The effect of natural long term packaging methods on antioxidant components and malondialdehyde content and seed viability *Moringa oleifera* oilseed

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Highlights

- Moringa seed with MC ≤ 5% can be kept at constant temperature ≤ 30 °C beyond 12 months.
- *Moringa* seed may continue synthesizing antioxidant during postharvest storage.
- Storage period the main factor influencing change in polyphenol content and antioxidant capacity.
- Packaging type and storage temperature influence flavonoid and lipid peroxidation.
- Increase in lipid peroxidation and flavonoids correlate with the loss of viability.

Abstract

There have been increased interest to propagate *Moringa oleifera because of its multipurpose uses.* However there are still no appropriate guidelines for long-term storage of Moringa seed because diverse results are reported in literature. Although progress has been made to understand the causes of seed deterioration, few studies have been made on natural long-term aging of seed. This study aimed to determine and compare the level of polyphenols, flavonoids, MDA and antioxidant

capacity in Moringa seed stored in paper and aluminum bags at -19°, 4°, 20° and 30˚C for 24 months as well as to investigated the relationship between these secondary compounds and Moringa seed viability. Seeds were evaluated in regular intervals of 6 months. There was minor difference between viability percentage of seed stored below 20˚C. Significant decline in viability was recorded in seed stored for 24 months in aluminium bags at 30˚C as a result of high moisture content (8%) and high temperature at which seed were exposed; While Seed stored at 30˚C in paper bags had low moisture content (5%) and retain a high viability percentage . Storage duration was the main factor affecting the changes on polyphenols and antioxidant activity levels. Although storage duration played a major role on the change in flavonoid and MDA content, the influence of storage temperature and the seed moisture content was also evident on some treatments. The change in investigated secondary compounds in seed did not always reflect on its viability percentage, but two relationships emerge from this study: The lowest moisture content, slow increase in MDA content, the lowest flavonoids content and the highest germination % in seed stored in paper bags at 20 and 30°C at 24 months; and the highest MDA content, highest increase in flavonoids and lowest viability percentage observed in seed stored in aluminium bags at 30°C for 24 months. It is recommended that for long-term storage (≥12 months) Moringa seed be stored with low moisture content (≤5%) at constant temperature below 30°C.

Keywords: Antioxidants capacity; Flavonoids; Malondialdehyde; Polyphenols; Seed viability; storage conditions

1 INTRODUCTION

Seed ageing during long-term storage can cause deterioration in seed quality and eventually death. Orthodox seed has thus developed several resistance mechanisms responsible for increased longevity to overcome unfavourable environmental conditions and optimize plant rejuvenation from seed (Arc *et al*., 2011). In this dry state, the metabolic activity of seed drops drastically to a very low level (quiescence) while retaining the ability to germinate for substantial periods (Buitink and Leprince, 2008). However, the low water content in seed is also a restraining factor for certain biochemical activities that are essential to limit cellular damage that occur during ageing and enable seed to survive during long-term storage (Arc et al., 2011). On the other hand, the low moisture content can still support auto-oxidation which generates free radicals and reactive oxygen species (ROS) in organelles (McDonald, 1999; Kibinza et al., 2006).

Seeds are known to contain an antioxidative system (enzymatic and non-enzymatic) that plays a crucial role in protection of membranes and other cellular macromolecules in dry seeds. Low molecular weight antioxidants (ascobate, phenolic compounds, glutathione) which are directly able to scavenge ROS and free radicals, are particularly important. Phenolic compounds have structural chemistry that is similar to that of compounds with free radical scavenging activities (Rice-Evans et al., 1997); they act as antioxidants and protect the embryo against oxidative stress (Puckaka and Ratajckak, 2005). Other phenolic compounds, especially flavonoids, are also known to protect membranes and inner tissue against ROS and limit lipid peroxidation (Hoekstra et al., 2001; Stevenson and Hurst, 2007).

Studies (Tommasi et al., 2006; Puckaka and Ratajckak, 2005; Choudhury and Mandi, 2012) have related the decline of seed viability, associated with ageing, to the decrease in antioxidant defence mechanisms as well as the decrease in some secondary metabolic compounds that have antioxidative properties. Malondialdehyde (MDA) produced during reactive oxygen species reaction, is an indicator of oxidative damage (Bailly et al. 1996). It has been suggested that seed germination can only occur when the ROS level is kept below a critical threshold (Bailly, 2004). ROS accumulation affects many cellular functions by damaging nucleic acids, oxidizing proteins and membrane lipids (Masoumi et al, 2010).

Although progress has been made, the causes of seed deterioration is still not well elucidated. In addition, seed aging is often studied by using various accelerated aging tests (Bijanzadeh et al. 2016, Hussein et al., 2011; Prochazkova and Bezdeckova, 2009; Vijay et al., 2015; Barreto & Garcia, 2017). Few studies have been made on natural long-term aging of seed. Investigating the biochemical changes in seed subjected to natural long-term storage may provide important insight into the process of seed deterioration.

M. oleifera belong to the monogeneric family Moringaceae. It is a fast-growing deciduous or evergreen tree that can reach up to 15 m in height (Ferreira et al., 2008). It is one of the most useful tropical trees. The leaves, flowers, fruits and roots of this tree are used as vegetables (Anwar & Bhangar, 2003). The young leaves are rich sources of vitamins (A, B and C), minerals and amino acids. The leaves are also used as plant growth enhancers as well as forage material for cattle (Foidl et al., 2001). A number of medicinal and therapeutic properties have been attributed to different parts of this multipurpose tree, which include treatment of ascites, rheumatism,

venomous bites and as a cardiac stimulant (Dahot, 1988). Moringa seeds are one of the best natural coagulants discovered so far (Ndabigengesere et al., 1995). The seed oil content is about 30.8-41.4%. Its oil can be used as cooking oil and industrially as a fine machine lubricant (Rahman et al 2009) and has been identified as a potential source of biodiesel by Rashid et al. (2008).

Moringa seed are said to be orthodox (Panday et al, 2011). However many studies (Madinur, 2007; De oliveira et al, 2004; Ruíz-Pérez et al, 2017) have reported loss in *M. oleifera* seed viability within 6-12 months, depending on the storage conditions. Fewer studies (Jahn, 1986; Bezerra *et al*., 2004) have speculated on the possibility of storing Moringa seed for more than a year. There is still a need for an appropriate guideline for long-term storage of Moringa seed. Successful seed storage requires understanding of post-harvest physiology of the seeds. According to the study done by Moravec et al. (2008) Moringa seed tissues appears to present no major obstacle to water uptake or loss and can easily exchange water with their gaseous environment. Temperature, seed moisture content (MC), and duration of storage are the main factors affecting seed deterioration (Spanò et al., 2004). Storing seed under controlled conditions may provide useful information to delay Moringa seed deterioration. The aim of this study was to determine and compare the level of polyphenols, flavonoids, MDA and antioxidant capacity in seed stored in different packaging types and temperatures for 24 months. Furthermore, to investigate the relationship between these secondary compounds and Moringa seed viability.

2 MATERIALS AND METHODS

2.1 Plant material and storage conditions

Seeds from the fruits harvested from an eight year old Moringa orchard at the Hatfield Experimental Farm of the University of Pretoria (25˚45S, 28˚16E) during June to October 2012 and 2013 were used. Seeds were stored following a factorial 2 x 4 x 3 experiment with two types of storage containers (paper bags (P)) and sealed, water and moisture proof aluminium bags (A)), four temperatures (-19°, 4°, 20° and 30˚C) and three storage periods (6, 12, 18 and 24 months). Each treatment was done in triplicates.

2.2 *Moisture content and seed viability*

Low constant temperature oven method (103±2ºC) was used to determine the seed moisture content (MC) of each seed treatment before and again after storage. Since *M. oleifera* is not listed in the ISTA methods, instead of cutting and grinding, seed were gently crushed with a hammer, because of their high content in oil. Three replicates of ±5 g each were used per treatment and the moisture content expressed on the fresh weight basis.

Seeds were assessed prior to storage and after storage at 6, 12 and 24 months. Four replicates of 50 seeds were used for each treatment. Seeds were germinated according to 'between paper' method (ISTA 2006) and incubated in the dark at alternative temperatures of 20/30°C. The seed was considered geminated when normal seedlings were produced (ISTA, 2006). The first assessment was done after 7 days and repeated after 14 days. A tetrazolium test (TTS) was conducted on non-germinated seeds to establish if they were still viable.

2.3 Preparation of crude extracts of Moringa oleifera

After removal of the seed coat from seeds of all treatment sample, 5 g (± 20 seeds) of seeds from each treatment were freeze-dried for a week and immediately ground into powder in liquid nitrogen. The obtained powder was kept in eppendorf and the stored at -80°C until further analysis. The powder from each sample (200 mg) was homogenized in 1 ml of cold 80% ethanol solution and centrifuged at 15000 g for 15 min at 4°C. After this 700 ml of the supernatant was recovered, transferred to a new eppendorf tube and another 700 ml of cold 80% ethanol solution was added. The supernatant was used to determine the total antioxidant capacity, polyphenol, flavonoid and malondialdehyde content.

2.3.1 Polyphenols

The total polyphenol content was determined using the Folin-Ciocalteu reagent method described by Zhang et al. (2006). Modifications were done with regards to solvent use for extraction (ethanol was used) and instead of phloroglucinol, gallic acid was used for the standard curve. A diluted solution (10X) of Folin-Ciocalteau was added to 20 µl of seed crude extract in a microplate. The mixture was allowed to react for 5 min after which 40 µl of 10% saturated sodium carbonate solution was added. This was then left to stand for one hour in the dark. The absorbance of the reaction mixture was read at 725 nm using the microplate spectrophometer (Spectra max plus 384, Sunny, CA, USA). The gallic acid standard curve was used to calculate the phenolic content. The total polyphenol content was expressed as gallic equivalents in mg/g of seed material on a dry weight basis.

2.3.2 Flavonoids

The flavonoid content was measured according to the method of Chang *et al*. (2002) with slight modification. The reagent mixture consisted of 60 µl of absolute ethanol, 10 µl of 10% aluminium chloride, 10 µl of 1 M of potassium acetate and 80 µl of distilled water. The reagent was added to 40 µl of seed extract and the mixture was incubated at room temperature for 30 min. The absorbance was read at 415 nm with the microplate spectrophometer (Spectra max plus 384, Sunny, CA, USA). The total flavonoid content was expressed as quercetin equivalent in mg/g of seed material on a dry weight basis.

2.3.3 Total antioxidant capacity

The total reducing capacity was measured according to the method described by Benzie and Strain (1996) known as FRAP (Ferric reducing antioxidant power) with few modifications. The assay is based on the measurement of the reduction of ferric to ferrous ions by biological material. The reaction mixture of 6 ml was made with 5 ml of 0.3 M acetate buffer, 0.5 ml of 100 mM TPTZ (2,4,6-tri (2-pyridyl)-1,3,5-triazine) and 0.5 ml of 200 mM of $FeCl_3$. The total antioxidant was measured by adding 100 µl of the mix reagent to 100 µl of sample in a 96 well microplate. The reaction was left for 20 min and the absorbance determined with a microplate spectrophometer (Spectra max plus 384, Sunny, CA, USA) at 600 nm. A standard curve was prepared using TROLOX (6 Hydroxy-2,5,7,8 tetramethylchrommane-2-carboxylic acid). The total antioxidant capacity was expressed as mg TROLOX equivalents in mg/g of seed material on a dry weight basis.

2.3.4 Malondialdehyde content

The malondialdehyde content (MDA) was measured using the method of Hodges *et al*. (1999) and Murshed *et al*. (2008) with some modifications. The reagent consisted of 100 ml of 20% of Trichloroacetic acid (TCA) and 0.65 g of thiobarbituric acid. The reaction mixture consisted of 0.5 ml of seed extract supernantant and 1 ml of reagent in a 1.5 ml eppendorf tube. The mixture was then heated in the block-heater (Weather corp HB-2, Taiwan) at 80°C for one hour. Thereafter the tube was centrifuged at 12000 g for 1 min at room temperature and 200 µl aliquots of the mixture were used for absorbance readings at 440 nm, 532 nm and 600 nm with a microplate spectrophometer (Spectra max plus 384, Sunny, CA, USA). The final malondialdehyde quantity was calculated according to the formula: [6.45 x (A532-A600) – 0.56 x A450].

2.4 Data analysis

Data are presented as mean ± Standard Error (SE) of four independent experiments (n = 4). The significance of the effect of storage temperature, packaging type and storage periods as well as their interactions were estimated by three ways analysis of variance (ANOVA) with the aid of Statiscal Analysis System (SASv9.4, 2013). Statistical significance was defined as $P < 0.05$ for all tests. Pearson correlations were used to determine the relationship between viability and measured antioxidants compounds.

3 RESULTS

3.1 Moisture content and seed germination

The average moisture content of seed before germination and the germination percentages is given in Fig. 1

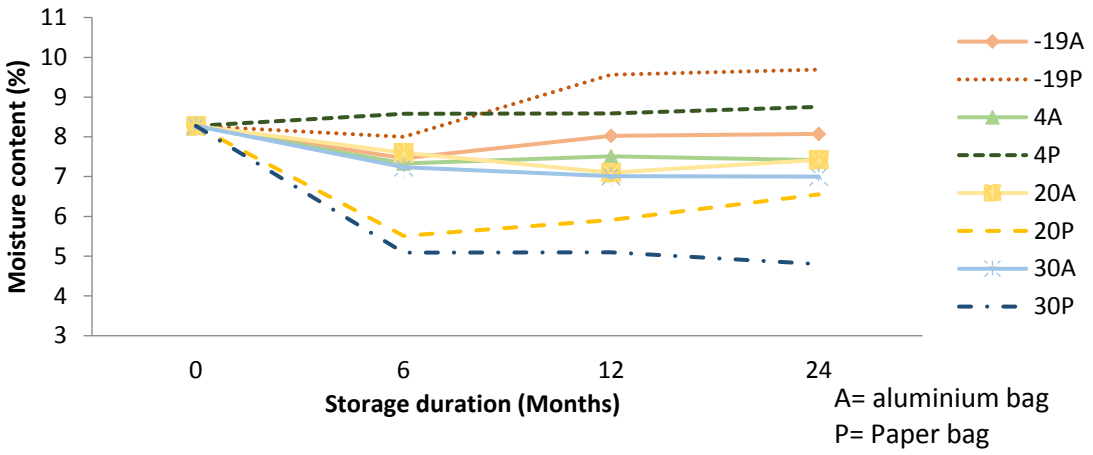

Figure 1: Moisture content of *M. oleifera* seed stored in aluminium (A) and paper bags (P) at -19, 4, 20 and 30°C for 6, 12 and 24 months

The moisture content of seed at zero month of storage (control) was ± 8%. Seed stored in aluminum bags maintained more less the same MC throughout the storage (Fig. 1). The minor fluctuation on moisture content of seed stored in alumunium bags could be due the fact that packaging were not vacuum sealed. The moisture content of seed stored in paper bags at low temperatures (4 and 19 ˚C) increased (±9%) while those of seed stored at high temperatures (20 and 30˚C) decreased (±5%).

The germination percentage (%) of seed stored at low temperatures showed minimal difference between treatments and were generally not significantly different from the control (Fig. 2). At 24 months of storage the germination % of seed stored in aluminum bags at 20˚C had significantly declined while those stored in paper bags at 20˚C remained more or less unchanged throughout the storage. Seeds stored at 30˚C in paper bags maintained a high germination % throughout the storage. The lowest germination percentage was recorded in seed stored in aluminum at 30˚C.

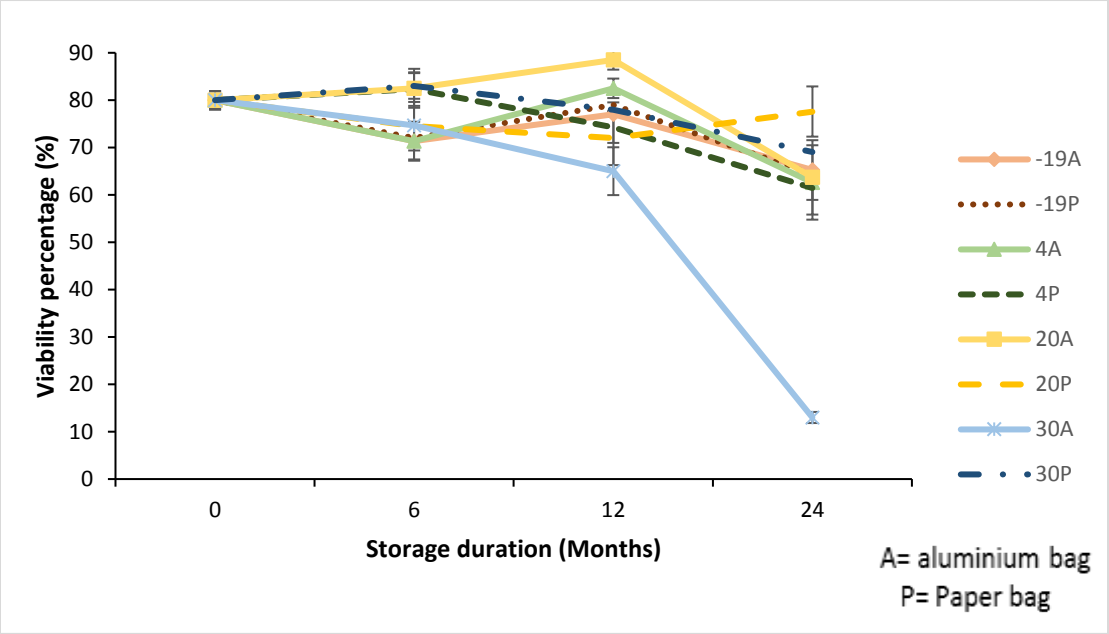

Figure 2. Viability percentage of *M. oleifera* seed stored for 24 months in aluminum and paper bags at temperatures -19, 4, 20 and 30˚C. Error bars represent ± standard error.

3.2 Polyphenols

The polyphenol content of freshly harvested (control) seed was 0.044 mg GA/g. There was generally no difference in polyphenols content between temperature and packaging treatments (Fig. 3). Storage period was the main factor affecting the level of polyphenol in seed. Up until 12 months of storage, the level of polyphenols was not significantly different from the control. A 26-fold increase was observed at 18 months of storage irrespective of packaging type and temperature (Fig. 3). At 24 months of storage the level of polyphenol content decreased significantly with the highest decrease recorded in seed stored at 20°C in paper bags but yet still higher than the levels at 12 months and below.

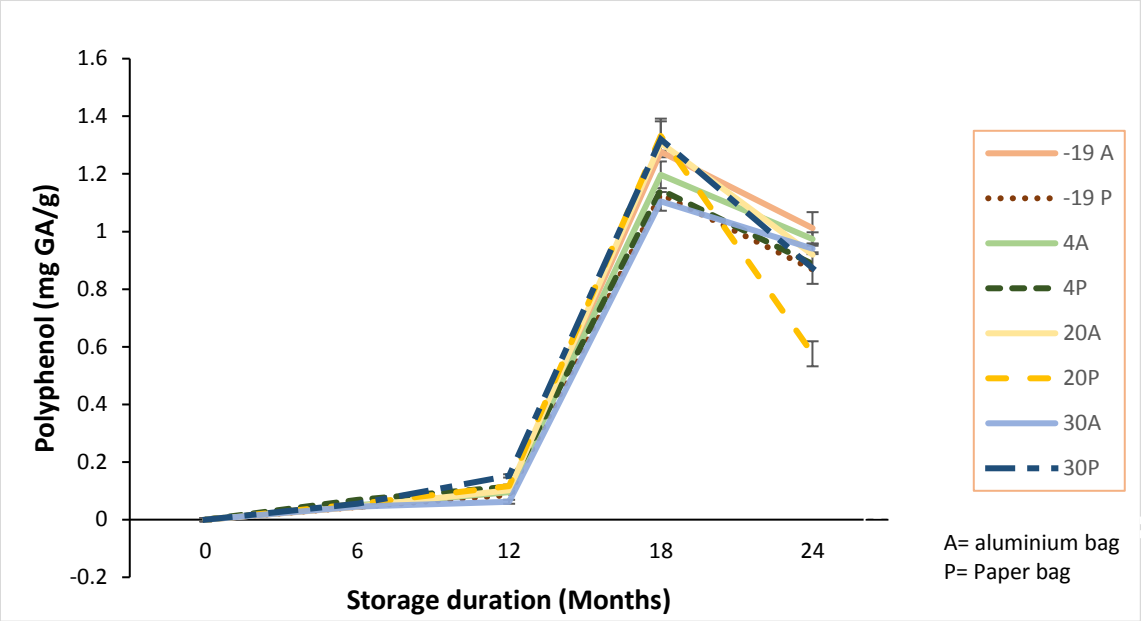

Figure 3. Polyphenol content seed stored for 24 months in aluminum and paper bags at temperatures -19, 4, 20 and 30˚C. Error bars represent ± standard error.

3.3 Flavonoids

The flavonoid content of freshly harvested seed was 0.113 mg GA/g. The flavonoid content increased progressively from 6 months upward but the increase was only statistically significant from 18 months (Fig. 4). Although storage period seem to be the main factor influencing the increase in flavonoids, some differences were noticed between temperature-packaging treatments of the same storage period. The increase in seed stored at low temperatures (-19° and 4°C) was generally smaller than of seed at high temperatures (20° and 30°C) up until 18 months of storage. At 24 months, storage temperature and packaging type influenced the seed flavonoid content. The flavonoids content continued to increase for most treatments but for seed stored at high temperatures in paper bags (20° and 30°C), a significant decrease was recorded.

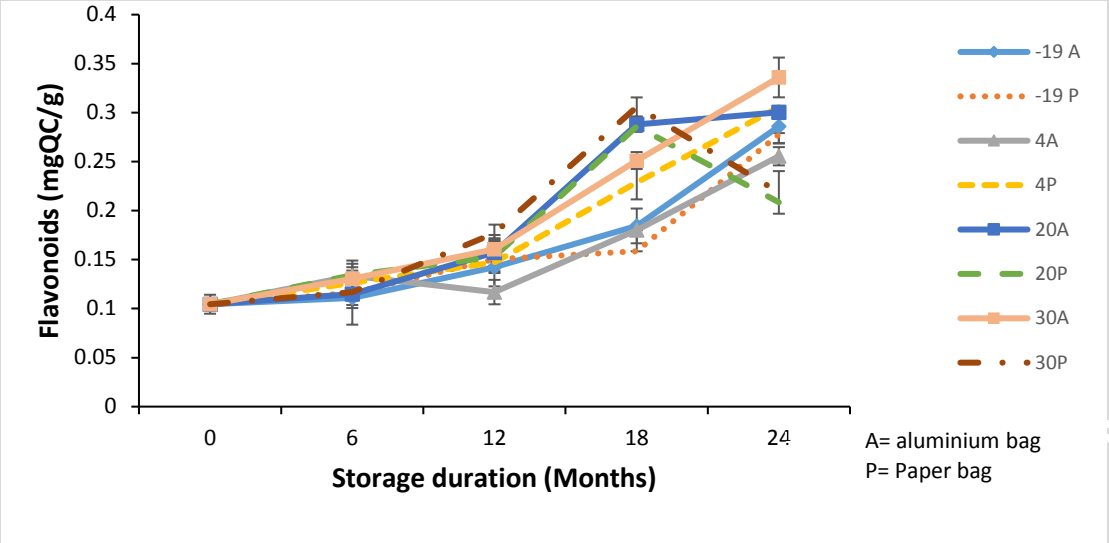

Figure 4. Flavonoids content seed stored for 24 months in aluminum and paper bags at temperatures -19, 4, 20 and 30˚C. Error bars represent ± standard error.

3.4 Total antioxidant capacity (FRAP method)

The total antioxidant capacity of freshly harvested seed (0 Months) was 0.52 µmol (trolox)/gFW. Temperature and packaging type had no significant effect on antioxidant capacity. Storage period was the only factor affecting the antioxidant capacity. The total antioxidant capacity remained unchanged for the first six months and increase significantly by about 3.5-fold (1.825 µmol (trolox)/gFW) after 12 months (Fig. 5). From 18 months, the total antioxidant capacity decreased significantly (below the initial value**,** 0.401 µmol (trolox)/gFW).

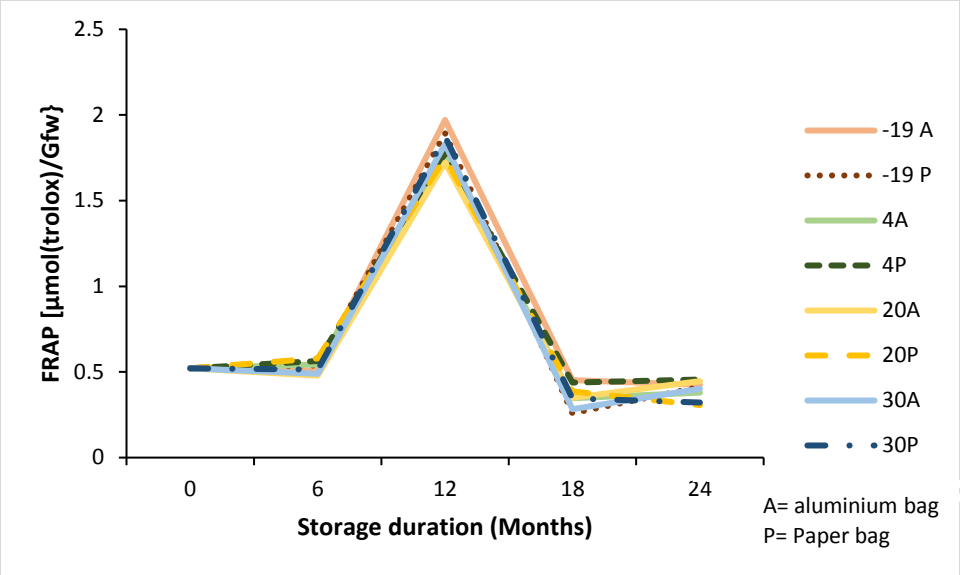

Figure 5. Antioxidant capacity (FRAP) seed stored for 24 months in aluminum and paper bags at temperatures -19, 4, 20 and 30˚C.

3.5 Malondialdehyde content (MDA)

The MDA of freshly harvested seed was 6.65 (MDA)/gFW. Storage period and storage conditions influenced the change in MDA content of the seed (Fig. 6). A slight increase was recorded up until 12 months regardless of the packaging type and the temperature. From 18 months of storage there was a significant increase in MDA for all treatments except for seed stored at -19°C in both packaging types and at 4°C in aluminium bags (Fig. 6). At 24 months, seed stored in aluminum bags at 30°C had the highest content in MDA, follow by seed stored in paper bags at 4°C.

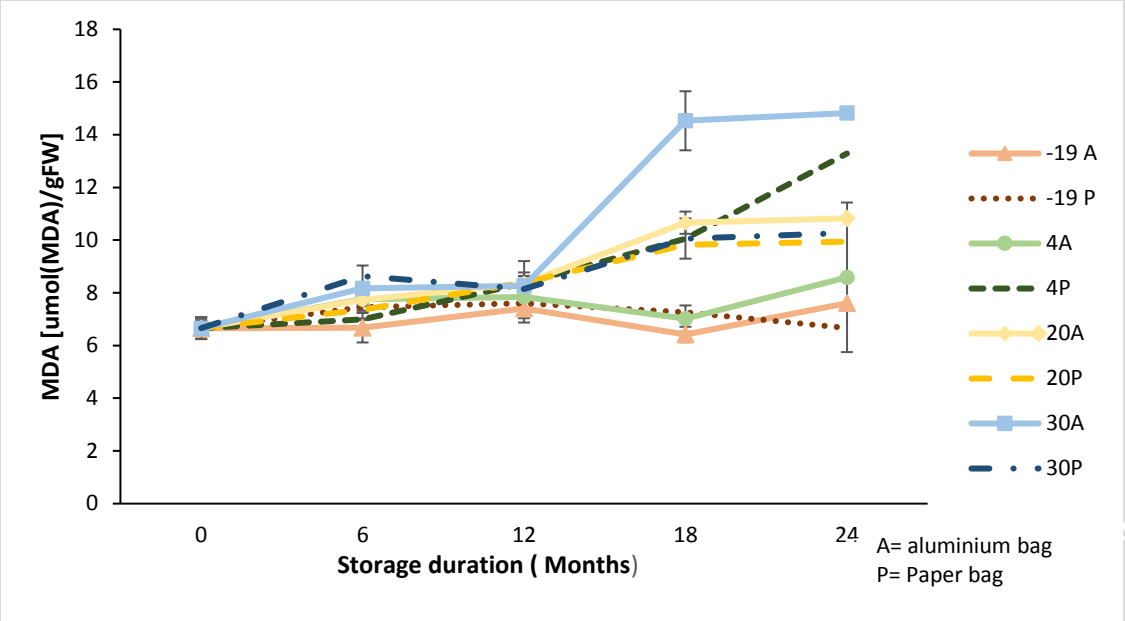

Figure 6. Malondialdehyde content (MDA) seed stored for 24 months in aluminum and paper bags at temperatures -19, 4, 20 and 30˚C. Error bars represent ± standard error

3.6 Relationship between polyphenols, flavonoids, total antioxidant capacity, MDA and seed viability

Up until 12 months of storage there seem be no relationship between the investigated biochemical compounds and the germination percentages as the changes were generally minimal. The possible relationship between the mentioned parameters could only be established at 24 months. There was no correlation between Frap and germination percentage as well as between polyphenols and germination% as the change in this parameters appears to be mainly age dependent. Moderate correlations were found between flavonoids and germination% (r^2 = -0.606) and MDA and germination (r^2 = -0.5038). These relationships are represented in figures 7 and 8 respectively.

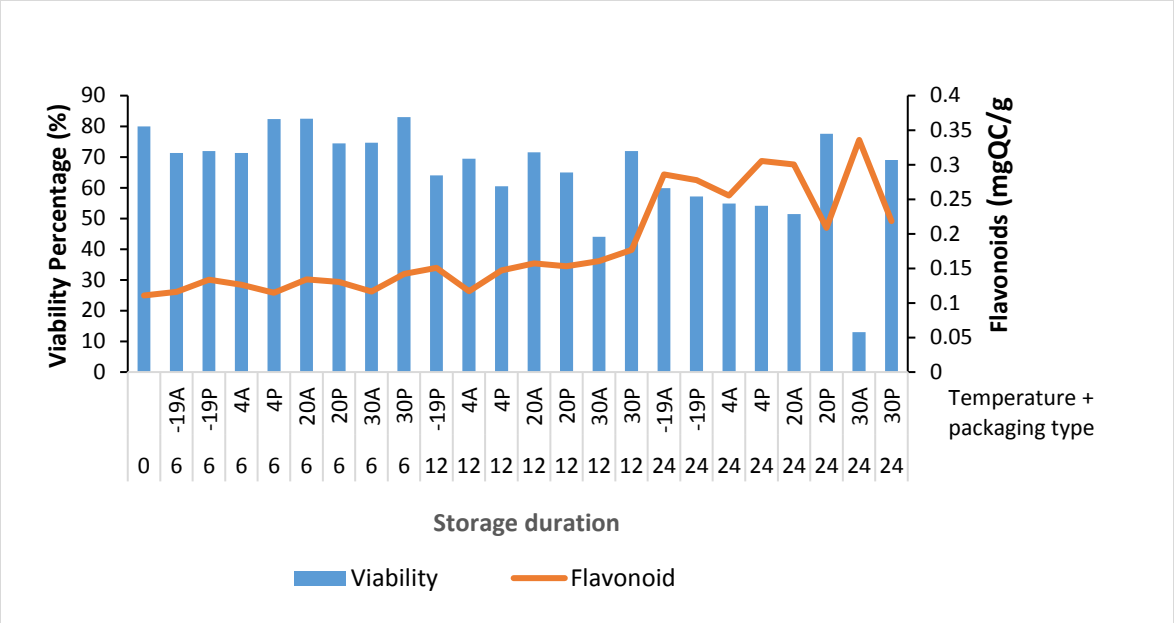

Figure 7. Relationship between flavonoid content and viability percentage of seed stored for 12 and 24 months in aluminum and paper bags at temperatures -19, 4, 20 and 30˚C.

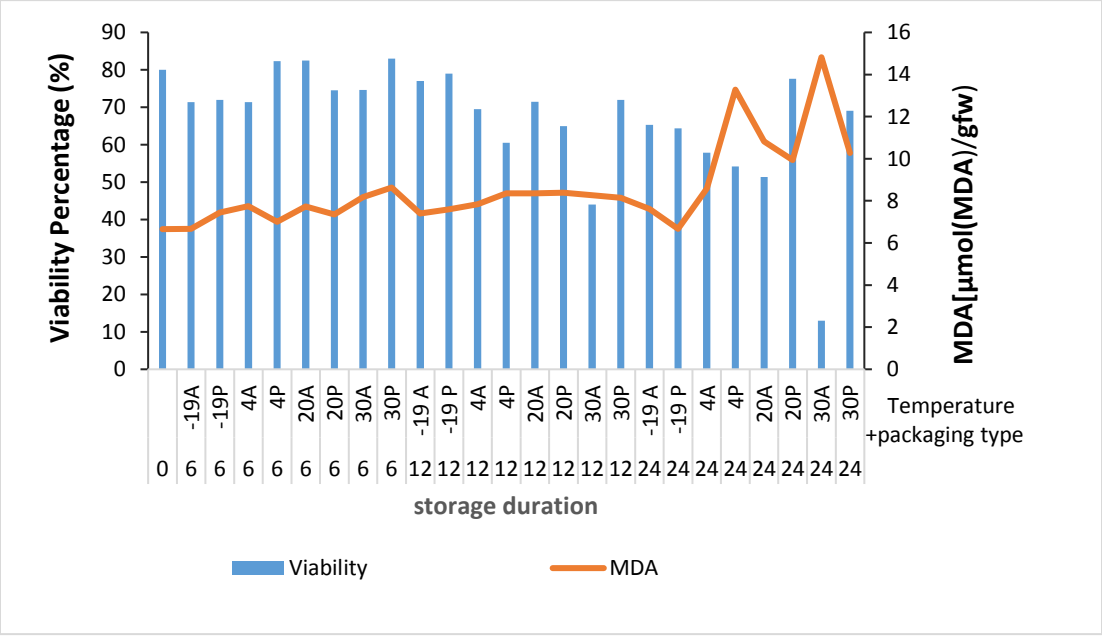

Figure 8: Relationship between malondialdehyde content and viability percentage of seed stored for 12 and 24 months in aluminum and paper bags at temperatures -19, 4, 20 and 30˚C.

4 DISCUSSION

4.1 Moisture content and seed germination

Generally in sealed moisture-vapour proof containers, seed moisture content remains constant, whereas in open storage or moisture permeable containers it changes with changes in the relative humidity of the environment where they are stored (Rao *et al*., 2006). That explains the significant decrease drop of MC in seed stored at 20°C and 30°C in paper bags. It is also the reason behind the observed increase of MC of seed stored in incubator at low temperature and high relative humidity. Drumstick tree seeds readily take up and lose water to their environment; both processes of imbibition and desiccation appeared to be completed within 24 h or less under conditions of free water for imbibition and extremely dry, still-air conditions for desiccation (Moravec et al., 2008). From the time courses presented in this study, it appears that the seed tissues of this species present no major barriers to water uptake or loss.

Other studies have found Moringa initial viability to be ± 80% and reported an improvement in germination after priming or storing Moringa seed for short period at temperature between 25 and 35°C (Nouman et al., 2013; Mubvuma et al., 2013). Our previous study (Fotouo et al., 2015) suggested dormancy in Moringa seed. This could the explanation for the slightly low viability obtain with fresh seed and seed stored at low temperature. It was observed that at 30°C, seed stored in paper bags had a high germination percentage even after storage of 24 months, while the germination of seed stored in aluminium bags declined as storage period increased. The high germination observed for seed stored in paper bag at 20°C and 30°C (low moisture content) is in agreement with the findings of Moravec et al. (2008) who suggested that ± 4% MC may be ideal for long term storage of Moringa. The significant decrease in germination percentage in seed stored

in aluminium bags at 30°C is as a result of a combination between high moisture content (± 8% mc) and high temperature. This explanation is also valid for seed stored in aluminium at 20°C. Although the moisture content of seed stored at 4°C and -19°C was also high in both packaging types, the deterioration was prevented by the low temperature. Pradhan and Badola (2012) found that seed of *Swertia chiragita* stored in sealed specimen tubes for up to 24 months maintained a higher percent germination at 4°C than seed stored at -15°C. They concluded that 4°C was a more efficient temperature for long-term storage than -15°C. In this study no significant difference was found between seed stored at 4°C and that stored at -19°C.

4.2 Changes in polyphenols, flavonoid, FRAP and MDA content

Other studies **(**Puckaka and Ratajckak, 2005; Bolling et al., 2010; Tsantili et al. 2011**)** found either an increase or decrease in secondary compounds with antioxidants properties in fruit or seeds during storage. In the present study, flavonoids increased throughout the storage while FRAP and polyphenols increased up until 12 and 18 months respectively. Ageing time seems to be the main factor influencing the increase except at 24 months of storage where there were noticeable differences between some temperature-packaging treatments. Seed moisture content which is determined by packaging type and storage temperature appears to be the main drive behind the differences observed in flavonoids between treatments. Similarly Bolling et al. (2010) reported progressive increase in flavonoids, FRAP and total phenol values in almond stored at 4° and 23°C. They found no difference between storage for total phenol and FRAP. The increase was mainly as a result of storage duration; no difference was found between temperatures except for total flavonoids were a significance difference was found between temperatures at 15 months of storage. It was suggested that polyphenol synthesis may continue after harvest in seed as has been reported

to occur in other fruits (Bolling et al. 2010). However in some studies an opposite trend was found. Tsantili et al. (2011) found a decrease in total phenol, total flavonoids and FRAP in pistachio nut stored in packaging atmosphere at 1°C and 20°C for 12 months. The increase and/or decrease in flavonoid, polyphenol and antioxidant activity is probably due to their ability to control toxic ROS that are formed during storage when enzymatic antioxidant activity is almost absent. Antioxidant response to ROS or to environmental stress can be characterized by two phases: first, the increase in the synthesis of antioxidants that inhibit the accumulation of ROS an there by preventing bio-molecular damage in the cell. This is followed by a second phase in which the level of antioxidants drops. As the level of ROS increases, antioxidants degradation exceeds its synthesis and the levels decrease and consequently ROS might continue to accumulate above the critical threshold (Munneé-Bosch. 2005; Shahidi et al., 2006)

An increase in MDA during seed storage was reported in many studies (Rao et al. 2006; Choudhury and Mandi, 2012) and was indicated to increase gradually with the loss of seed viability. Similarly to the present study, De Oleiveira (2017) found that lipase activity of Moringa seed stored 4°C and 27°C did not change until 12 months of storage but observed a degradation of storage lipids at 18 months of storage. The increase in MDA is often aggravated by the exposure of seed to high temperature and humidity. This explains the higher increase of MDA in seed stored in aluminium bags at 20° and 30°C as well as in paper bags at 4°C. Seed in aluminium bags maintained the high initial moisture content (7-8%) throughout storage and were exposed to high temperature. The moisture content of seed stored at 20° and 30°C in paper bags decreased during storage ($\leq$ 5.55%); explaining the lower increase of MDA throughout the storage compared to those stored in aluminium at the same temperatures. The moisture content of seed stored in paper bags at 4°C

increased slightly (8.68%) during storage leading to the increase of MDA. Although an increase in moisture content was also observed in seed stored in paper bags at -19°C, a higher increase in MDA was probably prevented by the freezing temperature that may have decreased the activities of enzymes responsible for lipid peroxidation (Choe and Min, 2006). Based on fatty acid composition of oil (77% m11onounsaturated, 2% polyunsaturated and 22% saturated) extracted from *M. oleifera* seed (Lalas and Tsaknis, 2002), it is clear that the seed contains less polyunsaturated fatty acids which are prone to lipid peroxidation. This might be the reason behind the slow increase of MDA in stored Moringa seed compared to other species such as cotton (Goel and Sheoran, 2003) and *Oryza sativa* L. (Choudhury and Mandi, 2012) where an increase of more than two fold (compared to initial level) was recorded within 24 months or less of storage.

4.3 Relationship between polyphenols, flavonoids, total antioxidant capacity, MDA and germination percentage

A closer look at different treatments revealed that seed stored in paper bags at 20 and 30°C had the lowest moisture content, the lowest flavonoids content and the highest germination % at 24 months of storage. Flavonoids have been suggested to prevent seed deterioration by removing reactive oxygen species (ROS) and thereby preventing ROS from damaging cellular bio-molecules (Choudhury et al., 2012). Although there is no clear explanation behind this relationship, it is possible that the low moisture content lead to low water activity, restraining the accumulation of ROS and the synthesis of flavonoid in seed.

Another evident relationship is between the high MDA content and the low germination percentage observed in seed stored in aluminium bags at 30°C for 24 months (Fig 7). Seed stored in aluminium

bags at 30°C for 24 months also had the highest increase in flavonoids (Fig 8). The impairment of the antioxidant system causes an increase in lipid peroxidation and lipid peroxidation can induce membrane damage and electrolytes leakage from the cells leading seed deterioration. As mentioned above, high temperature and high moisture content accelerate lipid peroxidation in seed. According Esmaeili *et al*. (2015), flavonoids guard organisms against lipid peroxidation, explaining the highly significant correlation between the flavonoids and MDA. This also implies that the high content of flavonoids found in seed stored in aluminium bags at 30°C for 24 months (Fig.1) was as a response to increased lipid peroxidation that led to the loss of seed viability. This is supported by the study of De Oleiveira (2017) where he reported that the loss of viability and vigour was accompanied by the degradation of storage lipids after storing Moringa seed for 18 months.

In summary, minor differences were found between viability of seed stored below 20°C. It is recommended that Moringa seed be stored with low moisture (≤5%) for long-term storage at temperature below 30°C. Regarding biochemical analysis, the difference in trends observed in different seed species may depend on whether they have the ability to continue producing secondary compounds after harvest/during storage, or the production stops during seed maturation. In this study the polyphenol content and the antioxidant capacity were mainly affected by storage time. Seed moisture content influenced flavonoids and MDA content. The change in the investigated biochemicals in seed did not always reflect on its germination percentage. However, a possible relationship was established between seed moisture content, flavonoids content, MDA content and seed germination percentage. This relationship is evidence that an increase in lipid peroxidation contributes to seed deterioration and that flavonoids may play a major role in

protecting seed from the deleterious effect of lipid peroxidation. In future studies should consider storing Moringa seed for longer than 24 months in order to improve r the knowledge on the process of seed deterioration.

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