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Influence of three alkalizing agents on some functional properties of cassava starch modified using epichlorohydrin

B. Daramola* and E.A. Bamidele

Dept. of Food Technology Federal Polytechnic, PMB 5351, Ado-Ekiti, Ekiti State, Nigeria

Abstract

Influence of three alkalizing agents namely, potassium hydroxide (KOH), ammonium hydroxide (NH₄OH), and dimethyl sulphoxide (DMSO) during modification as modification facilitator on some functional properties of cassava starch modified using epichlorohydrin (EPI) at room temperature was studied. Assessment of the principal functional property; pasting properties and associated pasting indices of technological importance, notably peak viscosity and set back viscosity. The low peak viscosity (RVU) of the cassava EPI-modified starch ranged from 119.67-178.17; 128.50-161.17 for samples alkalized using KOH and DMSO respectively in comparison to the high peak viscosity (RVU) of the native starch. NH₄OH was less effective alkalizing agent under the conditions used in this study. Cassava-EPI modified starch showed improved paste stability expressed in breakdown viscosity (RVU) values of 22.08-48.75 and 37.92-55.58 for KOH and DMSO alkalized samples respectively. Evaluation of other functional properties such as swelling power, apparent viscosity and paste clarity of the cassava-EPI modified starch showed alkaline-type dependent cross linkage activity of EPI. Assessment of all the functional properties of starch accomplished under the conditions employed in this study revealed that irrespective of concentration of EPI added, activity of alkalizing reagents is in the order: KOH>DMSO>NH₄OH.

Keywords: Cassava starch; Alkalizing agents; Epichlorohydrin modification; Pasting properties; Functional properties

Introduction

Starch, the most important reserve carbohydrate in the plant world has limited industrial application in its native form regardless of its source because of their inherent nature such as water insolubility and tendency to form unstable paste and gels (Wang *et al.*, 2009). Therefore there is need to treat starch with view to diminish negative attributes and/or enhance positive attributes. The treatment is termed 'modification'. Starch modification can be broadly grouped into four classes, namely physical, chemical, enzymic and biological modifications (Bemiller and Lafayette, 1997). Of all the modification methods, chemical route offers the largest number of modification opportunities thus used in this study.

Cross-linked starch, a product of a type of chemical modification has versatile utilities, they are used for thickening foods to impart texture in a process where the viscosity might be lost because of exposure to high acidity (low pH), high temp, longer heating times or strong agitation (Ruttenberg, 1980). Cross-linked starch could serve as feed stock for preparation of other speciality starches which could find application as: Flocculants, waste water treatment additives, soil conditioners, tablet binders, adhesives, coating and biodegradable plastics (Biswas *et al.*, 2008). This by no means exhausts the list. Broadly, factors affecting chemical modification of starch can be grouped into two. The first group

consist of actualization factors which covers modification types and associated reagents as well as facilitation or initiator reagents. This does not include catalysts. It is important to note that without the facilitator, modification cannot take place without destruction of the starch granule integrity hence important during starch modification, because the facilitator opens the hydroxyl moiety in glucan prior to substitution or cross-linkage hence important in modification actualization and precedes modification intensification. The second group of factors affecting starch modification is concerned with process intensification this includes, catalyst (chemical or radiation), reagent concentration, time of contact, temperature, stirring rate, solid solvent ratio and use of solvent free method among others.

In view of the importance of alkalizing reagents in facilitating or potentiating modification of starch by modification reagents, the aim of this study is to explore the influence of three alkalizing reagents on modification of cassava starch using EPI.

Materials and methods

Materials

Cassava roots of TME 30572 variety, aged 12 months at harvest were obtained from Igbira farm in the suburb of

*Corresponding author: E-mail: daramola_bode@yahoo.com

Federal Polytechnic, Ado-Ekiti, Nigeria. The roots were of an improved variety (low cyanide and high yield).

Extraction of native cassava starch

Native cassava starch was extracted according to the classical method described by Osunsami (1987).

Preparation of cassava- EPI modified starch

The method for the preparation of Cassava- EPI modified starch was conceptualised from the method of Kokini and Hirsch (2002).

The scheme is presented in Figure 1.

Methods of Analysis

Determination of pasting properties using rapid visco analyzer (RVA)

The pasting properties of cassava- EPI modified starch and native cassava starch samples were characterized using RVA as described by Delcour *et al.* (2000).

Five gram starch samples were added to water at a ratio of 1:2 (w/v). The sample slurry was heated from 28 °C to 150 °C at 4 °C/min and all experiments were carried out in triplicate. The RVA-d was operated with 2.5 g of 9.9 % starch-in-water suspensions. The temperature profile included a 2 min isothermal step at 50 °C, a linear temperature increase to 95 °C in 7 min, a holding step (8 min at 95 °C), a cooling step (7 min) with a linear temperature decrease to 50 °C, and a final isothermal step at 50 °C. Measurements agreed within 5 RVU over the entire profile. Pasting peaks and parameters that were of paramount importance were identified and determined for technological interpretation.

Moisture content determination

Moisture content was determined in accordance with the methods of AOAC (Anonymous 1990).

Determination of swelling power

This was determined in accordance with the method described by Leach *et al.* (1959) with a modification for small samples, at three temperatures (60 °C, 70 °C and 80 °C).

Pastes clarity determination

This was determined from percent light transmittance (%T) of starch paste as described by Craig *et al.* (1989).

Swelling characteristics determination

Sample swelling characteristics was determined subjectively by a procedure adapted from Teramoto *et al.* (2003). Simply, 0.50mg of native and EPI modified cassava selected samples was added to 100ml of solvents and the suspension or solution was stirred overnight at room temperature. Solvents or solution added in the tests were KOH, lactic acid, H₂SO₄, H₃PO₄, Na₂CO₃, Na-acetate and water. The pH of the solvents and solutions was as recorded beside each solvent. Granule dissolution or swell was observed and recorded when noticed.

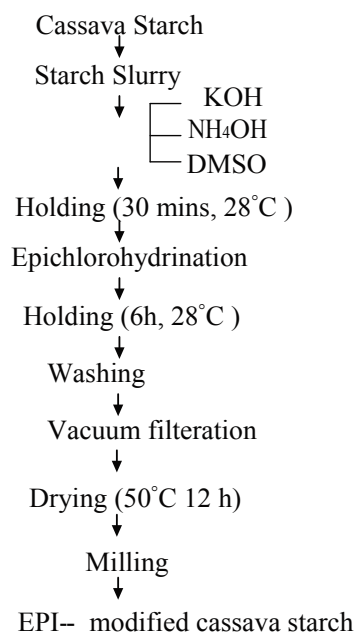


Fig. 1. Flow diagram for the preparation of EPI – modified cassava starch

Results and Discussion

Cross-linked starch is an example of chemically modified starches. Chemical modification is intended to facilitate intra and intermolecular bonds at random locations in the starch granules for their stabilization. Cross-linkage could be in nature of ether or ester inter-molecular linkages between the hydroxyl groups of starch molecules using cross linking agent such as EPI, POCl₃ and STMP (Singh *et al.*, 2007; Carmona-Garcia *et al.*, 2009). Cross-linkage is only accomplished using a facilitator called alkalizing agents. The results and discussion of the subject is presented hereunder.

The pasting characteristics of cassava starch modified using EPI under the influence of three alkalizing agents and the native cassava starch were evaluated. Pasting values of technological importance extrapolated from pasting curves of cassava of native and treated (modified) are shown in Table (I).

Table I. Rapid visco analysis readings of EPI-modified cassava starch and native cassava starch

Sample	Alkalizing agent (ml/mM)	EPI (mL)	PV (RVU)	TV (RVU)	BDV (RVU)	FV (RVU)	SBV _{F-T} (RVU)	SBV _{F-P} (RVU)	PT (min)	Pt (°C)
A	5 KOH	1	178.17	139.17	39.00	231.42	92.25	53.25	4.85	75.58
D	10	3	119.67	70.92	48.75	126.50	55.58	6.83	4.58	73.15
G	20	5	128.50	106.42	22.08	229.33	122.92	100.83	5.12	75.63
J	30	7	163.17	135.00	28.17	243.58	108.58	80.41	5.63	74.48
B	5 NH ₄ OH	1	267.75	225.58	42.17	319.17	93.58	51.42	4.96	76.59
E	10	3	274.17	94.33	179.83	137.58	43.25	-136.59	5.05	72.58
H	20	5	251.00	173.92	77.08	321.00	147.08	70.00	4.96	77.44
K	30	7	245.50	159.25	86.25	294.33	135.08	49.33	5.52	75.63
C	5 DMSO	1	133.50	85.05	48.42	145.25	60.17	11.75	4.78	74.42
F	10	3	139.83	84.25	155.58	124.33	40.08	-15.50	5.18	74.49
L	30	7	128.50	87.42	41.08	186.42	99.00	57.92	5.18	78.56
U	CONTROL	-	217.58	162.42	55.17	280.58	118.17	63.00	5.12	74.48

PV=Peak viscosity, TV=Trough viscosity BDV = Peak viscosity – trough viscosity, FV = Final viscosity, SBV_{F-T} = Final viscosity – trough viscosity, SBV_{F-P} = Final viscosity – peak viscosity, PT=Pasting time, Pt=Pasting temperature, RVU=Rapid visco analyzer unit

EPI-modified cassava starch effected using KOH and DMSO as catalyst were characterised with lower peak viscosities (119.67 – 178.17; (128.50-161.17) to the high viscosity (217.58) RVA of the native starch for KOH and DMSO respectively modification lend reduction in peak viscosity in comparison to the native starch. This type of modification is referred to as cross-linkage. Cross-linkages may reduce interactions of starch molecules with water molecules thereby showing low viscosity values during pasting in comparison to the native starch (Majzoubi *et al.*, 2009). This peak viscosity reduction is a characteristic of starch modification using EPI. There are plethora references that attest this some which are: Kokini and Hirsch (2002). Cross-linked starches are resistant to loss of viscosities at low pH. The property suggests that the product can be a useful bodying agents for acid fruit fillings in which the pH is often adjusted to a range of 3-4 (Stauffer, 1990). However, NH₄OH appeared to be ineffective in lowering the peak viscosity of the starch samples under the protocol employed in this study.

In addition, the trough viscosity (TRV) of the EPI-modified cassava starch was low, ranged from 70.92 – 139.17 RVU;84.25-123.25 RVU for KOH and DMSO alkalized facilitated modification respectively in comparison to the high (162 RVU) TRV of the cassava native starch. The technology implication of this is that the cross-linked or EPI-modified starch samples are characterized by low viscosity loss in comparison to high viscosity loss in the native starch. The stability of cassava-EPI-modified starch is expressed by

breakdown viscosity (PV-TR). Similarly, the breakdown viscosity BDV of the cassava-EPI-modified starch was low, ranged from 22.08-39.00 RVU; 37.92-55.58 RVU for KOH and DMSO alkalized facilitated modification respectively in comparison to high BDV (55.17 RVU) of the cassava native starch. Added to this, the final viscosities of the EPI-modified cassava starch samples were low, ranged from 126.50 RVU to243.58 RVU; 124.33-247.33 RVU for KOH and DMSO alkalized facilitated modification respectively in comparison to high 280.58 RVU final viscosity of the cassava native starch sample. Also, the set-back viscosity of the EPI-modified cassava starch were low (55.58-108.58 RVU; 40.08-99.00 in comparison to the high (118.17RVU) setback viscosity of the cassava native starch sample. However, the result of set-back viscosity for samples with identity G and I appeared to be an exception afore claimed. Low setback viscosity is an indication of suitability of cooked paste against retrogradation (Mazurs *et al* 1957). This low setback viscosity is as a result of the cross-linkage activity of EPI on cassava starch facilitated by the employed alkalines. Examination of setback viscosity from PV showed that sample with identity D was characterized with the least SBV from peak (6.83 RVU) in comparison to SBV from peak with 63.00 RVU of the cassava native starch. Both the peak time of pasting temp of the EPI-cassava modified starch and native starch appear similar.

However, EPI-cassava modified starch under the influence of NH₄OH as alkalizing agent showed no cross-linkage properties.

Nevertheless, on inspection of PV, TR, BDV, ESB of the EPI-cassava-modified starch in comparison to the cassava native starch does not categorically imply that NH_4OH could not act as alkaline to EPI for cross-linkage of starch. This assertion is informed by the fact that some of the setback viscosities of the samples (B.K) are low in comparison to the setback viscosity of the cassava native starch. Therefore the use of the alkaline need further studies prior to making a conclusive statement about its usage as alkalizing agent for cassava starch modification using EPI. Therefore, it could be safely asserted that KOH and DMSO effectively catalysed cross-linkage of cassava starch using EPI as cross-linking agent.

Moisture content

The moisture content (Table II) of EPI-modified cassava starch and native cassava starch were similar. This was because the starch samples were dried under similar conditions, thus given rise to similar amounts of total solids in the samples. Shildneck and Smith (1967) reported that the moisture content of starches is usually related to a large extent to the type of starch and the method and conditions of drying. Therefore type of alkaline used should not affect the moisture content of both native and modified samples as found in this study.

Paste clarity (% T)

The paste clarity of the EPI-modified cassava starch and the native cassava starch is shown in Table II. The paste clarity of the EPI-modified cassava starch samples is low in comparison to the high paste clarity of the native or non-modified starch. This could be explained in terms of the cross-linkage effected by EPI on the starch granules which was stable to destruction during application of heat which translated to low paste clarity in comparison to the high paste clarity of the cassava native starch. The high paste clarity of the cassava native starch could be explain in terms of heating that breaks the starch molecular chains which ultimately thereby lending high % transmittance. Considering the result present herein, it appears there is no clear marked difference on the effect of the type of alkaline on the % paste clarity.

Apparent viscosity

The apparent viscosity of the EPI-modified cassava and the native cassava sample is shown in Table (II). The values varied, however on comparison to the control samples, it is clear that low apparent viscosity is very prevalent in samples prepared using KOH as alkalizing agent while the effect of the other alkalizing agents were marginal. The very low apparent viscosity of the samples were as result of the temperature 75°C employed in the methodology. Cross linked starch has lower intrinsic viscosity than the native starch. This can be explained

by the fact that cross-linkage cause reduction in the distance between the starch molecules consequently formation of more compact structure. Therefore such molecules may have lower intrinsic viscosity since they have less friction with the solvent and other molecules in the solution.(Majzoobi *et al.*, 2009).

Cross-linked starch delays full gelatinization so that little viscosity developed in the early stages of last treatment. The low viscosity facilitates heat transfer hence the sterification temperature is reached more quickly. The cross-linked starch thickens at the high temp of the later stage of heating and given a higher final product viscosity since it does not break down (Rutenberg, 1980).

Swelling power

The swelling power of the EPI- modified cassava starch and the native starch examined at the temperatures of 40°C , 60°C , 80°C is shown in Table (II). A cursory look at the result showed that there was no marked difference between the treated samples and the native samples. This implies the energy supplied was not adequate to effect gelatinization of the starch granules. However, when the temperature was increased to 80°C , there was a notable difference in the swelling of the native starch in comparison to the EPI- treated samples. The treated samples notably samples (D,G,T) showed restriction to swelling in comparison to the control sample that dissolve to form paste. This is similar to earlier result on heating uncross linked waxy maize starches by Kokini and Hirsch (2002). The restriction to swelling by the treated samples can be described as starch cross linkage. Cross-linked starch is required in any starch food in which process conditions in term of low pH, extreme high temperature, and agitation that could cause the product to loose viscosity. Such product include pie-filling, this is because the starch to be used for pie-filling should be able to maintain viscosity at the baking temperature (180°C - 220°C). Food products that could require cross-linked starch are candy and gum drops. Cross-linkage prevents the starch granules from fully swelling and ultimately disintegrating (Kokini and Hirsch, 2002). Comparison of the swelling power of the treated samples from the perspective of alkaline used revealed that KOH was more effective in cross-linkage of starch than the other alkaline agents under the conditions employed in this study. The result herein suggests that alkalization by NH_4OH could facilitate cross-linkage of cassava starch using EPI.

Swelling characteristics of native and EPI-modified cassava starch and in some solvents/solution

The result of swelling or dissolution of granules of the cassava native starch and selected EPI-modified cassava starch samples in tested solvent and solution is shown in Table (III).

Table II. Some physiochemical property and swelling power of Epi-modified cassava starch and native cassava starch

Sample	Alkalizing agent (ml/mM)	EPI mL	Moisture content %	Swelling Power (g paste/g dry sample)			Paste clarity %	Apparent viscosity Cp
				45°C	60°C	75°C		
A	5 KOH	1	8.00	1.12	1.19	2.10	52.48	4.0
D	10	3	7.50	1.25	1.21	1.56	53.70	1.8
G	20	5	7.50	1.28	1.13	2.06	58.89	1.7
J	30	7	7.00	1.12	1.32	1.76	51.29	2.0
B	5 NH ₄ OH	1	7.00	0.98	1.06	3.33	53.70	5.0
E	10	3	7.70	1.15	1.30	3.68	54.95	17.0
H	20	5	8.50	1.09	1.15	3.38	54.95	1.8
K	30	7	8.00	1.09	1.21	4.29	53.70	18.5
C	5 DMSO	1	6.00	1.07	1.27	3.94	53.70	22.0
F	10	3	7.00	1.12	1.21	4.18	53.70	1.2
I	20	5	7.50	1.08	1.19	4.17	53.70	16.0
L	30	7	8.50	1.09	1.13	4.14	53.70	3.0
U	CONTROL		8.00	0.96	1.13	4.80	75.87	32.0

The pH of the solvent or solution gives insight to the severity of the medium employed. Neither the cassava native starch nor the EPI modified samples swell nor dissolved in distilled water. This is a characteristic of native starch due to the crystallinity and hydrogen bonding between glucose molecules within the molecular chains of starch and the EPI-modified cassava starch

samples because of its cross linkage characteristics should also display similar properties. However, all the native samples showed granules swelling or dissolution in all the solvent or solution tested with the exception of Na₂CO₃ solution. The various swollen degree and slight dissolution caused by the tested

Table III. Swelling characteristics of native and EPI-modified cassava starch in some solvents/solution

Solvent	Sample Identity				pH
	U	D	L	K	
Water	--	--	--	--	7.5
KOH-1	+++	--	-	-	12.2
KOH-2	+++	-	-	-	13.0
Lactic acid-3	+	-	-	--	2.1
Lactic acid-4	+	-	-	-	2.0
H ₃ PO ₄ -5	+	-	-	-	1.7
H ₃ PO ₄ -6	+	-	-	-	1.6
Na ₂ CO ₃ -7	-	--	--	--	10.3
Na-acetate-8	+	--	-	-	8.0
H ₂ SO ₄ -9	+++	-	-	-	1.4
H ₂ SO ₄ -10	+++	-	-	-	1.2

+++ = slight dissolution, ++ = much granule swollen, + = granule swollen, - = slight granule swollen, -- = no granule swelling noticeable

solvents or solution on the cassava native starch could be explained in terms of protonation either from acid or base employed which facilitate hydrolysis of starch molecular chains. Depending on severity of the solution it could cause swelling or granule rupture or dissolution. However, the result of the examined EPI modified cassava starch samples showed no granule swollen noticeable as well as no granule dissolution. The resistant to granule swollen or granule dissolution by the EPI modified cassava starch samples was as a result of the cross linkage in the starch granules irrespective of the alkaline use for modification using EPI.

Conclusion

Three alkalizing reagents namely, potassium hydroxide, ammonium hydroxide, and DMSO potentiate the activity of EPI as a cross-linking reagent during the modification of cassava starch. Assessment of selected functional properties of the cassava EPI-modified starch showed that irrespective of the concentration of EPI added, modification of starch using EPI was influenced by the alkalizing reagents in the order: KOH>DMSO>NH₄OH. The finding in this study provides insight to alkalization activity of the reagent during cassava starch modification using EPI.

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