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Browning Phenomenon in Stored Raw Cowpea (*Vigna unguiculata*) Flour

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ABSTRACT

Direct evidence (decreases in 'L'-values) is established for a browning phenomenon in raw cowpea flour. Changes in the contents of reducing sugar, and lipids with free amino groups with changes in water activity (A_w) and temperature, during a 6-month storage, suggest the participation of these entities in the browning observed. The observed trend in pH changes appeared to be related, at least in part, to browning reactions.

INTRODUCTION

Browning in stored foods can be due to Maillard reactions, enzymic browning reactions, ascorbic acid oxidation and caramelization (Richardson, 1976).

Interest in these reactions in foods has centered on their beneficial and detrimental effects on food quality. They improve food flavours and aromas (Hodge *et al.*, 1972). Deleterious effects on food flavours and aromas have been reported by Ferretti and Flanagan (1972). Loss of amino acids (Finot *et al.*, 1968) and decreased amino acid availability which lead to loss of nutritive value (Bender, 1977) are some of the negative effects of browning in foods, apart from the loss of colour naturally associated with a given food which, in itself, can affect consumer acceptability.

The prime position of legumes as a source of protein in developing countries and the growing importance of convenience, or 'semi-convenience', foods in these countries call for a knowledge of their storage chemistry. This should go some way to providing a basis for the

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maintenance of their wholesomeness post harvest. The present studies on browning and associated factors have been carried out with the above in view.

EXPERIMENTAL

Cowpea (*Vigna unguiculata*) seeds obtained from the National Seed Co., New Orleans, USA, were processed into flour by a milling operation using a Hobart Company mill, model number 3430. The flour was such as to be able to pass through a No. 1 Standard Sieve with a Tyler Equivalent of 16 mesh.

Four hundred grams each of the raw flour were weighed into 500-ml glass beakers kept in three desiccators. Water activities of 0·11, 0·33 and 0·75, respectively, were established in the flour according to the method described by Rockland (1960). The desiccators containing the samples were then stored at a uniform temperature of 25°C in a temperature-controlled storage room.

Similarly, three desiccators, containing 400 g flour each, weighed into 500-ml glass beakers, were stored at 5, 25 and 40°C, respectively, at a uniform A_w of 0·75 in temperature-controlled storage rooms.

The duration of storage was 6 months and the contents of reducing sugar and lipids with free amino groups, pH and the colour of the flour were monitored on a monthly basis.

A Hunter Colorimeter (Hunter Associates Laboratory, Inc., VA) was used in the colour assessment. Forty grams of the flour were added to the colorimeter cup such that there were no transparent areas at its bottom. After standardizing the equipment with a white plate standard supplied with it, '*L*' values, which were used to follow loss of whiteness of the samples, were obtained by direct read-out from a digitalized panel attached to the equipment.

The reducing sugar content of the flour was determined by the official method of the Association of Official Analytical Chemists (AOAC, 1970).

The method of Siakotos (1967) was employed in the determination of the flour content of lipids with free amino groups.

For the pH determinations, 5·0 g of flour were weighed accurately into a 100-ml glass beaker and 50 ml of deionized distilled water added. The flour was then stirred into the water with a glass rod and, thereafter, left to stand for 30 min. This allowed the aqueous phase to separate from the solid phase, while acid was extracted. pH readings were obtained on a digital pH meter (Leeds and Northrup Model No. 7421) by allowing the pH electrode to dip down into the sample slurry which was stirred with a glass rod just before electrode immersion.

The ascorbic acid content of the flour was also determined prior to storage, using the method described by the Association of Vitamin Chemists (1966). Since repeated pre-storage measurements did not indicate the presence of the vitamin in the flour, no further measurements were done in the course of storage. A final check for ascorbic acid at the end of storage also gave negative results.

RESULTS AND DISCUSSION

The small but persistent and consistent changes in the '*L*' values of the cowpea flour with changes in water activity, temperature and length of storage period (Table 1), are indicative of increasing browning with increasing water activity, temperature and length of storage period. The

TABLE 1
Effects of Water Activity and Temperature on the '*L*' Values of Raw Cowpea Flour During 6 months' Storage

Month	A_w[a]			*Temperature (°C)*[b]		
	0·11	*0·33*	*0·75*	*5*	*25*	*40*
0	81·94	81·94	81·94	81·94	81·94	81·94
1	81·45	81·45	81·20	81·70	81·60	44·60
2	81·25	81·20	80·60	81·50	81·00	38·70
3	81·30	81·00	80·20	81·25	80·15	31·10
4	81·00	80·90	80·20	81·00	80·10	27·70
5	81·00	80·55	80·05	80·60	80·05	23·40
6	80·65	80·15	79·60	80·20	79·55	15·00

[a] At 25°C.
[b] At $A_w = 0{\cdot}75$.

results agree with previous findings (Labuza *et al.*, 1970). Even a visual observation at the end of the 6-month storage indicated that browning had occurred in the cowpea flour. The browning may have been of the Maillard type (Hurrell, 1980). No role can be assigned to ascorbic acid browning since the raw cowpea flour did not contain any measurable amount of ascorbic acid.

Reactive glucose and fructose molecules released by amylases, and carbonyl compounds from cowpea lipid oxidation (Ukhun, 1984), probably participated in the browning observed. Enzymic browning may also have been initiated by polyphenolase enzymes.

TABLE 2
Effects of Water Activity and Temperature on the Reducing Sugar Content (%) of Raw Cowpea Flour During 6 months' Storage

Month	A_w[a]			*Temperature* (°C)[b]		
	0·11	*0·33*	*0·75*	*5*	*25*	*40*
0	0·40	0·40	0·40	0·40	0·40	0·40
1	0·39	0·37	0·36	0·39	0·36	0·29
2	0·36	0·35	0·34	0·39	0·33	0·20
3	0·33	0·31	0·28	0·37	0·30	0·13
4	0·31	0·27	0·23	0·35	0·27	0·09
5	0·28	0·24	0·19	0·36	0·22	0·07
6	0·26	0·24	0·18	0·36	0·20	0·04

[a] At 25°C.
[b] At $A_w = 0{\cdot}75$.

The decreasing amounts of reducing sugar of the cowpea flour with increasing water activity, temperature and storage time, as shown in Table 2, are consistent with increasing browning reactions. Increasing starch synthesis, mediated by synthetase enzymes in the cowpea flour, may also partly account for the observed trend in reducing sugar content (Amir *et al.*, 1971).

Lipids such as phosphatidyl ethanolamine and phosphatidyl serine which have free and reactive amino groups can, obviously, participate in Maillard browning reactions (Lea, 1956). The trends in Tables 3 and 4, which show

TABLE 3
Effects of Water Activity and Temperature on the Content of Lipids with Free Amino Groups (μg per gram of sample) of Raw Cowpea Flour During 6 months' Storage

Month	A_w[a]			*Temperature* (°C)[b]		
	0·11	*0·33*	*0·75*	*5*	*25*	*40*
0	127	127	127	127	127	127
1	126	126	126	127	126	125
2	126	127	126	127	127	124
3	124	123	122	126	124	122
4	122	120	118	125	122	116
5	123	120	116	125	118	111
6	122	120	116	123	117	111

[a] At 25°C.
[b] At $A_w = 0{\cdot}75$.

TABLE 4
Effects of Water Activity and Temperature on the pH of Raw Cowpea Flour During 6 months' Storage

Month	A_w[a]			*Temperature (°C)*[b]		
	0·11	*0·33*	*0·75*	*5*	*25*	*40*
0	6·56	6·56	6·56	6·56	6·56	6·56
1	6·62	6·53	6·51	6·50	6·48	5·59
2	6·40	6·33	6·27	6·36	6·33	5·04
3	6·35	6·35	6·26	6·31	6·28	4·98
4	6·30	6·31	6·04	6·31	6·00	5·20
5	5·89	5·88	5·60	6·27	5·90	4·81
6	5·81	5·59	5·01	5·94	5·26	4·65

[a] At 25°C.
[b] At $A_w = 0·75$.

decreasing contents of lipids with free amino groups, with increasing water activity, temperature and length of storage, would seem to indicate such involvement. This speculation is supported further by the high correlation coefficients (r) between the 'L' values of the cowpea flours and their contents of lipids with free amino groups, as shown in Table 5.

The trend in the changes of pH with changes in water activity, temperature and storage time suggests that at least one factor in these changes is the Maillard browning phenomenon. Predictably, a condensation reaction—the initial step of Maillard browning—between the free and reactive amino groups of phospholipids and the carbonyl groups of compounds such as glucose, fructose, malonaldehyde, etc., should translate

TABLE 5
Correlation Coefficient Between the 'L' Values and the Content of Lipids with Free Amino Groups in Raw Cowpea Flour Stored at Different Water Activities and Temperatures for 6 Months

A_w[a]			*Temperature (°C)*[b]		
0·11	*0·33*	*0·75*	*5*	*25*	*40*
0·84[c]	0·87[d]	0·84[d]	0·09	0·87[c]	0·79[c]

[a] At 25°C.
[b] At $A_w = 0·75$.
[c] t-value significant (α level 0·05, 5 df).
[d] t-value significant (α level 0·01, 5 df).

TABLE 6
Correlation Coefficients Between the pH and the Content of Lipids with Free Amino Groups of Raw Cowpea Flour Stored at Different Water Activities for 6 Months

A_w[a]			Temperature (°C)[b]		
0·11	*0·33*	*0·75*	*5*	*25*	*40*
0·84[c]	0·77[c]	0·87[c]	0·92[d]	0·93[d]	0·72

[a] At 25°C.
[b] At $A_w = 0{\cdot}75$.
[c] t-value significant (α level 0·05, 5 df).
[d] t-value significant (α level 0·01, 5 df).

to some decrease in pH of the flours. Their contents of lipids with free amino groups, as presented in Table 6, also lend credence to this speculation.

CONCLUSION

Browning of cowpea flour might be expected to lead to losses of reducing sugar, lipids with free amino groups and to decrease in pH, during storage.

Appropriate storage conditions of low water activity and temperature should help to ameliorate browning in the flour although this should be weighed against the problem of lipid oxidation which a low water activity can be predicted to accelerate.

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