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Full Length Research Paper

Biochemical response of sweet potato to bemul-wax coating combined with calcium chloride treatment during ambient storage

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Sweet potato (*Ipomoea batatas* Linn) tuber is a very nutritious but highly perishable crop that is subject to high wastages due to non-availability of appropriate storage techniques. This work assessed the effectiveness of treating the tubers with calcium chloride dip (CCD), bemul-wax (B-wax) and their combinations (CCD/B-wax) at ambient storage conditions (24.9 ± 4.0°C and 44.6 ± 18.4% RH). Some biochemical parameters of each treated sample were assessed and compared with the control for 20 days storage period. There were significant reductions ($P < 0.05$) in weight loss by B-wax and CCD/B-wax. CCD and CCD/B-wax caused significant reductions in pH, total amino acid and pectin esterase. Also, all the treatments caused significant reductions in ascorbic acid, phenylalanine ammonia lyase, α-Amylase and alcohol dehydrogenase activities. There were significant reductions in polyphenol oxidase activity by B-wax. In conclusion, while all the treatments proved to be effective to varying degrees, CCD/B-wax seemed to be the most effective in preventing spoilage in the tuber.

Key words: Biochemistry, storage, bemul-wax, calcium chloride dip, combined agent, sweet potato.

INTRODUCTION

Sweet potato is a good source of crude fibre (implicated in the prevention of cancer), carbohydrate, carotene, protein and some vitamins (IITA, 1996), as well as calcium, phosphorus, potassium and sodium, compared with other root and tuber crops (FAO, 1990). It is undoubtedly more nutritious than other roots and tuber crops like yams, cassava and Irish potato (FAO, 1990). In addition to the various nutrients that can be attained from sweet potato, the tuber has also been reported to have healing properties as an antioxidant food (Meteljan, 2006).

Despite its nutritional importance, the tubers have a short storage life, generally, less than four weeks in the tropics. Their skin is easily damaged during harvest and post-harvest handling leaving the crop highly perishable (IITA, 1996). Insect damage of the leaves and tuber on the field, dehydration and rottenness of the tubers, high moisture content leading to its high perishability, lack of storage skills which discourages production, sprouting and chilling injury during low temperature storage are some of the pre and post-harvest problems associated with sweet potatoes cultivated in Nigeria (NSPRI, 2002). About 35 to 95% (FAO, 1988) of the 3318000 MT annual production of sweet potato in Nigeria (FAO, 2008) is been wasted. The principal postharvest problems with sweet potato storage are fungal rots, weevil damage and physiological changes (IITA, 1996). The only available storage methods for sweet potatoes are by leaving the crop in the ground and harvesting it only when needed and trench storage (FAO, 1991). However, the extent of postharvest losses associated with sweet potatoes warrants the need for improved methods for their storage. This led to the adoption of two technologies already in use for fruits and vegetables, for storing sweet potato tubers. A bio-wax (bemul-wax) was reported to have

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developed from cassava starch (Afolabi et al., 2003) for shelf life extension of agro-produce and consequently, to promote their exportation. The bemul-wax emulsion had been reported to preserve sweet oranges (*Citrus sinensis* (L) Osbeck*)*, with no negative effect on its sensory and biochemical qualities during a four months period of storage (Afolabi, 2009). Many fruit and vegetable transformation processes involve treatment with calcium to preserve their firmness, reduce respiratory rates and ethylene evolution and decrease storage rots (Matoo, 1991). The combination of these two techniques (calcium chloride dip and waxing) had not been tested for storage of sweet potatoes before. Various biochemical changes are associated with fruit physiological disorders (Mirakabadi et al., 2006; Pérez-Tello et al., 2009). Understanding the biochemistry of sweet potato tubers in response to the stated treatments at ambient conditions, will enhance the knowledge of the approach required for its shelf-life extension under optimum conditions. This work describes the effect of the bemul-wax (a bioemulsion), calcium chloride dip and the combination of the two treatments on the tuber biochemistry, with special reference to spoilage and defence – related enzymes and nutrients, during ambient storage. The outcome of this work would be used in assessing the efficacy of the selected treatments in extending the shelf-life of sweet potatoes. It is expected that the effects manifested in response to the treatments may further enhance under its optimum storage conditions.

MATERIALS AND METHODS

Chemicals

The following chemicals used in this study were products of Sigma Chemical Ltd, U.S.A: potato starch, polyethylene glycol (6000Carbowax), L-phenylalanine, dithiothreitol, NADH, acetaldehyde, pectin and pyro-catechol. Calcium chloride and L-leucine were from BDH Chemicals Limited, Poole, England.

Sample

Matured, wholesome, white variety of sweet potatoes (about 4 months old from the day of planting) was purchased from a farm in Offa, Kwara State of Nigeria. Tubers weighing about 500 to 700 g each (100 tubers were used for each group) were washed with clean water and thereafter treated as follows: (1) Coating with bemul-wax only (B-wax): the sweet potatoes were dipped into undiluted (100% concentrated) bemul-wax emulsion for 2 min, removed and dried with the aid of electric fans; (2) calcium chloride dip (CCD): the tubers were dipped in 3% w/v calcium chloride solution for 60 min as described by Pooviah (1986) for apples; (3) combination of dipping in calcium chloride (3%w/v) solution and bemul-wax coating (CCD/B-wax): the tubers were first dipped in 25 L of 3% (w/v) calcium chloride solution for 60 min, allowed to dry and thereafter, subjected to coating with bemul-wax as described in 1; (4) tubers that were not subjected to any treatment at all were used as the control (C-A). The tubers for each group were gently arranged in vegetables plastic crates to avoid them from bruising each other prior to storage. Each treatment including the control was stored in a grass thatch roofed storage barn (with the lower part enclosed with brick wall and upper part enclosed with wooden network to allow ventilation) under ambient conditions (24.9 ± 4.0°C; 44.6 ±18.4%RH). The changes in temperature and relative humidity of the storage environment were monitored with the aid of a thermohydrograph.

Five (earlier marked) sweet potatoes were used for the determination of physiological loss in weight (PLW) at four days interval for 20 days of storage. Three tubers were also withdrawn for biochemical analysis at four days interval for 20 days of the storage. The choice of the 20 days storage period was chosen from preliminary study that indicated the onset of rottening and manisfestation of shriveling. An unsual high degree of weight loss was noticed beyond the 20 days storage. The samples were shredded with a plastic grater and stored at -16°C until analysis was carried out. Activity of some selected enzymes related to food spoilage and its defence including pectin methyl esterase (PME), phenylalanine ammonia lyase (PAL), α-Amylase, alcohol dehydrogenase (ADH) and polyphenol oxidase (PPO) were assessed to identify the effect of the treatments on cell wall deterioration, wound development, sprouting, fermentation and browning, respectively. In addition, some biochemical components (pH, ascorbic acid and total amino acid) contributing to tuber quality were monitored. Three replications were used for each analysis.

pH

The pH of tubers was measured by slight modifications to the method of Thompson (1984). 20 g portion of the shredded samples was homogenized with 25 ml distilled water using mortar and pestle to increase the surface area. The homogenized samples were filtered through a double-layered muslin cloth and the collected filtrate was read with a pH meter (Jenway model 3310).

Pectin methyl esterase activity was determined by the method described by Hagerman and Austin (1986). α-Amylase activity was determined using the method of Bidderback (1971), as described by Okolie and Ugochuckwu (1988). Alcohol dehydrogenase (ADH) activity was determined by the extraction and assay procedure described by Ke et al. (1994). Phenylalanine ammonia lyase (PAL) activity was measured by the extraction and assay method of Abell and Shen (1987), as described by Morrison et al. (1994). Polyphenol oxidase was extracted and assayed using the method of Adamson and Abigor (1980). Assay for non-enzymatic browning was carried out as described by Lee et al. (1991) and Sting and Rouseff (1986). Ascorbic acid was extracted by the method of Tono and Fujita (1982), while the assay was carried out using the method of Jagota and Dani (1982), as modified by Oloyede and Afolabi (2002). Total amino acid component of protein was assayed by the method of Mark et al. (1985) as modified by Barbehenn (1995).

Statistical analysis

One factor randomized complete block design was used for this experiment. Data were analyzed using analysis of variance (ANOVA) MegaStat statistical software package. Treatments that differed at the $P = 0.05$ level were subjected to student T-test calculation using the same statistical software. The results were expressed as mean ± standard error of mean.

RESULTS AND DISCUSSION

Both B-wax alone and the CCD/B-wax treatments significantly ($P < 0.05$) reduced weight loss of the sweet potatoes for 8 days and 16 days under ambient

Table 1. Physiological loss in weight of treated sweet potato under ambient temperature storage.

Treatment	Duration of storage (Day)				
	4	8	12	16	20
	Weight loss (%)[1]				
C-A	7.071 ± 0.740[a]	11.043 ± 1.222[a]	14.226 ± 1.770[a]	18.097 ± 2.695[a]	15.949 ± 1.598[a]
CCD	6.430 ± 0.725[a]	9.991 ± 1.259[a]	12.721 ± 1.852[a]	15.984 ± 2.786[a]	16.511 ± 2.742[a]
B-wax	5.236 ± 0.700[b]	7.845 ± 1.152[b]	11.055 ± 2.174[a]	15.295 ± 3.877[a]	9.875 ± 0.319[b]
CCD/B-wax	4.524 ± 0.843[b]	7.062 ± 1.147[b]	9.499 ± 1.316[b]	12.853 ± 1.766[b]	16.731 ± 3.168[a]

[1]Each value is a mean of three determinations ± standard error of mean; values along the same column with different superscripts are significantly different ($P < 0.05$).

Table 2. pH of treated sweet potato at ambient temperature storage.

Treatment	Duration of storage (Day)					
	0	4	8	12	16	20
	pH value					
C-A	5.780 ± 0.000	6.036 ± 0.030[a]	6.525 ± 0.012[a]	6.213 ± 0.021[a]	6.203 ± 0.033[a]	6.018 ± 0.032[a]
CCD	5.780 ± 0.000	6.083 ± 0.028[a]	5.995 ± 0.020[b]	5.805 ± 0.004[b]	5.850 ± 0.024[b]	5.795 ± 0.094[b]
B- wax	5.780 ± 0.000	6.503 ± 0.059[b]	6.355 ± 0.012[c]	6.420 ± 0.024[c]	6.050 ± 0.008[c]	5.775 ± 0.003[c]
CCD/B- wax	5.780 ± 0.000	5.904 ± 0.026[c]	6.055 ± 0.004[b]	6.257 ± 0.003[d]	6.100 ± 0.008[d]	5.845 ± 0.003[d]

[1]Each value is a mean of three determinations ± standard error of mean; values along the same column with different superscripts are significantly different ($P < 0.05$).

temperature, respectively (Table 1). The significant reduction in the PLW by both b-wax and the CCD/B-wax treatments during ambient temperature storage may be a reflection of the effectiveness of the treatments in preventing physical damage to the cell wall of the tuber (Table 1). Thus, the CCD/B-wax treatment exhibited the most pronounced effect in preventing physical damage to the tuber stored at ambient temperature (Table 1). Physiological deterioration is associated with stress induced by water loss (Kader, 1986; Weichmann 1987; FAO, 1995) and the water loss is enhanced when there is cell membrane and cell wall integrity damage (Nagaragan et al., 2005). Thus, both B-wax and the CCD/B-wax were able to prevent physical damage which in turn prevented water loss from the tuber. It may however be pointed out that, the ability to prevent the water loss in tubers may largely be due to the involvement of B-wax coating rather than the CCD treatment. The B-wax forms a thin film around the tubers creating a permeability barrier to moisture migration and possibly for some gases like ethylene, oxygen and carbon dioxide (Shewfelt, 1986; Hoa et al., 2002). Additionally, the weight retained in the potato tubers treated with b-wax coating and the CCD/B-wax, could be an economic gain to marketers during export of the tubers.

A significant ($P < 0.05$) decrease in pH of the CCD and the CCD/B-wax treated tubers were observed for 16 and 8 days, respectively compared with control (Table 2). However, a significant increase ($P < 0.05$) in pH was observed for both samples treated with b-wax coatings during the first 12 days ambient temperature storage. pH is an important physiological characteristic of a tissue, which is related to the level of acidity/alkalinity due to the release or absorption of hydrogen ions. In this study, the reduction in pH of the tubers treated with CCD and the CCD/B-wax at ambient storage may have resulted in increased acidity in the tuber (Table 2). The increase in acidity may be associated with the production of organic acid possibly via the TCA cycle or fermentation (Steinkraus, 1996). Thus, both treatments involving calcium chloride dip may have lead to production of organic acids and thereby reduction in pH (Osuji, 1985; Ke et al., 1994; Steinkraus, 1996). The creation of a low pH value may reduce the activity of spoilage enzymes (Jay, 1985) under ambient storage.

Tubers treated with CCD alone and the CCD/B- wax manifested significantly lower ($P < 0.05$) pectin methyl esterase activity (Table 3) on the 4th day at ambient storage. Thereafter, a significantly higher ($P < 0.05$) PME activity was evident with all the treated tubers when compared with the control till the end of the storage period. Spoilage of plant tubers is enhanced when there is breakdown of cell wall materials (Hagerman and Austin, 1986; Awad and Young, 1979; Hobson, 1963), which requires the action of pectin methyl esterase enzyme. In this study, it was evident that cell wall firmness/ texture was preserved for the first four days with calcium chloride dip (CCD) and the combined treatment (CCD/B-wax) at ambient temperature storage (Table 3). It is possible that these agents were effective in preventing

Table 3. Pectin methyl esterase activity of treated sweet potato at ambient temperature storage.

Treatment	Duration of storage (Day)					
	0	4	8	12	16	20
	Pectin methyl esterase activity x 10^{-3} (µmole of acid produced/min/g fresh weight)					
C-A	1.063 ± 0.153	1.671 ± 0.017^{a}	0.513 ± 0.141^{a}	0.787 ± 0.126^{a}	0.446 ± 0.175^{a}	0.479 ± 0.09^{a}
CCD	1.063 ± 0.153	0.940 ± 0.104^{b}	0.627 ± 0.070^{a}	0.573 ± 0.163^{a}	0.766 ± 0.371^{a}	0.893 ± 0.058^{b}
B- wax	1.063 ± 0.153	1.757 ± 0.064^{a}	0.687 ± 0.079^{a}	0.886 ± 0.104^{a}	0.713 ± 0.057^{b}	0.520 ± 0.046^{c}
CCD/B- wax	1.063 ± 20.153	0.938 ± 0.125^{c}	0.580 ± 0.061^{a}	0.652 ± 0.214^{a}	0.959 ± 0.122^{c}	0.726 ± 0.027^{d}

[1]Each value is a mean of three determinations ± Standard error of mean; values along the same column with different superscripts are significantly different ($P < 0.05$).

Table 4. α-amylase activity of treated sweet potato at ambient temperature storage.

Treatment	Duration of storage (Day)					
	0	4	8	12	16	20
	α- Amylase activity (µmol of starch consumed/min/g fresh weight) x 10^{-1}					
C-A	0.204 ± 0.060	0.613 ± 0.013^{a}	0.599 ± 0.027^{a}	0.603 ± 0.005^{a}	0.598 ± 0.045^{a}	0.629 ± 0.021^{a}
CCD	0.204 ± 0.060	0.393 ± 0.005^{a}	0.393 ± 0.024^{b}	0.438 ± 0.032^{b}	0.422 ± 0.014^{b}	0.633 ± 0.021^{a}
B- wax	0.204 ± 0.060	0.583 ± 0.065^{a}	0.544 ± 0.017^{a}	0.608 ± 0.007^{a}	0.496 ± 0.039^{c}	0.457 ± 0.007^{b}
CCD/B- wax	0.204 ± 0.060	0.289 ± 0.060^{b}	0.335 ± 0.014^{c}	0.463 ± 0.016^{b}	0.343 ± 0.023^{d}	0.499 ± 0.017^{b}

[1]Each value is a mean of three determinations ± standard error of mean; [2] Values along the same column with different superscripts are significantly different ($P < 0.05$).

spoilage up to 4 days under ambient conditions. The expected reduction of spoilage may be attributed to the incorporation of Ca^{2+} ions into the COOH group of cell wall pectin through calcium chloride treatment (Conway et al., 1994; Alonso et al., 1995; Lee et al., 2007). The effect of this action was however lost subsequently, leading to the early spoilage of the tubers. The significant reduction in pectin esterase activity during the first four days under ambient storage in tubers treated with calcium chloride indicates some inhibitory effect of the Ca^{2+} on the enzyme (Conway et al., 1994; Alonso et al., 1995). The early increase in pectin esterase activity in the control and b-wax coated tubers was sufficient to initiate spoilage leading to their loss of cell wall integrity, and consequently to shorten storage life under ambient conditions.

A significant decrease ($P < 0.05$) in α-Amylase activity compared with the control was observed for all the treated tubers during the ambient storage (Table 4), while CCD/B-wax treatment was the most effective among them throughout the 20 days storage, and b-wax and CCD could only achieve that for 4 and 16 days, respectively. α-Amylase is an enzyme that regulates the break down of starch to simple sugars in plant. The sugars are employed by the plants to sustain its metabolic activities during storage.

The metabolic activity may results in sprouting of the tubers (Osuji, 1985), which is an indication of spoilage. In this study, α- Amylase activities were observed to be generally reduced in all the treated tubers during the ambient storage (Table 4). The reduction in α- amylase activity is an indication of the ability of the various treatments (CCD, b-wax, and CCD/B-wax) in slowing down the breakdown of starch to sugars and may have a consequential effect of reducing the sweetness of the tubers (Martin, 1987). It is to be noted that of all the treatments, the CCD/B-wax was observed to be the best in preventing sprouting in sweet potato tubers. The reduction in α-Amylase activity in particular by treatments that involved calcium chloride dip could be exploited in reducing the sweetness of the sweet potato tuber. Excess calcium ion had been reported to irreversibly unfold α- Amylase at relatively high temperatures like that of the ambient temperature utilized in this study (Nielsen et al., 2003), while on the contrary, depletion of calcium ion was identified to result in the irreversible inactivation at low temperature. Among the attempts made by several researchers to reduce the level of sweetness of sweet potato, only genetic modification through crossbreeding and gene manipulation had been successful (Martin, 1987). These findings can be considered as a novel, non-genetic approach in achieving the same objective.

Also, b-wax, CCD and the CCD/B-wax treated tubers manifested a significant decrease ($P < 0.05$) in PAL activity for 12, 4 and 20 days at ambient storage, respectively (Table 5). However, there was an initial rise in phenylalanine ammonia lyase activity in the control and CCD treated tubers at the 4th day (Table 5). PAL is the enzyme responsible for the healing of wounds by building the polyphenol content of plants cell wal l (FAO, 1995).

Table 5. Phenylalanine ammonia lyase activity of treated sweet potato at ambient temperature storage.

Treatment	Duration of storage (Day)					
	0	4	8	12	16	20
	Phenylalanine ammonia lyase (µmol of cinnamic acid produced/h/g fresh weight)					
C-A	0.212 ± 0.012	0.452 ± 0.014[a]	0.144 ± 0.036[a]	0.162 ± 0.013[a]	0.142 ± 0.001[a]	0.149 ± 0.002[a]
CCD	0.212 ± 0.012	0.269 ± 0.007[b]	0.187 ± 0.009[a]	0.149 ± 0.029[a]	0.137 ± 0.008[a]	0.185 ± 0.034[a]
B- wax	0.212 ± 0.012	0.178 ± 0.028[c]	0.117 ± 0.049[a]	0.136 ± 0.048[a]	0.082 ± 0.012[b]	0.056 ± 0.004[b]
CCD/B- wax	0.212 ± 0.012	0.103 ± 0.021[d]	0.102 ± 0.013[a]	0.104 ± 0.016[a]	0.017 ± 0.001[c]	0.044 ± 0.034[c]

[1] Each value is a mean of three determinations ± standard error of mean; values along the same column with different superscripts are significantly different ($P < 0.05$).

Table 6. Alcohol dehydrogenase activity of treated sweet potato at ambient storage temperature.

Treatment	Duration of storage (Day)					
	0	4	8	12	16	20
	Alcohol dehydrogenase activity x 10^{-2} (µmole of acetaldehyde consumed/min/g fresh weight)					
C-A	0.471 ± 0.056	0.601 ± 0.206[a]	0.535 ± 0.081[a]	0.249 ± 0.106[a]	0.308 ± 0.080[a]	0.313 ± 0.070[a]
CCD	0.471 ± 0.056	0.766 ± 0.082[b]	1.138 ± 0.220[b]	0.492 ± 0.058[b]	0.291 ± 0.025[a]	0.151 ± 0.052[b]
B- wax	0.471 ± 0.056	0.435 ± 0.000[c]	0.296 ± 0.041[c]	0.202 ± 0.018[a]	0.326 ± 0.142[a]	0.173 ± 0.014[b]
CCD/B- wax	0.471 ± 0.056	0.283 ± 0.062[d]	0.210 ± 0.003[d]	0.218 ± 0.121[a]	0.304 ± 0.047[a]	0.164 ± 0.021[b]

[1]Each value is a mean of three determinations ± standard error of mean; values along the same column with different superscripts are significantly different ($P < 0.05$).

The activity of PAL is always induced to generate healing in response to any wound. Thus, the significant reduction ($P < 0.05$) in PAL activity by all the treatments gave an indication that, the injury of the treated tubers may have been delayed by a period of 12, 4 and 20 days compared with the control for b-wax, CCD and the CCD/B-wax treatments, respectively. The PAL activity reduced in the control tubers to a level that was the same as the calcium chloride treated tubers after 4 days storage. The initial rise in PAL activity for the control tubers and tubers treated with calcium chloride dip on the 4th day (Table 5) may be due to the increased metabolic activity arising from the need for the increase in production of more polyphenols, so as to protect/repair the tubers from damage (FAO, 1995; Sankat and Maharaj, 1997). It may also be in response to the initial physiological deteriorations as a result of their high physiological loss in weight (FAO, 1995) as PAL had been reported to increase in response to physiological damage (FAO, 1995). Wound signal formation is suppressed and the wound response processes are reduced, as the normal internal environment of the plant is reinstated. This may explain the gradual decrease in PAL activity after 4 days of storage. Also, the general reduction in PAL activities in B-wax coated tubers (B-wax and CCD/B-wax) throughout the storage period can be attributed to the low permeability of the B-wax to oxygen thereby, limiting the PAL enzyme from oxygen. The activity of PAL had been positively correlated to the concentration of oxygen during storage (Kleiber et al., 2005; Shirsat and Nair, 1976).

ADH enzyme activity in CCD treated tubers was significantly higher ($P < 0.05$) for 12 days compared with that in the control. However, ADH activity was significantly lower ($P < 0.05$) throughout the period of storage (Table 6) for tubers treated with b-wax and CCD/B-wax. The generally high ADH activity detected in CCD treated tubers may be an indication of the occurrence of alcoholic fermentation in such tubers (Table 6), which may culminate in the development of off-flavor/foul odor in those tubers at ambient storage (Ke et al., 1994; Osuji, 1985).

Considering polyphenol oxidase (PPO) activity (Table 7), significantly higher ($P < 0.05$) values were observed with tubers treated with CCD and the CCD/B-wax, for the first eight days of storage. The general reduction in PPO activity on the 4th day of storage could be due to the consequential effect of just harvesting the crop. This is similar to the reduction in PPO activity 2 to 3 days of storage of freshly harvested avocado fruit (Hofman and Husband, 1987). On the contrary, it was observed that on a general note, a significant decrease ($P < 0.05$) in its activity was found for b-wax coated tubers for 12 days at the ambient storage. The reduction of PPO activity in B-wax coated tubers (B-wax and CCD/B-wax) was similar to our earlier observations for PAL activity. Polyphenol oxidase is an enzyme in plant that plays a critical role in the defense system against pathogens or wounds in plant tissues (Aurthur and McLemore, 1956; Mayer and Harel, 1979; DeEll *et* al., 2003; Suliman et al., 2004; Thipyapong

Table 7. Polyphenol oxidase activity of treated sweet potato at ambient temperature storage.

Treatment	Duration of storage (Day)					
	0	4	8	12	16	20
	Polyphenol oxidase activity x 10^{-2} (µmole of cathecol consumed/min/g fresh weight)					
C-A	0.790 ± 0.020	0.496 ± 0.038^{a}	0.907 ± 0.027^{a}	0.988 ± 0.172^{a}	0.786 ± 0.008^{a}	0.584 ± 0.079^{a}
CCD	0.790 ± 0.020	0.606 ± 0.025^{a}	1.045 ± 0.019^{b}	0.816 ± 0.030^{a}	0.773 ± 0.017^{b}	0.717 ± 0.021^{b}
B- wax	0.790 ± 0.020	0.578 ± 0.062^{a}	0.634 ± 0.010^{c}	0.678 ± 0.056^{b}	0.685 ± 0.013^{c}	0.636 ± 0.032^{a}
CCD/B- wax	0.790 ± 0.020	0.716 ± 0.005^{b}	1.113 ± 0.005^{b}	0.938 ± 0.014^{a}	0.794 ± 0.020^{a}	0.792 ± 0.081^{b}

[1]Each value is a mean of three determinations ± standard error of mean; values along the same column with different superscripts are significantly different (P < 0.05).

Table 8. Non Enzymatic Browning of treated sweet potato at ambient temperature storage.

Treatment	Duration of storage (Day)					
	0	4	8	12	16	20
	Optical density value					
C-A	0.063 ± 0.014	0.086 ± 0.036	0.085 ± 0.002^{a}	0.072 ± 0.013^{a}	0.113 ± 0.020^{a}	0.078 ± 0.003^{a}
CCD	0.063 ± 0.014	0.117 ± 0.032	0.112 ± 0.027^{a}	0.181 ± 0.010^{b}	0.174 ± 0.010^{b}	0.160 ± 0.016^{b}
B- wax	0.063 ± 0.014	0.079 ± 0.007	0.104 ± 0.013^{b}	0.0717 ± 0.003^{a}	0.107 ± 0.013^{a}	0.090 ± 0.008^{c}
CCD/B- wax	0.063 ± 0.014	0.058 ± 0.018	0.091 ± 0.010^{a}	0.110 ± 0.008^{c}	0.093 ± 0.007^{a}	0.096 ± 0.010^{d}

[1]Each value is a mean of three determinations ± standard error of mean; values along the same column with different superscripts are significantly different (P < 0.05).

et al., 2007). It catalyses the reaction that leads to browning in sweet potato and other crops. The observations (Table 7) indicated that b-wax coatings is likely to minimize browning in sweet potato tubers for 12 days storage, while this would likely occur after eight days in the case of CCD and the CCD/B-wax treated tubers. The reduction in browning reaction is an indication of the ability of the treated tubers to fight invasion by the spoilage agents (microorganisms) (Mayer and Harel, 1979; Glennie, 1981; DeEll et al., 2003; Mayer, 2006).

Compared with the control, the b-wax, CCD and the CCD/B-wax treated tubers exhibited significantly higher (P < 0.05) non enzymatic browning for 4, 20 and 4 days storage, respectively (Table 8). Non-enzymatic browning is a measure of brown coloration due to a reaction between sugars and amine part of a protein (BeMiller and Whistler, 1996). The CCD, B-wax and CCD/B-wax treatments were definitely ineffective for inhibiting non-enzymatic browning in sweet potato at ambient storage having contributed to its enhancement by 105.10, 15.35 and 23.08%, respectively at the end of the 20 days storage. The effectiveness of B-wax in reducing non-enzymatic browning compared with the other treatments could be attributed to its ability to reduce oxygen in the sweet potato tissue. The presence of oxygen is a requirement for non-enzymatic browning reactions to proceed (Dyer et al., 1991).

Generally, there were initial increases in ascorbic acid levels for all the treated tubers in the first eight days (Table 9); similar increase in ascorbic acid during storage had been reported (Weighmann, 1987; Fonseca and Jones, 2004). It is opined that, the storage conditions could have been favourably disposed towards the conversion of other carbohydrates to ascorbic acid (Loewus and Kelly, 1961; Conklin, 2001; Agius et al., 2003). This suggestion was also supported by the general increase in α–Amylase activities for all the treated tubers reported in this study compared with that of the fresh tubers (Table 6), which indicated increase in the synthesis of more simple sugars that could be converted to ascorbic acid during the storage. The ascorbic acid levels (Table 9) for tubers treated with CCD and CCD/B-wax were significantly low (P < 0.05) throughout the 20 days ambient storage when compared with the control. The significant increases (P < 0.05) in ascorbic acid levels of the tubers coated with B-wax at the 4th day of ambient storage were followed by a general significant reduction (Table 9). Ascorbic acid is an antioxidant vitamin/nutrient in food. The maintenance or increase of its level in a plant system will prevent stress induced spoilage and thereby extend the shelf-life. Stress induced spoilage may have occurred in CCD and the CCD/B-wax treated tubers as shown by the significant reduction (P < 0.05) in their ascorbic acid levels throughout the 20 days ambient storage. The reduction in ascorbic acid level was not the case in B-wax coated tubers as the coating could have decreased the oxygen that could serve as substrate for ascorbic acid oxidase. It is this enzyme that is responsible for the degradation of ascorbic acid (Esaka et al., 1992). Several reasons could be attributed to the

Table 9. Ascorbic acid levels of treated sweet potato at ambient temperature storage.

Treatment	Duration of storage (Day)					
	0	4	8	12	16	20
	Ascorbic acid level (µg/g fresh weight)					
C-A	182.744 ± 11.452	353.322 ± 4.589[a]	428.649 ± 3.546[a]	353.984 ± 56.143[a]	311.256 ± 2.699[a]	264.727 ± 18.894[a]
CCD	182.744 ± 11.452	360.256 ± 33.193[a]	250.512 ± 11.067[b]	280.265 ± 25.912[a]	245.223 ± 19.704[a]	244.397 ± 0.000[b]
B- wax	182.744 ± 11.452	516.298 ± 9.447[b]	346.711 ± 9.987[c]	305.719 ± 0.270[a]	341.422 ± 25.102[b]	208.198 ± 5.398[c]
CCD/B- wax	182.744 ± 11.452	230.347 ± 8.907[c]	183.405 ± 7.288[d]	296.132 ± 21.863[a]	249.190 ± 27.532[a]	246.215 ± 6.748[a]

[1]Each value is a mean of three determinations ± standard error of mean; [2] values along the same column with different superscripts are significantly different ($P < 0.05$).

reduction in ascorbic acid, among which is its interaction with amino acids during maillard reaction leading to non enzymatic browning of the tubers (Bemiller and Whistler, 1996). Ascorbic acid had also been linked to be a point substrate for PPO enzymes leading to more enzymatic browning (Arthur and McLemore, 1956) and increase in other oxidases activity (for example, ascorbate peroxidase that uses two molecule of ascorbic acid to reduce hydrogen peroxide to water) during ripening and maturation of fruits had also been reported (Lee and Howard, 1999; Osasuna-Garcia et al., 1998) to contribute to ascorbic acid degradation. The increase in oxidation had earlier been reported to contribute to ascorbic acid decline.

Also, levels of total amino acid in tubers treated with calcium chloride dip and the CCD/B-wax treatments were significantly lower ($P < 0.05$) when compared with the control throughout the 20 days storage period (Table 10). B-wax treatment was found to significantly increase ($P < 0.05$) total amino acid level of the sweet potato only on 4th day ambient storage period compared with the control. Significantly lower levels of amino acid composition for CCD and the CCD/B-wax treated tubers, indicate loss of protein amino acids of the treated tubers. The amino acids of the proteins in both CCD and the CCD/B-wax treated sweet potatoes stored at ambient temperature may react with aldehyde or keto groups of reducing sugars through non enzymatic reactions yielding brown pigments (Kumar et al., 1999). The pronouncedly higher non-enzymatic browning in CCD treated tubers (Table 8), in which the lost of amino acids are most pronounced further gives credence to this observation. This was not so pronounced in CCD/B-wax treated tubers (Table 8) due to synergistic effect of B-wax on that of the CCD treatment.

In summary, the data from this study indicates that bemul-wax alone and the bemul wax/calcium chloride dip combined treatments were effective in reducing fresh weight loss of sweet potato at ambient temperature storage. Thus, application of both treatments on sweet potatoes will provide more economic gain to exporters. The creation of a lower tissue pH by the calcium chloride and the bemul wax/calcium chloride dip combined treatments had reduced the activity of the spoilage enzymes as well as reduced the susceptibility of the tubers to attack by microorganisms. A non-genetic approach to reduced sweetness development and the reduction of sprouting of sweet potatoes were manifested by the calcium chloride and bemul-wax treatments. The CCD/B-wax treatment was observed to be the best in preventing sprouting in sweet potato tubers. The reduced rate of loss of firmness/texture of the cell wall of the sweet potato tubers as deduced from the low activity of pectin esterase is an indication that, CCD and the CCD/B-wax treatments are effective in minimizing spoilage. Both CCD and the CCD/B-wax treatments however, led to loss of amino acids during the ambient storage.

Also, the reduction in enzymatic browning reaction was achieved by all the treatments at ambient temperature storage indicating their ability to fight invasion by the spoilage agents. Both the bemul-wax and the combined agent treatments showed ability to reduce fermentation as manifested by the reduction of ADH activity. However, the combined agent was most effective in reducing fermentation by implication spoilage. Stress induced spoilage of the tubers treated with calcium chloride dip and the combined agent may be attributed to the rapid loss of antioxidant nutrients as is the case with ascorbic acid.

Table 10. Total amino acid levels of treated sweet potato at ambient temperature storage.

Treatment	Duration of storage (Day)					
	0	4	8	12	16	20
	Amino acid level (mg/g dry weight)					
C-A	8.823 ± 0.276	12.146 ± 0.290[a]	9.924 ± 0.379[a]	2.141 ± 0.451[a]	1.227 ± 0.253[a]	1.425 ± 0.330[a]
CCD	8.823 ± 0.276	4.740 ± 0.505[b]	4.491 ± 0.206[b]	1.299 ± 0.146[b]	0.159 ± 0.016[b]	2.354 ± 0.518[b]
B- wax	8.823 ± 0.276	15.347 ± 0.107[c]	8.575 ± 0.325[c]	1.704 ± 0.266[a]	1.451 ± 0.257[a]	1.011 ± 0.101[c]
CCD/B- wax	8.823 ± 0.276	6.284 ± 0.0672[d]	4.147 ± 0.337[d]	1.167 ± 0.426[c]	1.001 ± 0.230[a]	0.695 ± 0.113[d]

[1]Each value is a mean of three determinations ± standard error of mean; values along the same column with different superscripts are significantly different ($P < 0.05$).

Finally, it was considered that while all the treatments proved to be effective to varying degrees, the combination of both calcium chloride dip and bemul-wax seemed to be the most effective at the ambient temperature storage.

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