NUSANTARA BIOSCIENCE
Vol. 6, No. 2, pp. 126-131
November 2014

ISSN: 2087-3948
E-ISSN: 2087-3956
DOI: 10.13057/nusbiosci/n060204

Biodeterioration of stored castor (*Ricinus communis*) seeds by *Aspergillus tamarii*

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Manuscript received: 16 May 2014. Revision accepted: 10 July 2014.

Abstract. *Negedu A, Joseph Ab, Umoh VJ, Atawodi SE, Rai MK. 2014. Biodeterioration of stored castor (*Ricinus communis*) seeds by* Aspergillus tamarii*. Nusantara Bioscience 6: 126-131.* Biodeteriorative changes induced by *Aspergillus tamarii* in seeds of castor stored for a period of 180 days were investigated. Using Sabouroud Dextrose Agar (SDA) and direct plating methods, mycoflora associated with stored castor seeds were examined. The lipolytic ability of the isolates was determined based on free fatty acid production (quantitative). The effect of *Aspergillus tamarii* on moisture, total fat, crude protein, nitrogen free extract, ash contents as well as free fatty acid and peroxide values were monitored bimonthly for six months during storage in the laboratory. In storage, *Aspergillus tamarii* caused significant ($P \leq 0.05$) increases in free fatty acids, peroxide value, moisture content, crude protein, ash and crude fibre contents and significantly ($P \leq 0.05$) reduced the total fat and soluble sugar content (NFE) of the inoculated seeds compared to the uninoculated control. *Aspergillus tamarii* associated with stored castor seeds caused deteriorative changes in the seeds during storage period.

Key words: Biodeteriorative changes, *Aspergillus tamarii*, seeds, castor, mycoflora.

INTRODUCTION

Castor seeds are obtained from the castor plant (*Ricinus communis* L.). The seeds contain economically important oil (Roetheli et al. 1991) which has been reported to have one thousand patented industrial applications and it has already been utilized in the production of over 300 products in the textile, leather, cosmetics, plastic, Rubber, automobile, pharmaceutical and engineering industries (Gobin et al. 2001; Anjani et al. 2004). Agricultural produce such as soybeans, sunflower, safflower, *Irvingia gabonensis* and allied products are vulnerable to contamination, colonization and subsequent deterioration by fungi which can cause damage up to 80-100% to the produce (Anjani et al. 2004).

Deterioration, a biochemical change that effect considerable changes in seeds and their constituents, as well as the free fatty acid and peroxide values of the seed oil, had been attributed to the growth of fungi on the seeds. For instance, fungal-colonized soyabean seeds, maize seeds and groundnuts had been reported to have caused reduction in protein, oil and carbohydrate contents (Bhattacharya and Raha 2004) and Negedu et al. (2010) reported increased free fatty acid content and peroxide value of stored soya bean oil.

Although, literature revealed that several researchers had reported reduction in oil, protein and carbohydrate content, as well as increased free fatty acid and peroxide values in fungal-infected oil seeds, but little or no information appeared to be available on the biochemical changes induced in castor seeds by associated fungi. Therefore, the experiment was designed to evaluate the ability of fungi causing deterioration by using *Aspergillus tamarii* as a test fungus on Nigerian castor seeds.

MATERIALS AND METHODS

Collection of seed samples. Shortly after harvesting and sun-drying, two wild popular castor seed types (large- and small-sized) were purchased from local farmers in Ankpa and Kabba areas of Kogi State, Nigeria. Visibly mouldy as well as necrotic lesioned seeds were removed after which whole seeds that failed to pass through ¾ X ¾ inch mesh were used as substrates.

Sample preparation. Three hundred grams of the approximately uniform seeds were weighted into 1 liter plastic jars and plugged with cotton wool. The seed samples were prepared in triplicates, with a forth jar as control. All the jars containing seeds were autoclaved at 121^{o}C for 15 minutes at 15cm^2. After cooling, 25ml of hot sterile-demineralized water was added to the seeds in the jars to equilibrate the moisture content to about 18% w/w and shaken intermittently.

Isolation of fungus from castor seeds. *Aspergillus tamarii* Kita was isolated from naturally infected stored castor seeds employing the method described by Bankole et al. (2005) and confirmed by the Global Plant Clinic of the Commonwealth Agriculture Bureau International (CABI), London.

Inoculation of seeds. Using a sterile cork borer of 5mm diameter, the fungus on agar plate was aseptically introduced into the autoclaved seeds (Bankole et al. 2004). The jars were incubated at room temperature (28±2°C) for 180 days with intermittent shaking.

Proximate composition and biochemical changes in castor seeds. The free fatty acid, peroxide value and proximate composition of the seeds were bimonthly evaluated using standard methods of AOAC (1995). The biochemical changes that occurred in both the experimentally-infected seeds and the control were compared at p = 0. 05 and means separated using Duncan's Multiple Range Test.

Data analysis. Data were expressed as mean± standard error of M (SEM). The data were subjected to one-way analysis of variance (ANOVA). SPSS soft ware was used to analyze the data and P< 0. 05 was considered statistically significant.

RESULTS AND DISCUSSION

Table 1. shows the effect of fungal growth on the parameters studied of castor seeds after 0, 60, 120, 180 days of storage, both uninoculated and inoculated by *Aspergillus tamarii*, parameters studied includes moisture, crude protein, fat, crude fiber, ash content, nitrogen fibre, free fatty acid, and peroxide value.

The moisture content

The initial moisture content (7.20±0.21%) of the inoculated seeds decreased and subsequently increased to a higher value (8.46±0.16%) till end of storage period (Figure 1). A similar trend was observed in the un-inoculated seeds (control). Statistical analysis however, revealed that at the end of storage period (180 days), the difference between the moisture content value of the inoculated and Uninoculated seeds was statistically significant (P≤0.05).

This observation confirmed with the findings of Bhattacharya and Raha (2004) who reported initial increase in moisture content of fungi-infected stored grains (groundnuts and soybeans), followed by a gradual decrease during prolonged storage. However, the result of this study was at variance with that of Ward and Diener (1961) who observed decreases in the moisture content of fungi-infected groundnuts within the experimental period. The variance in the results could be attributed to the fact that moisture content values do not necessarily reflect the average. However, the values for some grains may be above the average and since fungi grow where the moisture levels are favorable and not necessarily according to average moisture content of grains (Sweets 2000), it is likely that these grains could be responsible for the observed rise in moisture content *vis-à-vis* the metabolic activities. The subsequent decrease in the moisture content of the seeds as the storage period was prolonged (Figure 1.) may be attributed to the denaturation of protein causing reduced water holding capacity of the seed tissues. This may have resulted from physical changes in structure of the seeds due to ageing process during storage and enzymatic reactions. It is also possible that, during the drying period in the field and storage, and consequent enzymatic activities within the tissues of the seeds remained at higher potentials, thereby activating the enzymes (lipoxygenases) of the seeds and resulting in accelerated moisture migration together with thermogenesis (Majunder 2007). Since the test fungus inoculated into the castor seeds was the same species that would colonize the stored castor seeds under normal conditions, there are therefore strong indications that the test fungus was responsible for the deterioration of stored castor seeds.

The crude protein content

The initial crude protein value (2.18±0.03%) of the inoculated seeds increased to a higher value (26.72±1.44%) and subsequently decreased to a lower value (22.10±0.45%) at the end of the storage period (Figure 2). A similar trend was observed in the un-inoculated seeds (control). Statistical analysis revealed that at the end of storage period, the difference between the value of crude protein content of the inoculated and un-inoculated seeds was not statistically significant (P≥0.05).

The increase in the crude protein content of the inoculated seeds was significantly higher than that of the uninoculated at the early days of storage before decreasing as the storage period was prolonged (Figure 2). It is in confirmation with earlier findings of Ataga and Ota-Ibe (2006) who reported similar increase in crude protein content of stored fungal-infected seeds of *Irvingia gabonensis*. The initial increase in the level of crude

Table 1. Effect of fungal growth on the parameters studied of castor seeds after 180 days of storage

Parameters Studied	Duration of storage (in days)							
	0		60		120		180	
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
Moisture	3.61±1.01	3.61±1.01	6.34^{a} ±0.13	6.47^{a} ±0.13	5.34^{a} ±0.14	5.39^{a} ±0.14	7.95^{b} ±0.16	8.46^{a} ±0.16
Crude protein	21.28±0.03	21.28±0.03	20.83^{a} ±1.44	26.72^{b} ±1.44	21.86^{b} ±0.32	25.82^{ab}±0.32	20.93^{a} ±0.45	22.10^{a} ±0.45
Fat	47.55±0.42	47.55±0.42	31.24^{a} ±1.82	22.09^{b} ±1.82	28.12^{a} ±0.94	19.82^{b} ±0.94	26.65^{a} ±1.18	17.08^{b} ±1.18
Crude fibre	10.68±2.11	10.68±2.11	23.87^{b} ±.071	27.95^{a} ±.071	32.19^{a} ±1.21	34.34^{a} ±1.21	35.50^{a} ±2.46	149.10^{a} ±2.46
Ash content	3.61±1.01	3.61±1.01	4.45±^{a} 0.17	4.20^{a} ±0.17	5.64^{a} ±0.98	4.29^{a} ±0.9±8	4.74^{b} ±0.21	6.48^{a} ±0.21
Nitrogen fibre	11.79±1.11	11.79±1.11	24.03^{a} ±1.06	25.55^{a} ±1.04	17.25^{a} ±1.49	16.38^{a} ±1.49	15.34^{a} ±0.56	6.48^{a} ±0.56
Free fatty acid	9.21±0.02	9.21±0.02	19.51^{b} ±0.80	28.80^{a} ±0.80	38.61^{b} ±1.92	65.79^{a} ±1.92	23.28±2.43	36.41±2.43
Peroxide value	3.07±0.50	3.07±0.50	5.81^{b} ±0.30	12.34^{a} ±0.30	17.09^{b} ±0.89	34.97^{a} ±0.89	9.63^{b} ±0.85	16.91^{a} ±0.85

Note: Means with the same letters are not significantly different at P=0.05.

protein may be attributed to bioconversion of sugars into microbial protein and due to synthesis of certain amino acids (Bilgrami et al. 1976). However, the declined trend at the later part of the storage period (Figure 2), could be attributed to proteolytic enzymes possibly breaking down the protein into indole, ammonia and hydrogen sulphide which may be responsible for the odor of deteriorating seeds. Also, the decrease may be due to the consumption of the protein by test fungus. This assumption is supported by the report of Bhattacharya and Raha (2004) on soybeans and groundnuts.

The total fat content

The initial total fat content (47.55±0.42%) in the inoculated seeds decreased to a lower value (17.08±1.18%) at the end of the storage period (Figure 3). A similar trend was observed in the un-inoculated seeds (decreased from 47.55±0.42% to 26.65±1.18%). Statistical analysis revealed that the difference in the value of total fat content between the inoculated and un-inoculated seeds was statistically significant ($P \leq 0.05$)

The significantly lower levels of total fat content in the colonized seeds over control (Figure 3). It is in agreement with the findings of Bhattacharya and Raha (2004) who reported decrease in the fat contents of soybeans, maize and rice as result of fungal growth on the seeds. The decrease might have resulted from the fat utilization by the storage fungi. Bankole et al. (2005) recorded a progressive decline in total fat content of melon seeds after three months of storage. The reduction in quality and quantity of oil rich crops is generally attributed to utilization of total fat by the colonizing fungi (Ataga and Ota-Ibe 2006).

The crude fiber

The initial fiber content value (10.68±2.11%) of the inoculated seeds increased sharply to a higher value (149.10%±24.65%) at the end of storage period (Figure 4), while a gradual increase in the crude fiber content value occurred in the un-inoculated seeds (control) (from 10.68±2.11% to 35.50±24.65%). Statistical analysis however, revealed that between the crude fiber content value of the inoculated and un-inoculated seeds, the difference was not significant ($P \geq 0.05$).

The level of crude fiber content was significantly higher in the inoculated seeds than the un-inoculated (Figure 4.). This result confirmed with the findings of Horn (2005), who observed increased crude fiber content of fungal-infected peanuts in storage. The increase in the crude fiber content may be attributed to the ability of the fungus to produce necessary enzymes required for the hydrolysis of the structural carbohydrates such as cellulose and hemicellulose into their component monomers. Some of the polysaccharides might have been retained as crude fiber leading to the higher value in the crude fiber content recorded in the fungal colonized seeds. This reasoning is supported by Hamlyn (1998) who reported that fungi have the ability to produce a variety of enzymes which would help to hydrolyse non-starch polysaccharides in the substrate into their component monomers. In addition, it was possible that the growing test fungus on the castor seeds caused the bioconversion of some of the polysaccharides in the seeds to soluble sugars while the non-converted polysaccharides remained as crude fiber which resulted in the increased fiber content level.

The total ash content

The initial ash content value (3.61±1.01%) of the inoculated seeds gradually increased and slightly decreased before rising to a higher value (6.48±0.21%) at the end of storage period (Figure 4). In the control seeds (un-inoculated seeds), an initial rise was followed by a fall in the ash content value (from 3.61±1.01 to 4.74±0.21%) till end of storage period. However, statistical analysis showed that between the inoculated and un-inoculated seeds, the difference in the ash content values was statistically significant ($P \leq 0.05$).

The level of ash content which remained unchanged in both the inoculated and uninoculated at the early period of storage, however increased slightly in the inoculated compared to the uninoculated as the storage time prolonged (Figure 5). This result agreed with the report of Ataga and Ota–Ibe (2006) on seeds of *Irvingia gabonensis.* The increase could be attributed to non-or less utilization of minerals present in the medium leading to proportionate increase. Increase could also be due the presence of certain mineral elements (such as K and P) in the mycelium of the test fungus growing on the stored seeds.

The nitrogen free extract content (NFE)

The initial value (11.79±1.11%) of nitrogen free extract of the inoculated seeds increased to a higher value (25.55±1.04%) and subsequently decreased to a lower value (14.49±0.56%) at the end of storage period (Figure 6). A similar trend was observed in the nitrogen free extract value of the un-inoculated seeds. Statistical analysis revealed that between the inoculated and un-inoculated seeds, the difference in the value of nitrogen free extract at the end of storage period was not statistically significant ($P \geq 0.05$).

The initial rise in level of NFE obtained in the un-inoculated seeds declined appreciably as the storage time was prolonged (Figure 6). This result disagreed with Bhattacharya and Raha (2004), who reported a decreasing value of soluble carbohydrates content (nitrogen free extract) of fungal–infected seeds of groundnut and soybeans. This initial increase in the NFE after inoculation could be attributed to the hydrolysis of polysaccarides (hemicellulose, cellulose, etc.) in the castor seeds into soluble carbohydrates (NFE) by the test fungus. This reasoning is supported by Hamlyn (1998) who reported the ability of fungi to produce a variety of enzymes (such as cellulase, hemicellulase, catalase, xylanase and pectinase) needed for the hydrolysis of the polymeric components.

However, the decline in the level of the NFE (soluble sugars) at a later stage could be attributed to the uptake of the products of hydrolysis of polysaccharides by the fungus. It could also be that some of the simple sugars formed were utilized in respiration. This could have led to reduction in the level of NFE. It could then be inferred that the test fungus growing on the seeds played effective roles in the deterioration of the experimental seeds in storage.

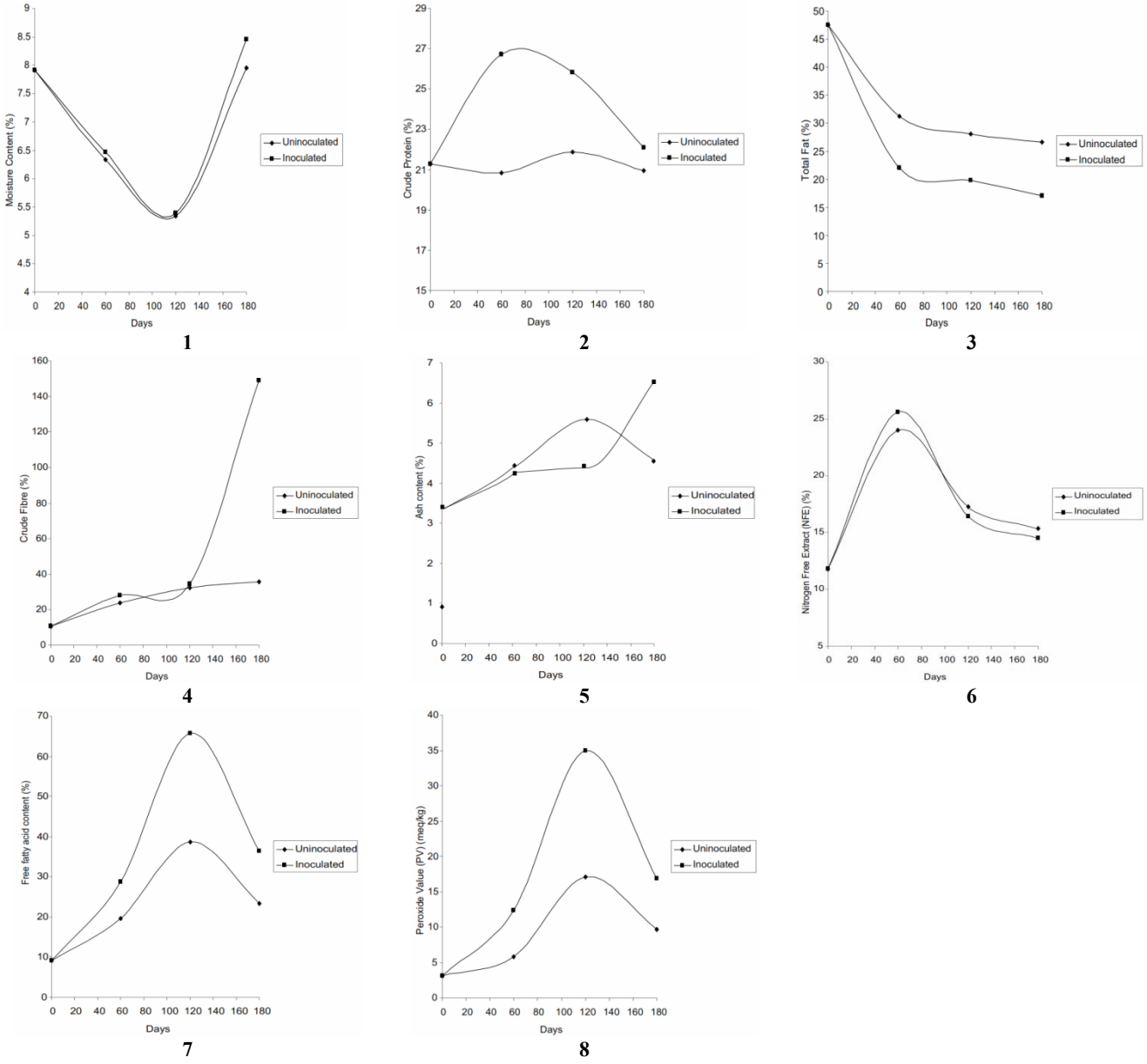

Figure 1. Effects of fungal growth on moisture content of castor seed after 180 days of storage
Figure 2. Effect of fungal growth on crude protein content of castor seeds after 180 days of storage
Figure 3. Effect of fungal growth on the total fat content of castor seeds after 180 days of storage
Figure 4. Effect of fungal growth on crude fibre content of castor seeds after 180 days of storage
Figure 5. Effect of growth of the test fungus on ash content of castor seeds after 180 days of storage.
Figure 6. Effects of the test fungal growth on the nitrogen free extract content (NFE) of castor seeds after 180 days of storage
Figure 7. Effect of fungal growth on free fatty acid content of castor seeds after 180 days of storage
Figure 8. Effect of the test fungal growth on peroxide value of castor seeds after 180 days of storage

The free fatty acid (ffa) content

The initial free fatty acid value (9.21±0.02%) of the inoculated seeds increased to a higher value (65.79±1.92%) and subsequently decreased to a lower level (36.41±2.43%) at the end of storage period (Figure 7). A similar trend was observed in the uninoculated seeds (control) at the end of the storage period. Statistical analysis revealed that at the end of the storage period, the difference in the value of free fatty acid between the inoculated and un-inoculated seeds was statistically significant ($P \leq 0.05$).

This observation agreed with the report of Aboaba and Amasike (1991) and Bankole et al. *(*2005) on melon seeds. In the same vein, increase in the level of free fatty acid had been reported by Oso (1979) on stored palm fruits. This increase in the level of free fatty acid could be as a result of the conversion of the oil into fatty acids as observed by St. Angelo and Ory (1983). It could also be due to production of the free fatty acids at a rate faster than was utilized by the associated fungi growing on the seeds resulting in an increased ffa level. Earlier workers had reported that some

fungi utilized fatty acids as sole carbon sources for energy and growth (Sowunmi 1981; Negedu et al. 2010). Such fungi produced lipases which hydrolyzed the ester linkages of the oil to free the linked fatty acids thereby giving rise to an increased level of the free fatty acid.

The decrease in the level of the free fatty acid could also be as a result of low lipolytic action of the enzymes as the environmental conditions in the medium became unfavourable for enzymatic activities. Increases in the free fatty acid might have resulted from the hydrolysis of either the triglycerides or the more complex lipids such as phospholipids as observed by Beuchat (1976) who reported that some fungi are active lipase producers, hydrolysing triglycerides to yield free fatty acids that accumulated to various levels depending on the substrates.

The peroxide value

The initial peroxide value (3.07±0.50Meq/kg) of the inoculated seeds increased to a higher value (34.97±0.89Meq/kg) and subsequently decreased to a lower value (16.91±0.85Meq/kg) at the end of the storage period. (Figure 8). A similar trend was observed in the uninoculated seeds (control). Statistical analysis revealed that between the inoculated and un-inoculated seeds the difference in the peroxide values was statistically significant ($P \leq 0.05$).

A significant increase in the level of the peroxide value was obtained in the colonized seeds compared to the control (Figure 8). This was followed by a decline thereby not corresponding to the extent of oxidation as deterioration continued with prolonged storage of the seeds. Similar observations had been made by previous authors Adebiyi et al. (2002) on fungal-infected groundnuts, Bankole et al. (2004) on melon seeds and Negedu et al. (2012). Those workers recorded increased level of peroxide values as a result of the growth of fungi on the oilseeds. The rise in the value of peroxide value could be due to the incorporation of oxygen into the oil by the formation of hydroperoxides which are themselves unstable and readily decompose into more stable products such as aldehydes, ketones (Budin et al. 1993). It had been established that lipid oxidation proceed rapidly in dry systems (Budin et al. 1993). The rise in the oxidation value (peroxide value) and the occasional decrease is in contrast to the findings of Sowunmi (1981) who reported a continuous increase in oxidation value in stored palm oil.

This rise followed by decline which did not correspond to the extent of deterioration could be attributed to the decomposition or further oxidation of these peroxides into secondary products such as aldehydes, ketones, epoxides. These could be largely responsible for the off-flavor and odor characteristics of stale oily products or deteriorated seeds (Arumughan et al. 1984). This could also be explained on the basis of possible conversion of the free fatty acid to compounds such as hydroperoxyfattyacids through peroxidation, since the hydroperoxyfattyacids are not very stable compounds and are converted into a set of more stable ones such as aldehydes, ketones (Gaillard 1975). From this study, it was observed that the lipolytic fungus while contributing to the deterioration of the castor seeds by increasing the amount of free fatty acid, also affected oxidative state of the oil in the seeds (Figure 8).

The observed steady rise in the peroxide value (Figure 8) may also mean that the formation of peroxide was a steady process at the initial stage, while the decline resulted from the transitory nature of the peroxides formed, which decomposed to products of secondary reaction (Amoo and Asoore 2006). Although Sowunmi (1981) had observed that mycelial growth has no significant effect on the oxidation of palm oil because, in his studies, the control oil sample oxidized to about the same extent with the colonized samples, however in this study, the fungus studied might have induced auto-oxidation, because the peroxide value of the colonized seeds was higher than that observed in the control. The variance may have been due to difference in crop genotype and fatty acid profile (Balesevic-Tubic et al. 2007) and the strain of fungi involved. At the initial stage of oxidation, hydroperoxides might have formed at a much faster rate than the rate of their decomposition leading to an increase in their levels as oxidation proceeded in the stored seeds. The hydroperoxides which have no odor or off-flavor decomposed as oxidation progressed to give volatile compounds that have resulted in the strong off-flavor and odor (Ezebor et al. 2005), indicating that the fungus were involved in the deterioration of castor seeds.

CONCLUSION

In conclusion, the study has revealed that the growth of fungus (*Aspergillus tamarii*) induced bio-deteriorative changes in the seed constituents such as total protein, total fat, nitrogen free extract (NFE) as well as free fatty acid and peroxide values. All these together reduce the economic value of the seeds and therefore, castor seed value chain operators (producers, processors, marketers etc.) are advised to prevent the growth and development of fungi (moulds) in seeds along the entire castor seed value chain.

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