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Effect of Chemical Modification on Starch of Some Legume Flours

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Abstract: Chemical modification of the starch of gourd seed, white melon, yellow melon, bennissed and bulma cottonseed were carried out. Infra-red spectrophotometer was run to confirm the products obtained after chemical modification. Hypochlorite oxidation and acetylation of the starch samples were studied for pasting stability using Brabender amylograph, freeze – thaw stability, starch paste clarity and water binding capacity. The results obtained for modified starch were compared with the native starch. The paste clarities of the oxidized starch were better than the acetylated and unmodified starches. The acetylated and unmodified starches allowed 90% transmittance at 650nm. Acetylation, markedly decreased the tendency for syneresis and improved the freeze thaw stability. This may be due to bulky acetyl groups which caused stearic hindrance. Modification had influenced the stability of the starch studied. The oxidized starch has better stability than those of the corresponding acetylated and unmodified starch.

Key words: Effect, chemical, modification, starch, legume, flours

Introduction

Starch is the only qualitatively important digestible polysaccharide and has been regarded as nutritionally superior to low molecular weight carbohydrate or sugars (ASP, 1996). The modern food processing industries are increasingly dependent on the use of both native and modified starches (and gums as well) for manufacture of various fabricated food products. Starch is an important ingredient for the food industries. Starches with specific properties are necessary to impart functionality desirable attributes (Tharanahan *et al.,* 1990) and to impart viscosity to foods. The clarity of starch paste is one of its importance attributes. Starch used to thicken fruit pie filling is preferably transparent but starch used in spoonable salad dressing should be opaque. Clarity varies considerable with the sources of starch and can be altered by chemical modification of the starch (Stuart *et al.*, 1989). A number of modifications / derivatizations via chemical treatments can be effected resulting in products suitable for specific purposes in the food industries (Lee *et al.*, 1993). Some physical and chemical properties of taro starch have been reported by (Sugimoto *et al.,* 1986). Mild oxidation of starches with hypochlorite yields a system with greater stability and clarity and less tendency towards gelatinization and retrogradation (Ihekoronye and Ngoddy, 1985). The present work was undertaken to study the effect of acetylation and oxidation on gourd seed, white melon, yellow melon, bennissed and bulma cotton starches and some of the starch properties such as starch paste clarity, freeze-thaw stability, starch paste stability, water binding capacity and the effect of acetyl and carboxyl groups introduced on these functional properties. The infra red (IR) spectroscopic study was carried out to confirm the groups that have been incorporated during modification processes.

Materials and Methods

Gourd seeds, oblong species of white melon, yellow melon and benniseed were purchased from Ojo Oba market in Akure while bulma cotton seeds were harvested at University of Ado-Ekiti, Nigeria. The seeds were thoroughly screened to remove bad ones and the remaining good seeds were blended into fine flour using Kenwood mixer and kept in polythene bags and stored in refrigerator until used.

Isolation of starch from the samples: The method of Tharanathan *et al.* (1990) was used for the isolation of starch from the sample flours. Each sample flour was extracted using soxhlet extractor with a mixture of hexane- $CHCl_3$ – CH_3OH (1:2:1, v/v/v) at reflux temperature. The crude starch was recovered when the defatted flour was steeped in water containing $HgCl_2$ (100ppm) for 16h at room temperature and mascirated in a blender. The crude starch granules were separated by filteration through 150-200µm mesh sieves and centrifuged at 5000rpm for 10min.

The crude starch granules were purified by treating with dil NaOH (0.IM,5 min at room temperature) and 0.IM NaCl – toluene, after each treatment the granules were sedimented by centrifugation and the sediment were thoroughly washed with water. The final sediment was further washed twice with methanol and air dried.

Modification of the starch samples

Preparation of Hypochlorite (oxidized) starch: A 10g of each starch was dispersed in 50cm^3 distilled water. The pH of the slurry was adjusted to 9.0 using 3% NaOH. 1g of NaOCl was added slowly for a period of 90 minutes and constantly monitoring the pH 9.0 and simultaneously cooling was done with crushed ice and

Table 1: Starch paste clarity (%T)

	Starch 1% (w/v)				
	Gourd seed	White melon	Yellow melon	Benniseed	Bulma cotton seed
Native Starch					
Remark	90, High clarity	80, High clarity	55, moderate High clarity	35, low clarity	32, low clarity
Observation	No Whiteness	No Whiteness	Low Whiteness	clarity/opaque/ High whiteness	clarity/opaque/ High whiteness
Acetylated starch					
Remark	92, High clarity	90, High clarity	60, moderate clarity	52, moderate clarity	50, moderate clarity
Observation	No Whiteness	No Whiteness	low Whiteness	low Whiteness	low Whiteness
Oxidized Starch					
Remark	95, High clarity	94, High clarity	62, moderate clarity	54, moderate clarity	52, moderate clarity
Observation	No Whiteness	No Whiteness	low Whiteness	low Whiteness	low Whiteness

Table 2: Water binding capacity % of the starches

Sample	Native starch (%)	Acetylated starch (%)	Oxidized starch (%)
Gourd seed	85	95	96
White melon	81	91	94
Yellow melon	78	90	92
Benniseed	83	88	90
Bulma cotton seed	80	86	89

NaCl. The reaction proceeded for four hours after NaOCl addition was completed. The pH of the mixture was then adjusted to 7.0 with 0.5M HCl and the slurry latter filtered through whatman No.1 filter paper. The residue was then washed four times with distilled water and air dried at room temperature.

Preparation of acetylated starch: Starch (10g) was dispersed in 50cm^3 of distilled water and then constantly stirred for 30 minutes. The slurry was adjusted to pH 8.0 with NaOH, 1.2g of acetic anhydride was then added to the slurry. After the addition of the acetic anhydride the reaction was allowed to proceed for another five minutes. The pH of the slurry was adjusted to 4.5 with 0.5M HCl and filtered through whatman No. 1 filter paper. The residue obtained was washed four times with distilled water to remove completely some acids that may be present in the product and finally air dried at room temperature.

Determination of starch paste clarity: The method of Stuart *et al.* (1989) using light transmittance (%) of starch paste was followed. Paste (1%) was produced when starch (0.05g) was suspended in 5ml of distilled water in screw cap tubes and then placed in a boiling water bath for 30min. The tubes were thoroughly shaken every 5min. After cooling to room temperature was done for 5min, the percent transmittance at 650nm was determined against a water blank in a spectronic 21 spectrophotometer. Value for %T were not significantly different after 24 hr at room temperature.

Determination of freeze – thaw stability: The freeze – thaw stability was determined according to the method of Rege and Pai (1996) with slight modification. The starch pastes were prepared by heating 8% w/v starches in distilled water at 95^{o}C for 30 minutes with constant stirring. The suspension was subjected to alternate freezing and thawing by holding at – 10^{o}C for 18 hours and at 30^{o}C for 6 hours followed by centrifuging at 3000rpm for 10 minutes. The amount of water separated was expressed as the percentage of water separated after each cycle of alternate freezing and thawing.

Determination of starch paste stability: The method of Alves *et al.* (1999) with slight modification was employed. The starch samples were determined using Brabender visco amylograph. The starch stability was expressed as the peak viscosity (V_p)minus viscosity after heating for 30 minutes as 95^{o}C Vr (Vp - Vr).

Determination of water binding capacity: The procedure described by Sugimoto *et al.* (1986) was used with slight modification. Starch (5.0g) was added to 75ml distilled water in 100ml centrifuge bottle. The bottle was stoppered and agitated on magnetic stirrer for 1hr, then centrifuged for 10min. at 2,200 x g. The water was decanted and the bottle was allowed to further drain for 10min and weighed. The amount of water held by the starch was determined. The binding capacity was calculated from the formula: Bound water (g) x 100/5.0.

Determination of starch granular density: The granular densities of the starch samples were determined using the liquid displacement method. Five grammes of each starch sample was added to petridish and dried to a constant weight at 45^{o}C. Magnesium stearate (0.05g) was added as a lubricant and the mixture was carefully mixed to avoid falling off of the starch particles. Lubricated starch granules was poured through a glass funnel into a measuring cylinder which already contained 50ml n-hexane as immersion liquid and from the volume of n – hexame displaced, the volume of starch granule was taken. Granule density was calculated from the equation below.

Table 3: Freeze – thaw stability (%)

		No of Freeze thaw cycles				
% water separated		1	2	3	4	5
Gourd seed						
	Native	6.0	26	33.5	36	39
	Oxidized	0.5	27	32	35	36
	Acetylated	9.5	14.5	17.0	19.0	21.0
White melon						
	Native	1.0	2.0	3.0	3.5	4.0
	Oxidized	2.0	6.0	11.0	14.5	16.0
	Acetylated	3.0	5.0	6.0	6.5	7.0
Yellow melon						
	Native	4.0	6.0	8.0	9.0	9.5
	Oxidized	1.0	4.0	6.0	8.5	9.0
	Acetylated	6.0	9.0	11.0	14.0	14.5
Benniseed						
	Native	5.5	6.0	7.0	8.0	9.0
	Oxidized	3.0	16.0	24.0	26.0	29.0
	Acetylated	9.0	12.0	15.0	17.0	19.0
Bulme cotton seed						
	Native	0.5	1.0	1.5	3.0	3.5
	Oxidized	0	2.0	3.0	4.0	5.0
	Acetylated	1.0	2.0	2.1	2.1	2.1

$$= \quad [w / V_2 - V_1]$$

Where $V_2 - V_1$ is the displaced volume, w is the weight of starch sample.

The infrared spectroscopy was determined using infrared spectrophotometer Perkin – Elmer model.

Results and Discussion

Starch is a digestible polysaccharide. Starch may be linear (amylose) and branched (amylopectin) it is a polysaccharide units of glucose with hydroxyl groups (OH) as major functional group which appears in the region ($3650cm^{-1}$ – $3200cm^{-1}$) which disappeared when the starch was oxidized. There was introduction of carboxyl group and the spectral now processed peaks around ($1752cm^{-1}$ – $1680cm^{-1}$). When the starch was acetylated, there were introduction of acetyl groups which had peaks around the region ($1750cm^{-1}$ – $1730cm^{-1}$).

The results obtained from the infrared spectroscopy charts confirmed that the hydroxyl groups present in structure of the native starch had disappeared and there had been introduction of carboxyl and acetyl groups in the derivatized starches.

The results of starch clarity of both native and modified starches are presented in Table 1. The percent transmittance (%T) was measured as function of wavelength for various starch pastes. The percentage transmittance of native and derivatized starch pastes was classified into three categories depending on their behavior in light (I) high clarity and almost no whiteness which is due to little or no refraction because of lack of swollen granular remnants and little reflection of light (ii) moderate clarity and high whiteness, due to little refraction (few granular remnants) and high reflection of light (iii) low clarity and whiteness due to high refraction of light by swollen granular remnants but little reflection of light (3). The results in Table 1, showed that the clarity of starch pastes can be improved by modifying their molecules as observed by Rutterberg and Solarek (1984).

The paste clarities of the oxidized starches were better than the acetylated and native starches, allowed 90% transmittance at 650nm, which was improved when oxidized and appeared clearer and not white allowing 95% transmittance at 650nm. The value 95%T obtained for gourd seed starch is compared to that of potato starch 96%T at 650nm reported by Stuart *et al.* (1989). Stuart *et al.* (1989) observed that more opaque paste gave a lower %T. This implies that native starches of benniseed and bulma cotton at 35%T and 32%T are more opaque than other starches studied. The high clarity observed for oxidized starch pastes signified that the starch granules of these samples are fragile during pasting and remnants of granules are absent from the paste (Callghan and Lelieve, 1985; Banks and Greenwood, 1975). When the beam of light impinges on the aqueous suspension of native starch granule of bennissed and bulma starch pastes, light is scattered at the surface of the granules because the surface is large compared to the wavelength of the light. Much of the scattered light is reflected back to the observer and the native granule appeared white or cloudy and opaque. During gelatinization, these granules swell and more of light begins to pass through the granules instead of being reflected. This is because the starch molecules dissociate and the ability of the granules to reflect light diminishes, however, the transmitted light passing through smaller granules is refracted, and the degree of refraction decreases with increasing swelling of the granules.

Table 4: Starch paste stability (B.U)

Samples 8% w/v	Native starch Vp - Vr (B.U)	Acetylated starch Vp - Vr (B.U)	Oxidized starch Vp - Vr (B.U)
Gourd seed	15	45	10
White melon	65	65	5
Yellow melon	65	20	20
Benniseed	35	40	25
Bulma cotton	15	20	15

Vp - Vr = Stability of starch. Vp = peak viscosity (B.U)
Vr = viscosity after 30 minutes holding at 95°C (B.U)

Table 5: Granular density of the starch samples (g/cm^3)

Sample(s)	Starch granular density (g/cm^3)
Gourd seed	1.450 ± 0.300
White melon	1.350 ± 0.200
Yellow melon	1.115 ± 0.100
Benniseed	1.255 ± 0.200
Bulma cotton seed	1.475 ± 0.100

Error as Standard Deviation

The results of water binding capacity are shown in Table 2. The amount of bound water determined in this way was both absorbed by the granules and adsorbed on their surfaces. The values obtained for oxidized starches were higher than those reported by Medcalf and Gilles (1965) for native starches of Durum wheats (93%), selkirik (Fargo) (86%). Consequently, the water binding capacity of the starch samples studied agreed quite well with those of native starches reported by Medcalf and Gilles (1965) using similar technique. Leach *et al.* (1959) observed that hypochlorite oxidation is a means for weakening the internal structure of the granule. This makes the starches to be more susceptible to water molecules.

The results of freeze-thaw stability of both native and modified starches are shown in Table 3. There were remarkable decreases and fair increases in freeze-thaw stabilities after five freeze-thaw cycles, which indicates that gourd and benniseed starches did not tolerate five, freeze-thaw cycles. This poor freeze-thaw stabilities make them unsuitable for use in custards and pie filling which are frozen stored. But bulma cotton starch has fairly good freeze-thaw stability and this compared favourably with the chickpea starch reported by Rege and Pai (1996) Acetylation, however, markedly decreased the tendency for syneresis and improved the stability of freeze-thaw. This may be due to the steric hindrance exhibited by the bulky acetyl groups which obstructed the proper alignment of starch chain for maximum retrogradation.

Acetylation showed some useful changes in the properties of the starches of gourd, white melon, yellow melon, benniseed and bulma cotton by increasing substantially their freeze-thaw stabilities. The starches of gourd and bennissed did not tolerate even one freeze-thaw cycle showing extensive syneresis.

Table 3 further showed that there were 6% and 5% water separation after the first cycle, 26% and 18% after second and 39% after 5 cycles. Amongst the starches studied bulma cotton starches had better freeze-thaw stability.

The trend in freeze-thaw stability is bulma cotton starch > yellow melon > white melon > benniseed > gourd starch respectively.

The results of starch paste stability are presented in Table 4. Derivatization of starches influenced stability of starches. Peak height shows the maximum viscosity (Vp) obtained and when compared with the viscosity at 30min. height (Vr) gives an indication of the relative stability (Vp - Vr) of the starch pastes (Maningat, 1986). The value obtained for bulma starch (15BU) indicates that it has relatively high stability compared to other samples under consideration. The lower the values of Vp – Vr, the better the stability of the starches. The oxidized starches have better stability than the corresponding acetylated starch but the acetylated starches were better than that of native starches. The superior stability of oxidized starch pastes compared to those of unmodified starches may be due to the presence of carboxyl groups introduced into the starch molecules during hypochlorite oxidation. Since carboxyl groups are bulkier than the hydroxyl groups, they tend to displace hydroxyl groups which sterically hindered the tendency of amylose to associate and retrograde. Hypochlorite is likewise a highly affective means for weakening internal structure of the granules (Sugimoto *et al.*,1986).

The densities of the starch samples are shown in the Table 5. There are differences in densities between the starch samples studied, this may be due to different varieties and granule structures of the starches.

The values are gourd starch (1.450± 0.300g/cm^3), white melon (1.350 ± 0.200g/cm^3), yellow melon (1.115± 0.100g/cm^3) and bulma starch seems to have the highest density of 1.475 ± 0.100 g/cm^3 while yellow melon has the lowest value of 1.115 ± 0.100g/cm^3. These values for density presently reported are lower than those varieties of hard red spring wheats (1.473 – 1.491g/cm^3) reported by Medcalf and Gilles (1965) but the value of bulma starch is higher than those of varieties of Durum wheats (Medcalf and Gilles, 1965) but quite similar to that of crim wheats (1.475g/cm^3) reported by Medcalf and Gilles (1965). This enables us to explain why the starches are more susceptible to enzyme attack and have greater swelling capacities than Durum wheat starches and Hard red spring wheat starches (Medcalf and Gilles, 1965). The granule density influences the rate and extent of packing experienced by a material during the various unit of operation. The result of the particle density showed that yellow melon starch is denser than other samples studied and this explain the usefulness of starch of yellow melon in tabletting in the

pharmaceutical industry.

From the results, it can be concluded that the applicability and potentiality of the starch samples studied were enhanced by the chemical modifications and thus enable their properties compared favourably with conventional cereal starches and dues to relative abundance of the samples they are recommended for food and allied industries.

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