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Investigation of mycoflora on *dagaa* (*Rastrineobola argentea*) as affected by washing and drying methods

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ABSTRACT

Objective: To determine the fungal characteristics and asses possible aflatoxin contamination during field sun-drying and marketing stages of sun-dried *dagaa* (*Rastrineobola argentea*) (a small pelagic fish found in Lake Victoria) and to investigate the effect of selected pre-washing treatments and drying temperatures on the mycoflora characteristics of *dagaa.*

Methodology and results: Mould, yeast and aflatoxins analyses were carried out on *dagaa* that had been sampled from the sun-drying and market stages. Analyses were also done on *dagaa* that had been oven-dried at 30, 40 and 50^{o}C after pre-washing with salted (3% NaCl), chlorinated (100ppm) solutions and potable tap water (control). The mean mould counts in the sun-dried *dagaa* from market were 3.63 log cfu/g. No aflatoxins were detected. The mould counts were below 1 log cfu/g in all the pre-wash treatments dried at 30, 40 and 50^{o}C. At 40^{o}C, the *dagaa* washed with salted water and chlorinated water had significantly less ($p<0.05$) yeast counts than those washed using potable tap water at 1.35 log cfu/g , 1.38 log cfu/g and 1.48 cfu/g respectively.

Conclusion and application of findings: This study demonstrates the importance of proper processing and handling of fish in order to safeguard public health. The study established that field sun-drying predisposes *dagaa* to contamination by mycotoxic flora. The low counts of mould growth in the oven-dried *dagaa* when compared to the open field sun-dried *dagaa* is attributed to enhanced hygiene due to the incorporated washing steps and during drying and storage.The lowest yeast and mould counts were obtained in the *dagaa* subjected to salted (3% NaCl) pre-wash and subsequently dried at 50^{o}C for 15hrs. This process can be achieved at the local community level through use of solar driers or improved kiln ovens whereas common salt is accessible to the households involved in fish processing. The findings of this study will increase the knowledge base towards adoption of improved handling and drying methods hence minimize mould growth and possible aflatoxin contamination in the dried *dagaa* sub-sector.

Key words: fish; *dagaa*; moulds; yeast; aflatoxins

INTRODUCTION

In Kenya, the fisheries sector contributes about 0.5% of the Gross Domestic Product (GDP) (Nyeko, 2008). Lake Victoria provides about 95 % of the total fish landed in Kenya of which the main ones are *dagaa* (*Rastrineobola argentea* 62.9 %), nile perch (*Lates niloticus* L. 29.9%), tilapia (*Oreochromis*

niloticus L. 5.3%), *Fulu (*Haplochromines1%) and others (0.8%) (Odongkara, 2008). Therefore, *dagaa* plays a significant role in the livelihoods of artisanal fisherfolk communities in terms of employment, income and provision of nutrition.

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 highly attributed to physical losses, colour change, bacterial and mould spoilage (Mndeme, 1998). After being harvested, *dagaa* are entirely processed by sun-drying. However, this process leads to irregular and unpredictable quality as a result of slow drying. The harvesting and handling of the *dagaa* is a potential source of bacterial contamination due to lack of basic infrastructure such as the chilling and hygiene facilities at the landing, processing and marketing sites. Besides bacteria, fungal growth also occurs frequently during processing, storage and marketing of *dagaa* and poses a potential health hazard due to possible mycotoxin contamination.

The most predominant mould genera that have been reported in various dried fish products include the *Aspergillus spp, Penicillium spp, Rhizopus spp, Mucor spp, Fusarium spp, Wallemia spp,* and *Cladosporium spp (*Prasad *et al*., 1987; Munimbazi & Bullerman, 1996; Chakrabarti & Varma, 1999; Jonsyn & Lahai, 2006; Obeyamiji *et al*., 2008). According to Marth (1998), the particular yeast species of interest in the spoilage of meat and fish products include *Candida, Crytoloccus, Deboromyces, Hansenula, Pichia, Rhodotorula ,Saccharomyces, Sporobomyces,Torulopsis* and *Trichosppora*.

Mycotoxins are currently of concern to global food safety because of their ubiquity and potential deleterious effect on human and animal health (Bullerman, 1986). Considerable importance has been attached to aflatoxins because of their carcinogenic, mutagenic and teratogenic nature (Gourama & Bullerman, 1995; CDC, 2004; CDC, 2005). Production of aflatoxins is primarily associated with the growth of *Aspergillus flavus* and *Aspergillus parasiticus* (Abarca *et al*., 1994). Several studies on the assessment of the risk potential of dried fish have reported detection of aflatoxins as potential natural contaminants (Jonsyn & Lahai, 1992; Mugula & Lyimo, 1992; Ali *et al*., 2005; Bukola *et al*., 2008).

However, the growth of the *Aspergillus spp* and the production of aflatoxins are dependent on factors such as the fungal strain, competing flora, substrates, temperature and relative humidity conditions (Wheeler & Hocking, 1993; Gourama & Bullerman, 1995; Pitt & Miscamble, 1995; Santour *et al*., 2002). In Kenya, there is limited information available on the likely risk of dried fish products such as *dagaa*, which is the staple food source amongst the fisherfolk communities. There is, therefore, an urgent need for continuous surveillance in order to protect public health and information on proper processing conditions of dried *dagaa* products to avoid fungal growth, which results in the rapid spoilage and possible contamination with mycotoxins.

This study was, therefore, undertaken to determine the mould and yeast population and asses possible aflatoxin contamination at the field drying and marketing stages of sun-dried *dagaa* .The study also investigated the effect of selected pre-washing treatments i.e. salted (3% sodium chloride), chlorinated solutions (100ppm) and potable tap water (control) and drying temperatures (30, 40 and 50^{o}C) on the mycofloral population of oven-dried *dagaa.*

MATERIALS AND METHODS

This study was conducted in two phases as described below:

Sampling of field sun-dried and market *dagaa*: Three batches of each of *dagaa* that had been sun-dried for 1 day, 2 days, 3 days, and 4 days (one kg each) were collected randomly from fish processors based at the Dunga, Tako and Block drying sites, Kisumu town. These sites are officially recognized by the Ministry of Fisheries Development (Kenya) under the Beach Management Unit Programme. Three other batches of market *dagaa* (one kg each) that had been sun-dried for 4 days by identified traders and held in the retail market for one week, were

randomly sampled from three traders at Kibuye fish market, which is the largest retail market in Kisumu. Ordinarily, sun-dried *dagaa* stock would last for 1 week in the retail market, therefore the sun-dried *dagaa* were held at the prevailing market conditions. The conditions were monitored and temperature ranged 19-33^{o}C, whereas relative humidity ranged between 70 - 84%. The samples were transported on the same day in a cool box to the Department of Food Science and Technology at Jomo Kenyatta University of Agriculture and Technology (JKUAT).

Preparation 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. The fresh *dagaa* were washed with prepared solutions and oven-dried under different temperatures as shown in Figure 1. About 800g of the fresh *dagaa* 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^{o}C) wash solutions through the sieve. The washed *dagaa* were allowed to drain excess liquid and subsequently oven-dried at 30, 40 and 50^{o}C using an 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 hr at 30^{o}C, 23 hr at 40^{o}C and 15 hr at 50^{o}C. The dried *dagaa* samples were then packaged in low-density polyethylene (LDPE) bags and stored at prevailing ambient temperature, which ranged from 19 - 33^{o}C, whereas the prevailing relative humidity varied between 69 - 84%. These were monitored daily using maximum/minimum mercury thermometer and dry/wet bulb hygrometer, respectively during the 9 days storage period.

Mould and yeast count and characterization: Yeasts and moulds were enumerated by the surface plate method using potato dextrose agar (PDA) (Harrigan, 1998). A sample of 25g was homogenized in 225ml of buffered peptone water (BPW) using Waring Laboratory blender, and then 10-fold serial dilutions were prepared to 10^{-6}. Aliquots of appropriate sample dilutions were spread onto PDA agar supplemented with 75 ppm chloramphenical antibiotic and incubated at 25^{o}C for 5 days. Further purification of mould growth was done on malt extract agar (MEA) and subsequently taxonomic identification of all different mould colonies was done to the genera level by macroscopic and microscopic assessment of the sporulating bodies and mycelial growth according to Samson *et al*. (1981).

Analyses of aflatoxins contamination: Aflatoxins were analysed using the solvent efficient thin layer chromatography (TLC) method according to AOAC method 993.17 (AOAC, 1995). About 50g of ground sample was extracted with 200ml of methanol-water mixture (85:15) and filtered. Then 40ml of the extract was partitioned in a separating funnel using 40 ml of 10% NaCl and 25ml of hexane. Aflatoxins were then extracted with duplicate 25ml of chloroform from the aqueous phase and evaporated to dryness in a steam bath.

Aflatoxin extracts were dissolved in 3ml of dichloromethane and purified in a glass column packing (22 x 300 mm) of 10g silica gel 60M and 0.5g Na_2SO_4 .The packing of the column was done as described in the AOAC method 968.22 (AOAC, 1995). The column was initially conditioned with 30ml of hexane and 30ml of dichloromethane. Aflatoxins were then eluted with 3 portions of 30ml chloroform-acetone mixture (9:1) and the collected eluate evaporated to dryness on a steam bath under nitrogen stream. Aflatoxins were then recovered with chloroform and spotted onto silicagel TLC plates before development with chloroform - acetone mixture (9:1). Aflatoxin B1, B2, G1 and G2 reference standards were spotted across the plates. Long wave UV light at 635nm (FUNA UV Light SL-800G) was used to examine the TLC plates so as to establish the presence of aflatoxins.

Statistical analysis: 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, 40 and 50^{o}C) (DT) and 5 storage periods (day 1, 3, 5, 7, 9) (SP). Treatments of dried *dagaa* were arranged as a 3WT × 3DT × 5SP factorial design. 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 (Costat, 1990).

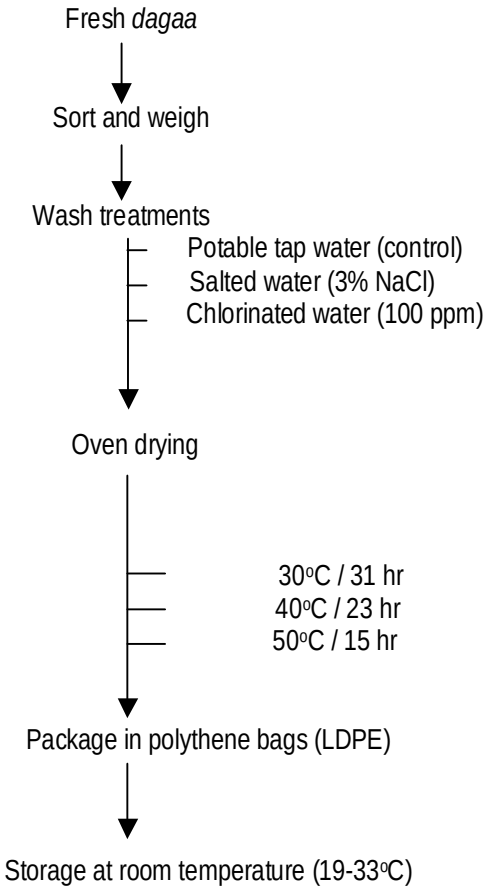

Figure 1: Process flow for experimental washing and oven drying of *dagaa*.

RESULTS AND DISCUSSION

The mould counts observed in the market *dagaa* samples (3.63 log cfu/g) were significantly higher ($p<0.05$) than the counts in the fresh *dagaa* (2.27 log cfu/g) (Figure 2). A significant ($p<0.05$) increase in mould counts was observed in the samples dried for 1 day (3.74 log cfu/g). However, a slight decline was recorded in samples dried for longer periods. The substantially high mould counts observed in the sun-dried *dagaa* sampled from market could have arisen from the favourable relative humidity (70 - 84%), temperature (25 – 35°C) conditions (Arun *et al*., 1987) and repeated handling during the storage and marketing period.

The predominant mould genera isolated included *Aspergillus spp., Cladosporium spp.,* and *Penicillium spp*. Other mould growths identified, though not predominant, in the sun-dried *dagaa* included *Mucor spp., Rhizopus spp., Curvularea spp*., and *Alternaria spp*. Most of these moulds can grow well on substrates of relatively low moisture content. Some of the most prevalent *Aspergillus spp* that have been isolated from fish products by other authors include *A. flavus, A. niger, A. sydowi*, *A. ochraceus* and *A. tamarii* (Jonsyn & Lahai, 1992; Munimbazi & Bullerman, 1996). *Aspergillus spp* are closely associated with the soil. Therefore, it is possible that the sun drying of *dagaa* on the bare ground surfaces could have exposed dried fish to high mould infestation particularly from *Aspergillus spp*.

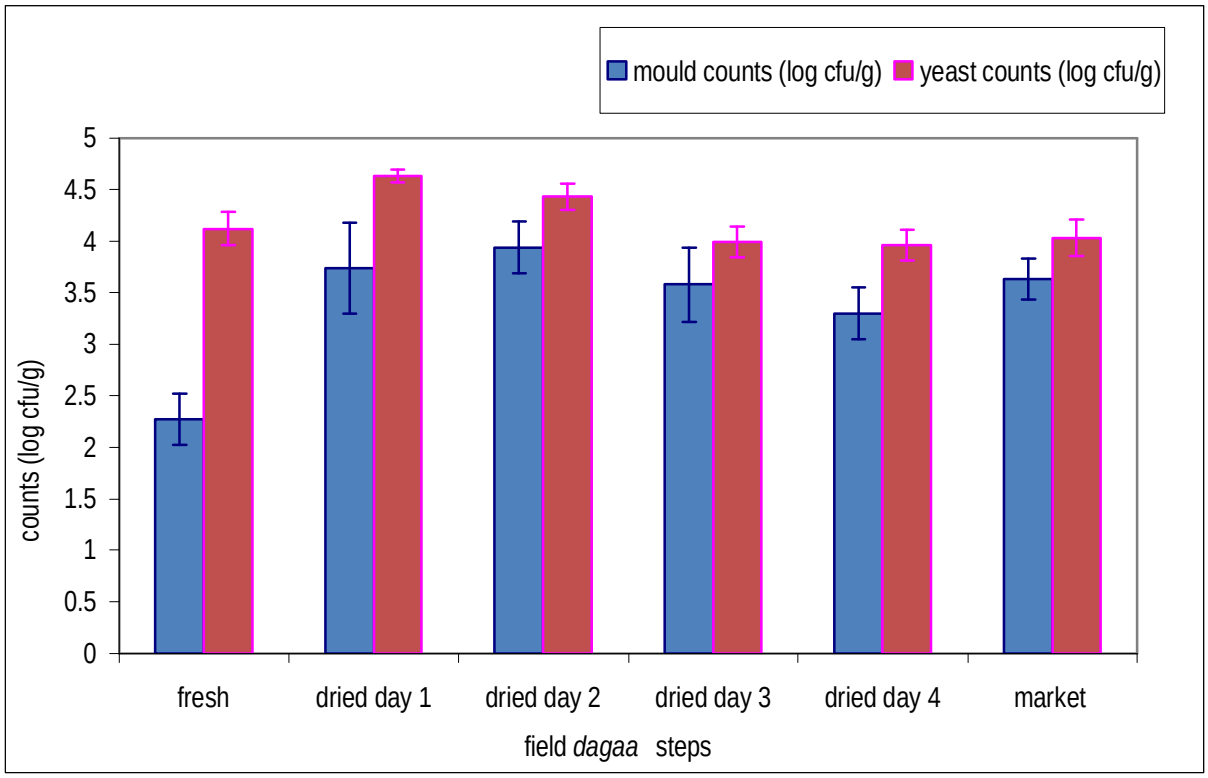

Figure 2: Mould and yeast counts of fresh, sundried and market *dagaa*.

The mould counts in all the oven-dried *dagaa* treatments (salted, chlorinated and potable tap water) were below 1 log cfu/g after drying at 30, 40 and 50°C (Table 1). However, the chlorinated and salted-wash treatments generally had lower mould counts than the fish washed with potable tap water at all the drying temperatures assessed. Generally, higher mould counts (though below 1 log cfu/g) were observed after drying at 30°C, when compared to drying at 40 and 50°C for all the wash treatments.

In the storage study, slight increases in mould counts were observed in all the oven-dried *dagaa* treatments by day 9, however, resulting to counts of below 1 log cfu/g (Table 1). The predominant mould species isolated from the oven-dried *dagaa* included *Cladosporium spp, Rhizopus spp,* and *Mucor spp*. Some minimal mould growth of *Aspergillus spp* was also observed particularly in the 30°C treatments. In comparison to the field sun-dried *dagaa,* the low counts of mould growth in the oven-dried *dagaa* could be attributed to enhanced hygiene due to the incorporated washing steps and better handling during drying and storage.

The yeast counts in the field sun-dried *dagaa* samples obtained from the market (4.03 log cfu/g) were not significantly ($p<0.05$) different from the counts in fresh samples (4.12 log cfu/g) (Figure 2). A significant ($p<0.05$) increase in yeast count was observed in the samples dried for 1 day. However, a significant ($p<0.05$) decline in counts was recorded during the subsequent drying periods up to day 4. The yeast counts in the *dagaa* sampled from market marginally exceeded the 4 log cfu/g acceptable limit (Huss, 1994).This is an indication that the field sun-dried *dagaa* could be highly susceptible to yeast spoilage. Yeast contributes to spoilage through production of lipolytic and proteolytic enzymes, giving rise to off flavours and odours (Marth, 1998). In this study, isolation of yeasts was done to the morphological level in terms of colour of the colonies. The colonies observed in the field sun-dried *dagaa* showed colourations varying from cream, pink, yellow and white. These results call for more study into the identification and the role of the yeast groups isolated.

After oven-drying at 40°C, the salted (1.35 log cfu/g) and chlorinated-wash treatments (1.38 log cfu) showed significantly ($p<0.05$) lower yeast counts when compared to the control-wash treatments (1.48 log cfu/g) (Table 2). A similar trend was observed at 50°C. However, at 30°C, the salted-wash treatments (1.35 log cfu/g) had significantly ($p<0.05$) lower counts than the chlorinated (1.75 log cfu/g) and control-wash (1.80 log

cfu/g) treatments. Both salt and chlorine are often used as food preservative and sanitizer, respectively, due to their antimicrobial activity. The salted, chlorinated and control-wash treatments dried at 40 and 50°C exhibited significantly ($p<0.05$) lower yeast count than equivalent wash treatments at 30°C. This is because the higher temperatures led to destruction of yeasts due to heat, as they are heat labile.

In the storage stability study, there was a significant ($p<0.05$) increase in yeast counts in all the wash and drying treatments by day 9 of the storage period (Table 2). The yeast counts ranged from 1.26 log cfu/g in the 50°C salted-wash treatments to 1.96 log cfu/g in the 30°C control-wash treatments. This could be attributed to the corresponding increase in moisture content of the dried *dagaa* as established by the author in a separate unpublished study and the fluctuating ambient temperature conditions of the storage environment (19 – 33°C). In contrast to the observations in the field sun-dried *dagaa*, all the yeast colonies isolated from the oven-dried *dagaa* were white in colour. Based on our results, the condition that exhibited the lowest yeast and mould counts was obtained in the *dagaa* subjected to salted (3% NaCl) pre-wash before drying at 50°C for 15hrs.

Although a potential risk of aflatoxin contamination was conceivable, no aflatoxin was detected in both the field sun-dried and oven-dried *dagaa*. Although no aflatoxins were detected in the present study, the presence of *Aspergillus spp* is expected to influence the safety of sun-dried *dagaa* if improperly stored. Several factors have been identified as critical towards production of aflatoxins. Aflatoxin production has also been reported to occur at favourable temperature conditions of 28 - 30°C and relative humidity of 90% (Arun *et al*., 1987). Aflatoxins are mainly associated with *A. flavus* and *A. Parasiticus* (Abarca *et al*., 1994; Gourama & Bullerman, 1995). Other factors include interaction between competing flora (Wheeler & Hocking, 1993) and water activity (Pitt & Miscamble, 1995; Santour *et al*., 2002).

In conclusion, the lowest yeast and mould counts were obtained in the *dagaa* subjected to salted (3% NaCl) pre-wash and subsequently dried at 50°C for 15hrs. This process can be achieved at the local fisherfolk community level through use of solar driers or improved kiln ovens whereas common salt is accessible to most of the households involved in processing of dried *dagaa*. This study demonstrates the need to handle process and store dried fish products under proper conditions so as to reduce the risk of contamination with mycotoxigenic fungi and subsequent aflatoxin occurrence. 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. Other areas that would benefit from further research include assessment of the combined effect of competing flora, water activity, temperature and substrate condition on the kinetics of growth and aflatoxin production on dried fish products by *Aspergillus spp*.

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Table 1: Changes in mould 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	<1	<1	<1	<1	<1	<1	<1	ND[2]	ND
day 3	<1	<1	<1	<1	<1	<1	<1	<1	<1
day 5	<1	<1	<1	<1	<1	<1	<1	<1	<1
day 7	<1	<1	<1	<1	<1	<1	<1	<1	<1
day 9	<1	<1	<1	<1	<1	<1	<1	<1	<1

[1] Values are a mean of triplicate determinations. [2] ND = Not detected

Table 2: Changes in yeast counts (log cfu/g) after drying and during storage on 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	1.80 ± 0.04^{d}	1.35 ± 0.03^{c}	1.75 ± 0.03^{c}	1.48 ± 0.01^{c}	1.35 ± 0.03^{b}	1.38 ± 0.01^{c}	1.36 ± 0.03^{c}	1.10 ± 0.11^{c}	1.34 ± 0.03^{b}
day 3	1.85 ± 0.03^{c}	1.36 ± 0.03^{c}	1.73 ± 0.02^{c}	1.53 ± 0.01^{c}	1.34 ± 0.03^{b}	1.43 ± 0.02^{b}	1.38 ± 0.02^{c}	1.13 ± 0.29^{bc}	1.36 ± 0.01^{b}
day 5	1.87 ± 0.03^{bc}	1.54 ± 0.04^{b}	1.83 ± 0.07^{b}	1.64 ± 0.02^{b}	1.57 ± 0.01^{a}	1.45 ± 0.03^{b}	1.43 ± 0.03^{b}	1.18 ± 0.01^{b}	1.37 ± 0.29^{b}
day 7	1.89 ± 0.03^{b}	1.68 ± 0.01^{a}	1.84 ± 0.03^{b}	1.70 ± 0.14^{ab}	1.56 ± 0.03^{a}	1.55 ± 0.02^{a}	1.48 ± 0.02^{a}	1.26 ± 0.03^{a}	1.45 ± 0.37^{a}
day 9	1.96 ± 0.02^{a}	1.67 ± 0.03^{a}	1.93 ± 0.02^{a}	1.77 ± 0.02^{a}	1.55 ± 0.04^{a}	1.57 ± 0.04^{a}	1.45 ± 0.02^{b}	1.27 ± 0.03^{a}	1.46 ± 0.34^{a}
$LSD_{0.05}$	0.038	0.035	0.045	0.075	0.034	0.031	0.027	0.064	0.035

[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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