ml of TBA reagent (prepared by dissolving 200 mg of 2-thiobarbituric acid in 100 ml 1-butanol) were then added. The test tubes were covered, vortexed and then held in a water bath at 95 °C for 2 h and then cooled. Absorbance (As) was measured at 530 nm against a water blank. A reagent blank was run and absorbance (Ab) recorded. TBA value (mg of malonaldehyde per 100 g of tissue) was obtained by the formula

$$TBA = \frac{50 \times (As - Ab)}{200}$$

# 3.3. Total volatile nitrogen determination

A 10 g fish sample was mixed with 90 ml 6% perchloric acid, homogenised for 2 min. The mixture was filtered and 50 ml of the filtrate was steam distilled with 20% sodium hydroxide for 10 min. The vapours were collected in 3% boric acid and titrated with 0.01 M hydrochloric acid. A blank was also prepared with 50 ml perchloric acid only.

Total volatile nitrogen (TVN) was calculated as follows;

TVN (mg/100 g) = 
$$\frac{(V_1 - V_0) \times 0.14 \times 2 \times 100}{M}$$

Where  $V_1 = \text{Titre (ml) for samples}$   $V_0 = \text{Titre (ml) for blank}$ M = weight of sample in grams.

## 4. Microbial analysis

Total viable and psychrophilic counts were determined by the pour plate method as described by Harrigan and McCance (1976) using Plate Count Agar (Oxoid, CM325). The inoculated plates were incubated at 30 °C for 24 h and 5 °C for 72 h for total viable counts and psychrophilic counts respectively.

#### 4.1. Sensory evaluation

An untrained panel of 25 members was used to evaluate the odour of the Nile perch samples. Portions of 50 g flesh were cut from the fish samples and put in small cups. The panellists were asked to sniff the samples and score their intensity of the off-odour using a five point hedonic scale in which 0=no off-odour; 1=mild offodour; 2=moderate off-odour; 3=strong off-odour; 4=very strong off-odour.

# 4.2. Statistical analysis

Data for the different parameters were analysed using ANOVA. The least mean square was used to separate the means.

# 5. Results and discussion

Delay between fish capture and icing resulted in fast fish deterioration during holding of fish at ambient temperature and affected the subsequent stability of the fish when stored on ice (Figs. 1–5).

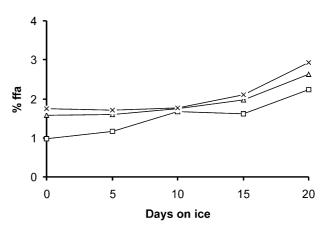


Fig. 1. Changes in free fatty acids in Nile perch samples stored on ice after 0 ( $\Box$ ), 3 ( $\Delta$ ), 6 ( $\times$ ), h delay before icing.

## 5.1. Free fatty acids

The presence of free fatty acids is due to oxidation and hydrolysis of lipids and is undesirable since the fatty acids may be converted to odorous volatiles (Lindsay, 1991). Holding samples at ambient temperature for 3 or 6 h led to significant (P < 0.05) increase in the concentration of free fatty acids (Fig. 1). It seems therefore that lipid hydrolysis is more rapid at the high ambient temperature than during storage on ice.

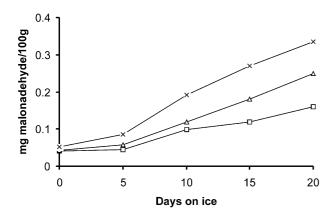


Fig. 2. Changes in thiobarbituric acid (TBA) in Nile perch samples stored on ice after 0 ( $\Box$ ), 3 ( $\Delta$ ), 6 (×), h delay before icing.

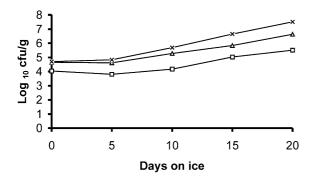


Fig. 3. Changes in mesophilic plate count of Nile perch samples stored on ice after 0 ( $\Box$ ), 3 ( $\Delta$ ), 6 (×), h delay before icing.

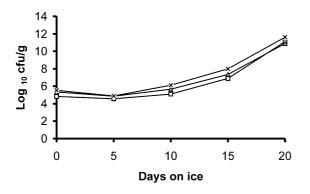


Fig. 4. Changes in psychrotrophic plate count of Nile perch samples stored on ice after 0 ( $\Box$ ), 3 ( $\Delta$ ), 6 (×), h delay before icing.

Upon icing, the rate of increase in concentration of free fatty acids was similar regardless of the pre-icing duration. Because of the initially higher concentration of free fatty acids in samples iced after 3 or 6 h, these samples contained consistently higher concentrations of free fatty acids than samples iced immediately throughout the period of storage on ice.

## 5.2. Thiobarbuturic acid concentration

The presence of TBA reactive substances is due to the second stage auto oxidation during which peroxides are oxidized to aldehydes and ketones (Lindsay, 1991). TBA values are, therefore, also a measure of the extent of lipolysis. High values are undesirable because they are associated with rancid odours. In this study, TBA values were not significantly altered by holding fish samples at ambient temperature for up to 6 h before icing. However, during storage on ice, the rate of increase of TBA was significantly higher for samples iced after 3 or 6 h holding at ambient temperature (Fig. 2). It seems during the pre-icing duration, intermediates accumulated and these were then converted to aldehydes and ketones during storage on ice.

# 5.3. Microbial counts

Delay of 3 or 6 h before icing led to significant (P < 0.05) increases in mesophilic plate counts of about 0.5 log cycle (Fig. 3). The mesophilic microbial count increased throughout the storage period and the counts were consistently higher for the samples iced after 3 or 6 h throughout the period of storage on ice than for samples iced immediately. The psychrotrophic counts were however, not significantly (P > 0.05) affected by pre-icing duration (Fig. 4). The psychrotrophic counts increased in all samples, during storage on ice, but the increase only commenced after the first 5 days on ice. It seems the first days of storage on ice constituted the lag phase for the psychrotrophs and substantial growth only started there after. Ababouch, Souibri, Rhaliby, Ouahdi, Battal, and Busta (1996) showed that sardines stored at ambient (21-27 °C) temperature experienced more rapid microbial growth than those stored in ice. There was no difference in growth rate of psychrotrophs and mesophiles in sardines stored in ice, while at ambient temperature, mesophiles multiplied more rapidly.

#### 5.4. Total volatile base nitrogen

Volatile bases result from degradation of proteins and non-protein nitrogenous compounds, mainly as a result of microbial activity (Connell, 1975). TVB-N is widely used as an indicator for fish deterioration (Olafsdottir et al., 1997). In this study, TVB-N concentration was not significantly (P > 0.05) affected by holding fish samples

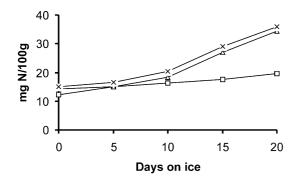


Fig. 5. Changes in TVB-N in Nile perch stored on ice after delays before icing of  $0 (\Box)$ ,  $3 (\Delta)$ ,  $6 (\times)$ , h delay before icing.

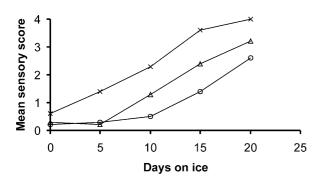


Fig. 6. Mean sensory off-odour scores of Nile perch stored on ice after delays before icing of  $0 (\Box)$ ,  $3 (\Delta)$ ,  $6 (\times)$ , h delay before icing.

at ambient temperature for up to 6 h (Fig. 5). However, the rate of accumulation of TVB-N during storage of fish on ice was higher in samples iced after 3 or 6 h, compared to those iced immediately. This is probably because TVB-N accumulation results from microbial activity and it occurs at relatively high microbial populations (Benjakul, Visessanguan, & Tueksuban, 2003). It is actually an indicator of imminent spoilage.

TVB-N accumulated rather slowly for the fish iced without delay but the rate of accumulation was much higher for samples iced after 3 or 6 h holding at ambient temperature. The rate of accumulation of TVB-N was positively correlated (r=0.92 for psychrotrophs and 0.94 for mesophiles, P < 0.05) to microbial counts, confirming that the degradation of nitrogenous compounds was a function of the micro-organisms. Ababouch et al. (1996) showed that sardines accumulated TVB-N faster when stored at ambient (21-27 °C) than when stored on ice probably because of the increased growth and activity of mesophiles at ambient temperature.

The TVB-N at which fish becomes unacceptable has been determined for some fish species. Some of the values reported are 25–35 mg/100 g for sardines (Ababouch et al., 1996; Marrakchi, Bennour, Bouchriti, Hamama, & Tagafait, 1990) and 30–40 mg/100 g for cold water fish species (Connell, 1975). None of the sample TVB-N values reached 40 mg/100 g which seems to suggest that all the samples were still acceptable. However, the TVB-N value at which Nile perch becomes unacceptable is not well established.

#### 5.5. Sensory quality

Samples iced immediately after landing did not show any off-odour during storage on ice until day 15. At day 20, these samples were scored as having a moderate offodour. The samples stored after 3 or 6 h holding at ambient temperature on the other hand were found to have a mild off odour at day 10 and 5, respectively. These samples were judged to have a moderate offodour at day 15 and 10. Sensory evaluation therefore revealed that the fish iced immediately upon landing deteriorated at a slower rate (Fig. 6) than the fish iced after 3 h holding at ambient temperature. Icing after 6 h holding at ambient temperature led to an even faster rate of deterioration.

#### 6. Conclusion

Delay between fish capture and icing leads to microbial proliferation, chemical and biochemical degradation of fish, leading to a reduction in the shelf-life of fish on ice. Longer pre-icing periods result in shorter fish shelf life. It is therefore recommended that fish be iced immediately after capture in order to maintain its freshness and to extend its shelf-life on ice.

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