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Effect of delayed processing on sensory quality, organoleptic test, nutritional composition and pH of fresh *Engraulicypris sardella* (Usipa) stored at ambient temperature

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Abstract

The study on nutrient composition (moisture, protein, fat and ash), sensory quality, pH and organoleptic test of fresh *Engraulicypris sardella* stored at ambient temperature was investigated at various time intervals. Acceptable freshness (sensory) quality of the fish was estimated at 6 hours after which, consumer liking declined significantly ($P<0.05$). However, when fish were deep fried in edible vegetable cooking oil for about 15 minutes, its acceptability extended up to 14 hours of ambient storage suggesting the importance of processing in value addition. Significant ($P<0.05$) reduction in all nutrients was observed after 6 hours of ambient storage. In freshly caught fish, pH was close to normal (6.40) but decreased sharply during the first 3 three hours to 6.35 then increased to 6.36 after 6 hours before drastically decreasing to its lowest point (6.28) at 12 hours then rising sharply to 6.41 at 14 hours of ambient storage (rejection time). The study demonstrated that optimum nutrient content in fresh *E. sardella* can be obtained if fish are consumed not later than 6 hours of ambient storage. Therefore, it is recommended that beyond 6 hours of ambient storage, fresh E. sardella should be presented in a processed form to extend consumers' liking of the fish.

Introduction

Engraulicypris sardella (Figure 1) is a small sized fish species widely consumed in Malawi due to its availability throughout the year and relatively cheaper prices affordable to many low income people (Per. obs). It is found throughout Lake Malawi and locally known as usipa. In the northern most part of the lake bordering Tanzania, it is named dagaa. *E. sardella* is a nutrient rich food fish due to its high levels of unsaturated fat and protein (Owaga *et al.*, 2010; Kabahenda *et al.*, 2011; IOC, 2012). Due to its small size, *E. sardella* is consumed whole with bones, hence a best source of minerals such as calcium, iron, zinc and sodium (Mumba and Jose, 2005; Effiong and Fakunle, 2011; Jiang *et al.*, 2015). As a fatty fish, spoilage is rapid under aerobic storage due to fat oxidation (Huss, 1995). Further, the marketing and handling chain involving fresh fish in Malawi is long and complex due to the high consumer demand (Kapute, 2008). After catch at night from the lake, fish are brought on to the beach early in the morning where it changes several hands starting from fishers to fish vendors also known as middle men, then wholesalers and eventually retailers before reaching the ultimate consumer. Fish are thus processed very late after catch. Delayed processing however, affects nutritional and organoleptic properties of fresh fish (Makawa *et al.*, 2014; Goliat *et al.*, 2016). The aim of this study was to assess the effect of delayed processing on sensory, organoleptic quality, nutrient composition (moisture, protein, fat and ash) and pH of fresh *E. Sardella* kept at ambient temperature.

Figure 1. Fresh *Engraulicypris sardella* (Left) for sensory analysis and deep frying in edible vegetable cooking oil (Right) for organoleptic testing

Materials and Methods

Fish sample collection and preparation

Fresh samples of *E. sardella* (about 3 kg) were purchased from Lake Malawi (Nkhata Bay) early in the morning immediately upon landing at the beach and carried to Mzuzu University laboratory in a cooler

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box preserved with block ice. At the laboratory, fish were displayed at ambient storage (around 27°C) while sprinkling with water periodically imitating the real local market situation. Traders sprinkle fresh fish with water to maintain the outward freshness appearance of the fish so that customers think they are buying fresh and better quality fish. Two portions of fresh fish were taken every 3 hours, one for sensory analysis and the other for organoleptic testing after being deep fried in edible vegetable cooking oil (Figure 1).

Sensory analysis

Sensory freshness quality of the fish was determined using the Quality Index Method (QIM) scheme (Table 1) which was developed following procedures used by Martinsdóttir *et al*. (2001) and Hyldig and Green-Petersen (2004). A panel of seven pre-trained people assessed changes in appearance of the skin, eyes (cornea), gills (including smell), backside and belly of the fish at a 3 hour interval.

Organoleptic testing

Fish samples were cleaned and deep fried for about 15 minutes in edible cooking vegetable oil (Figure 1) for organoleptic testing at a 3 hour interval. Panelists scored flavour and general acceptability by ranking 5=definitely like, 4=slightly like, 3=neither like nor dislike, 2=slightly dislike, and 1=definitely dislike. Consumer ethical conduct was followed by explaining the whole experimental process to the panelists before the organoleptic testing. Assessors were briefed about the type of fish species which they would taste and the source of the fish including the mode of processing e.g. the type of cooking oil used. Each panelist therefore participated in the study with full knowledge of the process. The study was also conducted within ranges when the fish were assumed to be in the conditions acceptable to consumers to allow organoleptic testing without endangering the panelists.

Proximate analysis

Fresh *E. sardella* fish samples were analysed for proximate composition (protein, fat, ash and moisture) following a procedure by AOAC (2005) every 3 hours until the experiment was terminated by the sensory panelists after rejecting the samples.

Determination of pH

To determine pH, a procedure earlier applied by Kapute *et al.* (2013) was used. A 10 g sample of ground fish paste was homogenized in 50 ml of distilled water then centrifuged using a magnetic stirrer, and the mixture was filtered using Whatman filter paper No.1. pH was measured by inserting a pH meter electrode into the homogenate after calibration using standard buffers of pH 7 and 4 at 25°C.

Table 1. Quality Index Method (QIM) scheme developed for the assessment of fresh *Engraulicypris sardella* at ambient storage

Quality parameter		Description	Scores
Appearance	Skin	Shiny silver	0
		Dark silver	1
	Scale	Firm	0
		Loose	1
Eyes	Cornea	Very clear (glass like)	0
		Reddish clear	1
		Reddish	2
Gills	Color	Bright red	0
		Pale red	1
		Dull red	2
		Brown	3
	Smell	Neutral	0
		Fishy	1
		Weedy	2
	Mucus	Clear	0
		Cloudy	1
		Milky	2
Texture	Backside	Firm & elastic / in rigor	0
		Soft	1
		Very soft/depression	2
	Belly	Firm	0
		Soft	1
		Bulging	2
Total QI score			15

Data analysis

The results from the QIM evaluation and proximate analysis were analysed in the statistical programme SPSS for Windows version 20 with one-way analysis of variance (ANOVA) for the normalized data. Means that were statistically different were separated using Duncan´s Multiple comparison test at 5% level of significance. Pearson correlations between moisture, protein, fat, ash, pH, QI scores, organoleptic scores and storage time were also performed in SPSS. A linear regression for quality index (QI) scores against storage time was fitted using Microsoft Excel for Windows 2013 where QI and time were explanatory and independent variables respectively.

Results

Sensory analysis and organoleptic tests

Quality Index scores increased significantly with storage period with a strong positive correlation (R^2=0.994) suggesting decrease in consumer acceptance of the fish (Figure 2). Fish were rejected by panelists after 14 hours of ambient storage when maximum QI score of 13.6 was attained (Figure 2). This was well supported by the striking difference

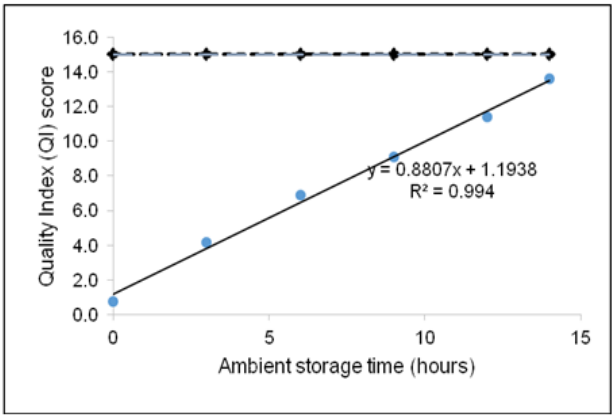

Figure 2. Quality Index scores of fresh *Engraulicypris sardella* stored at ambient temperature

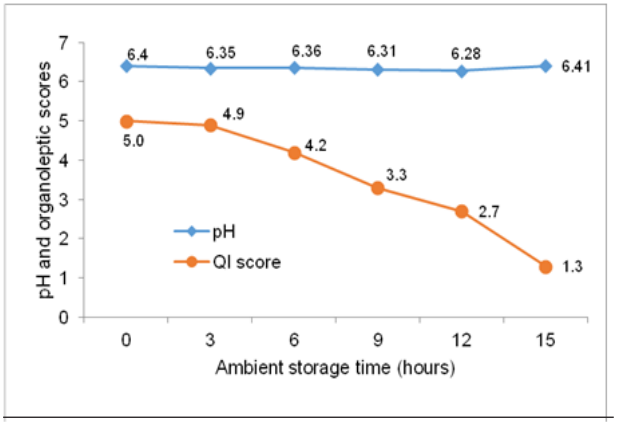

Figure 3. pH and Organoleptic quality index scores (QI) of fresh-deep fried *Engraulicypris sardella* stored at ambient temperature

in appearance between the sensory attributes at time zero and after 14 hours of ambient storage. Gills changed from bright red to dark red while the eye cornea showed some reddish colour at 14 hours from a clear appearance in a fresh fish. The belly of the fish which was stiff at zero hour ruptured when fish were kept up to 14 hours denoting a product completely unacceptable. Significant changes in QI scores were observed between 6 and 9 hours an indication that panelists observed notable changes in the freshness quality of the fish. This agreed with the organoleptic scores (Figure 3) where consumers' liking of the fried fish drastically decreased from 4.9 to 4.2 after 6 hours of ambient storage while the change from 0 to 3 hours was only 0.1 i.e. from 5.0 to 4.9. There was a strong negative correlation (-.973) between organoleptic scores and storage time suggesting decreasing consumer acceptability with time.

Proximate analysis

There were significant differences ($P<0.05$) in moisture, protein, fat and ash contents with a decreasing trend against ambient storage time (Table 2). The lowest moisture, protein, fat and ash were observed in samples at 15 hours of storage. Significant changes ($P<0.05$) in the nutrients were observed at 6 hours of storage time also agreeing with results from sensory and organoleptic tests. All nutrients highly negatively correlated with storage time, -.972, -.926, -916 and -.966 for moisture, ash, fat and protein respectively ($P<0.05$) denoting loss of nutrients with time in storage.

pH

pH of the samples (Figure 3) was close to normal for fresh fish (6.40) at zero hour but decreased sharply ($P<0.05$) during the first 3 three hours of storage to 6.35 then increased to 6.36 after 6 hours. pH then drastically decreased to its lowest point at 12 hours before rising sharply to 6.41 at 14 hours of ambient storage (rejection time).

Discussion

Although fish were rejected after 14 hours, it was apparent that assessors' notable sensory changes were reported after 6 hours indicative of the best freshness quality for fresh *E. sardella* kept at ambient temperature. The unreliability of using scales and skin appearance in assessing freshness quality in fish observed in this study has been earlier reported by Kapute *et al*. (2013) that these are usually affected by handling of the fish and not necessarily due to changes in freshness quality. Results nevertheless, confirm previous findings (Botta, 1995; Huss, 1995; Hyldig and Green-Petersen, 2004; Kapute *et al*., 2013) that gills (appearance and colour), and eyes (cornea) still remain reliable indicators for assessing freshness quality of fresh fish. However, unlike bigger sized fish such as Tilapia where gills are often used as a freshness quality attribute, this is not the case for *E. sardella* in an ideal local market situation due to its tiny size (pers. obs). *E. sardella* is sold in heaps and it is a common tradition that consumers rely on use of appearance of the eye (cornea) to determine freshness quality (pers. obs). Bursting of belly observed in this study after 15 hours of ambient storage is indicative of heavy digestive enzymatic activity present in the gut of the fish that causes decomposition in the fish (Huss, 1995; Singh *et al*., 2011). Declining acceptability of the fish could likely be attributed to the fishy odour and bitter flavour produced which is consistent with spoiling fish (Kapute *et al.,* 2013; Makawa *et al*., 2014). Frying improves the taste of food (Makawa *et al*., 2014; Joram and Kapute, 2016). It is likely that the fish could have been declared not fit for consumption much earlier than 14 hours had other methods of cooking such as boiling been used.

Table 2. Proximate composition of fresh *Engraulicypris sardella* at different ambient storage periods (hours)

Time interval	Parameter			
(hours)	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
0	93.55±0.08^{a}	63.60±0.12^{a}	21.56±0.35^{a}	19.65±0.41^{ab}
3	93.21±0.24^{b}	63.06±0.16^{b}	20.00±0.54^{b}	19.71±0.26^{a}
6	93.15±0.11^{b}	62.56±0.15^{c}	19.41±0.06^{bc}	19.67±0.04^{ab}
9	92.98±0.08^{bc}	62.63±0.40^{bc}	18.89±0.21^{cd}	19.35±0.03^{abc}
12	92.81±0.06^{c}	62.27±0.06^{cd}	18.77±0.22^{cd}	19.15±0.13^{bc}
15	92.76±0.08^{c}	61.92±0.07^{d}	18.56±0.03^{d}	18.84±0.03^{c}

Means without a common superscript in a column are significantly different ($P<0.05$). (Mean ± Std. deviation)

This may explain why dishonest fish traders resort to frying or smoking stale fish other than presenting them fresh or use normal cooking (pers. obs).

Proximate composition is a reliable objective indicator for determining nutritional value and quality of fish (Sutharshiny and Sivashanthini, 2011). Its importance in studies such as this cannot be underestimated because spoilage in fish, affects its nutritional quality (Aberoumad, 2013; Makawa *et al.*, 2014). Declining levels in nutrients of fresh *E. sardella* observed in this study may therefore be attributed to spoilage of the fish. Like for sensory and organoleptic results, rapid changes in nutrient reduction were observed between 6 and 9 hours of ambient storage (Table 2) suggesting that freshness quality was affected by spoilage of the fish as well as loss of nutrients.

Despite periodic sprinkling of water onto the fish, decrease in moisture with storage period earlier reported by Osibona and Ezekiel (2014) could be attributed to the difference in the moisture of the fish relative to its surrounding (Daramola *et al.*, 2007). Declining protein content has been linked to gradual degradation of the initial crude protein to more volatile products associated with autolytic deterioration of the endogenous enzymes and bacteria (Hultman and Rustad, 2004; Okeyo *et al.*, 2009; Ayeloja *et al.*, 2011). *E. sardella* is a fatty species (Mumba and Jose 2005) and reduction in fat could be due to oxidation and breakdown of poly-unsaturated fatty acids during ambient storage earlier reported by Daramola *et al.* (2007) and Makawa *et al.* (2014). Many Malawians consume *E. sardella* due to its plentiful supply and affordable prices on the market. Proper storage is therefore paramount to minimize or avoid loss of nutrients, mainly protein. Despite the gradual decrease in ash, results show that the changes were not significant indicating that *E. sardella* has high ash content. This may be attributed to the fact that ash content is mainly determined by bone to flesh ratio (Daramola *et al.*, 2007) and *E. sardella* as a small sized species falls into the same category. Ash is the inorganic residue that remains after water and organic matter have been removed and is an indicator of total amount of minerals in a food (Holma and Maalekuu, 2013).

Initial decrease in pH followed by a rise towards rejection point in fresh fish may be attributed to formation of lactic acids which occurs during the first hours of rigormotis after death of fish (Mørkøre *et al.*, 2010) earlier reported by Obemeata and Christopher (2012); Kapute *et al.* (2013) and Makawa *et al.* (2014). Sharp decline in pH observed between 6 and 9 hours that may suggest heightened autolytic activities appears to correspond to timing of significant changes in sensory and organoleptic results. Increase in pH towards and after sensory rejection may be as a result of accumulation of alkaline compounds as well as volatile bases produced by autolytic activities and metabolism of spoilage bacteria (Liu *et al.*, 2010). Rising pH towards the 12th hour of ambient storage may explain the rapid spoilage of the fish evidenced by high QI and low organoleptic scores (Kapute *et al.*, 2013). Increase in pH towards normal (7.0) creates favourable conditions for microbial activity resulting into spoilage of fish (Huss, 1995).

Conclusion

Freshness quality of *E. sardella* fish can remain acceptable between 3 and 6 hours. However, if processed (deep fried), time of acceptability increased up to 14 hours suggesting that processing improves organoleptic quality of fish. The study has also demonstrated that optimum nutrient content in *E. sardella* can be obtained if fish are consumed not later than 6 hours of ambient storage. This study therefore, recommends that fresh *E. sardella* should be presented in a processed form beyond 6 hours of ambient storage to extend consumers' liking of

the fish. Although microbiological analysis of the samples was not carried out, the assumption that microbes cannot survive in fish that are deep fried in cooking oil, may also help in protecting consumers from microbial food poisoning.

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