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Research Article

STORABILITY AND SEED QUALITY ASSESSMENT OF NIGER (*GUIZOTIA ABYSSINICA*) SEEDS STORED IN AMBIENT CONDITIONS

Jaya Singh[1,2]*., Seema Paroha[2] and Ravi Prakash Mishra[1]

[1]Department of P.G. Studies and Research in Biological Sciences, Rani Durgavati University, Jabalpur-482001, India

[2]Biochemistry Laboratory, Project Co-ordination Unit, All India Coordination Project (Sesame and Niger), Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur-482004 India

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ABSTRACT

The present study investigates the effect of storage on seed quality parameters of niger (*Guizotia abyssinica*), a marginal oilseed crop of Central India. The studied quality parameters were: percentage of vital molecules (oil content, protein and sugars), markers of seed deterioration (lipid peroxides, proline content) and activity of antioxidative enzymes (peroxidase, catalase) in the seeds stored for a period of seven years under ambient conditions. The results revealed that during storage, oil content reduced by 13%, protein by 18.4%, total sugar by 23.4% and reducing sugars by 45.6%. The seed deterioration markers increased as storage time increased. Proline content increased by 20.22% while malonaldehyde (lipid peroxide) increased by 32.6%. The antioxidative enzyme activity decreased by 45.5% for peroxidase and 36.2% for catalase respectively. Statistical analysis shows that niger seeds can be stored for one year without any significant loss of quality and upto 4 years with marginal loss of quality parameters.

INTRODUCTION

Oilseeds are important sources of edible oils of nutritional, industrial, and pharmaceutical importance. The extracted oilseeds are useful as animal fodder. Niger seed belongs to the same botanical family as sunflower. The genus *Guizotia* (Compositae) comprises six species and *Guizotia abyssinica* Cass. is the only species cultivated. Niger (*Guizotia abyssinica* L.F.) Cass. is an oil yielding plant native to Ethiopia and Malawi (Dimberu *et al*., 2015), though is being cultivated in some parts of India, especially in Madhya Pradesh (Dwivedi, 2014). Niger seed resembles sunflower seeds in shape, but is smaller in size and black, bears a fairly thick, adherent seed coat and contains good amounts of proteins, oil and soluble sugars. Niger seed has high oil content (30-35%). The seed oil is clear, edible and polyunsaturated oil with a nutty taste and sweet odor. Raw oil has low acidity and can be used directly for cooking with fatty acid composition similar to sunflower oil and has high content of linoleic acid (Ramadan and Mörsel, 2002). Nasirullah *et al*. (1982) have investigated niger seed samples collected from Maharashtra and Gujarat (India) to find the oil content ranged from 30.0 to 32.4% and protein from 26.0 to 30.6%. Niger seed oil has a commercial importance in India because of good oil quality and lower costs.

Niger is a minor crop in India, hence due to the less production of its seeds, storage of seeds is an important aspect owing to the high oil content as well as other nutritional values. During storage, seed quality can remain at the initial level or decline to a level that may make the seed unacceptable for commercial purposes and/or planting. Storage longevity may vary and is influenced by the initial quality of stored seed as well as storage conditions. It has been demonstrated that niger seed can be stored for up to a year without deterioration. Unfavourable storage conditions, i.e. moisture, temperature, humidity etc. accelerate the seed deterioration during storage (Heatherly and Elmore, 2004). Further, in oil crops, such as, niger, rancidity of the oil due to autooxidation of lipids during the storage period is an important consideration (Balašević-Tubić *et al*., 2005). Raw niger oil has low acidity and can be used directly for cooking. Normally it has a poor shelf life and will become rancid when stored for a long period (Dimberu *et al*., 2015). The objective of the present study is to identify the storage

Corresponding author:* **Jaya Singh
Department of P.G. Studies and Research in Biological Sciences, Rani Durgavati University, Jabalpur-482001, India

period of niger seeds and to ascertain the seed quality during the storage period.

MATERIALS AND METHODS

The niger seeds were harvested during 2009 to 2015 from the crops cultivated at the premises of Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur India and stored in sealed polythene bags under normal environmental conditions in different batches at the Department of Seed Technology, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, India. Storage temperature and relative humidity were monitored each day throughout the experiment but were not controlled. One sample lot from each storage year was used for the experiments. The seeds harvested freshly during 2015 were used as control seeds.

Determination of oil content

The oil content was measured by extracting the niger seeds by hexane using Soxhlet extraction method and was calculated as percent value per 100 g seed (AOAC, 1999). To determine the percentage of fat, the dried sample of plant was extracted with hexane (petroleum sprit, bp 40-60°C). It was then distilled off completely and dried. The oil weight and percentage of oil was calculated.

Determination of protein

The total nitrogen was calculated using Kjeldahl method. Briefly, the seed powder was digested in Kjeldahl digestion flask with sulphuric acid and mercuric oxide. The ammonia was distilled from the resultant clear digest and the nitrogen content was calculated using titrimetric method using standard NaOH solution. The nitrogen was converted to total protein by using a factor of 6.25 (AOAC, 1999).

Determination of total soluble sugar

For the estimation of soluble sugars the method proposed by Montgomery (1957) was adopted. Two hundred mg of seeds were homogenised in 80% (v/v) ethyl alcohol using a glass mortar and pestle. The homogenate was refluxed over a steam bath for 4 hours. The flask was cooled and the extract was centrifuged at 4000 x g for 10 minutes. The supernatant was collected and the residue was homogenised again in 80% (v/v) ethanol and refluxed for 1 hour. The extract was clarified by centrifugation; the supernatant was collected and combined with the original. The combined extract was dried and was dissolved in a known volume of distilled water and aliquots were taken from this for estimation.

From the sample, a known volume of aliquot was taken in a test tube and made up to 1.0 ml. To this, 0.1 ml of 80% (w/v) phenol was added and mixed well. Five millilitres of concentrated sulphuric acid was added to the tube quickly from a burette. After cooling, the optical density of the resultant solution was measured using green filter in a colorimeter. D-glucose was used as the standard.

Estimation of reducing sugars

An aliquot from the extract prepared for the estimation of total soluble sugar was used for the estimation of total reducing sugars according to the Nelson-Somogyi method (Nelson, 1944; Somogyi, 1945). Somogyi's Copper reagent was prepared by dissolving 24 g of anhydrous sodium carbonate and 12 g of sodium potassium tartrate in about 250 ml of distilled water. To this, 4 g of copper sulphate as a 10% (w /v) solution was added and mixed followed by the addition of 16 g of sodium bicarbonate. Then 180 g of sodium sulphate was dissolved in about 500 ml of distilled water and boiled to expel air. After cooling, the two solutions were mixed and the volume was made up to 1000 ml (Somogyi, 1952).

Nelson's arsenomolybdate reagent was prepared by dissolving 25 g of Ammonium heptamolybdate in 450 ml of water. Then 21 ml of sulphuric acid was added and mixed well. To the mixture 3.0 g of disodium hydrogen arsenate dissolved in 25 ml of distilled water was added. The solution was mixed well and incubated for 24 hours at 37°C (Nelson, 1944).

From the sample, a known volume of aliquot was pipetted out and was made up to 1.0 ml using distilled water. To this, 1.0 ml of Somogyi's copper reagent was added. The mixture was then heated for 20 minutes in boiling water. After cooling under tap water, 1.0 ml of Nelson's arsenomolybdate reagent was added with immediate mixing till the effervescence ceased. The intensity of colour was measured after proper dilution at 540 nm using a spectrophotometer. D-Glucose was used as the standard.

Determination of proline

Proline content in the niger seeds was estimated according to the method of Cha-um and Kirdmanee (2009). One hundred and fifty milligram fresh seeds were weighed out from the finely ground pooled samples and homogenised in 10 ml of 3% (w/v) aqueous sulfosalicylic acid. The homogenate was filtered through Whatman No. 1 filter paper and from the filtrate solution, 2 ml aliquot was taken in triplicates and equal volumes of glacial acetic acid and ninhydrin reagent (1.25 mg ninhydrin in 30 ml of glacial acetic acid and 20 ml of phosphoric acid) were added and mixed well. The tubes were heated in a boiling water bath for 1 h and then the reaction was terminated by placing the tubes in an ice-bath. For colour development, 4.0 ml of toluene was added to the reaction mixture and stirred well for 20-30 seconds. The coloured toluene layer was separated and brought to the room temperature. The colour intensity of the solution was measured at 520 nm using toluene as reagent blank in Spectrophotometer (EI, India). The absorbance of the reaction mixture was converted to the concentration by using standard curve of L-proline (SRL, India). The standard curve was prepared by taking 20, 40, 60, 80 and 100 mg equivalent L-proline dissolved in sulfosalicyclic acid.

Detrmination of lipid peroxides

The lipid peroxides were estimated using the method of Health & Packer (1968). One hundred mg of niger seeds were homogenized with 0.5 ml 0.1 % (w/v) trichloro acetic acid (TCA). The homogenate was centrifuged for 10 min (15000 x g, 4 °C) and the supernatant (0.5 ml) was mixed 1.5 ml 0.5% thiobarbituric acid (TBA) diluted in 20 % TCA and incubated in a water bath at 95 °C for 25 min. The reaction was terminated by incubating on ice. The absorbance was measured at 532 and 600 nm. Free malonaldehyde (MDA) concentration were calculated by subtracting OD_{600} values from the MDA-TBA complex values at 532 nm using the Lambert-Beer law

with an extinction coefficient εM= 155 $mM^{-1}cm^{-1}$. Results are presented as µmols MDA g^{-1} seed weight.

Estimation of Antioxidant Enzymes

Peroxidase Activity

The peroxidase activity was determined according to the procedure given by Abeles and Biles (1991). The peroxidase enzyme was extracted from the niger seeds stored for different time periods as described earlier. The powder of dry seeds was homogenized in 10 ml Tris/HCl buffer (50 µM, pH 7.5) and centrifuged at 16,000 x *g* at 0°C for 15 minutes in a refrigerated centrifuge (Remi, India). For the enzyme assay, 0.3 ml of the supernatant solution is mixed to 1.5 ml Phosphate buffer (100 µM, pH 6.0), 0.3 ml of guaiacol (10 µM) and 0.6 ml water. Finally, 0.3 ml H_2O_2 was added as substrate to initiate the enzyme activity. Immediately after the addition of H_2O_2, the activity was measured at 470 nm by direct spectrophotometry. The blank was prepared by adding 0.3 ml H_2O to the reaction mixture instead of enzyme extract. The changes in absorbance were recorded at 30 second interval for 3 minutes i.e., 30, 60, 90, 120, 150 and 180 seconds. Enzyme activity was determined by subtracting the initial absorbance from the absorbance at 180^{th} second. The unit activity of peroxidase enzyme was expressed as the change in absorbance per minute per gram weight of seeds. The enzyme activity was also expressed as specific activity, which is the activity per mg protein.

Catalase Activity

Two hundred microlitres of the extract supernatant (as shown above) were added to 4.3 ml of phosphate buffer (50 mM, pH 7.0) containing 3.125 mM of H_2O_2. Catalase activity was measured using H_2O_2 decay measuring absorbance at 240 nm and was expressed as nmol of H_2O_2 decay (mg protein)$^{-1}$ min^{-1} (Xu *et al*., 2008).

RESULTS

The niger seeds stored for a duration of seven years were investigated for the important parameters in order to check out the loss of quality as well as the seed deterioration.

Oil percent

The niger seeds cultivated at the premises of Jawaharlal Nehru Krishi Vishwavidyalaya were found to contain good amount of oil in seeds. the freshly harvested seeds from the year 2015 showed oil percent to be 38.35 ± 2.65. The oil percent decreased slowely for the seeds stored for 3 years and rapidly for the seeds stored for longer than 3 years (Fig 1). One way ANOVA revealed that the oil percent was not significantly different during the storage of six years i.e. seeds harvested during 2009 showed comparable amount of oil as of seeds harvested freshly during 2015 ($F_{4,14}$=0.4061, p<0.05).

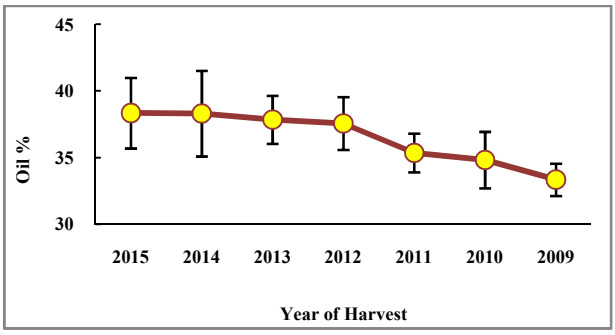

Fig 1 Oil percent in niger seeds stored for a period of six years. The data are presented as mean ± standard deviation (n=3).

Protein content

The protein content in the freshly harvested niger seeds was found to be 18.87 ± 0.81 percent. The protein content decreased constantly during storage (Fig 2). One way ANOVA revealed that the seeds harvested during 2009 and 2010 (stored for six and five years respectively) showed significant decrease in protein content ($F_{4,14}$=1.07, p<0.05). The seeds stored for less than five years showed no ignificantly difference in their protein content.

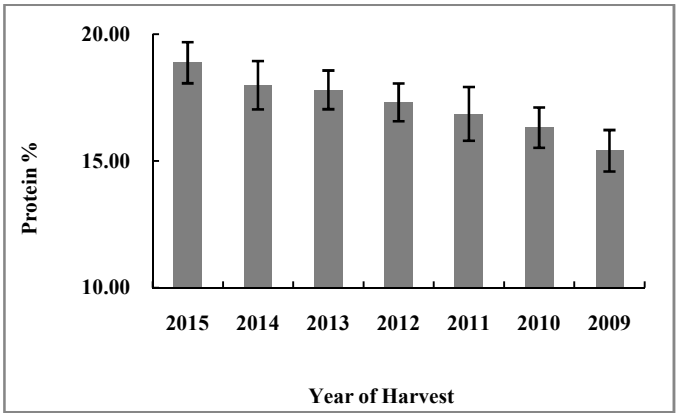

Fig 2 Protein percent in niger seeds stored for a period of six years. The data are presented as mean ± standard deviation (n=3).

Sugar Profile

The total soluble sugar in terms of glucose was calculated using the standard calibration curve of D- glucose (Y=0.0127X, R^2=0.9522). Total sugar in niger seeds were found to be 14.57 ± 0.82 % in control seeds i.e. seeds harvested freshly in 2015. The total soluble sugars decreased with increase in storage time (Fig 3). One way ANOVA shows siginicant decrease in total soluble sugar content after three years of storage ($F_{4,14}$=2.196, p<0.05) in comparison to control seeds. It means that seeds harvested in year 2009, 2010 and 2011 showed lower amount of total soluble sugars.

The reducing sugars were in the range of 3.92 ± 0.74 as evidenced by triplicate analysis of seeds harvested freshly during 2015. The decreasing trend was observed in amount of reducing sugars also (Fig 3). One way ANOVA revealed that amount of reducing sugars significantly decreased after the four years of storage ($F_{4,14}$=4.610, p<0.05).

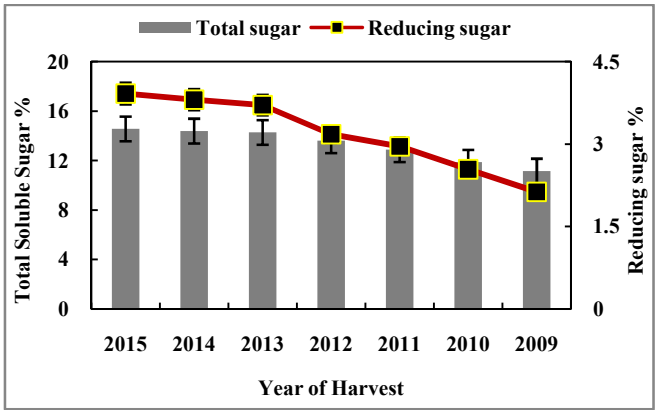

Fig 3 Total soluble sugars and reducing sugars percent in niger seeds stored for a period of six years. The data are presented as mean ± standard deviation (n=3).

Lipid peroxides

The lipid peroxides are indicative of seed deterioration. The MDA content was found to increase in niger seeds under increasing storage times (Fig 4). The control seeds showed MDA content as 1497.35 ± 119.7 per gram of seeds, which increased gradually up to 1985.5 ± 125 when seeds after six years of storage (seeds from harvest year 2009) were tested. The MDA content increased slowly upto five years of storage and than rapidly after fifth year. The seeds harvested in year 2009 and 2010 showed significant increase in MDA content (One way ANOVA, $F_{4,14}$=6.563, $p<0.05$).

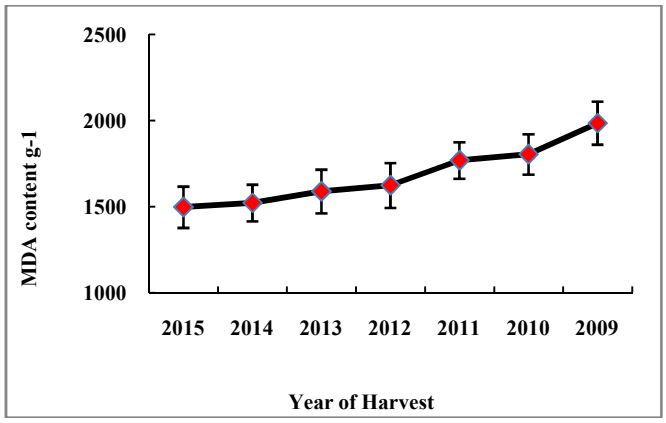

Fig 4 Lipid peroxides in terms of MDA content per gram seeds of niger stored for a period of seven years. The data are presented as mean ± standard deviation (n=3).

Proline content

Accumulation of proline is used as an index in selection for salt tolerance by means of interfering in osmotic adjustment which in turn is indicative of seed deterioration. The proline level was calculated using the standard calibration curve of L- proline (Y=0.2161X, R^2=0.9532). The proline content was found to be increased with storage time. The control seeds showed low levels of proline (98.89 ± 5.8 mg g^{-1} seed). Fig 5 shows that the increase was slow upto three years of storage and rapid afterwards. One way ANOVa revealed that the increase in proline content was not significant upto five years of storage and only seeds from year 2009 showed significantly elevanted levels of proline ($F_{4,14}$=2.965, $p<0.05$).

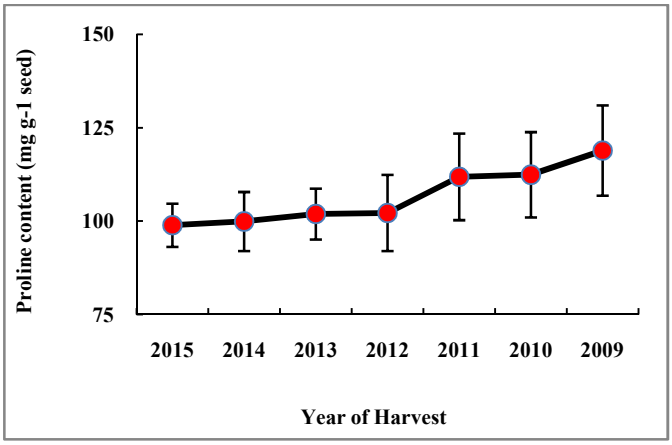

Fig 5 Proline content (mg proline per gram seeds) in niger stored for a period of seven years. The data are presented as mean ± standard deviation (n=3).

Antioxidant Enzymes

Antioxidative enzymes help in maintaining quality of oil by delaying the process of rancidity, hence are considered good parameters for the quality assessment of oilseeds. The present study evaluated peroxidase and catalase enzyme activity for the same.

Peroxidase activity

The enzyme activity of peroxidase was measured as Units per mg^{-1} seed protein. In control seeds, the peroxidase activity as measured as 16.05 ± 1.05 Units mg^{-1} protein. The peroxidase activity decreased with increase in storage time (Fig 6). Statistical analysis by one way ANOVA shows that the seeds harvested from 2009, 2010 and 2011 showed significantly low levels of peroxidase activity as compared to control seeds of year 2015 ($F_{4,14}$=20.96, $p<0.05$).

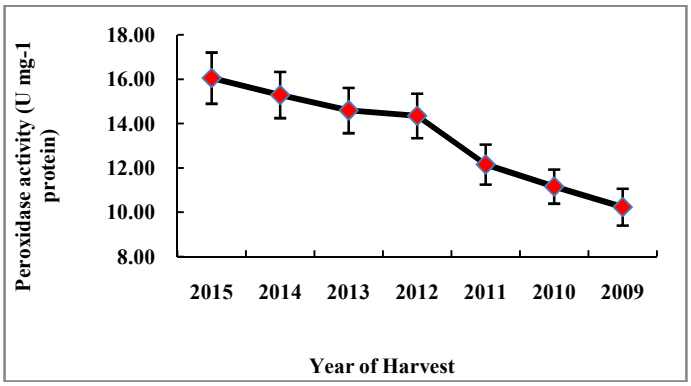

Fig 6 Peroxidase activity (unit mg^{-1} protein) in niger seeds stored for a period of seven years. The data are presented as mean ± standard deviation (n=3).

Catalase activity

The activity of catalase followed the trend observed with peroxidase activity (Fig 7). The statistical data revealed that the seeds from 2009 and 2010 showed significant decrease in catalase activity as compared to control, and the difference was highly significant. The seeds from year 2011 also showed significant difference in catalase activity in comparison to control, but the difference was marginal ($F_{4,14}$=25.01, $p<0.05$).

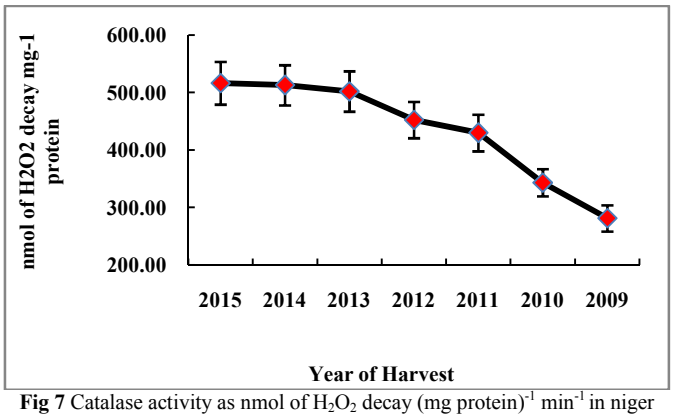

Fig 7 Catalase activity as nmol of H_2O_2 decay (mg protein)$^{-1}$ min^{-1} in niger seeds stored for a period of seven years. The data are presented as mean ± standard deviation (n=3).

DISCUSSION

Niger is one of the commercially important seed for marginal farmers in Central India as well as some parts of Africa, due to its high quality seed oil and its ability to grow in marginal conditions (Nema *et al*., 2006). Because of lower cultivation area, the storage of seeds for plantation and other commercial purposes becomes more important issue. Our group has earlier shown that the stored niger seeds had beeter germination rates in comparison to soybean during storage periods of more than 8 years (Singh *et al*., 2016).

Seeds of different species are believed to have characteristic shelf lives, although data confirming this are scarce. The exact phenonmenon for the same is still elusive, and no definite mechanism has been worked out till date. Overall, the seed deterioration rate is a combination of events and environmental conditions of seed storage. Apart from other storage conditions i.e., moisture, humidity, temperature etc, storage time also affects the seed quality. It has been reported that the free fatty acid contents tend to increase with increase in storage time, while oil content decreases with storage time in *Jatropha curcas* (Akowuah *et al*., 2012). Similar observations have been recorded with sunflower seeds, which are more closed to niger seeds physiologically (de Oliveira Lins *et al*., 2014). Further, the activities of antioxidative enzymes i.e., catalase and superoxide dismutase reduced with time, clearly indicating the seed deterioration. These studies are in line with our findings that shows the decrease in vital molecules i.e. oil, protein, and sugars in seeds during storage and increase in detriorative parameters such as lipid peroxides and proline content. The loss of antioxidative enzyme activities further promotes the oxidation of seed oil, which may hamper the oil quality. The study indicates that the niger seeds can be stored for a period of one year without significant loss of seed quality, and upto four years, with marginal loss of important seed quality parameters. Niger (*Guizotia abyssinica*) is one of the understudied and underexplored oilseed, but with potential to be an important oilseed crop for the deprevided farmers and poor soil conditions. Further studies that can contribute to the better use of the crop and its products should be highly welcomed.

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