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Colour Change: An Indicator of the Extent of Maillard Browning Reaction in Food System

F.I. BASSEY[1,*], M.S. CHINNAN[2], ENO E. EBENSO[3], C.A. EDEM[1] and C.M.A. IWEGBUE[4]

[1]Department of Pure and Applied Chemistry, University of Calabar, P.M.B. 1115, Calabar, Nigeria
[2]Department of Food Science and Technology, University of Georgia, 1109 Experiment Street, Griffin 30223-1797, Georgia
[3]Material Science Innovation and Modelling (MaSIM) Research Focus Area, North-West University (Mafikeng Campus), Private Bag X2046, Mmabatho 2735, South Africa
[4]Department of Chemistry, Delta State University, Nigeria

*Corresponding author: E-mail: fibassey@yahoo.ca

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The extent of Maillard browning reactions in cooking banana- and legumes-based weaning food as influenced by its storage environment was investigated, by measuring the intensity of browning of product using Gardner XL 800 tristimulus colorimeter equipped with a yellow standard tile (L* = 83.47, a* = -2.35, b* = 28.37). Sample was stored in glass jars and zip-loc bags at 20, 30 and 40 ºC, respectively for 3 months. The zip-loc bag samples were stored at 75 % relative humidity (RH) to simulate the relative humidity in tropical regions of West Africa. Increased moisture content and water activity, decreased pH, protein and sugar contents of sample stored in the zip-loc bags at 40 ºC for 12 weeks resulted in significantly darker product, with least L* value of 76.79 and highest b*(yellow) value of 24.58 and significantly higher value of a* (2.21) while samples stored in the jars had significantly different L*, a*, b*, hue and ΔE values at the end of the study. Results suggest that colour saturation intensified during storage for samples stored in the water permeable zip-loc bags at high temperatures may as a result of increased reactant mobility. Vapor barrier package (glass jar) was observed to significantly control Maillard reaction thus extending the shelf-life of the novel food.

Key Words: Food, Storage, Color, Maillard reaction.

INTRODUCTION

The application of heat during food processing inevitably gives rise to denaturation of protein and reactions between the constituents of food. One of the most important reactions that occur is the Maillard reaction. These reactions can also take place during storage, especially under adverse conditions of temperature and climate. The overall progress of the reaction can be followed by colour measurements[1]. Infant foods generally have high levels of proteins, which can react with reducing sugars leading to the formation of Maillard reaction products. Since infant food can be the sole source of lysine for babies, the Maillard reaction is undesirable in these products due to the destruction of amino acid on reaction with sugars. For example L-lysine, an essential amino acid is destroyed when it reacts with sugars.

The presence of an aldehyde group in sugars gives them great chemical reactivity. Browning in foods during heat treatment or storage is usually due to the reaction between D-glucose and a free amino acid or a free amino group of an amino acid that is part of the protein chain. This reaction is named Maillard or non enzymatic browning to differentiate it from the rapid enzyme catalyzed browning commonly observed in freshly cut fruits and vegetables and the end products are referred to as Maillard reaction products. The early stages of the Maillard reaction can be evaluated by the determination of furosine [ε-N-(furomethyl)-L-lysine. This is an amino acid formed during acid hydrolysis of the Amodori compounds such as fructosyl-lysine, lactosyl-lysine and maltolosyl-lysine, produced by the reaction of amino groups of lysine with glucose, lactose and maltose. Furosine is also a useful indicator of damage in milk. Prolonged heating or inadequate storage conditions are the main factors responsible for increases in the level of furosine[2]. Millard reactions can also be monitored by colour measurements[1,3,4].

Depending on their molecular weight, the coloured components resulting from this non enzymatic browning may be divided into two classes, namely, the low molecular weight products and the melanoidins, which are assumed to be nitrogen containing, high molecular weight coloured compounds with molecular weight up to 100000 Da. Reducing sugars react reversibly with amines to produce glycosalamine which undergoes an Amadori rearrangement, to give in the case of D-glucose, a derivative of 1-amino-1-deoxy-D-fructose.

Reaction continues, especially at pH 5 or lower, to give a 3-deoxyglucose that dehydrates leading to the formation of derivatives of furan. Under less acidic conditions, pH 5 and above, the reactive cyclic compounds such as hydroxy methyl furfural polymerize quickly to a dark coloured insoluble material containing nitrogen. Mutagenic heterocyclic amines formed by Maillard reactions have been isolated from some model systems. Maillard reaction products with 3(2*H*)-furanone structures have been reported to possess DNA-damaging properties.

A product should therefore no longer be sold to the consumer when the initial quality is lost. Quality losses include an unacceptable loss in nutrient value, an undesirable change in flavor or colour, or the development of an undesirable texture[5]. An extreme shift in the colour of a food, even though unaccompanied by change in flavor can make it completely unacceptable to consumers[3].

The objective of this study was to investigate the extent of Maillard reaction in a cooking banana- and legumes-based weaning food as influenced by its storage environment by measuring the intensity of browning using a Gardner XL 800 tristimulus colorimeter.

EXPERIMENTAL

A ready-to-eat weaning food was designed with the aid of "ESHA (Elizabeth Stuart Hands and Associates) Food Processor and Nutrient Analysis" computer software, prepared according to the method of Bassey[6]. Several trial formulations were proposed and evaluated by the ESHA software. A composite of 47 % cowpea, 40 % ripe banana and 13 % peanut was found to give the best nutrient and amino acid profile to meet the dietary requirements of the 0.5-0.9 year old infant.

The weaning food was prepared in batches within a period of 6 days in order to meet the Food and Drug Administration (FDA) requirements for food analysis. The appropriate amounts of precooked and dried cowpea, dried ripe banana and roasted peanut were weighed, mixed and co-milled with the aid of a plate mill. The banana was first reduced to a manageable size with the clearance between the plates adjusted to cut the banana chips into small chunks to allow for proper mixing before all three products were co-milled. The resultant product was fed back into the mill and the clearance between the plates further adjusted to allow more friction between the plates. The co-milled product was passed through the mill four times and each time the clearance between the plates was further reduced until the desired particle size was attained. All the lots processed for the 6 days received the same treatment. The different lots were combined and mixed with the aid of a Ribbon mixer (Model HD11/2-3SS, Munson Machinery Co. Inc., Utica, New York) to obtain homogeneous flour. A total of 8,890 g of sample was divided into two equal weights. Half of the sample was further divided into nine equal portions and placed in medium density poly ethylene bags and the other half also divided into nine equal portions and placed in air tight glass jars to simulate two different packaging systems. In order to investigate the effect of temperature and relative humidity, three different storage temperatures were chosen with temperatures pre set to 20, 30 and 40 ºC, respectively and a constant relative humidity of 75 % created with the aid of a saturated solution of sodium chloride. This was done to simulate the relative humidity in tropical regions of West Africa. Only samples in the Zip-loc bags were placed on a perforated rack over the saturated salt solution in a sealed plastic container. Each of the sets was brought out every four weeks for the analysis listed below; analysis was carried out for a total period of twelve weeks. All the samples were labeled for easy identification.

Colour: The colour as determined by the method of Murphy *et al.*[7]. The Gardner XL 800 tristimulus colorimeter (Pacific Scientific, Bethesda, Maryland, USA) equipped with a yellow standard tile (L* = 83.47, a* = -2.35, b* = 28.37) was used. Surface colour differences were minimized by reporting an average of four readings per sample. The L* a* b* values were recorded. Three replicates of each sample were analyzed. Derived attributes of chroma (C), hue angle (H) and total colour difference (ΔE) were determined using the following equations Murphy *et al.*[7]:

$$\text{Chroma} = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$\text{Hue angle} = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (2)$$

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3)$$

L* = lightness (0 = black, 100 = white), + b* (yellow), -b* (blue), + a*(red), -a*(green).

Water activity: The water activity as determined by the method of Murphy *et al.*[7] using a water activity meter (Aqua lab, model 3TE, Decagon Devices Inc., Pullman, Washington, USA). Each sample (2 g) was weighed into the measurement dish; the dish was placed in the drawer of the unit and the meter turned on. The measurements were conducted at room temperature and in triplicate.

pH: It was measured using the method of Murphy *et al.*[7] with the aid of an AR 15 pH meter (Accumet Research, Fisher Scientific, Pittsburgh, Pennysylvania, USA). Each sample (2 g) was mixed with 20 mL distilled water and shaken mechanically for 5 min. The pH of each sample was then determined in triplicate.

Moisture: The moisture was analyzed according to the method of the Association of Official Analytical Chemists (A.O.A.C., 1995)[8]. An Isotemp vacuum oven (model 281A, Fisher Scientific, Pittsburgh, Pennsylvania, USA) was used to determine moisture content. 3 g of sample was weighed into a pre-dried aluminium weighing dish and covered with a lid. This was then placed in the oven for 14 h with the temperature and pressure kept at 105 ºC and 25 mm Hg, respectively. The sample was transferred to a dessicator with the aid of a Kimwipe to cool and then weighed. The residue was recorded as total solids and stored in a desiccator for fat analysis while percentage moisture was calculated from loss in weight by the following formula:

$$\text{Moisture}(\%) = \frac{\text{Loss in weight}}{\text{Weight of the sample}} \times 100 \quad (4)$$

Each sample was analyzed in triplicate.

Data: All the data were analyzed statistically with the aid of the Statistical Analysis Software (SAS, 1990)[9]. Fisher's least significant difference test (LSD) was performed to determine which sample's means were significantly different, Difference were considered significant at $p < 0.05$ level.

RESULTS AND DISCUSSION

The extent of Maillard browning reactions in a cooking banana- and legumes-based weaning food as influenced by its storage environment was investigated, by measuring the intensity of browning of product using Gardner XL 800 tristimulus colorimeter equipped with a yellow standard tile (L* = 83.47, a* = -2.35, b* = 28.37). The colour data is shown in Table-1. After 12 weeks of storage, L*, a*, b*, calculated chroma, hue angle and delta E of the weaning food stored in the water permeable polyethylene bags at higher temperatures were all significantly different ($p \geq 0.05$) from those stored in the glass jars. Samples stored in the jars had significantly different l*, a*, b*, chroma and ΔE values from samples stored in the zip-loc bags especially those stored for longer period (12 weeks). Sample stored in Ziploc bags at 40 ºC for 12 weeks was significantly darker with the least L* value of 76.79 and the highest a*, b*, Chroma and delta E with values 2,21, 24.58, 24.64 and 5.69, respectively, than other samples stored in the zip-loc bags at 20, 30 and 40 ºC for 4 and 8 weeks. The moisture content and water activity (a_w) levels were higher at the end of the study for samples stored in the Zip loc bags with values of 7.8 and 0.49, respectively when compared with values for samples stored in glass jars. While pH was recorded as having the least value of 5.62 under this condition. Protein content on the other hand was observed to reduce from 16.89 (initial value) to 15.01 % at the end of the study for samples stored at 40 ºC in Zip loc bags. Sugar content was observed to decrease with storage time for all storage conditions though higher decreases were observed for samples stored in bags at 40 ºC (Table-2).

A less intense browning was observed in both weaning foods stored in jars and bags in the first 4 weeks of the study as indicated by the low chroma and ΔE values (Table-1). Sample stored in the zip-loc bags at 40 ºC for 12 weeks, had least L* value of 76.79 and highest b* (yellow) value of 24.58 and significantly higher value of a* (2.21).

Browning reactions can take place at temperature as low as 36 ºC. The water permeable polyethylene bags used in storing the weaning food as well as the accelerated storage conditions favored moisture migration from the environment as a result of the high relative humidity of the storage environment. This led to increased moisture content and water activity from 4.42-7.8 and 0.22-0.49, respectively at the end of the study. Increased moisture and water activity is known to lead to increased reactant mobility. Water activity above 0.3 is known to cause non-enzymatic (Maillard) browning if the product is susceptible to such reactions[5]. The rate of non-enzymatic reaction increases with increasing water activity, reaching a maximum at water activity (a_w) ranging from 0.6

TABLE-1
pH, MOISTURE, WATER ACTIVITY, PROTEIN AND COLOR MEASUREMENTAS AFFECTED BY STORAGE CONDITIONS AND TIME

Time	Bagging	Temp. (ºC)	pH	Colorl	Colora	Colorb	Hue	Chroma	ΔE	Moisture	aw	Protein
0 week	Jar	20	5.72	77.04	1.48	23.08	86.32	23.18	3.69	4.42	0.22	16.89
4th week	Jar	20	5.72	77.90	0.94	22.16	87.56	22.18	2.64	4.32	0.22	16.89
4th week	Jar	30	5.71	78.13	0.74	22.35	88.10	22.37	2.35	4.93	0.22	16.87
4th week	Jar	40	5.72	78.09	1.11	21.29	87.09	21.96	2.39	4.34	0.19	16.83
4th week	ziploc bag	20	5.74	78.45	1.21	21.27	86.74	21.30	2.67	5.26	0.26	16.72
4th week	ziploc bag	30	5.71	78.61	0.91	21.55	87.59	21.57	2.33	4.31	0.18	16.69
4th week	ziploc bag	40	5.70	78.38	1.35	21.82	86.45	21.86	1.81	6.05	0.33	16.67
8th week	Jar	20	5.86	78.93	1.54	22.20	86.01	22.26	2.01	4.18	0.18	16.83
8th week	Jar	30	5.91	78.37	1.20	22.39	86.92	22.43	3.14	4.95	0.26	16.78
8th week	Jar	40	5.88	78.32	1.19	22.27	86.95	22.31	1.62	4.21	0.18	16.72
8th week	ziploc bag	20	5.89	78.50	1.36	22.45	86.36	21.50	2.09	5.75	0.35	16.66
8th week	ziploc bag	30	5.87	78.04	1.48	22.46	86.23	22.51	1.59	4.24	0.19	16.62
8th week	ziploc bag	40	5.64	77.48	1.77	22.73	85.56	22.80	2.35	7.01	0.47	16.46
12th week	Jar	20	5.70	77.70	0.38	23.03	89.06	23.03	3.40	3.67	0.19	15.68
12th week	Jar	30	5.67	78.57	0.74	22.08	88.07	22.09	1.99	5.08	0.28	15.51
12th week	Jar	40	5.64	78.46	0.35	22.74	89.11	22.75	2.35	3.97	0.17	15.16
12th week	Ziplocbag	20	5.74	78.20	0.88	22.28	87.74	22.30	2.16	6.11	0.38	15.22
12th week	Ziplocbag	30	5.71	78.16	0.77	23.67	88.14	22.68	2.27	4.01	0.21	15.06
12th week	Ziplocbag	40	5.62	76.79	2.21	24.58	84.85	24.68	5.69	7.80	0.49	15.01

Note: L*= Lightness of the weaning food samplesfrom 0=black to 100=white,a*measures colours in the region of green to red, b*measures colours in the region of blue to yellow. Chroma = $\sqrt{a^{*2} + b^{*2}}$, hue angle = $\tan^{-1}$ (b*/a*), $\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)2}$.

TABLE 2
SUGAR PROFILE OF WEANING FOOD AT THE BEGINNING (ZERO TIME) AND END OF THE STUDY

Storage time	Packing mode/temp (ºC)	Fructose (%)	Glucose (%)	Sucrose (%)	Maltose (%)	Lactose (%)
Zero week	–	8.07	7.66	0.41	0.60	0.10
8 weeks	20 Bag	7.08	7.08	0.10	0.47	0.10
8 weeks	30 Bag	6.92	6.94	0.61	0.10	0.10
8 weeks	40 Bag	6.16	5.83	1.31	0.10	0.10
12 weeks	20 Jar	6.94	6.94	0.42	0.10	0.10
12 weeks	30 Jar	6.93	7.03	0.57	0.10	0.10
12 weeks	40 Jar	6.67	6.64	0.76	0.10	0.10

to 0.7[5]. The high relative humidity of the storage environment was therefore assumed to have influenced the rate of browning reactions of samples stored in the zip-loc bags. Vercet[10] also recorded an increased browning reaction in white chocolate as influenced by high relative humidity of the storage environment.

Hue angles of 0, 90 and 180° represent red, yellow and green hues respectively. Foods with hue angles between 0 and 90 tend toward orange-red colours whereas foods with angles between 90 and 180 are more greenish yellow[11]. The decreasing value in hue angles for these samples therefore indicates or suggests a shift from yellow to red colour. This observation indicates that colour saturation intensified during storage for samples in the zip-loc bags stored at high temperatures, giving rise to a reddish yellow colour. These observed changes in the colour of the weaning food, particularly for samples stored in the zip-loc bags at high temperatures is attributed to non enzymatic (Maillard browning) reactions since the weaning food contains all reactants required for Maillard browning; an amino bearing compound, (protein) a reducing sugar (D-glucose) and water[12].

A relationship between colour development resulting from Maillard reaction products, pH, temperature and packaging material has also been demonstrated by Felland and Koehler[13], Muego-Gnanasekharan and Ressurreccion[12] and Guerra-Hernandez *et al*.[1].

The higher browning and low protein values can be related to the lysine degradation which occurs in Maillard reaction. Guerra-Hernandez *et al*.[1] recorded loss of lysine in infant formulae during storage at high temperature. The decrease in sugar content with storage time and temperature can also be related to Maillard reaction since this is a reaction between sugars and amino acids.

The intensity of browning measured as ΔE showed high values for samples stored in the zip-loc bags for long period. This can be related to high moisture content and water activity indicating that these conditions favour Maillard browning reactions. Monsalve, *et al.*[14] and Felland and Koehler[13] recorded an increased oxidation and darker product (peanut butter) with increased water content. The low L*, high, a*, ΔE and chroma values are useful indicators of the extent of Maillard browning reaction in the weaning food under adverse storage conditions. Guerra-Hernandez *et al*.[1] observed similar increased intensity of browning (resulting from the formation of Maillard reaction products) in infant formula under adverse storage conditions. Patel, *et al.*[4] in their work on condensed milk also recorded a steady increase in brown pigment formation during storage with low order increases at temperatures up to 30 ºC but at a much higher order at temperature between 30 and 50 ºC.

The intensity of browning has also been related to the significant drop in pH of the system with storage time. The formation of Maillard browning intermediates/products was found to be favored under acidic conditions[4].

Conclusion

Samples stored in the jars had significantly different L*, a*, b*, hue and ΔE values at the end of the study compared to samples stored in zip-loc bags at a relative humidity of 75 %. Results suggest that colour saturation intensified during storage for samples stored in the water permeable zip-loc bags at high temperatures as a result of increased reactant mobility, hence vapor barrier package (glass jar) is observed to significantly control Maillard reaction thus extending the shelf-life of the novel food. This study is aimed at assessing the influence of storage environment in tropical regions like Nigeria and its effects on the shelf-life of locally processed infant formula for use as an intervention vehicle to combat malnutrition and infant mortality in developing countries, in general and in Nigeria in particular. This is necessary because of the inability of a majority of the populace (who are low income earners) to afford and use proprietary weaning foods for their babies.

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