

# Assessment of insect contamination, acid insoluble ash content and colour characteristics of traditionally sun-dried and oven-dried *dagaa* (*Rastrineobola argentea*)

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## ABSTRACT

**Objective:** To determine and investigate the influence of selected pre-washing treatments and drying temperatures on the colour characteristics, insect infestation and insoluble ash content of sun-dried *dagaa* (a small pelagic fish found in Lake Victoria).

**Methodology and results:** Insect infestation, colour (lightness L\*; redness a\*; yellowness b\*) and acid insoluble ash contents were analyzed on *dagaa* that were sun-dried or oven-dried at 30, 40 and 50°C after pre-washing with salted water (3% NaCl), chlorinated water (100ppm) solutions and potable tap water (control). Insect fragments including blowfly (*Lucilia spp*), Beetle (*Dermestes spp*), larvae and mites were present on the sun-dried *dagaa*. The acid insoluble ash in the sun-dried market samples (0.46% dry weight basis, dwb) was significantly higher ( $p < 0.05$ ) than in the fresh *dagaa* (0.11% dwb). The L\* values of fresh *dagaa* (47.98) were significantly ( $p < 0.05$ ) lower than in market samples (67.16). Chlorinated-wash treatments had significantly ( $p < 0.05$ ) higher L\* values compared to those washed in salted water and tap water (control).

**Conclusion and application of findings:** The study established that sun-drying *dagaa* in the field predisposes them to contamination by insects and grit. In the oven-dried *dagaa*, the chlorinated-wash treatments exhibited higher L\* values and lower a\* and b\* values than the control-wash treatments. The apparent increase in the L\* and b\* values and decline in a\* values could be attributed to the reactions of lipid oxidation products. This study emphasize the importance of improved handling and drying of *dagaa* in order to prevent occurrence of insects, grit and sand contamination; and colour degradation of *dagaa*. The knowledge will improve the capacity of local fishing communities on appropriate washing and drying methods for fish.

**Key words:** *dagaa*; colour, acid insoluble ash, insect contamination

## INTRODUCTION

Commercially important fish species in the Kenyan markets include Nile perch (*Lates niloticus* L.),

tilapia (*Oreochromis niloticus* L.) and *dagaa* (*Rastrineobola argentea*) (Abila, 1998). However, a

key feature of Lake Victoria's fisheries ecosystem is the abundance of the small pelagic fish species, *dagaa*, which constitutes the second largest volume (62.9%) of the total fisheries resources landed from L. Victoria, Kenya (Nyeko, 2008). Although *dagaa* landings are high, the value of the catch is very low. In Kenya, post harvest losses in the *dagaa* sub-sector is estimated at between 20 - 30% and even up to 50% during the rainy season (Ofulla *et al.*, 2007). This is strongly attributed to physical losses, colour change, insect infestation, grit and sand contamination (Mndeme, 1998).

After harvesting, most of the *dagaa* are sun-dried on grass mats, which are laid on the bare ground without protection from contaminants (Nyangambi, 1982; Mwambazi, 1992; Eyabi, 1998). Acid insoluble ash (AIA) is a common test used to assess sand and dirt contamination (Adidair, 1995). According to the Kenya Bureau of Standards, the maximum acceptable limit for acid insoluble ash in dried fish is 1.5% (KEBS, 1998). The rapid colour loss is attributed to the rather slow sun-drying process especially during rainy and humid seasons. Colour change in meat muscles is influenced by breakdown of the ferric molecule of the oxymyoglobin (Faustman *et al.*, 1992; Onyango, 1999) and lipid oxidation reactions (Smith and Hole, 1991; Seymour *et al.*, 1998). A common method for colour determination is the International Commission on Illumination (CIE) colour system based on lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) scale.

The extent of insect infestation in dried fish is influenced by factors such as length of storage,

salt content and the climatic conditions (Daniel & Etoh, 1983; Eyo & Awoyemi, 1998). On landing, wet fish is susceptible to infestation by blowflies such as *Chrysomya spp.*, *Lucilia spp.*, and *Wohlfartia spp.* (Taylor, 1982). However, during storage, beetles such as the De Geer (*Dermestes maculatus*, *Dermestes ater*, *Dermestes frischii*) and their respective larvae are predominant. The presence of mites in stored food is, however, indicative of moist storage conditions (Allotey *et al.*, 1998). The health hazards presented by insects arise from the pathogenic bacteria and fungi they transfer onto the fish products. The insect filth are also undesirable because of their offensive odour, presence of insect bodies, cast skin of larvae and their droppings.

As food security increasingly becomes an issue of national concern in Kenya, the fish industry has been identified as one of the sectors whose improvement would effectively contribute towards alleviation of food insecurity. There is therefore, need for improved handling and processing techniques that would mitigate losses and ensure effective utilization of sun-dried *dagaa* for human consumption.

This study was, therefore, undertaken to determine the occurrence of insect infestation, acid insoluble ash content and colour ( $L^*$ ,  $a^*$ ,  $b^*$ ) characteristics of sun-dried *dagaa*. The study also investigated the effect of selected pre-washing treatments and drying temperatures on the above characteristics on oven-dried *dagaa*.

## MATERIALS AND METHODS

This study was conducted in two phases as described below:

### (a) Sampling of sun-dried *dagaa* samples

Three batches of freshly caught *dagaa* samples of one kg each were collected randomly from fishermen at each of the three landing sites i.e. Dunga, Tako and Block sites located in Kisumu, Kenya. The samples from each site were mixed to obtain a composite sample. These sites are officially recognized by the Ministry of Fisheries Development under the Beach Management Unit Programme.

Three batches of sun-dried *dagaa* samples (one kg each) were collected randomly after drying for 1, 2, 3 and 4 consecutive days respectively from the drying sites located next to the three landing sites mentioned above. Three batches of dried *dagaa* (one kg each) that had been in the retail market for one week were randomly sampled from three traders at Kibuye market. This is the largest retail market in Kisumu town. Ordinarily, *dagaa* stock would last for 1 week in retail market, therefore the sun-dried *dagaa* were held for one week at the prevailing market conditions. These market samples had previously been sun-dried for a

period of four days by the identified traders who purchase them after drying from the three landing sites. The temperature and relative humidity conditions were monitored during sampling and ranges of 19-33°C and 70-84% realized respectively. The samples were transported on the same day in a cool box (4 - 9°C), to the Department of Food Science and Technology at Jomo Kenyatta University of Agriculture and Technology (JKUAT) for analysis of colour characteristics, insect infestation and acid insoluble ash content.

#### **(b) Oven-dried *dagaa* samples**

Three batches of freshly caught *dagaa* samples of one kg each were collected randomly from fishermen at each of the three landing sites i.e. Dunga, Tako and Block sites located in Kisumu, Kenya. The samples from each site were mixed to obtain a composite sample. The samples were transported on the same day in a cool box (4 - 9°C) to the laboratory at JKUAT.

In the laboratory, the fresh *dagaa* samples were washed with selected solutions and oven-dried under pre-determined temperatures as shown in Figure 1. *Dagaa* (800g) were washed with salted solution (3% sodium chloride), chlorinated solution (100 ppm) or potable tap water (control) in a 1:2.5 (w/v) ratio for fish to wash solutions. Each treatment was replicated three times. The washing operation involved placing the fresh *dagaa* in a standard mesh stainless steel sieve no. 8, and passing respective chilled (4 - 6°C) wash solutions through the sieve. The washed *dagaa* was allowed to drain excess liquid and subsequently oven-dried at 30, 40 or 50°C using the Eyela Windy oven (WFO - 1000ND, Tokyo Rikakikai Co. Ltd).

The ultimate drying duration for each of the selected temperatures was determined in the preliminary trials as the time it took to reduce the moisture content of the fish to below 10%, the standard requirement for dried fish products (KEBS, 1998). The durations realized

were: 31 h at 30°C, 23h at 40°C and 15h at 50°C. The dried *dagaa* samples were then packaged in low-density polyethylene (LDPE) bags and stored for 9 days at laboratory room ambient conditions.

**Determination of insect contamination:** Insect contamination was determined using the standard mesh sieve method (Adidair, 1995). The trap-offs were ultimately transferred to a beaker and filtered by suction using a Buchner funnel and a filter paper. The whole insects and equivalent fragments were identified according to the illustrations by Allotey *et al.* (1998).

**Determination of acid insoluble ash (AIA):** AIA content was determined according to the muffle furnace method described by Adidair (1995) based on the residual matter obtained relative to the sample weight

#### **Determination of L\* a\* b\* colour characteristics:**

The colour of fish samples was determined using tristimulus colorimeter (Minolta Chroma Meter CR-2006). *Dagaa* samples (20g) were ground and placed in a polyethylene cling film and readings were taken by placing the colorimeter onto the surface of the triplicate samples and results expressed in terms of the CIE L\* a\* b\* colour values. Measurements were standardised with a white calibrating tile (L\* 96.1; a\* + 46.5; b\* +12.8).

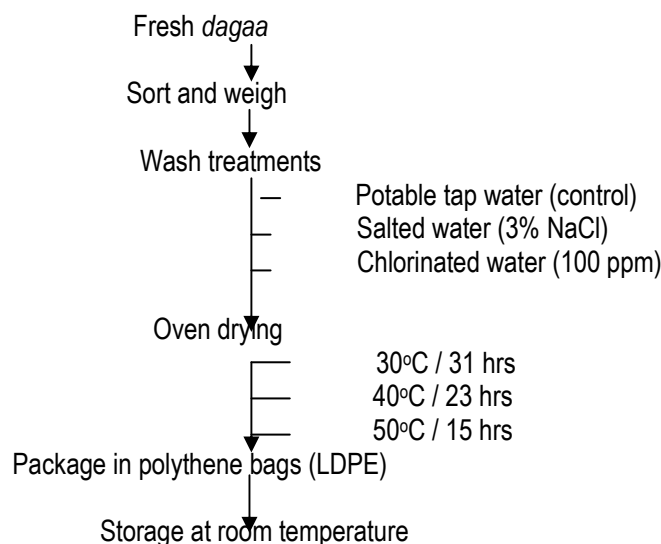
**Statistical analysis:** All treatments were conducted in triplicates. Oven drying experiments were planned in a randomised complete design involving 3 wash treatments (control, salted, chlorinated water) (WT), 3 drying temperatures (30, 40 and 50°C) (DT) and 5 storage periods (day 1, 3, 5, 7, 9) (SP). Treatments of dried *dagaa* were prepared using a 3WT × 3DT × 5SP factorial arrangement. The differences among treatments were tested by use of ANOVA while Duncan's multiple range test was used to determine significant differences between means at 5% (p<0.05) level of significance using COSTAT statistical package (Costat, 1990).

## **RESULTS AND DISCUSSION**

### **Insect contamination of sun-dried and oven-dried *dagaa*:**

The blowfly (*Lucilia spp*) counts varied between 4 flies in *dagaa* after day drying and 1 fly in the samples that had been dried for 4 days consecutively (Table 1). The beetles (*Dermestes spp*) and larvae were only isolated from the market samples that were on average one week old. According to Kenyan standards (KEBS, 1998), there is zero tolerance for insect contamination in fish products. It was observed that the market display

techniques of *dagaa* are either open or in stalls that are not insect proof, thus exposing the fish to the flying adults and crawling larvae. The oven-dried *dagaa* were physically intact with no visible insect filth. The risks of such infestation were reduced by the hygienic measures implemented during handling through various washing operations. The use of polythene packaging during storage also served as a barrier to attack from insects.



**Figure 1:** Flow diagram for the processing of oven-dried *dagaa*

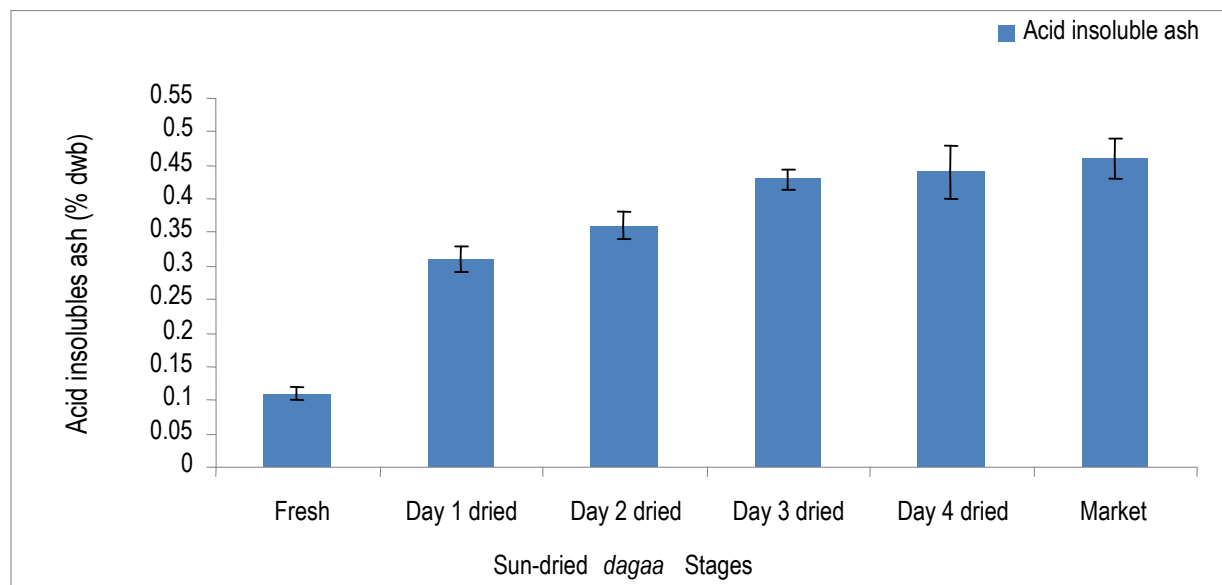
**Table 1:** Insect counts on sun-dried *dagaa* samples obtained as freshly caught, dried (obtained after 1, 2, 3 and 4 days of consecutive drying) and market samples.

Process stage	Blow fly ( <i>Lucilia spp</i> )	Mites	<i>Dermestes</i> larvae	Beetles ( <i>Dermestes</i> <i>spp</i> )	Total insect fragments
Fresh	ND <sup>2</sup>	ND	ND	ND	ND
day 1 dried	6	5	ND	ND	4
day 2 dried	4	3	ND	ND	6
day 3 dried	3	2	ND	ND	7
day 4 dried	1	2	ND	ND	6
Market	ND	1	2	2	4

<sup>1</sup> Values are means of triplicate determinations. Means in a column followed by the same letters are not significantly different ( $p < 0.05$ ). Counts expressed per 50g of sample. <sup>2</sup> ND = Not detected.

**Acid insoluble ash of sun-dried and oven-dried *dagaa*:** The acid insoluble ash values increased significantly ( $p < 0.05$ ) from 0.11% (dwb) in the freshly caught *dagaa* to 0.46% (dwb) in the market samples (Figure 2). The increasing trend observed along the handling chain, could be attributed to contamination by extraneous matter. At landing the fish are often sorted on the sandy beaches, and further whereas the drying

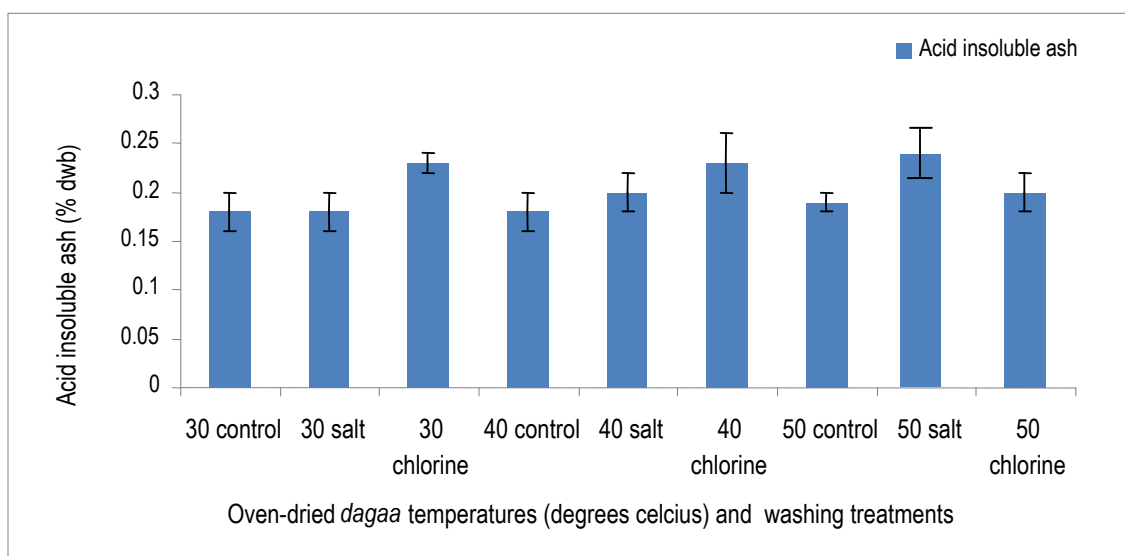
and marketing practices further expose the fish to sand and grit contamination. However, the levels shown in this study were below the 1.5% maximum acceptable limit for dried fish (KEBS, 1998). Other authors have reported higher insoluble ash content ranging between 0.12 – 2.51% both in fresh and dried fish (George et al., 1983; 1988)



**Figure 2:** Acid insoluble ash content of sun-dried *dagaa* samples obtained as freshly caught, dried (day 1, 2, 3 and 4 of consecutive drying) and market samples. Error bars =Standard Deviation; dwb = dry weight basis.

In the oven-dried *dagaa*, the acid insoluble ash content varied from 0.18% (dwb) in the 40°C control to 0.23% (dwb) in the 30°C chlorinated-wash treatments (Figure 3). No significant ( $p < 0.05$ ) differences were observed between all the washing and drying temperature treatments. However, the various washing procedures incorporated in this study caused a substantial removal

of sandy extraneous matter, from the surface of the fish prior to drying. The acid insoluble ash values observed for chlorinated treatments at 30°C and 40°C were generally higher when compared to 50°C. This could be attributed to the volatile nature of the hyperchlorite compounds hence its increased loss at 50°C.

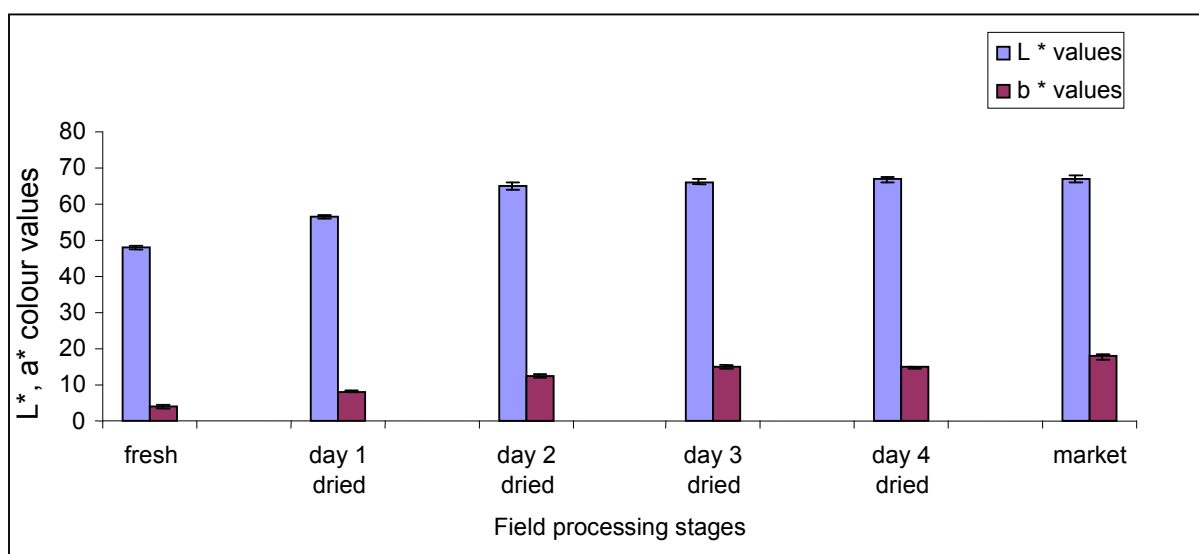


**Figure 3:** Acid insoluble ash content of oven-dried *dagaa* dried at 30, 40 or 50°C after washing with tap water, salted or chlorinated solutions. Error bars =Standard Deviation, dwb = dry weight basis.

### Colour properties of sun-dried and oven-dried *dagaa*:

The lightness ( $L^*$ ) values increased significantly ( $p < 0.05$ ) from 47.98 in the freshly caught fish to 67.16 in the sun-dried market samples (Figure 4). The redness ( $a^*$ ) values declined significantly ( $p < 0.05$ ) from 1.41 in the fresh fish to 0.21 in the market samples (Figure 5). The yellowness ( $b^*$ ) increased significantly ( $p < 0.05$ ) from 3.96 to 17.98 for freshly caught fish and market samples respectively. The degradation of colour in meat and fish products from bright red to brown hues during processing and storage has been primarily associated with the oxidation of oxymyoglobin to ferric red metmyoglobin (Faustman et al., 1992; Onyango, 1999). This may account for the decline in  $a^*$  values along the process chain of sun-dried *dagaa*. However, in fish there is an additional interaction between lipid

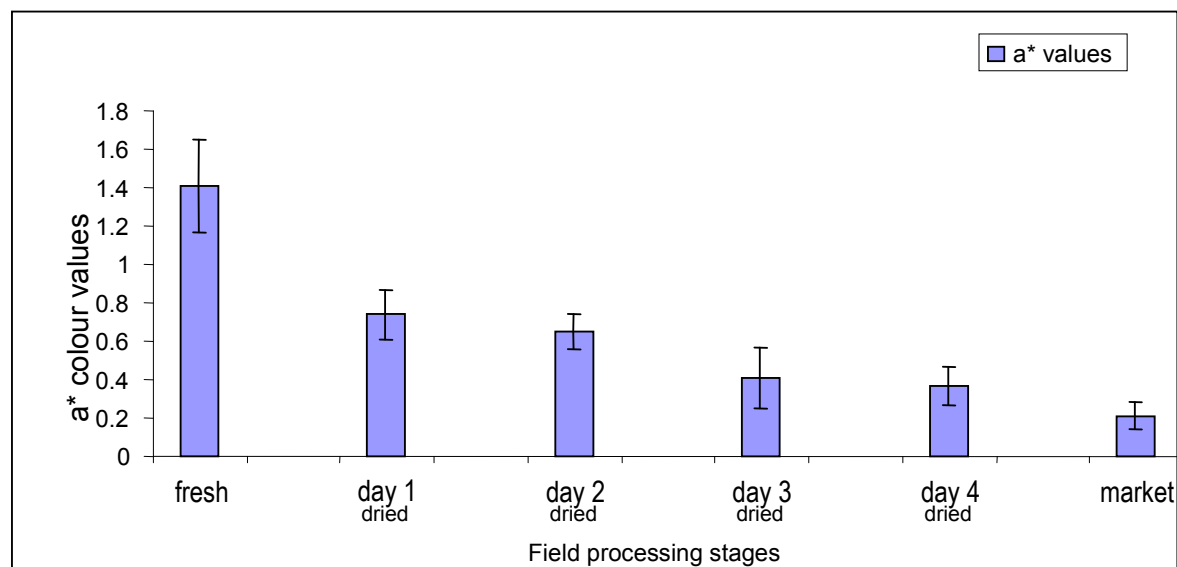
hydroperoxides with pigments and other macromolecules (Smith and Hole, 1991; Seymour et al., 1998). The uncontrolled exposure of sun-dried *dagaa* to light and air, during drying and marketing could have led to a series of alterations in its colour. The apparent increase in the lightness and browning most likely reflects the reactions of lipid oxidation products, such as aldehydes, with proteins, amino acids and nitrogen containing phospholipids which form browning and fluorescent products (Hasegawa et al., 1992; Perlo et al., 1995). In order to control the rapid colour loss, it would be recommended that emphasis should be laid on adoption of improved sun-drying technologies such as use of raised drying racks so that the drying process is completed in a shorter time.



**Figure 4:** The  $L^*$  and  $b^*$  colour values of sun-dried *dagaa* samples obtained as freshly caught, dried (day 1, 2, 3 and 4 of consecutive drying) and market samples. Error bars =Standard Deviation.

In the oven-dried *dagaa* study, the chlorinated-wash treatments showed significantly ( $p < 0.05$ ) higher  $L^*$  values compared to those washed with salted or tap water on drying at 30, 40 and 50°C at day one (Table 2), possibly due to the bleaching effect of the hypochlorous solution on the fish pigmentation. However,  $L^*$  values for the salted-wash treatments were significantly ( $p < 0.05$ ) higher at 40°C than values observed at 30 and 50°C. At 30°C,  $a^*$  values for the tap water wash treatments were significantly ( $p < 0.05$ ) higher when compared to the chlorinated and salt water wash treatments (Table 3). The browning of the dried

fish flesh has been reported to develop linearly with progressive lipid oxidation (You and Lee, 1982). This could be indicative of the loss of oxymyoglobin levels, which is red in colour. In a separate study by this author, it was realized that use of chlorinated water has a reduction effect on the rate of lipid oxidation when analyzed as thiobarbituric acid reactive substances, when compared to salted solution and tap water (Owaga et al., 2009). This observation has been attributed to the hypochlorite inhibiting the activity of lipid oxidation enzymes.



**Figure 5:** The  $a^*$  colour values of sun-dried *dagaa* samples obtained as freshly caught, dried (day 1, 2, 3 and 4 of consecutive drying) and market samples. Error bars =Standard Deviation.

The salted water wash treatments showed significantly ( $p < 0.05$ ) higher  $b^*$  values relative to the chlorinated and tap water wash treatments when dried at 30°C (Table 4). However, the salted water wash treatments indicated comparatively higher  $b^*$  values than the tap water wash treatments probably due to the pro-oxidant effect sodium chloride on the rate of lipid oxidation (Owaga *et al.*, 2009). The  $b^*$  values of the tap, salted and chlorinated water wash treatments dried at 40 and 50°C were significantly ( $p < 0.05$ ) higher than the values obtained at 30°C. Higher temperatures have higher rates of lipid oxidation hence higher  $a^*$  values of dried *dagaa* at elevated temperatures.

In conclusion, the presence of insect contaminants in the *dagaa* dried by sun in the field was an indicator of unhygienic handling and storage conditions of the products. In contrast, no insect was detected in the oven-dried *dagaa*, thus demonstrating the effectiveness

of this processing method. It is therefore possible to produce oven-dried *dagaa* of high quality as long as hygienic measures that alleviate insect infestation and contamination of extraneous matter are implemented. This can be achieved at the local community level through development of appropriate design and modification of the current sun-drying methods with regard to the product quality and reduced processing time through use of raised-rack driers and solar-driers. The data obtained will increase the knowledge base for policy making in the artisanal fish sector concerning the use of improved handling and processing techniques of artisanal dried fish products. These policies and programmes, however, should be developed participatorily including the stakeholders namely fish traders, researchers, extension officers, other relevant officers from government and non-governmental institutions and the community.

#### ACKNOWLEDGEMENT

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**Table 2:** Changes in lightness (L\*) after drying and during storage of oven-dried *dagaa*<sup>1</sup>

Days	30°C			40°C			50°C		
	Control	Salted Wash	Chlorinated wash	Control	Salted wash	Chlorinated Wash	Control	Salted wash	Chlorinated wash
day 1	64.46± 0.36 <sup>d</sup>	67.44± 0.33 <sup>c</sup>	68.60± 0.23 <sup>c</sup>	67.17± 0.04 <sup>c</sup>	68.55± 0.21 <sup>b</sup>	69.68± 0.31 <sup>b</sup>	67.54±0.36 <sup>b</sup>	67.54± 0.29 <sup>b</sup>	67.58± 0.37 <sup>b</sup>
day 3	66.50± 0.27 <sup>c</sup>	68.39± 0.29 <sup>b</sup>	69.51± 0.39 <sup>b</sup>	68.53± 0.26 <sup>b</sup>	68.57± 0.29 <sup>b</sup>	70.56± 0.34 <sup>a</sup>	67.42± 0.30 <sup>b</sup>	67.51± 0.36 <sup>b</sup>	67.44± 0.25 <sup>b</sup>
day 5	68.50 ± 0.29 <sup>a</sup>	68.43± 0.29 <sup>b</sup>	68.58± 0.26 <sup>c</sup>	68.64± 0.36 <sup>b</sup>	67.44± 0.30 <sup>c</sup>	69.43± 0.21 <sup>b</sup>	67.53± 0.39 <sup>b</sup>	66.65± 0.17 <sup>c</sup>	68.33± 0.23 <sup>a</sup>
day 7	67.57± 0.03 <sup>b</sup>	70.51± 0.24 <sup>a</sup>	70.51± 0.24 <sup>a</sup>	69.50± 0.31 <sup>a</sup>	68.41± 0.24 <sup>b</sup>	68.75± 0.26 <sup>c</sup>	67.68± 0.35 <sup>b</sup>	67.58± 0.41 <sup>b</sup>	68.29± 0.28 <sup>a</sup>
day 9	67.51± 0.33 <sup>b</sup>	70.85± 0.16 <sup>a</sup>	70.67± 0.34 <sup>a</sup>	69.46± 0.25 <sup>a</sup>	70.50± 0.40 <sup>a</sup>	70.63± 0.31 <sup>a</sup>	68.37± 0.23 <sup>a</sup>	68.34± 0.26 <sup>a</sup>	68.41± 0.24 <sup>a</sup>

<sup>1</sup>Values are a mean of triplicate determinations ± Standard Deviation. Means in a column followed by the same letter are not significantly different (p<0.05).

**Table 3:** Changes in redness (a\*) after drying and during storage of oven-dried *dagaa*<sup>1</sup>

days	30°C			40°C			50°C		
	Control	Salted wash	Chlorinated wash	Control	Salted wash	Chlorinated Wash	Control	Salted wash	Chlorinated wash
day 1	0.72± 0.08 <sup>d</sup>	1.33± 0.15 <sup>a</sup>	0.40± 0.23 <sup>d</sup>	0.70± 0.18 <sup>c</sup>	0.81± 0.43 <sup>d</sup>	0.68± 0.22 <sup>bc</sup>	0.75± 0.13 <sup>c</sup>	0.88± 0.05 <sup>b</sup>	0.81± 0.11 <sup>c</sup>
day 3	0.92± 0.06 <sup>d</sup>	1.35± 0.27 <sup>a</sup>	0.52± 0.29 <sup>cd</sup>	0.73± 0.16 <sup>c</sup>	1.35± 0.27 <sup>c</sup>	0.47± 0.20 <sup>c</sup>	1.34± 0.33 <sup>b</sup>	1.44± 0.32 <sup>a</sup>	1.21± 0.24 <sup>b</sup>
day 5	1.43 ± 0.35 <sup>b</sup>	1.38± 0.24 <sup>a</sup>	0.74± 0.32 <sup>bc</sup>	0.83± 0.08 <sup>c</sup>	1.51± 0.35 <sup>bc</sup>	0.86± 0.09 <sup>b</sup>	1.25± 0.11 <sup>b</sup>	1.59± 0.36 <sup>a</sup>	1.28± 0.29 <sup>b</sup>
day 7	1.15± 0.08 <sup>c</sup>	1.29± 0.38 <sup>a</sup>	0.83± 0.08 <sup>b</sup>	1.59± 0.25 <sup>b</sup>	1.73± 0.19 <sup>ab</sup>	1.73± 0.22 <sup>a</sup>	1.41± 0.24 <sup>b</sup>	1.37± 0.20 <sup>a</sup>	1.12± 0.06 <sup>b</sup>
day 9	1.88± 0.08 <sup>a</sup>	1.50± 0.45 <sup>a</sup>	1.25± 0.18 <sup>a</sup>	1.88± 0.09 <sup>a</sup>	1.85± 0.07 <sup>a</sup>	1.62± 0.26 <sup>a</sup>	1.73± 0.16 <sup>a</sup>	1.52± 0.20 <sup>a</sup>	1.75± 0.12 <sup>a</sup>

<sup>1</sup>Values are a mean of triplicate determinations ± Standard Deviation. Means in a column followed by the same letter are not significantly different (p<0.05).



**Table 4:** Changes in yellowness ( $b^*$ ) after drying and during storage of oven-dried *dagaa*<sup>1</sup>

days	30°C			40°C			50°C		
	Control	Salted wash	Chlorinated wash	Control	Salted wash	Chlorinated wash	Control	Salted wash	Chlorinated wash
day 1	10.64± 0.29 <sup>b</sup>	12.13± 0.55 <sup>a</sup>	9.56± 0.36 <sup>d</sup>	11.57± 0.22 <sup>b</sup>	11.53± 0.39 <sup>d</sup>	10.79± 0.15 <sup>b</sup>	11.59± 0.34 <sup>c</sup>	11.42± 0.36 <sup>b</sup>	10.58± 0.24 <sup>b</sup>
day 3	12.36± 0.33 <sup>a</sup>	12.49± 0.29 <sup>a</sup>	11.63± 0.14 <sup>c</sup>	11.61± 0.23 <sup>b</sup>	12.82± 0.29 <sup>b</sup>	10.66± 0.22 <sup>b</sup>	12.53± 0.22 <sup>ab</sup>	12.48± 0.30 <sup>b</sup>	12.39± 0.55 <sup>a</sup>
day 5	12.33 ± 0.25 <sup>a</sup>	12.19± 0.30 <sup>a</sup>	12.34± 0.23 <sup>b</sup>	11.57± 0.31 <sup>b</sup>	12.46± 0.32 <sup>c</sup>	10.62± 0.28 <sup>b</sup>	12.66± 0.21 <sup>a</sup>	12.61± 0.27 <sup>c</sup>	12.29± 0.22 <sup>a</sup>
day 7	12.34± 0.30 <sup>a</sup>	12.28± 0.22 <sup>a</sup>	12.37± 0.23 <sup>b</sup>	12.61± 0.17 <sup>a</sup>	12.29± 0.14 <sup>c</sup>	12.45± 0.24 <sup>a</sup>	12.48± 0.33 <sup>ab</sup>	12.60± 0.26 <sup>b</sup>	12.43± 0.32 <sup>a</sup>
day 9	12.63± 0.28 <sup>a</sup>	12.58± 0.25 <sup>a</sup>	12.74± 0.20 <sup>a</sup>	12.64± 0.21 <sup>a</sup>	13.37± 0.22 <sup>a</sup>	12.61± 0.21 <sup>a</sup>	12.27± 0.13 <sup>b</sup>	12.45± 0.26 <sup>a</sup>	12.72± 0.28 <sup>a</sup>

<sup>1</sup>Values are a mean of triplicate determinations ± Standard Deviation. Means in a column followed by the same letter are not significantly different ( $p < 0.05$ )

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