academicJournals

Vol. 8(5), pp. 89-93 May, 2014
DOI:10.5897/AJPAC2014.0571
Article Number: 802AA7844885
ISSN 1996 - 0840

http://www.academicjournals.org/AJPAC

African Journal of Pure and Applied Chemistry

Full Length Research Paper

Colour of starch-iodine complex as index of retrogradability of starch pastes

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Received 23 April, 2014; Accepted 12 May, 2014

Starch retrogradation is mainly due to the amylose fraction of starch. Amylose quantification is usually done by iodine staining with which it forms a blue colour complex while starch retrogradability can be monitored by freeze-thaw stability measurements. In this work, spectroscopic determination of the blue value and visual monitoring of starch–iodine colour complex were used to study starch retrogradation. The results obtained were compared with that from freeze-thaw stability measurements. Native cassava starch, its carboxymethylated and cyanoethylated derivatives of different degrees of substitution (D.S) were used in the study. From the results, increase in starch derivatization reduced amylose ability to bind iodine, decreased the blue value with resultant decay in the blue colour of the starch-iodine complex. The blue black colour of the starch-iodine complex was lost in carboxymethyl starch at D.S of 0.145 and cyanoethyl starch at D.S of 0.141; at these degrees of substitution, the helical structure of amylose was no longer maintained by the starch molecules and retrogradation eliminated. Freeze-thaw stability study showed clear pastes with no evidence of retrogradation over 10 freeze-thaw cycles for derivatized starches above these degrees of substitutions. This showed a good agreement with that obtained from the colour of the starch-iodine complex. Hence derivatized starch products, which showed absence of blue black colour of starch-iodine complex would be freeze-thaw stable over a long period of cold storage. The disappearance of the blue colour of starch-iodine complex thus becomes an index of retrogradability and freeze-thaw stability of starch pastes on cold storage.

Key words: Starch pastes, retrogradability, blue value, starch-iodine colour.

INTRODUCTION

When pasted starch products are subjected to cold storage the starch macromolecules in the starch dispersions tend to re-associate and exude water, a process referred to as syneresis (Thomas and Atwell, 1999; Balagopolan et al., 1998). This re-association and subsequent crystallization from the dispersions (retrogradation) is a major problem limiting starch utilization. Retrogradation problem can minimized by derivatization of the starch hydroxyls through introducing groups which retard the side-by-side association of starch molecules thereby stabilizing the aqueous dispersion of the paste (Thomas and Atwell, 1999; Balagopolan et al., 1998; Yeh and Yeh, 1993). When starch is derivatized, there is a change in the molecular organization within the granules and in starch physicochemical properties (Ogunmola and Nwokocha, 2002); and since starch

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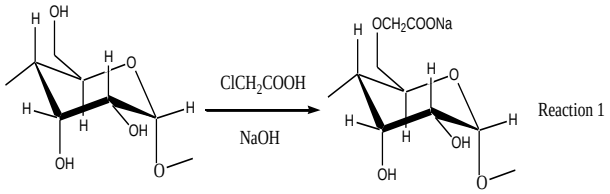
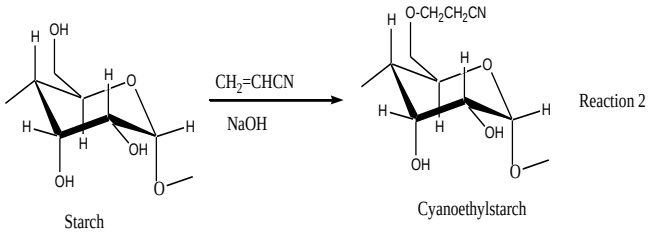
retrogradation is a phenomenon primarily associated with amylose molecules (Collison, 1968) (amylopectin retrogrades very slowly), the effective changes must have been those associated with amylose units. Starch reacts with iodine to form a blue colour (Thomas and Atwell, 1999). Studies on starch fractions have shown that the blue colour complex observed with iodine is due to the colour formation of amylose units (Radley, 1968). Amylopectin reacts with iodine to form a violet-red colour. The ability to form blue colour complex with iodine is gradually destroyed with increasing derivatization until the blue colour disappears (Radley, 1968). Similarly with increasing degree of derivatization, retrogradation is found to decrease until a certain degree of derivatization when retrogradation is completely removed. Since the blue colour of starch-iodine complex and retrogradation in starch pastes are primarily associated with amylose, it is possible to use the disappearance of the blue colour to monitor the retrogradability of aqueous starch pastes and thus provide an assay method of retrogradability in starch products evaluation.

MATERIALS AND METHODS

Preparation of cassava starch derivatives

Cassava starch used in this study was isolated in Chemistry Department, University of Ibadan, Nigeria. All reagents used including iodine crystals, potassium iodide, acetic acid, ethanol and sodium hydroxide are products of Sigma-Aldrich Chemical Co., St Louis, MO, USA) and of reagent grade. Carboxymethylation (Reaction 1) was achieved by treating aqueous starch dispersions with different concentrations of monosodium chloroacetate in presence of sodium hydroxide as the alkalizing agent. In similar manner, cyanoethylation (Reaction 2) was effected by treating aqueous starch dispersions with acrylonitrile using sodium hydroxide as the alkalizing agent. The detailed procedures are reported elsewhere (Ogunmola and Nwokocha, 2002; Nwokocha and Ogunmola, 2008). Derivatization can occur at any of the three hydroxyl groups of the glucopyranosyl unit.





Measurement of freeze-thaw stability of native, carboxymethyl and cyanoethyl starches

The freeze-thaw stability of the starch samples was determined on 5% starch pastes. 0.5 g dry starch samples were weighed into centrifuge tubes and 10 ml distilled water added and sealed. The samples were shaken to disperse the starch and then pasted in a Clifton water bath at 95°C for 30 min with occasional shaking throughout the heating regime. The samples were removed, cooled and subjected to alternate freezing (freezer temperature) and thawing (25°C) for 18 and 3 h, respectively and centrifuged at 5000 rpm for 15 min and the amount of water separated after each freeze-thaw cycle measured. The freeze-thaw stability was determined as percentage of water exuded per weight of paste.

Determination of the blue value of native, carboxymethyl and cyanoethyl starch-iodine complexes

0.1 g dry starch sample was weighed into a boiling tube, 1 ml ethanol (95%) was added followed by 9 ml of 1 M NaOH solution and heated in a boiling water bath for 10 min to solubilize the starch. The starch solution was cooled and quantitatively transferred into a 100 ml standard volumetric flask and the volume made up to 100 ml mark with distilled water. 2.5 ml of starch solution was taken into 50 ml standard flask; 0.5 ml of 1 M acetic acid was added followed by 1 ml of stock iodine (0.2 g I_2 / 2.0 g KI/ 100 ml) and the solution made up to the 50 ml mark with distilled water. The resulting colour was left for 20 min to fully develop before the absorbance reading was monitored at 620 nm with a Perkin-Elmer Lambda 3B double beam UV/ visible spectrophotometer. Iodine solution of same concentration as above but without starch sample was used in the reference cell. The colour of the starch- iodine complex was monitored visually. The blue value was calculated according to the method of Gilbert and Spragg (1964) using the formula:

$$Blue\ value = \frac{Absorbance\ at\ 620\,nm\ X\ 4}{Concentration\ (mg/dl)}$$

RESULTS AND DISCUSSION

Freeze-thaw stability of carboxymethyl and cyanoethyl starches

Tables 1 and 2 show the freeze-thaw stability of carboxymethyl and cyanoethyl starches respectively. Both modified starches exhibited unusual stability even at low degrees of substitution in comparison with native starch. In carboxymethyl starch, stability to syneresis was observed at D.S of 0.064 and above. At lower levels of substitution (D.S< 0.064), syneresis was observed from the 5th freeze-thaw cycle in D.S of 0.028 and 0.061 with maximum exudates achieved decreasing with increasing D.S. In cyanoethyl starch, maximum exudates of 11.50% were obtained after 10 freeze-thaw cycles in D.S of 0.038. However, D.S of 0.100 and higher levels were stable to freeze-thaw cycles. Native cassava starch (D.S. 0.0) could not survive the first freeze-thaw cycle and attained maximum exudates of 31.30% after seven freeze-thaw cycles. Thus derivatization introduced branching groups onto the amylose chains which

Table 1. Freeze–thaw stability of native and carboxymethyl starches at 5% (w/v) concentration.

No. freeze-thaw cycles (days)	0	1	2	3	4	5	6	7	8	9	10
D.S	Percentage water separated										
0.0	0.7	22.10	5.60	18.60	26.70	30.80	31.30	31.30	31.30	31.30	31.30
0.028	-	-	-	-	-	21.79	24.75	24.75	29.74	30.00	30.00
0.061	-	-	-	-	-	0.028	6.32	20.10	23.07	23.07	23.07
0.064	-	-	-	-	-	-	-	-	-	-	-
0.076	-	-	-	-	-	-	-	-	-	-	-
0.111	-	-	-	-	-	-	-	-	-	-	-
0.131	-	-	-	-	-	-	-	-	-	-	-
0.134	-	-	-	-	-	-	-	-	-	-	-
0.187	-	-	-	-	-	-	-	-	-	-	-
0.194	-	-	-	-	-	-	-	-	-	-	-
0.197	-	-	-	-	-	-	-	-	-	-	-
0.280	-	-	-	-	-	-	-	-	-	-	-

- clear paste with no water separation, D.S of native starch = 0.0.

Table 2. Freeze–thaw stability of native and cyanoethyl starches at 5% (w/v) concentration.

No. freeze-thaw cycles (days)	0	1	2	3	4	5	6	7	8	9	10
D.S	Percentage water separated										
0.0	0.7	5.60	18.60	22.10	26.70	30.80	31.30	31.30	31.30	31.30	31.30
0.038	-	-	6.90	8.14	8.56	10.15	10.51	10.51	11.03	11.08	11.50
0.100	-	-	-	-	-	-	-	-	-	-	-
0.141	-	-	-	-	-	-	-	-	-	-	-
0.168	-	-	-	-	-	-	-	-	-	-	-
0.199	-	-	-	-	-	-	-	-	-	-	-
0.255	-	-	-	-	-	-	-	-	-	-	-
0.303	-	-	-	-	-	-	-	-	-	-	-
0.368	-	-	-	-	-	-	-	-	-	-	-
0.603	-	-	-	-	-	-	-	-	-	-	-
0.644	-	-	-	-	-	-	-	-	-	-	-
0.699	-	-	-	-	-	-	-	-	-	-	-
0.963	-	-	-	-	-	-	-	-	-	-	-

- clear paste with no water separation, D.S of native starch = 0.0.

hindered reassociation of amylose and reduced their ability to retrograde.

Relationship between colour of starch-iodine complex and degree of substitution

From Tables 3 and 4, a general decrease in blue value was observed with increase in D.S. This implied a gradual derivatization of the amylose as the D.S increased. Also the helical structure of amylose in solution responsible for complex formation with iodine was gradually destroyed with increase in D.S. It was observed that the decrease in blue value did not vary uniformly with increase in D.S. This implied both amylose and amylopectin were randomly derivatized. The blue colour of the starch-iodine complex was gradually destroyed as D.S increased until a green colour was observed. In carboxymethyl starch, the transition to green colour occurred at a D.S of 0.145. Higher D.S gave various shades of green colour. At D.S of 0.145, the helical structure of amylose was destroyed to the extent that retrogradation was completely removed. Hence, D.S of 0.145 and above should produce pastes that are stable to cold storage. In cyanoethyl starch, the transition to green colour occurred at a D.S of 0.141. Higher D.S gave

Table 3. Relationship between the starch-iodine colour and degree of carboxymethylation.

Degree of carboxymethylation	Blue value	Starch-iodine colour
0.0	0.375±0.0005	Blue black
0.014	0.374±0.0007	Blue black
0.039	0.370±0.0010	Blue black
0.055	0.342±0.0015	Blue black
0.116	0.290±0.0004	Blue black
0.128	0.300±0.0011	Blue black
0.145	0.251±0.0013	Deep green
0.185	0.170±0.0006	Green
0.205	0.208±0.0004	Green
0.212	0.185±0.0004	Green
0.269	0.186±0.0002	Green
0.296	0.159±0.0004	Green

Table 4. Relationship between the starch-iodine colour complex and degree of cyanoethylation.

Degree of cyanoethylation	Blue value	Starch-iodine colour
0.0	0.375±0.0005	Blue black
0.038	0.268±0.0009	Blue black
0.100	0.266±0.0010	Blue black
0.141	0.266±0.0006	Green-blue
0.169	0.246±0.0005	Green-blue
0.199	0.114±0.0015	Green-blue
0.255	0.032±0.0006	Light green
0.274	0.081±0.0013	Light green
0.303	0.079±0.0013	Light green
0.368	0.214±0.0010	Green-blue
0.484	0.171±0.0012	Green
0.591	0.194±0.0008	Green-blue
0.603	0.075±0.0006	Light green
0.639	0.181±0.0005	Green-blue
0.644	0.149±0.0005	Green-blue
0.699	0.164±0.0010	Green-blue
0.963	0.127±0.0015	Green

various shades of green colour which in some cases had bluish tint. This has been attributed to crosslinking and surface derivatization which tends to occur when starch is cyanoethylated under high acrylonitrile concentration (Hefrieter, 1986). Due to surface derivatization and crosslinking some long segments amylose inside the granules were not accessible to the reagents and hence remained long enough to form blue complex with iodine, this was responsible for the blue tint observed at high cyanoethylation degree.

Comparison of results from freeze-thaw stability and colour of starch-iodine complex showed that stability was reached at slightly different points. The difference arose from the fact that each method monitored retrogradation by different parameters. Retrogradation by freeze-thaw stability measures re-association between adjacent starch molecules while retrogradation by colour of starch-iodine complex measures the extent of complexation of the amylose with iodine. At D.S less than 0.064 in carboxymethyl and 0.100 in cyanoethyl starches, re-association was significant enough to result in the crystallization of the starch molecules. At these degrees of substitution the amylose chains were long enough to enter into helical structure with iodine to give the blue colour complex. At higher D.S the associative forces were very weak to result in starch crystallization and at

D.S of 0.145 in carboxymethyl and 0.141 in cyanoethyl starches, the helical structure was no longer maintained resulting in destruction of the blue colour. The method based on complex formation of starch with iodine has the advantage of rapidity because the result can be obtained in less than an hour unlike the freeze-thaw stability method that requires at least a day to get result.

Conclusion

The stabilization of starch paste by derivatization was related to the colour of starch-iodine complex. The blue colour of starch-iodine complex decayed with increase in degree of substitution (D.S) of the derivatized starch until it completely disappeared. This corresponded to when freeze-thaw stability was achieved without any noticeable sign of retrogradation. The disappearance of the blue colour of starch-iodine complex thus becomes an index of retrogradability and freeze-thaw stability of starch on cold storage.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENT

This study was supported by University of Ibadan Research Grant 94/95.

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