ISSN No:-2456-2165

Effect of Storage and Packaging Materials on Color and Carotenoid Content of Orange-Fleshed Sweetpotato Flours

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Abstract:- The loss of Carotenes during storage of orange-fleshed sweetpotato (OFSP) flours is a major issue. This study evaluated the effect of storage and packaging materials on carotene content, color and water activity of OFSP flours. Flours from Vita and Kabode OFSP genotypes were packed in aluminum foil laminate (AFL), high density polyethylene and Kraft paper and stored under light and dark conditions for 4 mo. Results showed significant carotenoid losses (P < 0.001) and color value changes (P < 0.05) in stored OFSP flours under both light and dark storage conditions. The highest carotenoid loss was found in flours packed in Kraft paper (59.33%) while AFL (29.88%) was the least. A significant (P < 0.01) increase in water activity was observed in all packed samples regardless of storage environment. Therefore, the study suggests AFL as the best packaging material for stored OFSP flour due to the low loss of carotenes.

***Keywords:-** Sweet Potato Flour, Storage, Packaging Material, Color, Carotenoid.*

I. INTRODUCTION

Sweetpotato is an important food crop in Eastern and Southern Africa. It plays an important role as a food security crop, providing most of the dietary carbohydrate in South-East Africa especially in times of little rainfall because of its ability to tolerate drought (Hagenimana & Owori, 1996). High nutrient level such as energy, fiber, minerals, vitamins and antioxidants like phenolic acids, tocopherols and β-carotene have tremendously increased the popularity of sweetpotato (Teow et al. 2007). OFSP are particularly important because of high β-carotene content, a precursor for vitamin A, hence, a good and sustainable alternative for vitamin A deficiency mitigation.

In South-East Africa, traditional methods of OFSP utilization include boiling, roasting and drying (Hall et al., 1998). Boiled sweetpotato are taken as part of breakfast, snack or as main meal during lunch or dinner (Woolfe, 1992). Dried chips are milled into flour and used to make porridge, doughnuts, and flat bread (*chapati*) among others (Nungo et al., 2000; Woolfe, 1992). Although OFSP flour is associated with the high loss of carotenoids during storage, it is still a main raw material for product development both at local and commercial levels. Rodriguez-Amaya et al. (2011) emphasized the need to optimize the processing and storage conditions to minimize the loss of carotenoids in OFSP flour. The loss of carotenoids in dried OFSP products, like flour and chips, are mostly related to drying method, light, oxygen, water activity, and packaging material. No significant difference in carotenoid loss was observed when dried OFSP chips were dried under open air, oven, and solar driers. Total loss of carotenoids ranged from 16% to 33% with different drying techniques (Bechoff et al., 2009). The slight difference in carotenoid loss was attributed to exposure to direct sunlight during the open air drying method (Bechoff et al., 2009). Compared to dried chips, the high loss of carotenoids during storage were reported in OFSP flour (Hagenimana et al., 1999). The authors attributed high carotenoid loss of flour was due to the small particle size of flour increasing the surface area exposed to oxygen, hence, high carotenoid losses through oxidation. Lavelli et al. (2007) reported water activity of between 0.31 and 0.54 as having better carotene retention on freeze dried carrots stored at 40 ºC. These studies suggested a need to explore means of minimizing the loss of carotenoids in stored OFSP flour.

Packaging of OFSP flour is crucial to minimize carotenoid loss in storage. A good packaging material for flour/chips should protect it against light, oxygen and moisture. Packaging materials that are laminate, metallized, coated or aluminum foil are effective at protecting against light, moisture, and oxygen. Where possible, oxygen can be eliminated by creating a vacuum, flushing with inert gas such as nitrogen, or using an oxygen absorber. Sweetpotato flour was packaged and sealed in different forms of packaging such as porcelain and glass jars, tin cans, kraft paper bags, cotton bags, and polyethylene bags (van Hal, 2000) to protect against the aforementioned factors. No change in color was reported when sweetpotato flour was stored in thick gauge white polythene bag, possibly high density polyethylene (HDPE) compared to flour stored in enamel cans, plastic cans or calico bags (Tewe et al., 2003). In a separate study, Bechoff et al. (2010a) reported 83.7% carotenoid loss when dried OFSP chips of different thickness were stored traditionally in jute bags in a mud house for 4 mo.

Although it is general knowledge that prolonged storage of OFSP flour results in high carotenoid degradation, there is a lack of information on the effect of storage conditions and packaging materials on carotenoid retention of OFSP flour. This study was undertaken to evaluate the effect of storage conditions and packaging materials on color, water activity and carotenoid content of OFSP flours during a storage period of 4 mo. The study evaluated aluminum foil laminate (AFL), HPDE, and Kraft paper on carotenoid retention of OFSP flour made from Vita and Kabode genotypes stored in dark and light conditions.

II. MATERIALS AND METHODS

➢ *Source of OFSP Roots and Flour Processing*

OFSP flour was produced at Organi Ltd in Ringa, Kenya. Two OFSP genotypes, Vita and Kabode, were harvested 3.5 mo after planting for flour production. OFSP flour was produced following methods described by Abidin et al. (2015) with some modifications. The peeling step of flour production was skipped because OFSP flour is traditionally produced without peeling in Kenya. In brief, sweetpotato roots were sorted, cleaned and trimmed. The roots were washed again and then sliced into 2mm thick slices by a chipper. The slices were dried in a solar drier for 4 d to 8% moisture content and then milled by hammer mill into flour. The external average temperature and relative humidity during drying were 34°C and 54%, respectively. Temperature and relative humidity inside solar dryer were not recorded.

➢ *Packaging and Storage*

Three types of packaging materials namely Kraft paper (VIP Mills Ltd, Nairobi, Kenya, HDPE (General Industries Ltd, Nairobi, Kenya), and AFL (Packaging Industries Limited, Nadume Road, Nairobi, Kenya) were evaluated for their effect on carotene retention in relation to color. The thickness for HDPE and AFL was 80 and 90 microns respectively. Hundred gram flour from Vita and Kabode genotypes were packed in the aforementioned pouches and stored under light and dark conditions. The light storage of flour was included to simulate home storage condition. Vacuum sealing was applied to HDPE and AFL while Kraft paper had air removed manually and then glued. The two sets of experiments (dark and light) were replicated twice in a factorial experimental design. The storage study was conducted for 4 months and sampling for carotenoid content, moisture content, color, and water activity was done weekly for the first month, then biweekly for second month and monthly for the last 2 mo. Temperature and relative humidity were recorded with a data logger (Extech instruments, 9 Townsend W, Nashua, NH 03063, USA) during the entire storage period.

➢ *Carotenoid Extraction of OFSP Flour*

Procedure described by Muzhingi et al. (2008) was followed for carotenoid extraction with minor modifications. About 0.6 g of flour was weighed into an extraction tube and 5 mL of methanol was added. The sample was vortexed and incubated at 70°C for 10 min. After incubation, the sample was vortexed again and centrifuged at 3000 rpm for 5 min. The upper phase was transferred into a 25 mL volumetric flask and the residue was extracted four times with 5 mL Tetrahydrofuran (THF). The final volume of the extract was adjusted to 25 mL with THF. Exactly 2 mL of extract was purified with 2 mL water, 4 mL hexane and 1 mL methanol, then centrifuged at 3000 rpm for 5 min. The upper phase was transferred into another test tube and evaporated to complete dryness under liquid nitrogen. The dried extract was reconstituted with 2 mL of methanol: THF solution (85:15 v/v). About 1 mL of sample was transferred to HPLC vial for analysis. Carotenoid extraction was done under yellow light conditions to minimize degradation due to white light.

➢ *HPLC Analysis of Carotenoid*

Carotenoids were analyzed by reversed phase HPLC using a Waters 9562 system equipped with auto sampler injector, degasser, pump and Waters 9562-UV-visible photodiode array detector operating at 450 nm (Waters Corporation, Milford, MA). Separations were carried out on a 3-µm, 150 x 3.0 mm, Semibore column (YMC, Wilmington, NC). The isocratic mobile phase consisted of methanol:methyl tert-butyl ether:water (85:12:, v/v/v, 1.5% ammonium acetate (Phase A), 8:90:2, v/v/v, 1% ammonium acetate (Phase B). The flow rate was 0.4 mL/min and injection volume of the sample was 30 µL. Standard curves of pure all-trans β-carotene, 13-cis β-carotene and 9-cis β-carotene were used to quantify the carotenoids. Total carotenoid content was obtained as the summation of the individual carotenes.

➢ *Color Measurement*

Color was measured using a Lovibond LC100 Spectrophotometer (Wilford Industrial Estate, Ruddington Lane, Nottingham). Flour color was described based on L*, a* and b* where L* is a measure of lightness, a* defines components on the red–green axis, and b* defines components on the yellow-blue axis. Color images were taken at three different points per sample and mean was calculated for the parameters. In this study a* was correlated with β-carotene content.

➢ *Moisture Content Determination*

Moisture content was determined following AOAC method (AOAC, 1984) on the same day as carotenoid analysis. Five gram of sample was weighed into crucible and dried in the oven at 105°C until constant weight was achieved. Moisture content was determined as the difference between initial and final weight of sample expressed as a percentage.

➢ *Water Activity Measurement*

Water activity of flour was measured with an Aqualab instrument (Decagon Devices Inc., Hopkin Court, Pullman, WA). Calibration was done with LiCL at 0.5 $_{a}w$ and NaCL at 0.7$_{a}$w.

ISSN No:-2456-2165

➢ *Statistical Analysis*

The statistical analysis of data was performed by one way ANOVA using Genstat version 6.0 to determine significant differences among the treatments. The correlation coefficients and their probability levels were obtained from linear regression analysis. Significance differences among treatments was obtained by Tukey's HSD multiple rank test at $P < 0.05$.

III. RESULTS AND DISCUSSION

➢ *Identification and Quantification of Carotenoids*

All-trans β-carotene and its isomers, 13-cis β-carotene and 9-cis β-carotene, were the main pro-vitamin A carotenoids found in the sweetpotato flours (figure 1). Basing on the peak area, it is clear from figure 1 that all-trans β-carotene was the most predominant carotenoid found in the products. Vita roots had the total carotenoid content of 22.97 mg/100 g dry weight (DW), which was not significantly ($P > 0.05$) different from the value for Kabode roots (21.18 mg/100 g, DW). Processing of OFSP roots into flour resulted in significant loss of total carotenoid level. After drying, total carotenoid content of Vita flour was 17.21 mg/100 g (DW) representing 25.08% carotenoid loss while Kabode flour was 15.36 mg/100 g DW representing 27.48% carotenoid loss.

A slight decrease in 13-cis and 9-cis isomer contents was observed when sweetpotato roots were dried and milled into flour and during the entire storage period (data not shown). Achir et al. (2014) also reported 13-cis and 9-cis isomers as the major isomers of β-carotene degradation during storage of OFSP flour. The current findings are in contradiction to previous findings that indicated increased formation of β-carotene isomers under stressful conditions such as heating, UV exposure and storage (Chandler & Swartz, 1988). Bechoff et al. (2009) reported similar contents of β-carotene isomers before and after drying OFSP chips under hot air, solar drier and direct sun. The amount of isomers formed is related to heat and processing time (Doering et al., 1995). Hiranvarachat et al. (2008) showed that a minimum of 5 h at constant temperature (60°C) favored formation of 13-cis β-carotene isomers in oven dried diced carrots. In the current study flour was stored at ambient temperature hence, the carotene loss might have occurred due to oxidation rather than isomerization.

➢ *Effect of Packaging and Storage on Total Carotenoid Loss of OFSP Flour*

Significant differences ($P \leq 0.001$) in total carotenoid content were observed among the packaging materials in both storage conditions (figure 2). The highest carotenoid loss was found in Kraft paper, followed by HDPE, while AFL was the least. However in most cases, the carotenoid content of flour in HDPE was comparable to AFL during the entire storage period regardless of the storage environment.

Overall, during the four months storage period total carotenoid content of flour in Kraft paper was 7.84 mg/100 g DW for Vita flour and 6.24 mg/100 g DW for Kabode flour under light exposure (figure 2). Under dark conditions, the carotenoid contents for Vita and Kabode flours in Kraft paper were 7.98 mg/100 g DW and 6.4 mg/100 g DW, respectively (figure 2). Thus, the total carotenoid loss for Kraft paper under light condition was 54.43% (Vita flour) and 59.33% (Kabode flour). The total carotenoid loss under dark condition were 53.58% for Vita flour and 58.27% for Kabode flour. Emenhiser et al. (1999) attributed high β-carotene loss to oxygen permeability in sweetpotato chips stored under ambient conditions. The current results confirm oxygen as the main factor causing carotenoid degradation of flour during storage. Non-vacuum sealing of flour in Kraft paper led to high oxygen transmissivity into the package causing carotenoid oxidation, hence, losses.

When flour was packed in HDPE, the carotenoid content ranged from 13 mg/100 g DW to 10.7 mg/100 g DW after 4 mo under storage exposed to light while under dark storage it ranged from 14.97 to 8.9 mg/100 g DW (figure 2). This translates to 49.08% and 41.46% carotenoid loss for Kabode and Vita flour, respectively. In contrast, the percent carotenoid loss for flour in HDPE under dark conditions was 39.24% (Vita flour) and 40.64% (Kabode flour). No significant differences ($P > 0.05$) in carotenoid content were observed between the two storage environments. This further confirms that oxygen, and not light, was the main factor causing carotenoid degradation (Emenhiser et al., 1999). Cinar (2004) reported photoisomerization as having insignificant effect as opposed to oxidation. Additionally, the vacuum sealing of flour in HDPE paper compacted the pack, so it was only the small outer layer that was exposed to light while the rest of the inner layer was not affected.

AFL paper had the least carotenoid loss both under light and dark conditions. The carotenoid loss of flour in AFL pouches ranged from 41.5% to 35.03% under conditions exposed to light, while under dark conditions it ranged from 30.4% to 29.88%. The reason for low carotenoid loss of flours in AFL might be due to the opaque nature of AFL as well as vacuum sealing, which might have prevented carotenoid degradation through photoisomerization and oxidation, respectively. Beyond week 8, carotenoid loss was drastic. This might be due to accumulation of oxygen in the pouch, hence suggesting carotenoid loss through oxidation. Any packaging material has an oxygen transmission rate at a particular temperature and humidity. For instance the oxygen transmission rate of AFL at 23°C , 0% RH and 1 atm is 0.01 cc in^2/ day while water vapor transmission rate is 0.005 g in^2/day at 100% RH, 23°C and 1 atm (Emenhiser et al., 1999). During the storage time, average temperature and RH were 23°C and 24%, respectively.

Although not statistically significant ($P > 0.05$), flour from the Kabode genotype was more susceptible to carotenoid degradation under dark and light storage conditions. On the other hand, flours from Vita sweetpotato genotype were relatively stable and carotenoid degradation was slower. Similarly, there was no significant difference ($P > 0.05$) in carotenoid content between flours stored in dark and light conditions. The findings confirm oxygen has greater impact on carotenoid degradation through oxidation as previously reported by Bechoff et al. (2010a) and Emenhiser et al. (1999).

According to figure 3, it is clear that the greatest carotenoid degradation was observed during the first 3 weeks of storage both under light and dark conditions. From week 4, carotenoid degradation was occurring at a decreasing rate up to week 16 in all the samples. The results indicate that the first 3 weeks are critical in minimizing carotenoid loss during storage. Efforts to prevent carotenoid degradation during storage should target the first 3 weeks and any handling activities prior to storage. It can also be concluded from the results that HDPE and AFL are appropriate packaging for OFSP flours for high carotenoid retention. Where possible, storing of OFSP fours in HDPE and AFL pouches should be vacuum sealed to achieve maximum carotenoid retention. The biggest challenge of using AFL pouches to package OFSP lies in the high cost of the material and might, therefore, limit its use. For instance in Nairobi, Kenya HDPE costs KES 1200/100 pieces while AFL costs 2300/100 pieces. This explains why AFL is used to package high value crops like coffee. Perhaps governments should subsidize AFL pouches for packaging of OFSP flour.

➢ *Color*

A slight, but significant ($P < 0.05$), decrease in L*, a* and b* values was observed in all of the samples stored under light and dark conditions (figures 3 and 4). When OFSP flour was packed in Kraft paper, significant decreases in color values were observed during the entire storage period under both light and dark conditions. It is possible that light permeated into the package since it was brown in color. Non vacuum sealing of Kraft paper also might have led to high oxygen transmissivity into the pouches hence loss of color. Flours in HDPE also exhibited non-significant loss of color under both conditions, contrary to the expectation based on the transparency nature of HDPE. This is perhaps due to the compaction of flour by vacuum sealing which limited the surface area of flour exposed to light. Non-significant loss of color was also observed when flour was packed in AFL despite being opaque. The slight loss in color of the flour in AFL might be related to the effects of light during drying of chips. The a* values for Vita and Kabode flours were comparable. The b* and L* values of Kabode flour were slightly higher than Vita flour regardless of the packaging material. The high L* values of Kabode flours indicated that it was lighter than Vita flours. Generally, the loss of flour color experienced in this study was due to exposure to light during drying of OFSP chips in the solar dryer. Drying of OFSP chips in the solar dryer for 4 d exposed the product long enough to light to contribute to color loss. Effects of the light exposure continued during flour storage.

➢ *Correlation between Color Values and β-carotene of Flour*

Some studies have linked the a* and b* values to β-carotene content in sweetpotatoes (Takahata et.al., 1993; Bengtsson et al., 2008). The authors reported the a* values as having the highest correlation to β-carotene content in OFSP fresh roots with correlation coefficients of 0.9 and 0.96, respectively. However, in our study the a* value did not strongly correlate with the β-carotene content. The correlation coefficient for light ($r = 0.66$) and dark ($r = 0.68$) storage condition ($P < 0.001$) indicate a positive but slightly weak relationship between a* values and β-carotene content of the flours. During progressive storage of OFSP flours carotenoid loss was so rapid while color loss was gradual to constant values resulting in the weak correlation between a* value and β-carotene content. The gradual loss in color of stored flour is related to the packaging that prevented total access by light. Therefore, color can be used as an indicator of β-carotene content in fresh roots and exposed flours only. Where carotenoid rich food is processed and stored for a period of time, β-carotene content and color values can be affected by many factors. Gross (1991) suggested complexing of carotenoids with proteins, caramelization, phenolic action and enzymatic browning as the main factors responsible for color change in carotenoid rich processed products. In this study, enzymatic browning during preparation and drying of OFSP chips might influence color of flour after milling. Moreover, in this study flours were processed from unpeeled roots so the sweetpotato skin might have also affected flour color.

➢ *Water Activity*

The water activity of flours stored under conditions exposed to light increased from 0.432 to 0.601 during the 4 mo storage period (figure 5). A similar trend in water activity of flour during storage was observed when flours were stored under dark conditions (figure 5). Significant differences ($P \leq 0.001$) in water activity were observed among the treatments from week 4 to week 16 under both conditions although interaction between genotype, packaging materials and storage environment was not significant ($P \geq 0.05$). Flours in Kraft paper bags had the highest water activity, indicating migration of moisture from the environment into the package. It was also observed that the water activity of flours in HDPE and AFL increased gradually during the entire storage period. This implies that HDPE and AFL pouches provided a good barrier to moisture transmission during the storage of flour. The current findings are in agreement with findings by Sra et al. (2014) and Swain et al. (2013). Sra et al. (2014) reported a significant increase in water activity of stored, diced carrot slices from 0.365 to 0.432 during 6 mo of storage. According to Swain et al. (2013), aluminum foil and HDPE had a non-significant effect on water activity for both stored red and yellow capsicum. Therefore, HDPE and AFL are appropriate packaging materials for OFSP flour.

Correlations between water activity and β-carotene of stored flour were a negative but very strong relationship. The correlation coefficient between the two parameters for flour under conditions exposed to light was 0.88 while under dark conditions it was 0.89 ($P < 0.001$). The results indicate that an increase in water activity was associated with a significant decrease in β-carotene of flours during storage. Lavelli et al. (2007) reported that carotenoids are relatively stable over a water activity range of 0.31–0.57 in freeze-dried carrot. Thus, water activity can also be used as a reliable indicator in the assessment of β-carotene content in flour during storage.

Although data on sorption isotherms of stored OFSP flours was not collected, Bechoff (2010b) and Lavelli et al. (2007) have reported of the same in relation to water activity and carotenoid degradation. Bechoff (2010b) found that samples stored at $_aw$=0.13 showed greater losses of β-carotene, followed by those stored at $_aw$ = 0.30, 0.51 and 0.76. The study demonstrated that storage at high water activity ($_aw$=0.76) improved β-carotene retention, but such practice would not be recommended due to high probability of microbial spoilage. Bechoff (2010b) further reported a linear relationship between the β-carotene degradation rate and water activity (R2=0.952) which agrees with the current findings. The rapid carotene degradation at low water activities might have contributed to the total carotene breakdown at high water activities in the long run.

IV. CONCLUSIONS

The study has demonstrated that AFL with vacuum sealing is a suitable packaging material for storing OFSP flour with high carotenoid retention and minimal increase in product water activity. Packing of flour in AFL should be vacuum sealed for high carotenoid retention. It is also evident from the study that color is not the best measure of β-carotene content of stored OFSP flour. Increased water activity of OFSP flours was strongly, but negatively, associated with β-carotene content. The low water activity of flour in AFL makes the pouch the appropriate packaging for stored OFSP flour. Therefore, future studies should focus more on optimizing the storage conditions, particularly at household levels where OFSP flour is the main functional ingredient, to minimize the carotene loss that are mostly due to light and poor packaging. Deliberate efforts should also be made to make the communities aware of the optimal storage conditions of OFSP flour to minimize the loss of carotenoids during flour storage and to fully benefit from the nutrient.

ACKNOWLEDGEMENTS

This material is based upon work supported by the United State Agency for International Development, as part of the Feed the Future initiative, under the CGAIR Fund, award number BFS-G-11-00002, and the predecessor Fund the Food Security and Crisis Mitigation II grant, award number EEM-G-00-04-00013. Many thanks to CIP-SSA, and North Carolina State University for co-funding the study. The first author thanks Mr Daniel Mbogo for carotenoid extraction and HPLC analysis trainings prior to the study.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is not conflict of interest.

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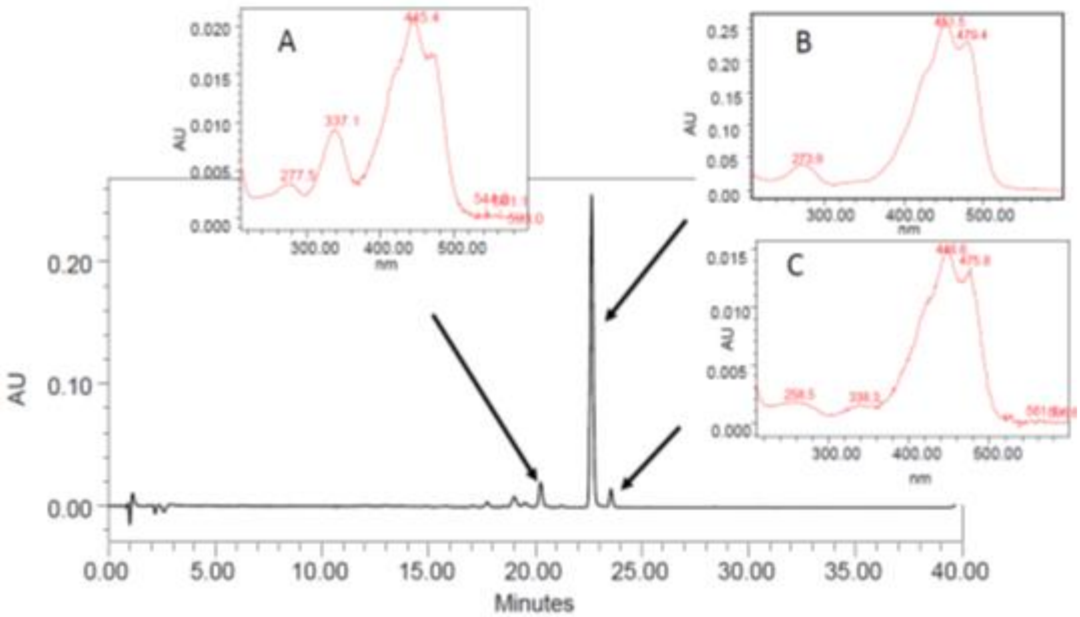

Fig 1:- Chromatogram peaks of 13-cis β-carotene (A), All trans β-carotene (B), and 9-cis β-carotene (C)

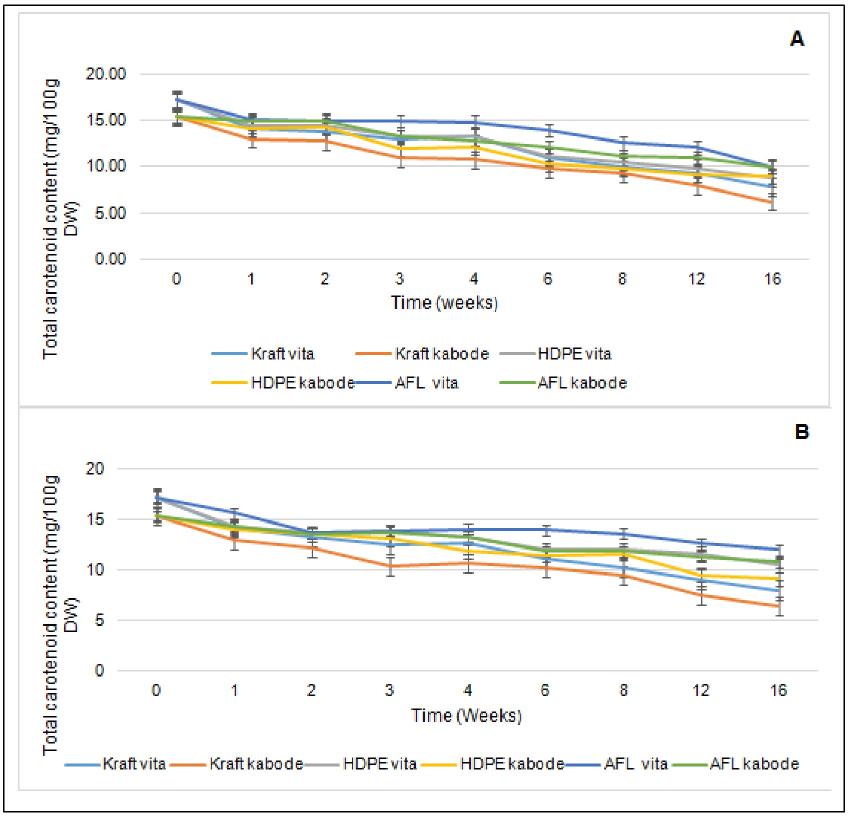

Fig 2:- Total Carotenoid content of OFSP flour stored under light condition (Panel A). Carotenoid content of OFSP flour stored under dark condition (Panel B)

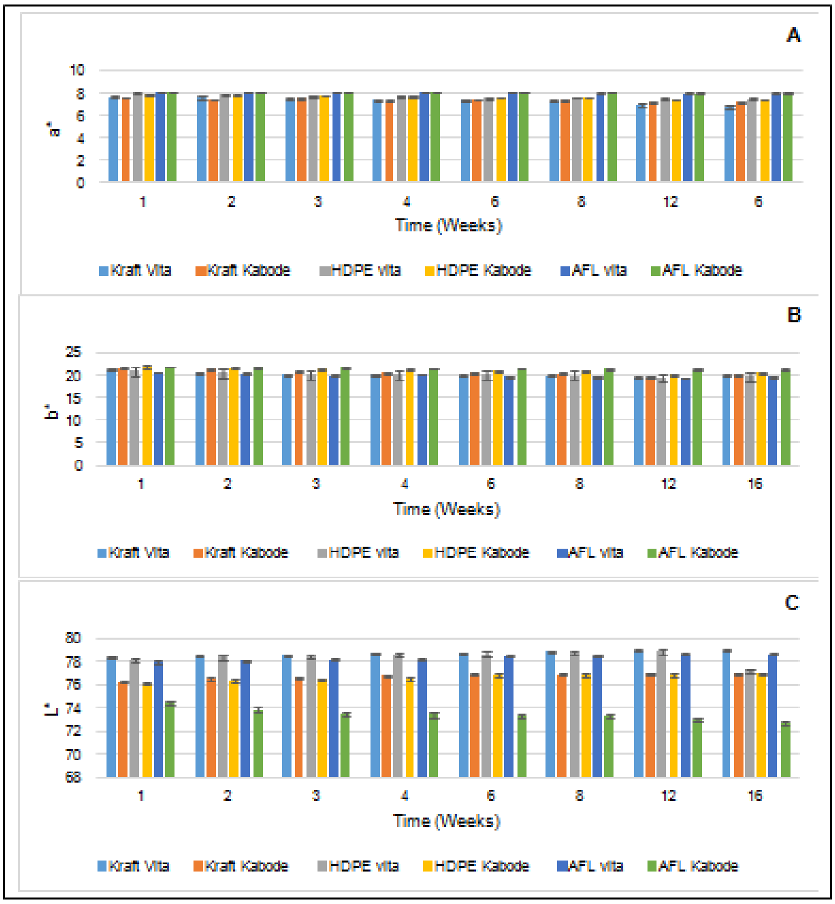

Fig 3:- The a* (Panel A), b* (Panel B) and L* (Panel C) of flour stored under light condition

ISSN No:-2456-2165

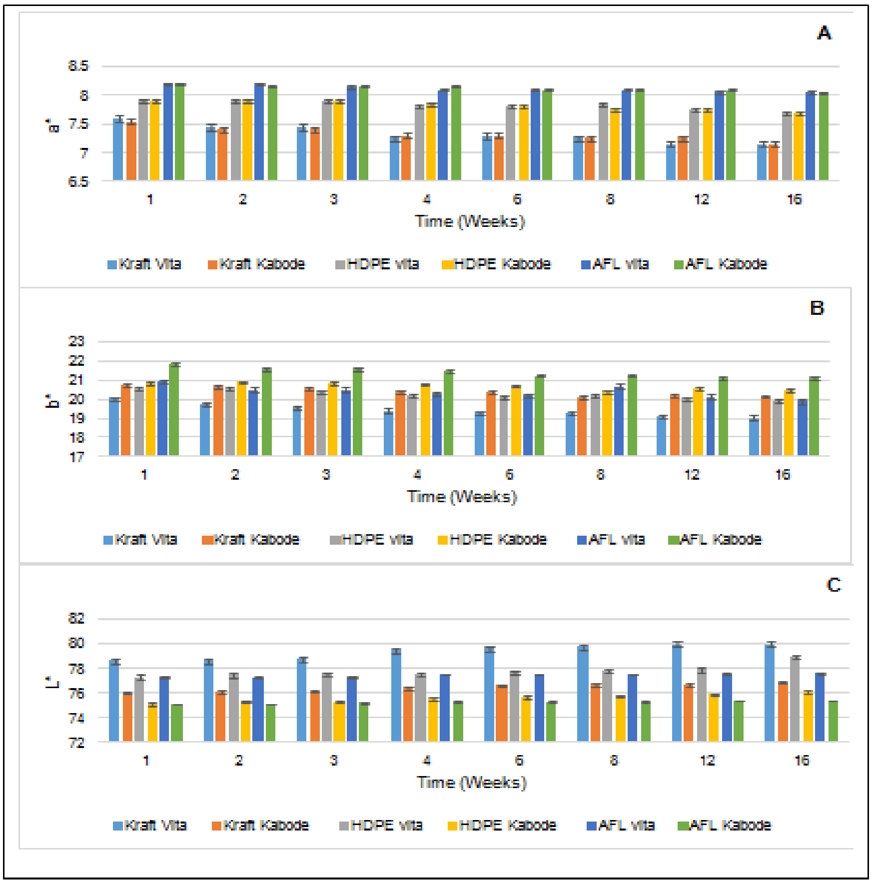

Fig 4:- The a* (Panel A), b* (Panel B) and L* (Panel C) of flour stored under dark condition

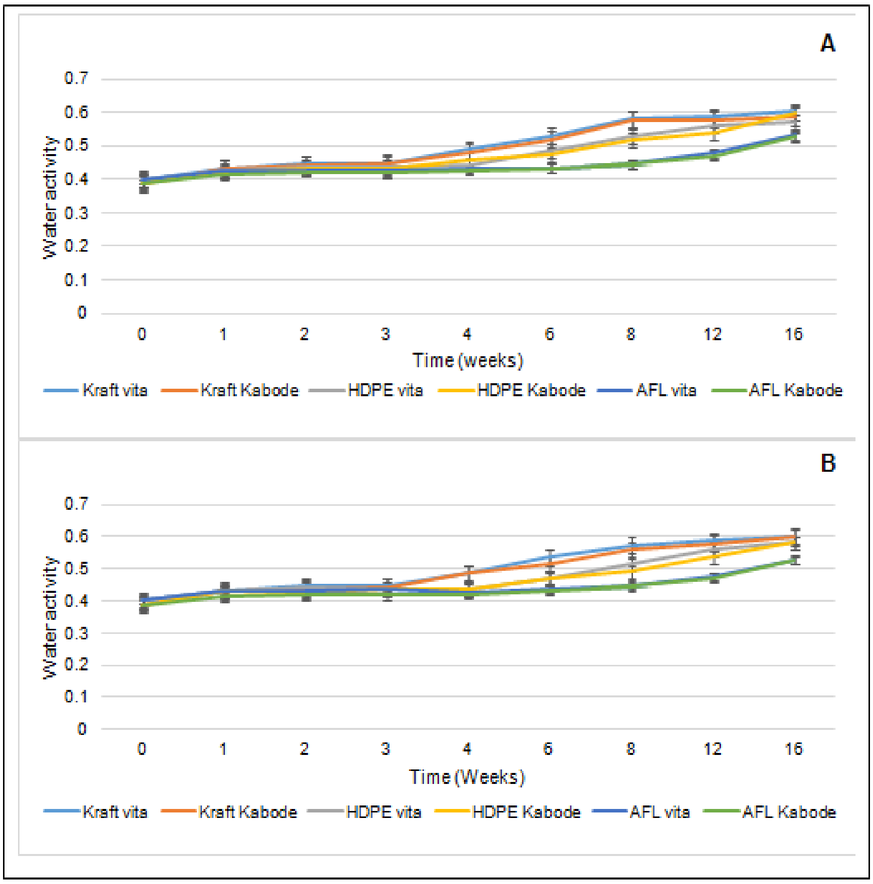

Fig 5:- Water activity of OFSP flour stored under light condition (Panel A) and dark condition (Panel B)