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EFFECT OF SELECTED WASHING TREATMENTS AND DRYING TEMPERATURES ON BIOCHEMICAL AND MICROBIOLOGICAL QUALITY OF *DAGAA* (*Rastrineobola argentea*)

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ABSTRACT

Dagaa (*Rastrineobola argentea)* is one of the most important fish foods for the low-income households in the Nyanza Province, Kenya. However, the off-flavour and off-odour that results from the traditional sun-drying process of sun-dried *dagaa* is a major disincentive to the use of the fish for human consumption, hence leading to utilization in animal feed. Chemical analyses for pH, Thiobarbituric reactive substances (TBARS), Total volatile bases-nitrogen (TVBN) and aerobic bacterial counts were carried out on *dagaa* sampled from various process steps within the open field sun-drying and market conditions. *Dagaa* was also oven-dried at 30^{o}C, 40^{o}C and 50^{o}C after washing with selected solutions namely salted (3% sodium chloride), chlorinated solutions (100ppm) and potable tap water (control). Results indicated that TBARS values increased significantly ($p<0.05$) from 1.39 mgMA/kg in fresh fish to 10.55 mgMA/kg in the market samples. The TVBN values increased significantly ($p<0.05$) from 9.42 mgMA/kg in fresh fish to 29.51 mg/ 100g in the market samples. The pH values declined significantly ($p<0.05$) from pH 6.72 in the fresh fish to pH 5.88 in the market samples .Lipid oxidation (TBARS) was significantly ($p<0.05$) higher in *dagaa* subjected to salted-wash treatments when compared to the chlorinated and control-wash treatments. The rate of lipid oxidation was significantly ($p<0.05$) higher at elevated temperatures of 50^{o}C relative to 30^{o}C and 40^{o}C conditions. The TVBN levels observed in the salted and chlorinated-wash treatments showed significantly ($p<0.05$) lower TVBN values when compared with the control-wash treatments. However, the values of TVBN obtained at 30^{o}C were significantly ($p<0.05$) higher when compared with the 40^{o}C and 50^{o}C drying temperature conditions. The salted-wash treatments resulted in lower pH values relative to the chlorinated and control-wash treatments on drying at 30^{o}C and 40^{o}C. In this study, the most appropriate treatment that showed the least TVBN and moderate TBARS values was drying the *dagaa* at 50^{o}C after washing with chlorinated solution.

Key words: *dagaa*, pH, TBARS, TVBN, bacteria

INTRODUCTION

Dagaa (*Rastrineobola argentea*) is a small pelagic fish that constitutes a large portion of Lake Victoria's fisheries ecosystem. *Dagaa* are entirely processed by sun drying process, which leads to significant losses in quality [1]. The characteristically undesirable flavor of the sun-dried *dagaa* is a major disincentive to the use of *dagaa* for human consumption, hence its utilization for production of fishmeal for animal feed.

Quality loss in *dagaa* is primarily associated with bacterial breakdown and lipid oxidation. The main pathway for the bacterial utilization of the nitrogenous compounds results in accumulation of compounds such as methyl mercaptan, hydrogen sulphide, dimethyl sulphide that are typical components of spoiling fish [2]. Determination of total volatile bases-nitrogen (TVBN) is commonly used to evaluate the extent of spoilage in fish [3, 4].

Lipid oxidation in fish can be manifested by changes in flavour, colour, texture and nutritive value [5, 6]. The degree of oxidation can be expressed at Thiobarbituric reactive substances (TBARS) value. Fish are excellent sources of unsaturated long chain free fatty acids, which make fish muscles highly susceptible to oxidation [7, 8]. Sodium chloride, which is an important food additive, has been reported to act generally as a pro-oxidant in muscle foods, thereby resulting in an increased level of lipid oxidation [9, 10, 11].

The constraints to effective and hygienic production of sun-dried *dagaa* indicate need for improved drying techniques. The knowledge that will be acquired in this study can be used to increase the supply of dried *dagaa* end products available for human consumption.

This study was, therefore, undertaken to determine the biochemical quality characteristics of the sun-dried *dagaa* and to also assess the effects of selected pre-washing treatments and drying temperatures combinations on the biochemical quality of 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 fresh *dagaa* samples of one kg each were collected randomly from fishermen at three landing sites namely Dunga, Tako and Block sites located in Kisumu. 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 days, respectively from the drying sites located next to the three landing sites. 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. The conditions were monitored and temperature ranged from 19-33^{o}C, whereas relative humidity ranged from 70-84%. The samples were transported on the same day in a cool box (4 - 9^{o}C), to the Department of Food Science and Technology at Jomo Kenyatta University of Agriculture and Technology.

(b) Sampling of oven-dried *dagaa* samples

Three batches of freshly caught *dagaa* samples of approximately one kg each, were collected randomly from fishermen at three landing sites namely Dunga, Tako and Block sites. The samples were transported on the same day in a cool box (4 - 9^{o}C) to the laboratory.

Washing and oven-drying of fresh *dagaa*

The fresh *dagaa* were washed with selected solutions and oven-dried under selected temperatures as shown in Figure 1. About 800g of the *dagaa* were washed with 2 litres of salt solution (3% NaCl), chlorinated solution (100 ppm) or potable tap water (control). 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^{o}C) wash solutions through the sieve. The washed *dagaa* were allowed to drain excess liquid and subsequently oven-dried at 30^{o}C, 40^{o}C and 50^{o}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%, which is the standard requirement for dried fish products [12]. The durations realized were: 30^{o}C (31hrs), 40^{o}C (23hrs) and 50^{o}C (15hrs). The dried *dagaa* samples were then packaged in low-density polyethylene (LDPE) bags and stored at ambient temperature, which ranged from 19 - 33^{o}C, whereas the relative humidity varied between 69 - 84%. These were monitored daily using maximum/minimum mercury thermometer and dry/wet bulb hygrometer, respectively.

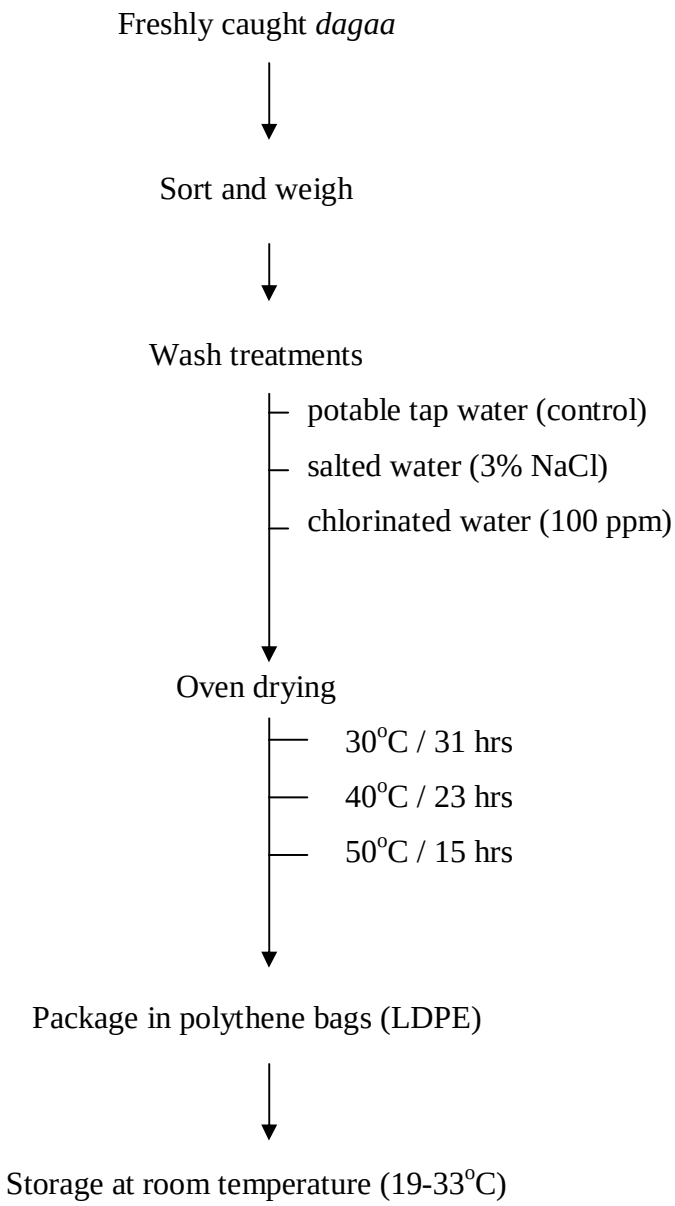

Figure 1: Flow diagram for oven-dried *dagaa.*

Chemical and microbial analyses

The fresh and sun-dried market samples were analysed for pH, TBARS, TVBN and aerobic bacterial counts. Samples of oven-dried *dagaa* were obtained at an interval of 2 days across a period of 9 days in storage and analysed for changes in pH, TBARS, TVBN and aerobic bacterial counts. The pH was determined by use of pH meter (Metrohm Ltd) [13]. The TBARS levels were determined by the extraction method [14]. The TVBN content was analysed by the distillation method [15]. Determination

of total viable count was done by the aerobic plate count method using plate count agar (PCA) according to AOAC method 966.23 [16].

Statistical analyses

All treatments were conducted in triplicates. Oven drying experiments were conducted in a randomised complete design involving 3 wash treatments (control, salted, chlorinated) (WT), 3 drying temperatures (30^{o}C, 40^{o}C, 50^{o}C) (DT) and 5 storage periods (day 1, 3, 5, 7, 9) (SP). Treatments of dried *dagaa* were prepared by a 3WT x 3DT x 5 SP factorial arrangement. The differences among treatments were measured 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. The statistical analysis was done by COSTAT statistical package [17].

RESULTS

The pH of sun-dried and oven-dried *dagaa*

The pH values were significantly lower ($p<0.05$) in fresh *dagaa* (pH 6.72) compared to sun dried market samples (pH 5.88) (Figure 2). At 30^{o}C and 40^{o}C temperatures, the salted-wash treatments showed significantly ($p<0.05$) lower pH values relative to the chlorinated and control-wash treatments (Table 1). After 9 days of storage, a significant decline ($p<0.05$) in the pH levels was observed in the oven-dried *dagaa*. Drying of *dagaa* at 30^{o}C, 40^{o}C and 50^{o}C did not show significant ($p<0.05$) difference between the control and chlorinated-wash treatments.

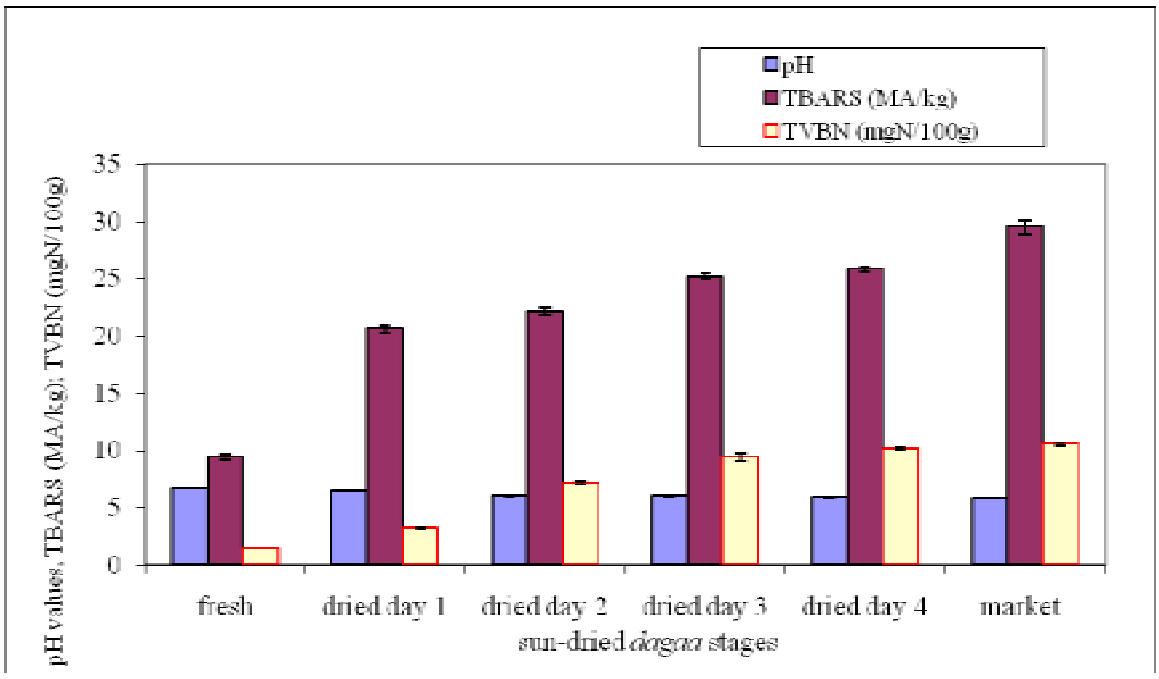

Figure 2: The pH, TVBN, TBARS of fresh sun-dried and sun-dried retail market *dagaa* samples.

The TBARS of sun-dried and oven-dried *dagaa*

The TBARS values were significantly ($p<0.05$) higher in the market samples (10.55 mg MA/kg) compared to 1.39 mg Malonaldehyde (MA) /kg in fresh fish (Figure 2).The salted-wash treatments showed significantly ($p<0.05$) higher accumulation of TBARS in the dried *dagaa* than the chlorinated and control-wash treatments on drying at 30^{o}C, 40^{o}C and 50^{o}C (Table 2). However, the chlorinated-wash treatments had a significantly ($p<0.05$) lower level of lipid oxidation than the control-wash treatments on drying at 30^{o}C and 40^{o}C. The TBARS levels for the salted, chlorinated and control-wash treatments were significantly ($p<0.05$) higher at elevated temperatures of 50^{o}C and 40^{o}C than at lower temperatures of 30^{o}C. A significant ($p<0.05$) increase in TBARS was observed during the 9 days storage of all oven-dried *dagaa*. Apart from the 40^{o}C salted, 50^{o}C control and 50^{o}C salted treatments, other oven-dried *dagaa* treatments had TBARS values below recommended limit of 6 mg MA/kg by day 9 of storage at ambient temperature conditions.

The TVBN of sun-dried and oven-dried *dagaa*

The TVBN values were significantly ($p<0.05$) higher in sun-dried market samples (29.51 mg/ 100g) compared to 9.42 mg /100g in the freshly caught samples (Figure 2). The salted and chlorinated-wash treatments showed significantly ($p<0.05$) lower TVBN values than the control-wash treatment at all drying temperatures of 30^{o}C, 40^{o}C and 50^{o}C (Table 3). The TVBN levels recorded at 30^{o}C were significantly ($p<0.05$) higher relative to those at temperatures of 40^{o}C and 50^{o}C in the control, salted and chlorinated-wash treatments. During the storage period, the increases in TVBN levels in all the sun-dried *dagaa* treatments were significant ($p<0.05$) by day 9.

Aerobic bacterial counts of sun-dried and oven-dried *dagaa*

The bacterial load in market samples (6.16 log cfu/g) was significantly higher ($p<0.05$) compared to day 4 sun-dried *dagaa* samples (5.58 log cfu/g). The salted-wash treatment showed a significantly ($p<0.05$) lower aerobic bacterial counts compared to the chlorinated and control-wash treatments on drying at 30^{o}C, 40^{o}C and 50^{o}C (Table 4). At 50^{o}C, there was significantly ($p<0.05$) lower bacterial counts than at 30^{o}C and 40^{o}C for the control, salted and chlorinated-wash treatments. During the 9-days storage period, the aerobic bacterial counts increased significantly ($p<0.05$) in all the oven- dried samples.

DISCUSSION

The pH of sun-dried *dagaa* and oven-dried *dagaa*

The pH of dried fish is important in the assessment of toughness, dryness and flavour of dried fish. The general decline of the pH values in sun-dried *dagaa* could be attributed to the concentration effect of the lactic acid by the lactobacilli bacteria [18, 19]. The salted wash treatments resulted in lower pH values possibly due to the leaching effect of salt on the salt soluble proteins.

The TBARS of sun-dried *dagaa* and oven-dried *dagaa*

Lipid oxidation determined as TBARS can be manifested by loss in flavour, colour, texture and nutritive quality in the dried fish products. The TBARS levels below 6 mg MA/kg in fish products is regarded as acceptable with regard to development of rancid flavour and odour [20]. Consequently, the sun-dried *dagaa* were rancid by day 3 of the drying process. The highly unsaturated nature of fish lipids in *dagaa* and the long holding during the sun-drying process is responsible for the rapid oxidative reactions [8, 21].

The salted-wash treatment resulted in higher accumulation of TBARS in the dried *dagaa* than the chlorinated and control wash treatments due to the pro-oxidant effect of salt on lipid oxidation [22]. Sodium chloride promotes the displacement of iron ions from the binding sites of heme compounds by interfering with the iron-protein interactions [9]. The TBARS values for chlorinated-wash samples were lower relative to the salted and control wash treatment, possibly as a result of inhibition of the lipolytic bacteria by chlorine (Table 4).

The TVBN of sun-dried *dagaa* and oven-dried *dagaa*

The rise in TVBN values of sun-dried *dagaa* is attributed to autolysis of the tissues by endogenous proteolytic enzymes, originating from the fish viscera as well as proteolytic microorganisms [23, 24, 25]. Based on the TVBN acceptability limit of 30 mgN /100g [15], the sun-dried market samples were regarded as marginally acceptable.

The salted and chlorinated-wash treatments showed lower TVBN values when compared with the *dagaa* subjected to control-wash treatment on drying at 30^{o}C, 40^{o}C and 50^{o}C. This is attributed to the inhibitory effects of chlorine and salt solutions on bacterial growth as reflected in respective lower bacterial counts (Table 4).The TVBN levels recorded at 30^{o}C were higher than the values obtained on drying at higher temperatures of 40^{o}C and 50^{o}C. This is because at lower temperatures, the bacterial proliferation was higher than at elevated temperatures. All the oven-dried *dagaa* were still considered acceptable by day 9 with regard to TVBN acceptable limits.

CONCLUSION

The salted treatments resulted in extensive lipid oxidation in the oven-dried *dagaa* product due to the pro-oxidative effect of sodium chloride (3% NaCl). However, the data demonstrated that chlorinated-wash (100ppm) might be an effective intervention strategy in reducing lipid oxidation evident in the lower TBARS values compared with the potable tap water (control) and salted-wash treatments. The TVBN values in the oven-dried *dagaa* were higher in treatments dried at 30^{o}C relative to the 40^{o}C and 50^{o}C conditions.

In the shelf stability study, it was noted that lipid oxidation could be a major quality problem in dried *dagaa* products. Therefore, the most appropriate treatment that showed the least TVBN and moderate TBARS values on completion of storage was

observed in the *dagaa* subjected to chlorinated wash and dried at 50^{o}C. These conditions can be achieved through use of chlorinated municipal water and properly designed solar driers.

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Table 1: Changes in pH after drying and during storage of oven-dried *dagaa*[1]

days	30 °C			40 °C			50 °C		
	control	Salted wash	Chlorinated wash	control	Salted wash	Chlorinated wash	control	Salted wash	Chlorinated wash
day 1	6.61± 0.06 [a]	6.45± 0.03 [a]	6.61± 0.03 [a]	6.51± 0.09 [b]	6.46± 0.06 [a]	6.54± 0.01 [a]	6.59± 0.09 [a]	6.52± 0.03 [a]	6.52± 0.07 [a]
day 3	6.55± 0.07 [ab]	6.42± 0.03 [a]	6.46± 0.08 [b]	6.65± 0.05 [a]	6.34± 0.02 [b]	6.50± 0.02 [b]	6.56± 0.03 [ab]	6.49± 0.08 [ab]	6.46± 0.03 [ab]
day 5	6.54 ± 0.03 [b]	6.41± 0.05 [ab]	6.42± 0.06 [bc]	6.51± 0.03 [b]	6.32± 0.05 [b]	6.47± 0.03 [bc]	6.51± 0.05 [bc]	6.53± 0.03 [a]	6.46± 0.08 [ab]
day 7	6.51± 0.05 [b]	6.40± 0.06 [ab]	6.38± 0.05 [c]	6.48± 0.04 [bc]	6.36± 0.02 [b]	6.43± 0.05 [c]	6.50± 0.05 [bc]	6.51± 0.04 [ab]	6.41± 0.05 [bc]
day 9	6.49± 0.06 [b]	6.36± 0.06 [b]	6.39± 0.05 [c]	6.44± 0.06 [c]	6.24± 0.08 [c]	6.38± 0.06 [d]	6.47± 0.07 [c]	6.44± 0.07 [b]	6.34± 0.04 [c]
$LSD_{0.05}$	0.061	0.053	0.068	0.056	0.064	0.046	0.072	0.066	0.071

[1] Values are means of triplicate determinations. Means in a column followed by the same letters are not significantly different ($p<0.05$).

Table 2: Changes in TBARS (mgMA/kg) after drying and during storage of oven-dried *dagaa*[1]

days	30 °C			40 °C			50 °C		
	control	Salted wash	Chlorinated wash	control	Salted wash	Chlorinated wash	control	Salted wash	Chlorinated wash
day 1	3.51± 0.34 [b]	4.33± 0.27 [c]	2.34± 0.35 [b]	4.36± 0.33 [c]	4.61± 0.2 [c]	3.35± 0.28 [d]	4.16± 0.22 [d]	4.84± 0.31 [e]	4.22± 0.25 [e]
day 3	4.55± 0.31 [a]	4.39± 0.23 [c]	3.37± 0.05 [a]	4.39± 0.25 [c]	4.71± 0.24 [c]	3.81± 0.17 [c]	4.56± 0.36 [c]	5.29± 0.32 [d]	4.48± 0.15 [d]
day 5	4.57 ± 0.37 [a]	4.81± 0.10 [b]	3.61± 0.03 [a]	5.06± 0.02 [b]	5.55± 0.09 [b]	4.41± 0.08 [b]	5.38± 0.28 [b]	6.19± 0.12 [c]	4.75± 0.09 [c]
day 7	4.71± 0.28 [a]	5.41± 0.38 [a]	3.42± 0.01 [a]	5.28± 0.20 [ab]	6.55± 0.30 [a]	4.42± 0.29 [b]	6.45± 0.28 [a]	6.73± 0.26 [b]	5.36± 0.29 [b]
day 9	4.82± 0.02 [a]	5.51± 0.31 [a]	3.44± 0.05 [a]	5.46± 0.29 [a]	6.75± 0.25 [a]	4.79± 0.12 [a]	6.69± 0.23 [a]	7.18± 0.32 [a]	5.77± 0.22 [a]
$LSD_{0.05}$	0.356	0.327	0.332	0.288	0.273	0.243	0.329	0.328	0.252

[1] Values are a mean of triplicate determinations. Means in a column followed by the same letter are not significantly different ($p<0.05$).

Table 3: Changes in TVBN (mgN/100g) after drying and during storage of oven-dried *dagaa*[1]

days	30 °C			40 °C			50 °C		
	control	Salted wash	Chlorinated wash	control	Salted wash	Chlorinated wash	control	Salted wash	Chlorinated wash
day 1	21.25± 0.73^{d}	15.59± 0.51^{d}	19.20± 0.75^{d}	16.58± 0.26^{d}	12.74± 0.55^{e}	14.23± 0.34^{d}	15.43± 0.23^{d}	11.61± 0.27^{d}	13.71± 0.38^{d}
day 3	23.71± 0.24^{c}	19.52± 0.29^{c}	20.23± 0.45^{c}	17.97± 0.51^{c}	13.81± 0.57^{d}	17.59± 0.58^{c}	17.60± 0.28^{c}	12.55± 0.55^{c}	14.74± 0.43^{c}
day 5	24.26 ± 0.67^{c}	21.25± 0.62^{b}	22.0± 0.45^{b}	19.22± 0.93^{b}	15.53± 0.26^{c}	17.61± 0.04^{c}	18.11± 0.41^{b}	14.52± 0.61^{b}	15.23± 0.50^{bc}
day 7	25.24± 0.42^{b}	22.48± 0.45^{a}	22.42± 0.52^{b}	20.31± 0.31^{a}	16.18± 0.52^{b}	20.33± 0.67^{b}	18.08± 0.45^{b}	15.08± 0.37^{b}	15.73± 0.60^{ab}
day 9	26.50± 0.42^{a}	22.29± 0.40^{a}	23.40± 0.29^{a}	20.72± 0.71^{a}	17.18± 0.38^{a}	22.42± 0.52^{a}	18.98± 0.54^{a}	15.76± 0.54^{a}	16.13± 0.60^{a}
LSD $_{0.05}$	0.627	0.557	0.613	0.711	0.560	0.619	0.474	0.573	0.606

[1] Values are a mean of triplicate determinations. Means in a column followed by the same letter are not significantly different ($p<0.05$).

Table 4: Changes in aerobic bacteria counts (log cfu/g) after drying and during storage of oven-dried *dagaa*[1]

days	30 °C			40 °C			50 °C		
	control	Salted wash	Chlorinated wash	control	Salted wash	Chlorinated wash	control	Salted wash	Chlorinated wash
day 1	5.62± 0.27[b]	4.93± 0.02[d]	5.56± 0.24[c]	4.97± 0.03[d]	4.57± 0.06[d]	4.94± 0.02[e]	4.39± 0.15[d]	3.17± 0.11[d]	4.45± 0.15[e]
day 3	5.83± 0.04[a]	4.95± 0.03[d]	5.77± 0.05[b]	5.64± 0.04[c]	5.21± 0.16[c]	5.37± 0.05[d]	4.79± 0.03[c]	4.14± 0.09[c]	4.74± 0.04[d]
day 5	5.88 ± 0.02[a]	5.66± 0.03[b]	5.80± 0.03[ab]	5.85± 0.03[b]	5.46± 0.09[b]	5.77± 0.05[c]	5.69± 0.06[b]	4.24± 0.05[c]	5.40± 0.06[c]
day 7	5.97± 0.02[a]	5.27± 0.08[c]	5.87± 0.01[ab]	5.86± 0.03[b]	5.62± 0.06[a]	5.82± 0.03[b]	5.83± 0.02[a]	5.31± 0.27[b]	5.56± 0.05[b]
day 9	5.88± 0.02[a]	5.91± 0.03[a]	5.93± 0.05[a]	5.91± 0.04[a]	5.70± 0.05[a]	5.87± 0.04[a]	5.88± 0.02[a]	5.58± 0.07[a]	5.83± 0.03[a]
$LSD_{0.05}$	0.148	0.051	0.134	0.039	0.111	0.047	0.091	0.017	0.093

[1] Values are a mean of triplicate determinations. Means in a column followed by the same letter are not significantly different ($p<0.05$)
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