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Research Paper

The effects of controlled atmospheric conditions in the microbial load of African star apple (*chrysophylum albidum*)

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This research was carried out to evaluate the effects of controlled atmospheric conditions in the microbial load of two varieties of African star apples (Nwanu and Nwaose).The two varieties (Nwanu and Nwaose) of freshly harvested ripe African star apples were sorted and cleaned by washing for removal of damaged fruits and allowed to drain. Each variety was divided into two different batches and stored separately in both refrigerated and unrefrigerated conditions. Three different packaging forms were applied to each batch as follows: covered in the absence of oxygen, uncovered and covered. The whole samples were analysed for microbial load both refrigerated and unrefrigerated. The analysis was carried out every three days for the first week and then weekly afterwards until the fruits got spoilt due to entrance of microorganisms. The microbial count increased from [25.5x105 ± 2.71x105 Cfu/ml] to [280.40x105 ± 56.48x105 Cfu/ml]. There was an increase in the Total Titratable Acidity from [2.2619% ± 0.13% anhydrous citric acid] to [1.6218% ± 0.66% anhydrous citric acid] within the same period. The microbial load in Nwaose [108.45x105Cfu/ml ± 103.16 x 105Cfu/ml] was significantly ($p<0.005$) higher than sweet star (Nwanu) [102.40x105cfu/ml±104.51x105Cfu/ml]. The microbial load for the samples stored under uncovered ambient temperature was highest [144.36x105Cfu/ml ± 128.40x105Cfu/ml] compared to the refrigerated samples stored in the absence of oxygen [70.14x105Cfu/ml ± 64.84 x 105Cfu/ml]. Control of microbial proliferation using low temperature and anaerobic conditions is important for successful storage of African star apple using controlled atmospheric condition. Hence, Controlled-atmosphere storage is one of the most important innovations in fruit and vegetable storage systems as the composition of gas in the storage affects their storage life.

Keywords: African star apple, Microbial load, Atmosphere, *Chrysophylum albidum*

INTRODUCTION

Controlled atmosphere (CA) storage is probably the most successful technology introduced to the fruit and vegetable industry in the 20th century. CA storage commonly uses low oxygen (O_2) levels and high carbon dioxide (CO_2) levels in the storage atmosphere combined with refrigeration. Even very early storage practices may have utilized a modified atmosphere enriched with CO_2 and depleted O_2 levels to extend storage life of fruits, vegetables, cereals, and other commodities (Dilley, 2006).

Controlled atmosphere storage was studied for first time in France by Jacques Etienne Berard in the early 1800s in France. He observed that fruits did not ripen in an atmosphere depleted of O_2 (Dalrymple, 1969).

A controlled atmosphere is an agricultural storage method in which the concentrations of oxygen, carbon dioxide and nitrogen, as well as the temperature and humidity of a storage room are regulated. Both dry commodities and fresh fruit and vegetables can be stored in controlled atmospheres. The method is most commonly used on apples and pears, where the combination of altered atmospheric conditions and reduced temperature allow prolonged storage with only a slow loss of quality (Annis *et al.,* 1993).

Controlled Atmosphere (CA) is a storage technique whereby the level of oxygen is reduced and CO_2 is increased. Quality and the freshness of fruit and vegetables are retained under Controlled Atmosphere conditions without the use of any chemicals. Under controlled atmospheric conditions, many products can be stored for 2 to 4 times longer than usual (Ige *et al*., 2007).

Vegetables and fruit respire; they take in oxygen (O_2) and give off carbon dioxide (CO_2). The storage of fruit and vegetables in a gas-tight environment allows the oxygen level in the air to be lowered and the level of CO_2 to increase. Long-term storage of vegetables and fruit actually involves inhibiting the ripening and ageing processes, thus retaining flavour and quality. Ripening is, in fact, postponed. This occurs as a result of modifying the gas conditions in the cool cell so that the respiration of fruit and vegetables is reduced (Akdemir *et al*., 2000).
Controlled-atmosphere storage is one of the most important innovations in fruit and vegetable storage systems as the composition of gas in the storage affects their storage life. The controlled atmospheric technology involves reduction of oxygen (O_2) and increasing carbon dioxide (CO_2) as compared to the ambient atmosphere. Sometimes it also involves removal of ethylene and addition of carbon monoxide. Controlled atmospheric condition entails continuous monitoring and precise adjustment of these gases within the storage container, to a predetermined level (Amusa *et al.,* 2007).

Moreover, Frazier and Westhoff, (1995) stated that storage at ambient temperature encourages proliferation of microorganism. This implies that with a combined treatment of pasteurization and refrigeration, apples can be stored up to six weeks (Chukwumalume *et al*., 2012).
Today, the marketing of apples and pears in virtually all fruit-growing regions of the world depends upon controlled atmosphere storage technology. Controlled atmosphere provides a means of regulating supplies of apples, pears and other produce. These fruits are harvested over a relatively short period, yet through the use of CA storage, they can now be made available to the public for most of the year. Several types of whole and minimally processed fruits and vegetables have been successfully stored in modified-atmosphere packages. It has been shown to be effective in extending the postharvest life and quality of a wide range of fruits and vegetables (Dilley, 2006).

The African star apple (*Chrysophylum albidum*) which is known as "udara" in Igbo language is a tropical fruit from a perennial tree and most widely consumed in south-east and south-west of Nigeria. It is usually abundant in dry season when it ripens and disappears during the rainy season. It is mostly sold and consumed fresh and it is not known to be of any other commercial industrial value despite its potential for use in jam/jelly making and alcohol (Falade, 2001).

The palatability of the fresh fruit lasts for 4-8 days after which it becomes highly objectionable to consume due to deterioration in aroma, colour, taste, appearance. Its natural microbiological quality apparently deteriorates during storage. The fresh fruit is a good source of ascorbic acid (Adisa, 2000).

Some varieties are known to have pronounced soured taste and as a result are not very popular, hence its lower commercial value. There is a need to make fruits like the African star apple available all year round or even extend their storage life long enough to be transported to distant market (Falade, 2001).

The phytochemical screening of *Chrysophyllum albidum* plant parts revealed the presence of all the secondary metabolite analysed in the leaves with the exception of tannin, saponin and the reducing sugar and also the stem analysed contain the following compound; alkaloids, steroids, cardiac glycosides, tannins and saponins anthraquinones and reducing sugar while flavonoids and terpeniods are found to be absent in the stem (Kamba and Hassan, 2011).

The presence of this secondary metabolite in this plant may contribute to the effects of this plant as remedy for various diseases raging from antimicrobial, anti-inflammatory, and anti-carcinogenic effect, this may be due to the presence of flavonoid in leaves which resulted to have played a vital role in the treatment of the above forms of diseases. The leaf has antiplatelet and hypoglycaemic properties (Adebayo *et al*., 2010).
In addition, its seeds are a source of oil, which is used for diverse purposes, Its rich sources of natural antioxidants have been established to promote health by acting against oxidative stress related disease such infections as; diabetics, cancer and coronary heart diseases (Burits and Bucar, 2002). Also the presence of Saponins and glycosides of both triterpenes and sterols having hypertensive and cardiac depressant properties, hence the presence of these metabolites in *Chrysophyllum albidum* leaves and stem tend to support its medical uses. Cardiac glycosides have been found to be effective in congestive heart failure (Aboaba, 2001). The presence of these metabolites in the plant confirmed the use of this plant in the treatment of various diseases.

The bark is used as a remedy for yellow fever and malaria, while the leaves are used as emollients and for the treatment of skin eruptions, diarrhoea and stomach ache, which are as a result of infections and inflammatory reactions (Adisa, 2000). Eleagnine, an alkaloid isolated from C. albidum seed cotyledon has been reported to have antinoceptive, anti– inflammatory and antioxidant activities (Idowu *et al.,* 2006). Methanol extract of C.albidum stem bark has been found to have antiplasmodial (Adewoye *et al*., 2010) and antimicrobial activities (Adewoye *et al*., 2011). Ajewole and Adeyeye (1991) also confirmed that unsaturated fatty acids are the main components of the oil (74%) and is desirable in the context of heart disease risk reduction. The residual cake also has potential for animal feed.

It has been discovered that fruits and leafy vegetables demand careful attention at every stage from before/after harvest, through to consumption, if product quality is to be achieved and maintained. Star apple fruit is one of the indigenous fruits that present special problems of both transport and preservation, because they are generally more perishable than most exotic fruits, under tropical conditions of high temperature, high moisture and numerous pests and diseases which attack the succulent mesocarp of the fruits. It is thus attacked by pests and diseases in addition to improper post-harvest handling of fruits that aggravate the quick deterioration commonly observed in this fruit (Adebisi, 1997).

High temperature-short time (HTST) treatments (60, 80, 90°C for 10s) and batch pasteurization (90°C for 10 min) were demonstrated to be effective on the reducing of patulin levels in apple cider but complete damage could not be reached (Kadakal, *et al.* 2002).

The controlled atmosphere storage and/or packaging are a possible means of achieving this shelf life extension. However, the effects of controlled atmosphere storage and packaging on the microbial qualities of the African star apple fruits have not been evaluated. It is therefore important to investigate the possibility of applying controlled storage and packaging for increased shelf life of fresh ripe African star apple fruits. Development of longer shelf life for fresh Udara will improve its availability and increase its commercial value. It will boost its value chain development. It will reduce losses and also help create better variety and make its natural nutrient available beyond its normal on-season. This study therefore evaluates the effects of controlled atmosphere storage and packaging on the microbial load of African star apple.

MATERIALS AND METHODS

Procurement of African star apple fruits

Two varieties of African star apple fruits (Nwanu and Nwaose) were harvested separately from their trees at Nnarabia, Ahiara Ahiazu Mbaise Local Government Area in Imo State, Nigeria, after 21 weeks of maturity. They were sorted to remove damaged ones and the ones that the stalk end has pulled out from inside the fruit exposing the inside area. The intact fruits were washed thoroughly and allowed to drain and dry.

Storage of African star apple fruits

A batch of each variety was divided into six (6) different portions. Out of the six portions, three were kept under ambient storage as follows: One portion was stored in a plastic basket without cover, another was stored in a glass bottle with air tight cover and the third was stored in a covered glass bottle with candle light that burned until all oxygen was consumed. The remaining three portions were stored in the same manner but kept in refrigerated storage (5±0.9°C). All samples from both varieties stored under-different packaging conditions in (27± 1°C) ambient or refrigerated storage (5±0.9°C). The time taken by the fruit from each portion before onset of spoilage was noted and recorded. There spoilage was determined by physical inspection by a five-member sensory panel of people. One piece of African star apple was withdrawn from each portion and used for Microbial analysis

METHOD OF ANALYSIS

Sterilization

The whole media and diluents used were sterilized using the autoclave at 1210C for 15minutes at 15 Psi. The glass wares were also sterilized using the autoclave.

Preparation of media

Nutrient Agar media was used in the isolation of total bacteria counts. According to the manufacturer’s instruction, 28 g of the media powder was dissolved completely in 1liter of distilled water in a round bottom flask. The mixture was stirred consciously for complete dispersion of the powder. The mouth of the round bottom flask was covered with cotton wool wrapped with foil and fully sealed with masking tape. It was further sterilized at 121°C for 15min in the autoclave. The media was then allowed to cool to 45°C before pouring into previously sterilized Petri-dishes. The solidified media were used without delay after the surface dried in a laboratory oven maintained at 40°C (Cheesbough, 2000).

Microbial analysis

The Fresh samples of the African star Apple peel was serially diluted 1:100 and plated from 10-1 using spread plate technique on Nutrient Agar media. There were three sets of samples (covered, uncovered and anaerobic) for both refrigerated and unrefrigerated conditions.

Sampling and counting of microbial growth on sample

The three (3) sets of samples for both refrigerated and unrefrigerated conditions were stored in different 10 ml volume of maccarthney bottle.1ml from each of the sample was serially diluted as 1:100 from 10-1 to 10-5 dilution factor.

Method of plating

The glass wares were all labelled. The spread plate technique was used as described by Bandler *et al.,* (1998). Plating was done in duplicate and the average determined. The number of Colony Forming Units (CFU) was counted by plate using a gallenhamp colony counter. The total number of organism per ml of sample was then calculated.

RESULTS AND DISCUSSION

The effect of the storage conditions on the microbial load of African star apple as shown in Table 1. Refrigeration storage is generally known to retard growth of micro-organism. The ambient stored covered (18.60 x 105±113.03 x 105cfu/ml) and the ambient stored anaerobic (116 x 105±112.72 x 105cfu/ml) were not significantly different ($p>0.05$) but were significantly different ($p<0.05$) from the ambient stored uncovered (144.36 x 105±128.40 x 105cfu/ml). Therefore, refrigeration and protective covering as well as storage in anaerobic conditions are vital factors for the control of microbial growth in the storage of African star apple. The effects of variety on microbial load of African star apple as shown in Table 2. Total Microbial Count for Nwose was significantly higher ($p<0.05$) than that of Nwanu. It could be that the Nwose variety provided a more conducive growth environment for microbes than Nwanu. The Nwose variety is significantly higher ($p<0.05$) in Total Titratable Acidity than Nwanu probably because of higher microbial activity resulting in production of more acids.The effect of duration of storage period on microbial load of stored African star apple is shown in Table 3. Microbial load significantly varied with time. The microbial count doubled at about the 6th day. The increase in microbial count must have contributed to the eventual deterioration and spoilage of the product. The storage in anaerobic conditions prolonged the storage life in respective of the variety and temperature of storage. The Nwanu and Nwose variety had comparable storage life for both the unrefrigerated and refrigerated samples. The increase in the microbial count coincided with the increase in Total Titritable Acidity. The microbes must have been generating acids from the fermentation of the nutrients found in the fruit.The shortest period of palatability occurred in the uncovered sample (of both the refrigerated and unrefrigerated). The uncovered samples also spoilt more rapidly for both varieties of African star apple. The palatability of refrigerated samples stayed 3 times longer than the unrefrigerated. The absence of oxygen and refrigeration reduce metabolic activities that lead to spoilage. Anaerobic conditions must have retarded the activities of all aerobic conditions and the growth of micro-organisms and some enzymatic reaction and other derivative reactions.

Table 1. The effect of the storage conditions on the microbial load of african star apple (*Chrysophyllum albidum*).

Storage Condition	Total Microbial Count Acidity (X105Cfu/ml)	Total Titratable (%anhydrous citric acid)
Ambient Covered	119.86 ± 113.03	1.680 ± 0.47
Ambient Uncovered	144.36 ± 128.40	1.446 ± 0.55
Ambient Anaerobic	115.12 ± 112.72	1.539 ± 0.50
Refrigerated Covered	78.86 ± 77.81	1.868 ± 0.61
Refrigerated Uncovered	106.36 ± 103.70	1.654 ± 0.87
Refrigerated Anaerobic	70.14 ± 64.84	1.656 ± 0.90
LSD	11.118	0.1595

Table 2. The effect of variety on the microbial load of African star apple (*Chrysophyllum albidum*).

Variety	Total Microbial Count (X105Cfu/ml)	Total Titratable Acidity (% anhydrous citric acid)
Nwannu	102.40 ± 104. 51	1.3412 ± 0.24
Nwaose	108.45 ± 103.16	1.9774 ± 0.55
LSD	6.419	0.001

Table 3. The Effect of duration of storage on microbial load of stored African star apple (*Chrysophyllum albidum*).

Duration of Storage (Days)	Total Microbial Count (X105Cfu/ml	Total Titratable Acidity (% anhydrous Citric Acid)
0	25.50 ± 2.71	2.2816 ± 0.14
2	33.42 ± 6.96	2.0814 ± 0.23
4	40.75 ± 10.37	1.9776 ± 0.65
6	52.00 ± 11.82	1.9375 ± 0.63
8	97.83 ± 41.66	1.8362 ± 0.93
14	206.16 ± 77.54	2.2145 ± 0.80
21	280.40 ± 56.48	1.6218 ± 0.66
LSD	12.007	0.283

Table 4. The effect of storage condition on how long the fruit remain palatable (palatability).

Storage Condition	Palatability (Days)	
	(Nwanu)	(Nwose)
Anaerobic Refrigerated	30	28
Covered Refrigerated	26	30
Uncovered Refrigerated	22	22
Anaerobic Ambient	11	11
Covered Ambient	10	10
Uncovered Ambient	8	8

The palatability of the refrigerated samples was retained for a longer period than the ambient stored fruit because the microbial activity was retarded by refrigeration.

Conclusion

Increase in microbial load is associated with spoilage of African star apple. Microbial activities are higher in Nwose variety than Nwanu variety. The population of micro-organisms in low temperature and anaerobic conditions were less than in ambient and aerobic conditions of storage. Storage of African star apple in refrigerated anaerobic conditions produced a two fold increase in storage life.

Recommendation

African star apple should be stored by control of microbial load as well as control of storage conditions under controlled atmosphere conditions with variation in temperature and gaseous conditions. Further research should be carried out to evaluate the effect of some pre-treatments such as blanching and waxing on the proliferation of microorganisms on stored African Star Apple.

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