ASCORBIC ACID AND MICROBIOLOGICAL ANALYSES OF EXTRA – COTYLEDONOUS DEPOSITS OF PRIDE OF BARBADOS (Caesalpina pulcherrima) STORED AT VARIOUS TEMPERATURES.

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ABSTRACT

Ascorbic acid and microbiological analyses of extra – cotyledonous deposits of Pride of Barbados (Caesalpina pulcherrima) stored at various temperatures were investigated.
2,6 - Dichlorophenolindophenol (dye) solution titration method was used in ascorbic acid determination while Nutrient and Sabouraud agar were used in microbiological analyses. Result obtained showed mean ascorbic acid content of 0.15mg/g ($P>0.05$) for extra – cotyledonous samples from pods of Pride of Barbados (PB) stored at - 21^{0}C for 7 days and lower mean values (ascorbic acid content) for exposed samples stored at other examined storage temperature conditions; 4^{0}C and 25 – 28^{0}C. Corynebacterium xerosis, Cladosporium spp. and Penicillium notatum were isolated from exposed extra – cotyledonous samples on the 6th and 7th days of storage under room temperature (25 - 28^{0}C). However, no growth was observed on samples from the pods of PB stored at - 21^{0}C, 4^{0}C and at room temperatures (25 - 28^{0}C) after 7 days of storage. Extra – cotyledonous deposits of Pride of Barbados, whenever collected, should be consumed immediately or stored appropriately in pods for as long as 7 days at - 21^{0}C for the preservation of the ascorbic acid (vitamin C) content and to avert the health hazard of contamination by pathogenic bacteria and fungi.

KEY WORDS: Ascorbic acid, microbiological analyses, extra – cotyledonous deposit.

INTRODUCTION

Pride of Barbados (Caesalpina pulcherrima) belongs to a class of crops known as legumes, which are important sources of proteins. Pride of Barbados is one of the common names of a small evergreen perennial shrub. It is a member of the leguminosae family, which is the second largest family among the dicotyledons. This shrub originates from the tropical West Indies and has alternate bipinnately divided leaves. The family is made up of approximately 650 genera and 1800 species, divided into three sub – families; caesalpinieae, mimoseae and papilonaeae. The flowers of Pride of Barbados are in terminal clusters; 5 – parted, yellow or yellow orange with log exerted red stamen. This plant flowers throughout the year and is usually 10 – 15 feet high. Other common names of Pride of Barbados include the following: dwarf Poinciana, bird of paradise, krere – krere, tabachin, tabaquin, Barbados flower – fence and peacock flower (Dutta, 1981;Burton, 1985). Pride of Barbados is usually found in some domestic and office environments in different parts of Nigeria due to its attractiveness and conduciveness. The seeds are laterally arranged in pods, which split into two halves when extremely dry. The mature

fresh seed is covered by a green testa under which is the translucent coat over the cotyledon known as the extra – cotyledonous deposit. This deposit is the only edible part of Pride of Barbados, which is usually consumed fresh (without boiling) by children and sometimes adults in some parts of Nigeria. However, it is not a staple food in Nigeria. Nutritional and anti – nutritional studies on extra – cotyledonous deposit of this legume have shown that it is not poisonous and could be tolerated as an additional nutritional supplement (Ihimire and Prohp, 2003; Prohp and Maduemezia, 2003; Prohp and Alaiya, 2002).
The objectives of this research are to determine the ascorbic acid (vitamin C) content and microbiological analyses of the extra – cotyledonous deposits of Pride of Barbados stored at various temperatures. This is with the view of understanding the best storage condition of this specimen for the preservation of its vitamin C content and for possible aversion of any microbial contaminations.

MATERIALS AND METHODS

Sample collection and preservation

Fresh and green mature pods of Pride of Barbados (Caesalpina pulcherrima) were obtained from Ambrose Alli University compound, Ekpoma, Edo State, Nigeria (plate 1).

The pods were aseptically dissected to unveil green seeds laterally positioned on either side of the opened pods. The green testa over the seed was carefully removed using sterile forceps and razor blade to avoid any microbial contamination. The translucent coat over the cotyledon was then separated as the extra – cotyledonous deposit (exposed). Samples of exposed extra – cotyledonous deposits of Pride of Barbados were stored in clean transparent cellophanes under room (25 - 28^0 C), freezing (- 21^{0}C) and refrigeration (4^{0}C) temperatures. Fresh green and matured pods of Pride of Barbados were also stored under the same conditions. Subsequently, samples were collected accordingly for ascorbic acid and microbiological analyses.

Determination of Ascorbic acid content

The ascorbic acid content of the exposed extra – cotyledonous deposit and that from pods stored at various temperatures was quantitated using 2,6 – dichlorophenolindophenol (dye) titration procedure of Lambert and Muir (1976) as reported by Shittu and Titus (2000).

Microbiological analysis

Preparation of media
Nutrient agar (NA) and Sabouraud dextrose agar (SDA) were prepared following manufacturer's instructions. Exposed extra – cotyledonous samples were stored in clean transparent cellophanes under room (25 - 28^0 C), freezing (- 21^{0}C) and refrigeration (4^{0}C) temperatures. Fresh green and matured pods of Pride of Barbados were also stored under the same conditions. Each day, extra – cotyledonous samples from the exposed conditions of different temperatures and from pods were collected aseptically and cultured on both media (Nutrient and Sabouraud agars). These were carried out consecutively for a period of 7 days. The isolates were characterized by Gram staining, sugar fermentation and motility tests (Conn, 1980).

RESULTS

Mean ascorbic acid content of fresh extra – cotyledonous (EC) deposit of Pride of Barbados before storage was 0.14 mg/g at P> 0.05, (Table 1).

Table 1:Ascorbic acid contents of fresh extra – cotyledonous deposits of Pride of Barbados (Caesalpina pulcherrima) before storage.

Extra – cotyledonous sample (fresh)	Ascorbic acid content (mg/g) ± S. E.M.
A	0.14 ± 0.09
B	0.16 ± 0.11
C	0.13 ± 0.07

Values are mean ± S. E. M of three separate determinations (n = 3)
A, B, C are fresh samples of exra – cotyledonous deposits of Pride of Barbados.

Table 2: Summary of mean ascorbic acid contents (mg/g) of exposed extra – cotyledonous (EC) deposits of Pride of Barbados (A) and EC deposits from pods (B) under various temperature conditions.

Storage temperatures	Exposed EC A	EC from pods B
25 - 28^{0}C	0.03 ± 0.02	0.05 ± 0.01
4^{0}C	0.04 ± 0.01	0.06 ± 0.02
- 21^{0}C	0.04 ± 0.01	0.15 ± 0.01

Values are mean ± S. E. M. of three separate (n = 3) determinations for day 1 through day 7.

Table 2 shows the mean ascorbic acid (vitamin C) (mg/g) content of EC in its exposed conditions and that from pods stored at various temperatures. Cornyebacterium xerosis, Cladosporium species and Penicillium notatum were isolated on the exposed samples at the

6th and 7th days of storage under room temperature. No microbial growth was observed on extra – cotyledonous samples from pods stored under refrigeration, freezing and room temperature for 7 days (Table 3).

Table 3.: Microbial load of exposed extra – cotyledonous deposits of Pride of Barbados (A) and extra – cotyledonous deposits from pods (B) under various temperature conditions.

Temperatures	25 - 28^{0}C		4^{0}C		- 21^{0}C	
Days	A	B	A	B	A	B
1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	-	-	-	-	-	-
4	-	-	-	-	-	-
5	-	-	-	-	-	-
6	+	-	-	-	-	-
7	+	-	-	-	-	-

+ = Microbial growth
- = No microbial growth.

DISCUSSION AND CONCLUSION

Mean ascorbic acid content of fresh extra – cotyledonous deposit of Pride of Barbados before storage was 0.14mg/g ($P > 0.05$). Sumati and Rajagopali (1985) reported the ascorbic acid content of some foodstuff consumed fresh (without boiling) as 210 – 310 (guava), 180 – 200 (cashew fruit), 25 – 55 (papaya, pineapple) and 30mg/g (orange). The same authors reported 50 and 100 – 150 mg/g for cauliflower/cluster beans and leafy vegetables respectively. Ascorbic acid is found most especially in foods of plant origin (Lioyd et al, 1978). Although dried cereals and pulses do not contain ascorbic acid, this compound is formed as they germinate (Passmore et al, 1979). In cells and body fluids ascorbic acid, which oxidizes easily, helps to maintain other biomolecules in their reduced state, necessary for appropriate metabolic functions (Hooges and Baker, 1973). This anti – oxidant activity of ascorbic acid is informed by its ability to alternate between the oxidized and reduced states (Webster, 1972). Ascorbic acid has also been implicated in the transfer of plasma iron to the liver, its incorporation into ferritin for storage (Mazur, 1961) and in the distribution of iron storage compound (Lipschitz et al, 1971). Scurvy is the symptom of deficiency of ascorbic acid. The recommended daily intake of ascorbic acid of 30mg represents a reasonable compromise between two points of view (Pyke, 1982). Extra – cotyledonous deposit of Pride of Barbados is very poor in ascorbic acid content to be consumed as a single source of this metabolically important compound.

When it is not convenient to consume the extra – cotyledonous deposits of Pride of Barbados after collection, it could be stored properly. This study shows that such storage should be carried out via pods kept under 4^0C or at -21^0C for as long as 7 days. It was observed that on the 6^{th} and 7^{th} days of storage of exposed extra – cotyledon of this plant, under room temperature (25 - 28^0C), microbial growths were obtained. These microorganisms isolated were Cladosporium spp., Penicillium notatum and Corynebacterium xerosis and are pathogenic to humans and animals that use plant as fodder (Table 3). However, these growths could be avoided by storing the Pride of Barbados pods under any of the above experimental conditions for as long as 7 days (Table 3). Extra-cotyledonous deposits are therefore better preserved in pods under refrigeration, freezing or room temperatures for as long as 7 days to avoid any form of microbial contamination. However, for the purpose of preserving the ascorbic acid (vitamin C) contents of the extra-cotyledon of Pride of Barbados whilst controlling any form of contamination, the pods of this plant are to be enclosed in clean transparent cellophanes and stored in the freezer at -21^0C for as long as 7 days (Table 2 and 3). Longer days of storage would decrease the ascorbic acid content due to the growth of microorganisms that depend on ascorbic acid for growth and development (Shittu and Titus, 2000). According to Tandon (1962) while some fungi are able to synthesize their own vitamins, others are incapable and therefore require external supply of vitamin for normal growth.

While extra-cotyledonous samples contain a small amount of ascorbic acid (when used as a single source of this compound), they are vulnerable to contamination by pathogenic bacteria and fungi, if appropriate storage conditions are compromised. And since Pride of Barbados is found in virtually most parts of Nigeria, it may be used as a complementary source of vitamin C.

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