Nigerian Journal of Botany, Volume 27(2), 241-250, December, 2014

INVESTIGATION INTO THE MICROORGANISMS ASSOCIATED WITH THE DETERIORATION OF AMARANTHUS HYBRIDUS (AMARANTH) (Linn) UNDER STORAGE.

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Received, 9th August, 2014 ; accepted, 30th October, 2014

ABSTRACT

The incidence of microorganisms and deterioration in vegetables may be expected to reflect the sanitary quality of the processing steps and the microbiological condition of the raw product at the time of processing. This study investigated microorganisms associated with the deterioration of *Amaranthus hybridus* under two different storage conditions: refrigeration (4°C) and at room temperature (28°C). Fresh samples of *Amaranthus hybridus* were obtained from three different sources (farm, market and vegetable vendors) in Lapai town, Niger State, Nigeria. Microorganisms associated with vegetables sampled were isolated on Potato Dextrose Agar (PDA) and identified. The results showed that samples stored at room temperature deteriorated completely at the 5th day of storage while those stored at the refrigerating temperature remained fresh. The deterioration was marked by loss of green colour to mushiness of the vegetables and defoliation. The bacteria isolated were *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeroginosa*, *Proteus mirabilis* and *Staphylococcus aureus*. The fungal isolates were *Aspergillus niger, Aspergillus flavus*, *Mucor pusillus* and *Aspergillus fumigatus*. The pH of the samples was found to increase with increase in number of days of storage at the two conditions of storage. The moisture content of samples stored at room condition increased and was found to have contributed to deterioration.

Key words: *Amaranthus hybridus*, deterioration, fungal and bacterial species

INTRODUCTION

Amaranthus hybridus L, popularly called "Amaranth or pigweed", is an annual herbaceous plant, 1-6 feet high. The leaves are alternate, petioled, 3 – 6 inches long, dull green, and rough, hairy, ovate or rhombic with wavy margins. The flowers are small, with greenish or red terminal panicles. Taproot is long, fleshy red or pink. The seeds are small and lenti-cellular in shape; with each seed averaging 1 – 1.5 mm in diameter and 1,000 seeds weighing 0.6 – 1.2 g. It is rather a common species in waste places, cultivated fields and barnyards. In Nigeria, *A. hybridus* leaves combined with condiments are used to prepare soup (Oke, 1983; Mepha *et al.*, 2007). In Congo, the leaves are eaten as spinach or green vegetables (Dhellot *et al.*, 2006). It is called "Aleho" in Hausa, "Inine" in Igbo, "Tete abalaye" in Yoruba (Fayemi, 1999).

Deterioration refers to any change in the condition of food in which the food becomes less palatable or even toxic. These changes may be accompanied by alteration in taste, smell or appearance (Effiuvwevwere, 2000).

The incidence of microorganisms in vegetables may be expected to reflect the sanitary quality of the processing steps and the microbiological condition of the raw product at the time of processing. For 100 years, vegetables contaminated in the field have been recognized as a source of human infection. Many of the

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viruses (Engle and Altoveras, 2000), bacteria and protozoan on vegetables which have caused food poisoning are derived from human faeces. However, pathogenic microorganisms of human origin may also be present in minimally processed vegetables as the minimal technological processing may be unable to remove the original contamination resulting from air, soil, water, insects, animals, workers, harvesting and transportation equipment. Certain fungal species such as *Aspergillus, Fusarium,* and *Penicillium* are commonly occurring filamentous fungi that grow in vegetables and their growth may result in production of toxins known as mycotoxins, which can cause ill-health in humans from allergic responses to immune suppression and cancer (Baiyewu *et al.*, 2007). The ability of public health agencies to identify through enhanced epidemiological and surveillance techniques, raw vegetables as probable sources of infectious microorganisms has undoubtedly resulted in increased numbers of outbreaks.

Storage fungi can cause decrease of germination capability, loss in weight, discoloration of kernels, heating and mustiness, chemical and nutritional changes, and mycotoxin contamination. They can change fat quality of peanuts by hydrolytic enzymes producing free fatty acids and glycerol. Altogether, these changes could lead to lower quality of leafy vegetables (Kendar and Rolle, 2004).

Amaranths are nutritious vegetables largely consumed in Nigeria; many tend to lose their value due to lack of adequate knowledge in deterioration and poor storage methods. This study aimed to determine the microorganisms and other physicochemical factors causing deterioration in *Amaranthus hybridus* under storage.

MATERIALS AND METHODS

Collection and Storage of samples.

Fresh samples of *Amaranthus hybridus* were obtained from three different places (farm, market and vegetable vendors) in Lapai town, Niger State, Nigeria, and were kept in sterile bags. Plant identity was confirmed by botanists in the Herbarium of the Department of Biological Sciences, Ibrahim Babadamasi Babangida University, Lapai. Only samples of good quality, free from disease injury, were used in this study. They were properly cleaned and stored in sterile open plastic containers in the laboratory under two different environmental conditions: at 4°Cin the refrigerator and 28± 2°C laboratory room temperature (Kitinoja and Kader, 2003).

Determination of pH of the vegetable samples

The change in pH of the stored vegetables was determined before and during storage period. Twenty grams (20 g) of the sample was weighed at days 1, 3, 5, 7and 9 into sterile beakers. The sample was crushed; 20 ml of sterile distilled water was added, and allowed to rest for 30 min after which the pH was determined. Three replicates of each were made.

Determination of degree of deterioration of samples during storage

The severity of deterioration during storage at days 1, 3. 5, 7 and 9 was determined. Change in colour, moldiness and defoliation of the leaves were observed and recorded using the rating scale 0 to 5(Bhuiyan and Croft, 2011).

Moisture content determination

The Association of Official Analytical Chemists (A.O.A.C) (1990) method was used to determine the moisture content of the samples before storage(day1) and at the end of storage period(day9). Evaporating dishes of known weights were used. One gramme of each sample was weighed into each dish. All weighed samples were kept in the oven at 70°C for 24 hours to dry up. The samples were brought out and weighed again. Loss in weight of sample was represented by loss in water content and was calculated thus:

Weight of dish = Xg
Initial weight of dish + sample = Yg
Initial weight of the sample = Y – X = Pg
Final weight of dish + sample = Zg
Final weight of the sample = Z – X g = Qg
% moisture loss = (P – Q)g / P X 1 X 100

Isolation of pathogenic fungi and bacteria species from the deteriorating samples

The edge of infected samples of *Amaranthus hybridus* collected from the farm, market and vendor labeled and L_1, L_2 and L_3, respectively, were excised and cut into 1mm pieces and surface-sterilized in 0.1% Mercury Chloride for one minute and rinsed in four successive changes of sterile distilled water and the sections were then blotted dry on clean, sterile paper towels. They were plated on Potato Dextrose Agar (PDA) and Nutrient Agar (NA) in 3 replicates and incubated for 36 hours at 32 ± 2°C under 12-h photoperiod as in the method of Rangaswami and Bagyaraj (2007).

Isolation and identification of the fungal and bacterial isolates

Isolation, characterization and identification of the microorganisms were carried out using colonial, morphological and biochemical characteristics. The fungal isolates were identified based on examination of the colonial heads, phlalides, conidiophores and presence or absence of foot cells or rhizoids. Growth colonies of stock isolates were repeatedly sub-cultured three times on PDA plates using aseptic techniques to obtain pure isolates of organisms. Fungi isolated were identified using Fungi Families of the World Mycological monographs by Samsonand Reenen-Hoekstra (1988). Stock cultures were prepared using slant Potatoes Dextrose Agar in sterile McCartney bottles and preserved at 4°C in a refrigerator (Amadi and Adebola 2008). Incidence of foliar diseases was monitored throughout the period of the experiment. The bacterial species were identified using the following biochemical tests: coagulase test, catalase test, mortility test, indole test and sugar fermentation test, according to the method of Rangaswami and Bagyaraj (2007).

RESULTS

Deterioration of stored vegetables

Table 1 shows the deterioration of vegetable samples stored at room temperature28± 2°C and at 4°C refrigeration. At day 1, there was no noticeable infection; leaves were green and fresh under both conditions of storage. At day 3, the same observations were made for samples stored at 4°C but at room temperature, the colour of leaves collected from farm and market changed to greenish yellow with less than 15% defoliation. However, the leaves of samples collected from vendors were completely yellow, with slight moldiness and 15 to 35% defoliation. At day 5, the samples stored in refrigerator were still green and looked fresh while samples collected from vendors and stored at room temperature turned blackish, heavily moldy and with over 67% defoliation. Followed closely were the samples collected from the market and stored at room temperature. At days 7 and 9, all samples in the refrigerator showed slight moldiness and the leaves turned yellowish green whereas samples under room temperature were moldy with almost complete defoliation.

pH Change during Storage

Generally, gradual change in pH of samples (Table 2) was observed under the two conditions in which the samples were stored. Samples collected from the farm and stored in refrigerator had initial pH of 5.87 before storage, which increased to 6.35 at the end of the storage period. The same trend was observed in other samples collected from the market.

Moisture Content (MC)

Increase in the moisture content of *Amaranthus hybridus* stored at room temperature (Table 3) was observed. Samples collected from farm had initial value of 80.12% MC but increased to 84.04% at the end of day 9. The MC of the samples collected from vendors was the least with initial value of 80.56% and final value of 80.82%.

Fungi and Bacteria species Isolated

A total of six fungal species from four different genera were isolated and identified (Table 4). These fungi were *Aspergillus niger, A. flavus, A. fumigatus, Penicillium notatum, Mucor pusillus and Rhizopus stolonifa.* These species were isolated from all the samples except *M. pusillus and P. notatum* that were not present in samples collected from farm and market. A total of five bacteria species (Table 5)from five genera were isolated and identified from samples collected (*Bacillus subtilis, Pseudomonas aeroginosa, Escherichia coli, Proteus mirabilis* and *Staphylococcus aureus)*. Out of these bacterial species, only *E. coli* and *Bacillus subtilis* were isolated from all the samples collected.

Table 1: Deterioration of vegetable Samples stored under two different conditions

Samples	Day 1		Day 3		Day 5		Day 7		Day 9	
	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
$L_{1(Farm)}$	0	0	0	1	0	2	2	4	2	4
$L_{2(Market)}$	0	0	0	1	0	3	2	4	2	4
$L_{3(Vendor)}$	0	0	0	2	0	4	2	4	2	4

T_1 = Refrigeration temperature 4°C T_2 = Room temperature 28 ± 2°C L_1, L_2 and L_3 = Leaves

Numbers in the table are from rating scale adapted from Bhuiyan and Croft, 2011.

Scale	Severity of deterioration
0	No noticeable infection, leaves are green and fresh
1	Leaves colour changed to greenish yellow/chlorosis with <15% defoliation
2	Leaves completely yellow, slight moldiness with 15-35% defoliation
3	Leaves turned yellowish brown, moldy and 35-67% defoliation
4	Leaves turned blackish, completely moldy and 67-100% defoliation

Table 2: pH of *A. hybridus* during storage at room temperature(28°C) and in refrigerator(4°C)

Sample		Days of storage				
		1	3	5	7	9
$L_{1(Farm)}$	At 4°C	5.87	5.71	6.02	6.27	6.35
	At 28°C	5.87	5.88	5.96	6.19	6.70
$L_{2(Market)}$	At 4°C	6.32	6.37	6.48	6.52	6.64
	At 28°C	6.32	6.93	7.03	7.13	7.19
$L_{3(Vendor)}$	At 4°C	5.76	5.79	6.02	6.12	6.17
	At 28°C	5.76	5.95	6.43	6.65	6.77

Table 3: Moisture content of *A. hybridus* stored at room temperature (28 ± 2°C)

Samples	Moisture content (%)	
	Initial	Final
$L_{1(Farm)}$	80.12	84.04
$L_{2(Market)}$	81.30	82.79
$L_{3(Vendor)}$	80.56	80.82

Table 4: Fungi species isolated from *Amaranthus hybridus* samples

Samples	*A. niger*	*A. flavus*	*P. notatum*	*M. pusillus*	*A. fumigatus*	*R. stolonifa*
$L_{1(Farm)}$	+	+	–	+	–	+
$L_{2(Market)}$	+	+	+	+	–	+
$L_{3(Vendor)}$	+	+	+	+	+	+

Keys: L_1, L_2 and L_3= Leaves + = *presence* , _ = *absence*

Table 5: Bacterial species isolated from *Amaranthus hybridus*

Samples	*Bacillus subtilis*	*Pseudomonas aeroginosa*	*Escherichia coli*	*Proteus mirabilis*	*Staphylococcus aureus*
L_1(F)	+	+	+	+	–
L_2(M)	+	+	+	–	–
L_3(V)	+	–	+	–	+

Keys: L_1, L_2 and L_3= Leaves + = *presence* , _ = *absence*

DISCUSSION

The results showed that deterioration began in *Amaranthus* samples collected from vendors and market and stored at room temperature before those collected from the farm. The vegetable samples stored at refrigerating temperature (4°C) took 7days before any evidence of deterioration was sighted. A lowering of the storage temperature has resulted in a considerable lengthening of the storage life. This result might probably be due to the arrest of the activities of the microorganisms at this temperature and the biological processes responsible for the break-down of the produce are greatly retarded by a lowering of the storage temperature. This was also reported by Kitinnoja and Kader (2003) that the microorganisms tend to be slow in their activities due to low temperature so that increase in the shelf life of the vegetables increased.

The high amount of microbial deterioration observed with samples taken from Lapai market, vendors and stored at room temperature might be because the environment in which the vegetable was exposed had been polluted with high fungal density. This is in agreement with the study of Adebanjo and Shopeju (1993) and Ofor *et al.*(2009), who separately reported that bacterial and fungal contamination of vegetables might be from polluted air from the surrounding, soil, irrigation water and handling processes by man before storage. The deterioration observed in this work was evident by loss of green colour to mushiness of the leaves and very high microbial count. The colour changes varied according to the storage condition. The samples stored at room temperature (28 ± 2°C, 60% relative humidity) changed colour from day 3 of storage while the same samples stored at refrigerating temperature (4°C) began from day 7. Different factors, such as respiration, transpiration, translocation and metabolic activity, might contribute to the break-down. Kendar and Rolle (2004) reported loss of green pigments as a post-harvest deterioration of leafy vegetables and microorganisms as agents of deterioration.

The fungal species isolated from this study have earlier been reported by Eaton and Groopman(1994) and Baiyewu *et al.*(2007). *A. niger* was the most commonly occurred among the fungi isolated. Earlier workers had reported this fungus as one that is commonly found on grape fruits (Chulze *et al.*,2006), apples (Oelofse *et al.*, 2006) and tomatoes (Yildz and Baysal,2006). Bali *et al.*(2008) reported that *A. niger* caused post harvest deterioration in orange and lime fruits in the field. Eboh and Okoh (1980) reported *Fusarium sp, Aspergillus flavus, Aspergillus niger* and *Mucor species* as the organisms found associated with decayed leafy vegetables. So, the deterioration of the *Amaranthus hybridus* samples was not unconnected with these isolates. Anon (1995) reported that the high moisture contents of *Amaranthus hybridus* coupled with its richness in minerals and vitamins must have encouraged the growth of this microbe on the vegetable leading to its deterioration. Other factors which may be responsible for the observed change in the deterioration rate might be the activity of various enzymes and the growth rate of micro-organisms at room temperatures.

CONCLUSION

Microorganisms naturally present on all foodstuffs can result from contamination from outside elements such as wind, soil, water, insects and handling during harvest. They can also become contaminated during growing, harvesting and transportation to the market (Akintobi *et al.*, 2011). The occurrence of fungal deterioration of vegetables was also recognised as a source of potential health hazard to man and animals. This is due to their production of mycotoxic compounds which are capable of causing mycotoxicoses in man following ingestion (Effiuvwevwere, 2000). It is, therefore, necessary and important that both the farmer who harvests the vegetables into bags for transportation, the marketers and consumers take necessary and appropriate precautions in preventing the contamination of vegetables offered for sales.

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