AJFAND ONLINE
Volume 10 No. 8
August 2010
ISSN 1684 5374
Peer Reviewed
AFRICAN JOURNAL OF FOOD AGRICULTURE NUTRITION AND DEVELOPMENT

CHANGES IN MICROSTRUCTURE, BETA CAROTENE CONTENT AND *IN VITRO* BIOACCESSIBILITY OF ORANGE-FLESHED SWEET POTATO ROOTS STORED UNDER DIFFERENT CONDITIONS

Tumuhimbise GA[1], Namutebi A[1] and JH Muyonga*[2]

John Muyonga

*Corresponding author email: muyongaj@agric.mak.ac.ug or hmuyonga@yahoo.com

[1]Department of Food Science and Technology, Makerere University, P.O Box 7062, Kampala-Uganda

AJFAND ONLINE
Volume 10 No. 8
August 2010
ISSN 1684 5374
Peer Reviewed
AFRICAN JOURNAL OF FOOD AGRICULTURE NUTRITION AND DEVELOPMENT

ABSTRACT

Orange-fleshed sweet potatoes {OFSP} (*Ipomoea batatas (L.) Lam*) contain high levels of beta carotene, an important provitamin A carotenoid. Stored sweet potato roots undergo many physiological changes that affect their beta carotene content and bioaccessibility as well as the tissue microstructure. This study investigated the changes in microstructure, beta carotene content and *in vitro* bioaccessibility of stored OFSP roots. Roots of two varieties of OFSP, *Ejumula* and *SPK004/6/6* were each stored under the following conditions: in a pit (17-21 ^{o}C, RH 90-100%), saw dust (19-23^{o}C, RH 86-100%), dark room (24.5-28 ^{o}C, RH 68-100%) and ambient (24-27 ^{o}C, RH 68-100%). Samples were drawn monthly from each of the storage treatments and analyzed for changes in beta carotene content, *in vitro* bioaccessibility and tissue microstructure. Stored roots of *Ejumula* variety contained significantly more ($P \leq 0.05$) beta carotene than those of the SPK004/6/6 variety. There was no significant difference ($P \geq 0.05$) between varieties in regards to beta carotene bioaccessibility. Roots stored in pits retained higher beta carotene content compared to roots stored under other conditions. *In vitro* bioaccessibility was significantly higher ($P \leq 0.05$) in roots stored in pits compared to roots stored under saw dust, dark room and ambient conditions. Samples of OFSP roots stored under ambient and dark room conditions retained the least amount of beta carotene and had the least amount of bioaccessible beta carotene. There was an increase in the level of cell wall lignification during storage of OFSP. The extent of lignification varied with storage method used. The roots stored under ambient and dark room conditions showed higher levels of lignification compared to those stored under sawdust and in pits. There were no differences in lignification between the different varieties studied. The study shows that storage of OFSP roots using methods that maintain low temperatures leads to higher retention of beta carotene and maintains higher *in vitro* bioaccessibility.

Key words: Storage, beta carotene, bioaccessibility, microstructure

INTRODUCTION

Sweet potato is one of the most important staple crops in developing countries [1]. Orange-fleshed sweet potatoes (OFSP) contain high levels of beta carotene, an important provitamin A carotenoid [2-5]. Orange-fleshed sweet potatoes can, therefore, help alleviate vitamin A deficiency [6]. However, carotenoids may be lost during potato root storage [7]. Traditional storage methods such as storage in bags, pits and open ground have not been evaluated to determine their impact on the retention of beta carotene content as well as its bioaccessibility. Bioaccessibility gives an estimate of the amount of beta carotene that would be potentially absorbed by the body after digestion [8]. Bioaccessibility of provitamin A carotenoids is known to be influenced by the tissue microstructure, among other factors [9].

During storage, there are many changes that take place in the tissue microstructure of the sweet potato roots. The physiological and compositional changes that take place include loss of moisture/water and modification of texture [10, 11]. During storage of sweet potato roots, starch is degraded into sugars by the action of endogenous amylase, thereby affecting the microstructure of the sweet potato root tubers [12]. The extent of these amylase moderated microstructural changes depend on temperature and water content [13, 14]. These factors vary with storage time. High temperatures, in particular, are known to increase respiration leading to lignification of the sweet potato cell walls during storage [15]. The purpose of this study was to establish the effect of storing OFSP roots under different conditions on their carotenoid content, microstructure and *in vitro* bioaccessibility.

MATERIALS AND METHODS

Sweet potato varieties

Two varieties (*Ejumula* and SPK004/6/6) of OFSP *(Ipomoea batatas (L) Lam*) were randomly harvested from a farm at Bombo, Luwero District of Uganda. The roots were harvested at 4.5 months.

Storage of OFSP

After harvest, sweet potatoes of 200-250g were sorted to remove physically, and pest or disease damaged roots. The sorted roots were cured naturally in the sun for four days by spreading them on the ground (26-29 ^{o}C, RH 80-95%). The sweet potatoes from each variety were divided into four portions. For each storage condition and variety, the roots were divided into three portions, each containing 24 roots. Pit stores were constructed by digging circular pits of 0.5m diameter and 0.5m depth. Pits were lined with dry spear grass (*Imperata cylindrica*) before sweet potato roots were placed there [7]. The sweet potatoes were then covered with dry spear grass before covering them with soil. The grass acted as an insulating material and ensured cool conditions in the pits (17^{o}C, RH 95-100%). The pits were then covered with grass-thatched roofing structures to prevent rain water from entering the storage pits.

The OFSP roots for dark room and ambient storage conditions were placed in woven polypropylene sacks, which allowed air circulation. One set of the polypropylene sacks was stored in a room at ambient conditions (24-28 ^{o}C, RH 78-100%) while another was stored in a well ventilated dark room (24.5-27 ^{o}C, RH 77-100%). Another portion of the OFSP roots was stored under sawdust. Sawdust was obtained from carpentries and then dried in the sun to a moisture content of about 10%. The dry saw dust was placed in well ventilated boxes (19-23 ^{o}C, RH 92%) containing the sweet potatoes and these were stored in a well ventilated room.

Sample preparation for analyses

For each of the storage methods, four roots were randomly selected. The roots were cut longitudinally and two opposite quarters of each removed. The sampled quarters were cut into thin slices (1-2 mm) and freeze-dried using a Virtis Genesis (American Lyophilizer, Inc., USA) freeze drier. The freeze-dried samples were packaged under nitrogen in polythene bags (125 micron) before storage at -50 ^{o}C. Before analysis, the freeze-dried OFSP samples were milled in a coffee grinder (Wagtech, UK) and made to pass through a 0.2 mm mesh. From each of the four roots sampled for the four storage methods, three samples were randomly picked for microscopic analysis.

Chemicals and standards

All chemicals, unless stated otherwise, were obtained from BDH (London, UK). The all-*trans*-beta carotene standard was obtained from CaroteNature GmbH (Lupisingen, Switzerland). Enzymes porcine pancreatin and pepsin as well as porcine bile extract were procured from Sigma Chemicals (St. Louis, MO). The water used for analytical work was double- distilled.

Moisture content

Moisture content was determined by drying 10g of sample in a forced air oven (Gallenkamp 300 Series, UK) at 105 ^{o}C to constant weight for about 20 hours.

Extraction of carotenoids

The OFSP dried samples (~0.2g) were weighed in triplicate into test tubes and reconstituted with 1 ml of deionised water for 20 min followed by addition of 2 ml of acetone containing 0.1% (w/v) butylated hydroxy toluene (BHT). Tubes were mixed by vortex for 3 min and sonicated for 15 min and then centrifuged in a MicroR centrifuge (Fisher Scientific, UK) for 3 min at 4750x*g*. The resulting supernatant was saved in a new test tube. The residue was extracted with 2 ml of acetone and centrifuged again. This was repeated up to 4 times until the residue was colorless. To the resulting acetone extract, 3 ml petroleum ether (40-60 ^{o}C) was added together with 5 ml deionised water to aid in the separation of the phases. The organic and water phases were separated by centrifugation at 4750x*g* for 4 min and the organic phase was pipetted into a new test tube. This step was repeated once. The pooled organic phases were collected in a round-bottomed flask and evaporated to dryness under nitrogen in a vacuum evaporator at 35 ^{o}C. The residue was then dissolved in 10 ml mobile phase methanol: methyl-tert-butyl-ether (1:1, v/v) in a flask and filtered through a 0.45 m pore size membrane filter before HPLC analysis. Precautionary measures such as exclusion of oxygen, protection

from light and avoiding temperatures above 40 °C were taken to prevent carotenoid losses during extraction and analysis.

HPLC analysis of carotenoids

Carotenoids were analysed by reversed phase High Performance Liquid Chromatography (HPLC) using a Gilson HPLC system (Gilson, USA) equipped with a pump, a degasser and a UV 6000LP photodiode array detector operating at 450nm. The data were stored and processed by means of PC1000 Version 3.5 Software. Absorption spectra were recorded between 250 and 500 nm. Separations were carried out on a ProntoSil C_{30} carotenoid column (5µm, 250mm x 4.6mm i.d). The mobile phase used for isocratic elution consisted of methanol: methyl tert-butyl ether: water (55:41:4, v/v/v). The flow rate was 1.3 ml/min and the injection volume was 20 µl. All *trans* -beta carotene (CaroteNature, Lupisingen, Switzerland) was dissolved in mobile phase and used as an external standard.

Determination of *in vitro* bioaccessibility

The *in vitro* bioaccessibility was applied to stored OFSP roots using an *in vitro* digestion model [16]. Since OFSP contains high starch content, the mouth digestion step was included [17]. At the end of the *in vitro* digestion, the micellar fraction was separated by centrifugation followed by filtration [18]. Briefly, 0.5g of freeze-dried OFSP powder was suspended in 10 ml distilled water containing 1% ascorbic acid (w/v) and then subjected to simulated gastric digestion at pH 2 and 37 °C in the presence of pepsin (5 mL of 0.5 % porcine pepsin solution in 0.1 molL^{-1} HCl). This was followed by simulated intestinal digestion in the presence of porcine pancreatin-bile extract mixture (4gL^{-1} of porcine pancreatin and bile salt extract of 25g L^{-1}) pH 7.5 for 2 h. After *in vitro* digestion, the digesta was filtered through a Millipore membrane (0.65µm pore size) following centrifugation in a MicroR centrifuge (Fisher Scientific, UK) at 5000x*g* for 20 min. The micellar fraction and the residue were analyzed for bioaccessible beta carotene.

Light microscopy

Tissues of dimensions 6 x 3.4 x 3.4 mm were sectioned from the outer parts of OFSP roots using a dissection blade. Tissues were first fixed in 10% formol saline solution [19]. The fixed tissues were then processed using an automatic Leica TP 1020 Histokinette tissue processor (Leica Microsystems, Germany). Samples were dehydrated using alcohol in an ascending order of concentration starting with 70%, 80%, 90%, 96%, 100%, 100%, 100% for 1½ h per concentration. Samples were later cleared in two changes of xylene for 1 h and 1 ½ hours. Lastly, samples were impregnated using two changes of molten paraffin wax at 50 °C for 2 h per change. After processing, the samples were embedded in paraffin wax, blocked and sectioned using a Leica RM 2235 rotary microtome (Leica Microsystems, Germany). Sections of 5-7 µm were cut and floated on a Leica H1120 water bath (Leica Microsystems, Germany). The wrinkle-free sections were picked on grease-free slides and then dried in the oven at 53 °C. The cut sections were de-waxed using two changes of xylene for 1-2 min per change. They were then dehydrated using alcohol, starting with two changes of alcohol of 95% and 80% for 3-5 min per change. The breakdown of cell-wall material was studied using Periodic Acid Schiffs (PAS)-reaction for visualization of totally insoluble carbohydrate. The

sections were stained with PAS for 15 min and then dehydrated using ethanol in an ascending order of concentration, starting with 95% and then two changes of absolute ethanol for 3-5 min per change. The sections were cleared in two changes of xylene for 1-2 min and then mounted using depex. After mounting, the slides were allowed to air dry and thereafter examined using a light microscope in objective 40 (Carl Zeiss, Germany).

Statistical analysis

The data obtained for moisture content, beta carotene content and bioaccessibility were analyzed using Stata statistical software (Stata Corporation, Texas, and USA). Comparison between sample treatments was done using analysis of variance (ANOVA) and means were separated using Bonferroni method. P-values ≤ 0.05 were considered significant.

RESULTS

Moisture content

The results showed that there was a big drop in moisture content in the first month of storage (Figure 1). The biggest drop of 3.9% in moisture content was observed in roots stored under ambient conditions while the least was 1.5% recorded in roots stored in pits in the first month of storage. There was no significant difference ($P \geq 0.05$) between moisture content of roots stored under ambient conditions and those stored in a dark room. The OFSP roots stored in the pit maintained significantly ($P \leq 0.05$) more moisture content than any other storage method.

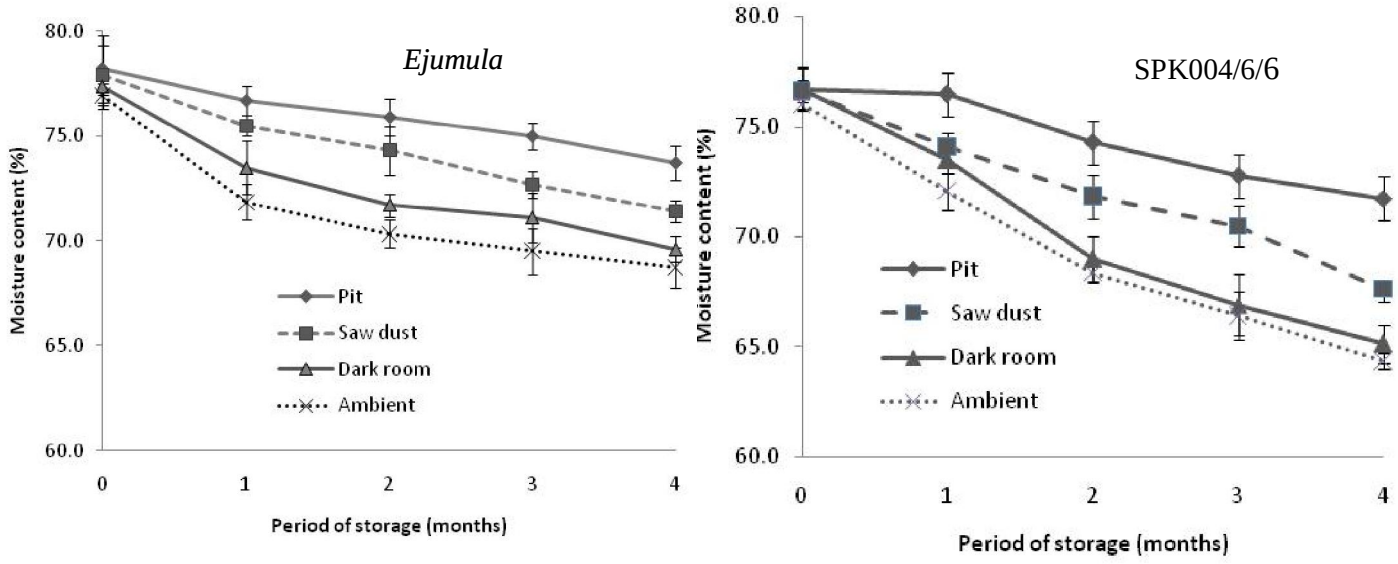

Figure 1: Changes in moisture content of *ejumula* and SPK004/6/6 OFSP varieties stored under different conditions

The amount of beta carotene in roots stored under different conditions tended to increase in the first month of storage. The amount of beta carotene in stored ejumula samples increased from 380.5 ± 2.46 to 412.8 ± 2.11 g/g dry matter) while in SPK004 it increased from 337.1 ± 15.38 to 344.4 ± 11.1 mg/g dry matter in the first month. However, the amount of beta carotene in stored roots decreased in subsequent months (Figure 2). There was no significant difference ($P \geq 0.05$) between the beta carotene content of roots stored in pits and those stored in saw dust. The beta carotene content in OFSP roots stored under ambient and dark room conditions was not significantly different ($P \geq 0.05$). Roots stored in pits maintained higher beta carotene content compared to roots stored under ambient and dark room conditions. There was a significant difference ($P \leq 0.05$) between the beta carotene content in *ejumula* and SPK004/6/6 varieties.

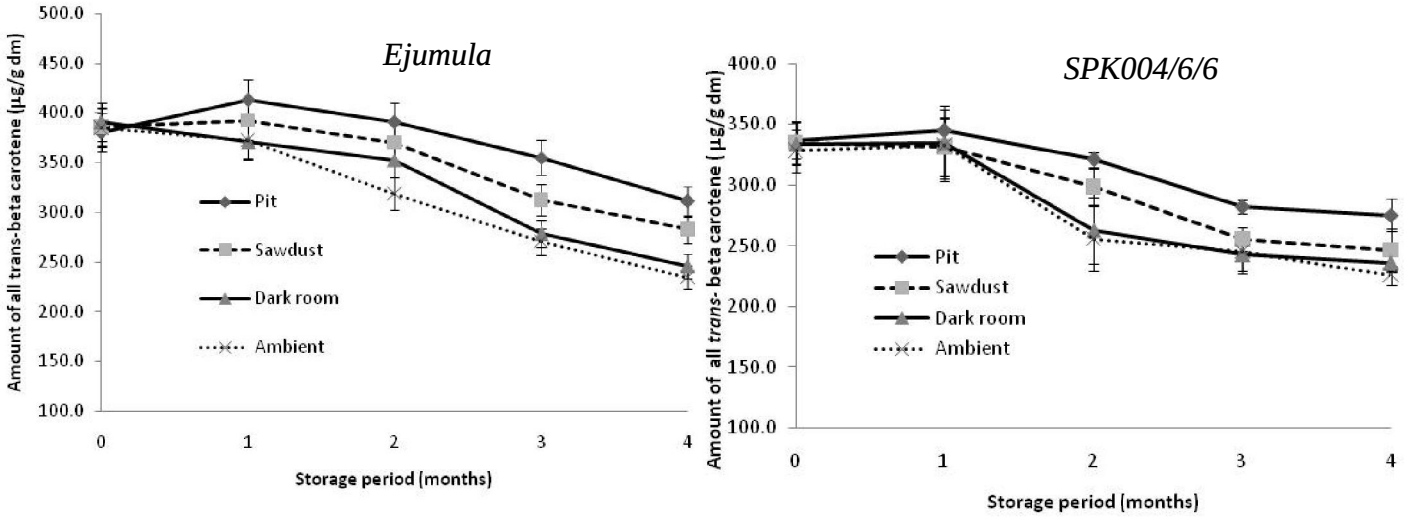

Figure 2: Changes in the content of all-*trans*-beta carotene (g/g dry matter) in *ejumula* and SPK 004/6/6 OFSP varieties stored under different conditions

Bioaccessible beta carotene was calculated as the percentage of the total amount recovered in the residue, and micellar aqueous fractions at the end of the simulated gastrointestinal digestion, and the initial amount in the fresh roots before storage. *In vitro* bioaccessibility of beta carotene in roots stored in pits was significantly higher ($P \leq 0.05$) than that recorded for roots stored under sawdust, dark room and ambient conditions (Figure 3). There were no significant differences in *in vitro* bioaccessibility of beta carotene among the storage conditions for the first month of storage. *In vitro* bioaccessibility of beta carotene in roots stored in sawdust was significantly higher ($P \leq 0.05$) than in roots stored under dark room and ambient conditions while there was no

significant difference ($P \geq 0.05$) in *in vitro* bioaccessibility of beta carotene in roots stored under dark room and ambient conditions. Both *Ejumula* and SPK004/6/6 varieties showed no significant difference ($P \leq 0.05$) in percent *in vitro* beta carotene bioaccessibility.

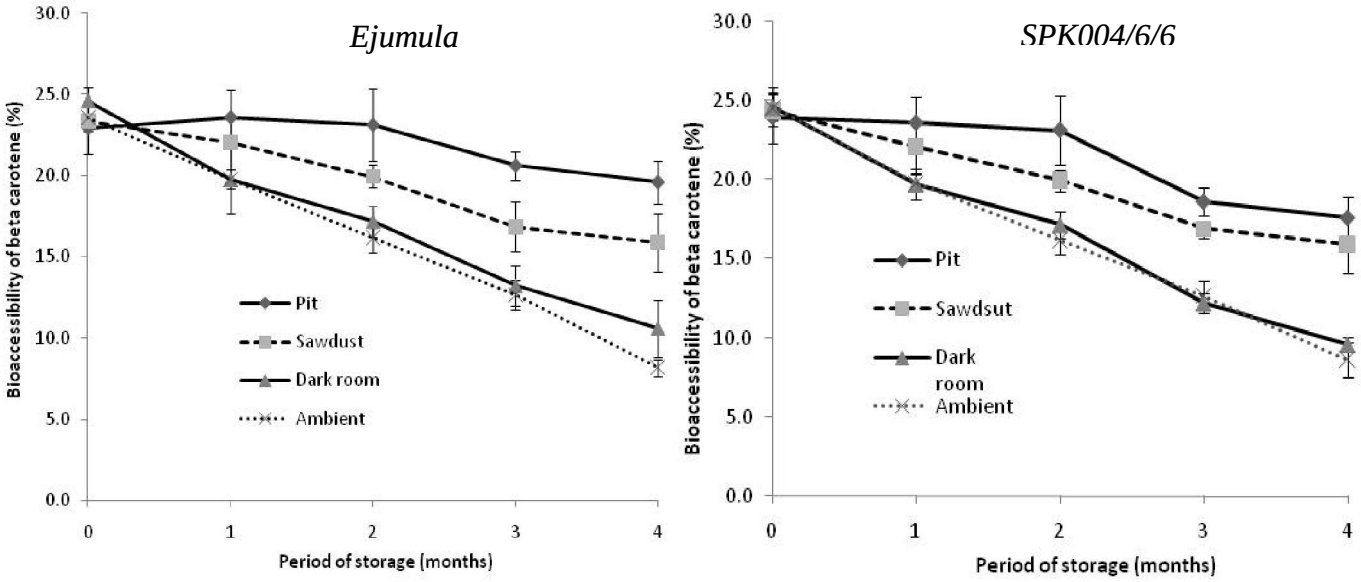

Figure 3: Changes in the *in vitro* bioaccessibility of all-*trans* -beta carotene in *ejumula* and SPK 004/6/6 varieties stored under different conditions

The storage parenchyma of the fresh *ejumula* and SPK004/6/6 was found to be composed of polyhedral cells with a diameter of approximately 98 m (Figure 4 a, b). The fresh OFSP root cells contained starch granules ranging from globular to ellipsoid and of varying sizes. The parenchyma of the sweet potato contained several intercellular spaces which were approximately 6.8 m in size. There were no marked differences between micrographs of fresh *ejumula* and SPK004/6/6 stored under different conditions.

AJFAND ONLINE
Volume 10 No. 8
August 2010
ISSN 1684 5374
Peer Reviewed
AFRICAN JOURNAL OF FOOD AGRICULTURE NUTRITION AND DEVELOPMENT

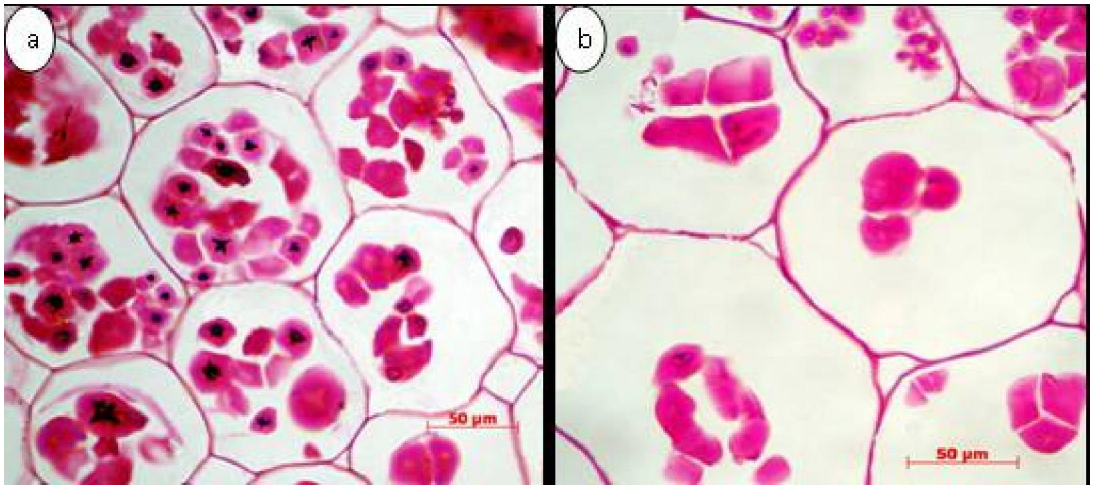

Figure 4: The micrographs of fresh *ejumula* (a) and SPK 004 (b) stained with Periodic Acid Shiff Reagent (PAS) and observed in light microscope

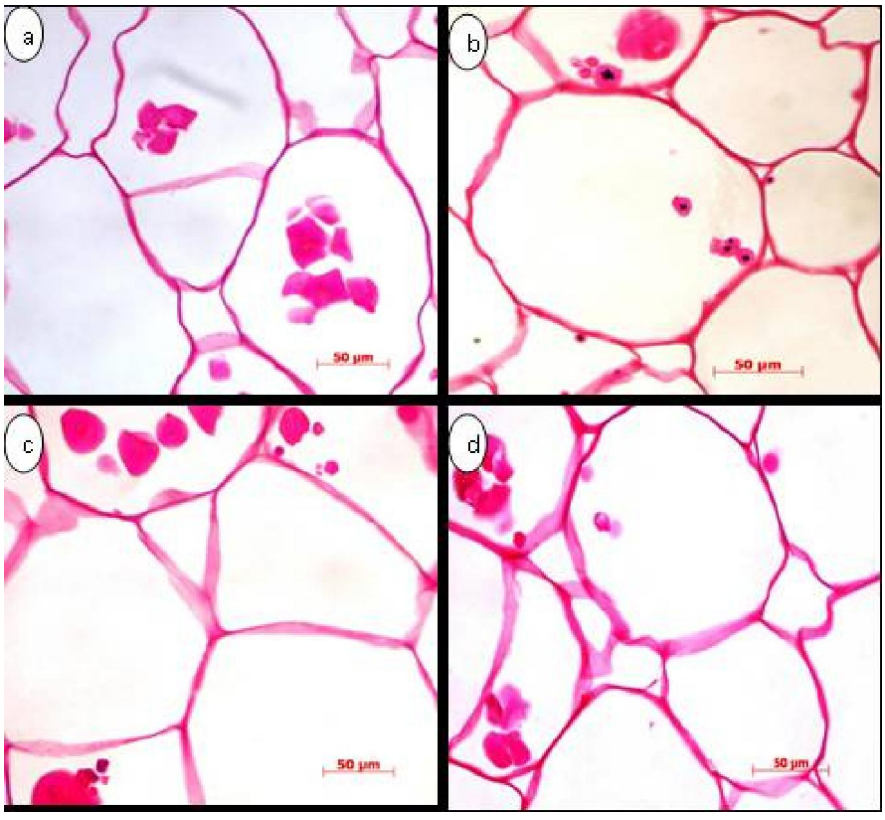

Figure 5: The micrographs showing the microstructure of stored *ejumula* tubers stored under (a) pit (b) sawdust (c) dark room (d) ambient conditions. The sections were stained using Periodic Acid Shiff Reagent (PAS) and observed using a light microscope

The micrographs showed that starch granules reduced in number during the storage period. The cell wall of the stored roots was thickened as it picked up more of the PAS stain as storage time increased. Roots stored under ambient and dark room conditions had more thickened cell walls compared to roots stored in pits and sawdust. Roots stored in pits revealed more intact starch granules than roots stored under sawdust, dark room or ambient conditions. Storage conditions affected the microstructure of *ejumula* and SPK 004/6/6 to the same extent.

DISCUSSION

Changes in all trans beta carotene content of *ejumula* and SPK 004/6/6 stored under different conditions

Carotenoids are known to decrease during storage of potato tubers [20]. However, other studies have reported an increase in beta carotene content which was attributed to the maturing of the sweet potatoes [21, 22]. It is generally known that in sweet potatoes, beta-carotene is synthesized and at the same time destroyed in the root itself during storage and, therefore, the final amount depends on the algebraic sum of the two processes [23]. The sweet potato roots stored in pits maintained a higher amount of beta carotene compared to those stored under ambient and dark room storage conditions, and this may be attributed to the low temperatures that prevailed in the pit stores. The storage temperatures in the sawdust storage condition were close to those in the pits. Factors such as heat and light have been observed to promote isomerisation of *trans* -carotenoids to the *cis* -form [24]. The lower beta carotene content in the roots stored under ambient and dark room conditions may have resulted from the higher temperatures which caused more thermal degradation. However, all the storage conditions maintained more than 100 g/g dry matter of beta carotene. This is the level used by sweet potato breeders to screen for varieties that can retain sufficient beta carotene after processing [25].

Changes in the *in vitro* beta carotene bioaccessibility and microstructure in sweet potato roots stored under different conditions

The reduction in bioaccessibility of beta carotene from stored OFSP may be attributed to loss in moisture content that may have resulted in hardening of the OFSP cell walls. The OFSP roots that were kept under low temperature conditions had higher percent *in vitro* bioaccessibility than the ones kept at higher temperatures. The rate of cell wall thickening is known to vary with storage time and temperature [26]. This is consistent with the higher *in vitro* bioaccessibility recorded in sweet potato roots stored in pits under lower temperatures than roots stored at ambient temperatures. The *in vitro* bioaccessibility of sweet potato roots stored under sawdust was also high, an observation attributed to the low temperature as compared to ambient conditions. The increased transpiration at ambient temperatures resulted in loss of moisture from the sweet potato roots, and this may have contributed to the increased lignification observed in the microstructure analysis. Similar results were reported for trifoliate yam tubers where the thickening of the cell wall and middle lamella increased with the temperature of storage [27]. The loss of moisture of sweet potato during storage may have influenced the

occurrence of polymerization and epimerization of cell wall microfibrils leading to the thickening of the cell walls and subsequent release of carotenoids during *in vitro* digestion. Since the rate of moisture loss in roots stored under ambient conditions was higher, the thickening of the cell walls was also higher and tended to limit the release of beta carotene during *in vitro* digestion.

CONCLUSION

The loss of beta carotene during storage of OFSP can be reduced by using storage techniques that maintain relatively low temperatures. Among the traditional methods commonly used in East Africa for sweet potato storage, pit storage maintained beta carotene content quite well while storage under ambient conditions significantly reduced beta carotene content and bioaccessibility. The changes in beta carotene bioaccessibility seem to correlate with changes in cell microstructure. Cell wall lignification seems to be associated with reduction in beta carotene bioaccessibility. This information could be useful in designing storage systems that ensure high *in vitro* bioaccessibility and content of beta carotene in OFSP.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the financial support from Carnergie Corporation of New York, Norwegian Agency for Development Cooperation (NORAD) and BTC/CTB.

REFERENCES

1. **Bovell-Benjamin AC and LT Steve** Sweet Potato: A review of its past, present and future role in human nutrition *Adv Food Nutr Res* 2007; **52**:1-59.

2. **Mwanga R, Odongo B, Niringiye C and A Alajo** Release of two orange-fleshed sweet potato cultivars,' SPK004' (Kakamega') and 'Ejumula', in Uganda. *HortScience* 2007; **42**:1728-1730.

3. **Faber M and PJ van Jaarsveld** The production of vitamin A-rich vegetables in home-gardens as a means of addressing vitamin A deficiency in rural African communities *J Sci Food Agric* 2007; **87**:366-377.

4. **Huang AS, Tanudjaja L and D Lum** Content of alpha-, beta carotene and dietary fiber in 18 sweet potato varieties grown in Hawaii. *J Food Comp Food Anal* 1999; **12**:147-151.

5. **Wu X, Sun C, Yang L, Zeng G, Liu Z and Y Li** Beta carotene content in sweet potato varieties from China and the effect of preparation on beta carotene retention in the Yanshu No. 5 *Inn Food Sci Em Tech* 2008; **9**:581-586.

6. **Low JW, Arimond M, Osman N, Cunguara B, Zano B and D Tschirley** A food-based approach introducing orange-fleshed sweet potatoes increased vitamin A intake and serum retinol concentrations in young children in rural Mozambique. *J Nutr* 2007; **137**:1320-7.

7. **Namutebi A, Nantabirwa H, Lemaga A, Kapinga R, Matovu M and S Tumwegamaire** Long-term storage of sweet potato by small-scale farmers through improved post harvest technologies. *Ug J Agric Sci* 2004; **9**:922-930.

8. **Tanumihardjo SA** Factors influencing the conversion of carotenoids to retinol: Bioavailability to conversion to bioefficacy *Int J Vit Res* 2002; **72**:40-45.

9. **Castenmiller JM and CE West** Bioavailability and bioconversion of carotenoids. *An Nutr Rev* 1998; **18**:19-38.

10. **Hu W and S Tanaka** Effects of heat treatment in the quality and storage life of sweet potato *J Sci Food Agric* 2007; **87**:313-319.

11. **Katundu GCM, Hendricks SL, Bower JP and M Siwela** Effect of traditional storage practices of small-scale organic farmers on potato quality. *J Sci Food Agric* 2007; **87**:1820-1825.

12. **Takahata Y, Takahiro N and S Tetsuo** Changes in carbohydrates and enzyme activities of sweetpotato lines during storage. *J Agric Food Chem* 1995; **43**:1923-1928.

13. **Picha DH** Carbohydrate changes in sweet potatoes during curing and storage. *J Am Soc Hort Sci* 1986; **111**:89-92.

14. **Hagenimana V, Simard E and LP Verina** Amylolytic activity in germinating sweetpotato (*Ipomoea batatas L.*) roots. *J Am Soc Hort Sci* 1994; **119**:313-320.

15. **Rees D, van Oirschot QEA, Mbilinyi LB, Muhanna M and KI Tomlins** In: Rees D, van Oirschot Q, Kapinga R, eds. Sweet potato post-harvest assessment: Experiences from East Africa. Chatham: Natural Resource Institute, 2003:85-92.

16. **Hedren E, Diaz V and U Svernburg** Estimation of carotenoid accessibility from carrots determined by an in vitro digestion method. *Eur J Clin Nutr* 2002; **56**:425-430.

17. **Oomen AG, Rombelberg CJ, Bruil MA, Dobbe CJ, Perreboom DP and AJ Sips** Development of an in vitro model for estimating the bioaccessibility of soil contaminants. *Arch Env Cont Toxicol* 2003; **44**:281-7.

18. **Veda S, Kamath A, Platel K, Begum K and K Srinivasan** Determination of bioaccessibility of β-carotene in vegetables by *in vitro* methods. *Mol Nutr Food Res* 2006; **50**:1047-1052.

19. **Ruzin SE** Microtechnique and microscopy. University of California, Berkeley: Oxford University Press, 1999.

20. **Griffiths DW, Dale MFB, Morris WL and G Ramsay** Effect of season and post harvest storage on the carotenoid content of *Solanum phureja* potato tubers. *J Agric Food Chem* 2007; **55**:379-385.

21. **K'osambo LM, Carey EE, Misra AK, Wilkes J and V Hagenimana** Influence of age, farming site and boiling on provitamin A content of sweet potato (*Ipomoea batatas (L.) Lam.*). *Food Comp Anal* 1998; **11**:305-321.

22. **Ravi VA, Aked J and C Balagopalan** Review on tropical roots and tuber crops. Storage methods and quality changes. *Crit Rev Food Sci Nutr* 1996; **36**:661-709.

23. **Yamamoto Y and Y Tomita** Studies on the bio-pigments and vitamins: corrective changes in the carotene, total carotenoids and constituents of sweet potatoes during storage. Kagoshima University Repository 1958; **3**:63-68.

24. **Rodriguez-Amaya DB** Carotenoids and food preparation: the retention of provitamin A carotenoids in prepared, processed and stored foods. Arlington, VA: John Snow Inc/OMNI Project, 1997.

25. **Hagenimana V, Carey EE, Gichuki ST, Oyunga MA and JK Imungi** Carotenoid contents in fresh dried and processed sweet potato products. *Ecol Food Nutr* 1999; **37**:455-473.

26. **Afoakwa EO and S Sefa-Dedeh** Changes in cell wall constituents and mechnical properties during post-harvest hardening of trifoliate yam *Dioscorea dumetorum* (Kunth) pax tubers. *Food Res Int* 2002; **35**:429-434.

27. **Afoakwa EO and S Sefa-Dedeh** Textural and microstructural changes associated with post-harvest hardening of trifoliate yam (*Dioscorea dumetorum*) pax tubers. *Food Chem* 2001; **77**:279-284.