Inhibition of enzymatic browning in *Dioscorea Alata* chips using natural anti-browning agents

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Abstract

Of all the yam varieties, *Dioscorea alata* is the most susceptible to enzymatic browning. A study was conducted on four natural anti-browning agents (lemon juice, water extract of *Moringa* seed, papaya latex and pectin) to monitor their anti-browning effect on *D. alata* chips. The effects of treatment time and concentration of anti-browning as well as the rate of colour change, were studied. The yam chips were soaked in solutions of anti-browning agents at concentrations of 40% and 50% v/v (for lemon juice) and 1% and 2%w/v (for *Moringa* seed extract, papaya latex and pectin) for 1, 3 and 5 minutes; solar dried for 2 days and their colour differences compared. Sensory evaluation of the treated samples was done using a colour reference sheet. All treatments, except pectin 2% at 3 minutes, significantly different (p>0.05) from the control exhibited significant antibrowning effects. Increase in treatment time increased the anti-browning potency of an ABA. Lower concentration had better antibrowning results in DPL, pectin and lemon. The result of the rate of colour change revealed that visual scoring and instrumental measurement produce data sets that differ in quality. Browning in water yam can therefore be controlled with natural anti-browning agents such as papaya latex, water extract of *Moringa* seed, pectin and lemon juice.

Introduction

The cosmetic quality of food is significantly impacted by colour which is the first attribute used by consumers in evaluating food quality (Guiwen *et al.*, 1995; Calvo, 2004). Colour may be influenced by naturally occurring pigments resulting from both enzymatic and non-enzymatic reactions (Marshall *et al.*, 2000). Maintaining the natural colour in processed and stored food has been a major challenge in food processing due to oxidative processes such as enzymatic browning. (Marshall *et al.*, 2000; Mohammadi *et al.*, 2008; Billaud *et al.*, 2003; Akissoe *et al.*, 2004). The phenomenon is a widespread problem in the food industry, as it leads to undesirable characteristics in food, decrease in food nutrients and quality, shelf life and market value thereby incurring economic losses and food insecurity (Saengnil *et al.*, 2005).

Browning and its control have been extensively studied and reported in many fruits and vegetables such as apples, pears, cabbages, bananas, and potatoes (Soliva-fortuny *et al.*, 2001; Rojas-Grau *et al.*, 2006). Most antibrowning agents used are chemicals of which sulphites are the most prominent. Sulphites are however banned in some countries because they have adverse health effects (Tong *et al.*, 1991; Lee *et al.*, 1995; Son *et al.*, 2000; Hang-ing *et al.*, 2005). According to Jiang *et al.* (2004), consumers are demanding a reduction in the overall use of chemicals on fresh products and alternative methods are being investigated to extend shelf life of fresh-cut fruits.

Yams are second to cassava as the most important tropical root crop due to the absence of cyanogenic compounds. Additionally, they are nutritionally better than cassava due to their higher vitamin C (40-120mg/g edible portion), crude protein (40-140g/kg dry matter) (Opara, 1999). According to Berghofer (2005), minimal processing is one way by which diversified products can be developed. However during processing, yam is prone enzymatic browning which must be controlled (Miyawaki, 2006). *Dioscorea alata* is a common but underutilized yam species in Ghana and browns quickly and intensely than other edible yam varieties (Ozo and Caygill, 2006) hence the motivation for its utilization in this work.

The aim of the study was to assess the potential anti-browning properties of dried papaya latex, water extract of *Moringa* seeds, pectin and lemon juice. The effect of concentration and treatment time of these materials on enzymatic browning in *D. alata* chips was studied. In addition, the rate of browning of *D. alata* was studied for the materials that gave the highest level of browning inhibition from sensory evaluation.

Methodology

Procurement of materials

D. alata (TDa /0099/208) tubers were obtained from Crop Research Institute, Femesua, Kumasi, partially ripened Lemon fruits from the Kumasi European Market, Ghana, powdered citrus pectin from Sigma Aldrich Inc., Germany and dried *Moringa oleifera* seeds from Oda in the Eastern region of Ghana.

Preparations of yam chips

Each tuber was washed, divided into 3 equal parts and a 2/3 fraction from the 'head' region was used for the experiment (Onayemi, 1986). The tubers were peeled, cut and chipped whilst submerged under tap water. Chipping of *D. alata* was done with a hand chipper into average sizes of 4cm x 3cm x 0.1cm.

Preparation of anti-browning solutions

Dried papaya latex solution. Incisions were made on four sides of the partially mature papaya fruits, from the stalk end to the tip and the papaya latex that exuded was collected into plastic bowls. Similar incisions were repeated on untapped surface of the same fruit three times at 3-4 days interval. Tapping of papaya latex was done early in the morning before 9.00 a.m. for each day. The liquid latex was solar dried for 2 days into flakes. Powder was prepared from dry flakes and stored in air tight plastic containers prior to use. Concentration of 1% and 2% w/v were prepared and used for the experiment. The solution was allowed to stand for 3 hours while shaken intermittently before being filtered off since the dried papaya latex powder is partially soluble in water.

Pectin, Lemon and Moringa Seed Extract solution. The following concentrations were selected and prepared for the study after preliminary test. Both 1% and 2% w/v solutions were prepared from pectin and *Moringa* seed powder respectively. For lemon juice, 40%v/v and 50%v/v were prepared. Warm distilled water (60°C) was used to allow for easy dissolution of the pectin. The *Moringa* seed extract was also agitated intermittently for 2 hours since the solute was partially soluble. The *Moringa* seeds extract had a vitamin C content of 87.93mg/100ml. The raw lemon juice had a pH and titratable acidity of 1.81 and 3.645g/100ml respectively.

Treatment of D.alata yam chips

The *D. alata* yam chips (average weight of 10.60g) were soaked in 200ml of the anti-browning agents (ABA) for three time treatments (1, 3 and 5 minutes). Yam chips were strained in a plastic colander to remove residual solution and placed in a solar drier for 48 hours. Treatments were conducted in triplicate. For control experiment distilled water was used.

Development of Colour chart

A colour reference sheet (Plate 1) was developed as a standard for measuring the level of browning of the *D. alata* chips. Yams were prepared as stated earlier. They were grouped into four with each group having three replicates. Yam chips were placed on a white cardboard and solar dried. Pictures of yam chips were taken from 0 min (the time taken out of water) to 3 hours at 30 mins interval. Pictures of the yam samples were taken after 2 days in the solar drier. Eight pictures which showed consecutive differences in browning were selected. The photographs were arranged in ascending order with respect to differences in browning level into a 7 – point ordinal scale where 0 = (no browning) and 7= (heavy browning).

Sensory analysis

The treated samples were presented to 25 trained panellists who scored the degree of browning using the colour reference sheet. The panellists compared the colour of treated yam chips with the colour of *D. alata* chips at different stages of browning on the reference sheet and assigned the corresponding score values from the reference which had the same/nearest browning level as the treated chips.

Tristimulus Measurement

Tristimulus measurement was conducted on fresh *D. alata* samples for the treatment combinations that gave the highest level of browning inhibition (indicated by a lowest mean score) from the sensory evaluation.

The measurement was done with a Minolta Chromameter (Minolta CR-300, Minolta Corp., Ramsey, NJ) at 0min, 20min, 40min, 1 hr, 2hr, 3 hr, 4hrs and 2 days after treatment. The data was recorded using the CIE-L*, a*, b* scale, where L* represents lightness, a* represents chromaticity on a green (–) to red (+) axis and b* represents chromaticity on a blue (–) to yellow (+) axis. Calibration of equipment was done against a standard white tile provided by the manufacturer (L* = 97.51, a* = +0.29. and b* = +1.88). The L*, a*, and b* values recorded are averages of three readings carried out at different point of the sample surfaces. The experiment was carried out at ambient temperature of 22-25°C.
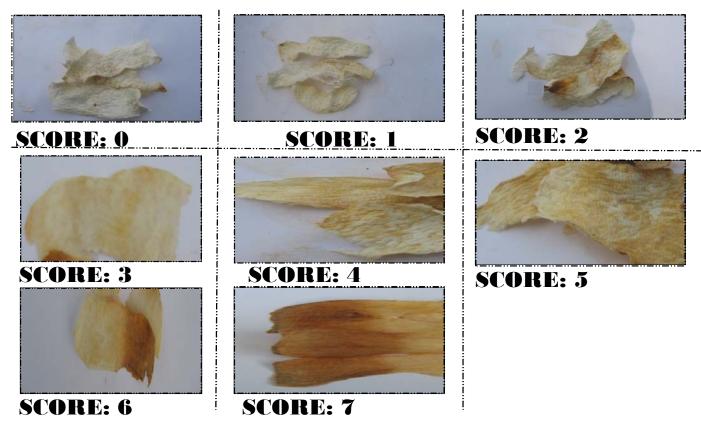

Plate 1. Colour Reference Chart

Data Analysis

The Statgraphics Centurion Statistical Software (version XV) was used to perform a multifactor analysis of variance on the means obtained from the sensory evaluation. Fisher's least significant difference (LSD) was used to discriminate among the means. The total colour change of the *D. alata* chips was determined from the L*, a* and b* data using the following equation (Iyudogan and Bayindirli, 2004):

Total colour change (ΔE) was evaluated as:

$$\Delta E = \sqrt{(L^*_t - L^*_{initial})^2 + (a^*_t - a^*_{initial})^2 + (b^*_t - b^*_{initial})^2}$$

"initial" refers to the colour reading of treated *D. alata* at 0 min after treatment.

The rate of browning was determined using Multiple Linear Regression of ΔE against time. The equation of the fitted model was of the form: $y = mx + c$ where the gradient, m also represents the rate of total colour change. Data analysis was done at a 5% level of significance ($\alpha = 0.05$).

Results and discussion

Potential anti-browning properties of materials

All treatments, except pectin 2% at 3 minutes, significantly different (p>0.05) from the control exhibited significant antibrowning effects (Table 2). This antibrowning effect could be due to the active agents- papain and malic acid in DPL (Ponting, 1960; Richard-Forget *et al.*, 1998); carboxyl groups in pectin as well as the coating ability of pectin (Marshall *et al.*, 2000; Oms-Oliu *et al.*, 2008); vitamin C, amino acids and antioxidants in *M. oleifera* seeds (Makkar and Becker, 1999; Anhwange *et al.*, 2004; Grubben and Denton, 2004; Andrew, 2006; Price, 2007) and ascorbic acid and citric acid in lemon (Tchone *et al.*, 2005).

Table 1. Mean of scores of *D. alata* chips treated with ABAs

ABA		Treatment Time		
		1min	3min	5min
DPL (Papain)	1%	2.87 (0.309)[a+A]	1.39 (0.867)[b+A]	1.71 (0.358)[a,bA]
	2%	1.93 (0.476)[a+A]	3.20 (0.910)[aB]	1.52 (0.457)[a,bA]
Pectin	1%	4.89 (0.387)[Aa]	1.43 (0.293)[b+A]	3.07 (0.105)[b,A]
	2%	3.88 (0.408)[+A]	4.11 (0.425)[+B]	3.48 (0.118)[+A]
Moringa	1%	3.77 (0.885)[a+A]	3.95 (0.508)[a,A]	2.16 (0.694)[b+A]
	2%	4.24 (0.120)[A]	3.63 (0.808)[A]	3.39 (0.122)[A]
Lemon	40%	3.48 (0.560)[+A]	2.59 (0.303)[+A]	2.09 (1.672)[A]
	50%	4.55 (1.086)[a,B]	2.84 (0.538)[b,A]	2.73 (0.340)[bA]
Control	-	5.17 (0.272)[a]	3.32 (0.223)[b]	3.95 (0.965)[b]

For each ABA (in rows), means with the same alphabet are not significantly different (p < 0.05).
All treatments without alphabets are significantly different (p>0.05) with other treatments in the same row.
Treatments tagged '+' are significantly different (p>0.05) with the control in the same column.
Alphabets in lower case denote significant difference (p>0.05) between treatments of the same ABA subjected to different treatment times.
Alphabets in upper case denote significant difference (p>0.05) between different concentrations of the same ABA (at constant time)

Effect of treatment time

There was no regular increase or decrease of antibrowning effect in samples treated with DPL, pectin, *Moringa* 1% and the control as treatment time increased. However, treatments at 5min exhibited significantly better (p>0.05) browning inhibition than those at 1min for all ABA except DPL. This could mean that increase in treatment time enhances the adsorption of the ABA (Huan Ng *et al.*, 2003) therefore increasing their performance. Nevertheless, DPL showed no significant difference in antibrowning effect as time increased probably because, the enzyme papain, which is the primary active agent in DPL (Richard-Forget *et al.*, 1998), is required in small amount for its activity (Chandrasekhar, 2002), thus increased adsorption had no significant effect on its browning inhibition.

This study shows that increase in treatment time increases the anti-browning potency of an ABA. However, depends on the type of ABA (Jiang *et al.,* 2004). Since time is an important resource in the food processing

industry, a case such as that of the DPL combination would require that the combination which gives effective browning inhibition with shorter treatment time will be preferred.

Effect of concentration

Lower concentration in papain (3 min), pectin (3 min) and lemon (1 min) where significantly different ($p>0.05$) from their corresponding higher concentrations of the same ABA (Table 1). Other treatments were not significantly different ($p<0.05$) with their corresponding concentrations.

The reduced antibrowning effect of higher concentration of pectin can be attributed to the light brown colour of pectin solution which increased with increase in concentration. Thus, its ability to act as a coating agent due to its gelling and thickening properties, results in the exhibition of this brown colour on the yam chips. Likewise, reduced antibrowning effect in lemon juice as concentration increased may be due to ascorbic acid browning (Davies, and Wedzicha, 1992) - a non-enzymatic browning of the ascorbic acid which involves intermediates similar to those found in maillard browning. It could be that an increase in the concentration of lemon juice had a corresponding increase in the ascorbic acid which in turn enhanced the ascorbic acid browning. It may also be due to the slightly yellow colour of lemon which may have been more evident on the chips at high concentration. In the case of DPL, the high effectiveness of papain at low concentration (Chandrasekhar, 2002) makes its use at high concentration wasteful. However, considering that DPL is an unrefined form of papain, there is room for more explanation on the reason for its reduced effect at high concentration.

Rate of colour change

The rate of colour change depicts the pace at which each treated yam chip changed colour until the 48 hr stoppage time. It does not necessarily correspond to the ΔE at the stoppage time. It can be used to assess the effectiveness of an ABA. Ideally, a treated sample ought to have a lower rate of colour change that the untreated due to inhibition effect of the ABA. Notwithstanding, DPL and Pectin 1% at 3min had higher rates than the control as depicted by 'm' (Table 2).

Table 2. Rate of colour change from E values

ABA	R^2 (%)	Equation of curve (y = mx + c)		
		m (x 10^{-3})	c	E at 48 hrs
DPL 1%:3min	97.6	4.85	1.70039	12.12
DPL 2%:5min	98.29	4.77	1.44834	13.03
Pectin 1%:3min	99.9	5.44	0.38843	13.03
Pectin 2%:5min	98.32	3.97	1.37698	11.31
Moringa 1%:5min	99.07	4.37	0.35323	12.99
Moringa 2%:5min	99.51	4.23	0.23429	12.78
Lemon 40%:5min	99.06	3.85	0.11004	10.3
Lemon 50%:5min	97.06	3.5	1.44522	8.96
Control:3min	98.78	4.59	0.93314	11.11

Pectin 1% at 3min and lemon 50% at 5min had the highest and lowest rate respectively. The discrepancy between this results and the result from sensory evaluation may due to the fact that visual scoring and instrumental measurement produce data sets that differ in quality (Nollet, 2004).

Conclusion

The materials used have antibrowning potential which can be used to prevent browning in *D. alata*. Treatment time increased the anti-browning potency of ABA, however, this depends on the type of ABA. Lower concentration had better antibrowning results in DPL, pectin and lemon. The result of the rate of colour change revealed that visual scoring and instrumental measurement produce data sets that differ in quality.

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