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Effect of Acetylation on the Physical and Functional Properties of Industrial and Laboratory Cassava (*Manihot esculenta* Crantz) Starches

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Authors' contributions

This work was carried out in collaboration between all authors. Author TNF designed the study and wrote part of the first draft of the manuscript. Author ASA managed the experimental process and wrote the manuscript. Author AAB managed the literature searches and contributed to the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Acetylated potato starch (APS) is commercially available and used widely in the food industries. It is imperative to study the physical and functional properties of acetylated cassava (*Manihot esculenta* Crantz) cultivar Tropical Manihot Series (TMS) 30572 and industrial starches for possible substitution/ replacement of expensive APS in food system.
Study Design: The properties of acetylated cassava starches were compared with those of commercially available acetylated potato starch (APS) and native cassava starches.
Place and Duration of Study: The experiment was performed in the Department of Food Science and Technology, Federal University of Technology, Akure Nigeria from June 2011 to January 2013.
Methodology: Industrial starch and starch extracted from cassava TMS 30572 were acetylated by

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standard procedure. The acetylated starches were analyzed for the physical as well as functional properties.
Results: The yields after acetylation ranged between 96-98% and 80-93% for TMS 30572 and industrial starches, respectively. Acetylated cassava starches showed improved physical and functional properties over the native cassava starch and these increased with increasing concentration of acetic anhydride in the reaction medium. At >2.50% acetylation, starch concentration of 5.5% had the same hot paste viscosity of 1500 cPa.s with commercial APS at 5% concentration. Also at 2.50% acetylation the starch was stable until the third freeze-thaw cycles and exhibited better stability than commercial APS.
Conclusion: Acetylation improved the yield of starch from cassava during processing. The industrial starch showed higher degree of acetylation than TMS 30572 starch under the same experimental condition. Acetylated cassava starches (at >2.50-2.70% acetylation) has improved functional properties and lesser tendency towards retrogradation thus could be a potential replacement to the more expensive APS as ingredient in food system.

Keywords: Cassava cultivar; food industry; freeze-thaw stability; retrogradation.

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is widely cultivated along the tropical belt for its starchy tubers which are used as food, feed or as an industrial raw material. Starches are used in a wide range of food products and its incorporation into food system is primarily governed by factors such as gelation, pasting properties, apparent viscosity, solubility, swelling power and clarity [1].

Only few percentages of the world crop of starch are used in their native state. Modification is usually carried out to introduce the desired properties and or remove certain inherent undesirable characteristics of the native starches. Genetic and chemical modification produces functionally tailored starch products that meet specific application in the food industries and this has led to expanded usage of starch and its products [2,3]. Modified (acetylated) starch cannot be readily broken down by digestive enzymes [4], thereby making it a desirable functional resistant starch with great health benefits [5].

Acetylation involves esterification of the hydroxyl functional group of starch to produce starch with altered polarity, lower pasting temperature and improved paste clarity and freeze-thaw properties. The detrimental syneresis and retrogradation effects are greatly reduced in acetylated starch thus making it very useful in frozen foods [6-8].

There is limited information on the effects of using graded amount of acetic anhydride to modify cassava starch and the properties of such modified starches. The present study aims at extraction and modification of cassava starch by acetylation technique using graded levels of acetic anhydride, and investigation of the changes in the functional and physical properties of the starch due to acetylation in comparison to the observed properties with the commercially available acetylated potato starch in order to determine the suitability of the cheaply available cassava starch as alternative to APS in the food industry.

2. MATERIALS AND METHODS

2.1 Source of Materials

Cassava cultivar variety TMS 30572 was planted and harvested after 18 months on the research farm of The Federal College of Agriculture, Akure, Nigeria. The industrial cassava starch was obtained from Matna Food Company Limited, Akure, Ondo State, Nigeria. A 2.5% acetylated potato starch was obtained from FAN MILK Plc. Ibadan, Nigeria.

2.2 Starch Extraction

Cassava starch TMS 30572 was extracted from cassava tubers according to the procedure described by Kordylas [9]. The starch obtained was dried in a hot air oven (Labcon air oven model) at 55±2°C for 48 h, then pulverized and sieved using 254 µm sieve.

2.3 Starch Acetylation

Starch acetylation was carried out using the method of Wurzburg [10] as modified by Golachowski [7]. Two hundred grams (200 g; dry

weight basis) of native cassava starch or TMS 30572 cultivar was dispersed in distilled water the pH was adjusted to 8.0 with 3% NaOH. Predetermined volume of acetic anhydride was added to the slurry at a rate of 1 mL/min. The pH was finally adjusted to 5.4 with 10% HCl. The starch was centrifuged at 1,000 x g and the residue washed with distilled water and dried at 30ºC for 24 h to determine the yield. The dried acetylated starch samples were then pulverized, sieved to pass through 254 µm sieve packaged in plastic containers and kept in cool dry place for further analyses. In order to determine the degree of acetylation, 10 g of the acetylated starch (dry weight basis) was added to 65 mL of distilled water followed by 25 mL of 0.5M NaOH with continuous mixing using a magnetic stirrer for 30 min as described by Golachowski [7].

2.4 Determination of Bulk Density and Sedimentation Volume

The bulk density was determined using the procedure of Narayana & Narasinga [11] with slight modification. An empty calibrated measuring cylinder was weighed and the weight recorded as W1. Cassava starch was then added to the measuring cylinder and the volume occupied was measured as V while the new weight was recorded as W2. The Bulk density was calculated as shown in the equation below.

$$\text{Bulk Density (g/mL)} = \frac{W2-W1}{V}$$

Sedimentation volume was determined as described by Raja et al. [12] and modified by Fagbemi et al. [3]. Briefly, 10 g of the cassava starch was weighed into a measuring cylinder with 20 mL of distilled water. The content was mixed thoroughly while adding more water until a final volume of 100 mL. The mixture was left to stand for a minimum of 3 h or till when no particle is suspended in the supernatant. The volume of the sediment is then read and recorded.

2.5 Determination of Water and Oil Absorption Capacity

A suspension of 1 g of each starch (dry weight basis) was made in 10 mL of distilled water [or 10 mL of oil (executive chef® oil with density of 0.92 g/mL)]. The water and oil absorption capacities of the starch samples were determined as described Omowaye-Taiwo et al [13] adapted from Sathe et al. [14]. Briefly, 1.0 g of the cassava starch was weighed and poured into a beaker containing 10 mL of water or oil. The mixture was stirred using magnetic stirrer for 5 min and the suspension was centrifuged for 15 min at 3,500 × g. The volume of the supernatant was measured. The water or oil absorbed was calculated as the difference between the weight of the initial volume of water or oil used and the weight of the final volume of the supernatant.

2.6 Determination of Viscosity, Swelling Power and Solubility

Starch suspension of 5% (w/v) in distilled water was heated to 90ºC in a water bath with continuous stirring. The paste was transferred to a rotatory viscometer and paste viscosity was measured at 10ºC interval as the paste cools from 90ºC down to 30ºC of the cooling phase. The paste viscosity was expressed as centi Pascal second using the method of Amani et al. [15]. The method described by Osundahunsi & Mueller [16] was then used to determine the solubility and swelling power of the starches. Briefly, 2% (w/v) of cassava starch (dry weight basis) in distilled water was heated in a water bath at 50ºC for 30 min with thorough stirring using glass rod. The centrifuge tube containing the mixture was then cooled to 25ºC and centrifuged for 10 min at 2,500 x g. The same procedure was repeated at 60ºC, 70ºC, 80ºC and 90ºC. From the supernatant, 5 mL was pipetted into a pre-weighed glass Petri dish and evaporated over a steam bath followed by oven drying at 110ºC for 3 h. The weight of the paste was used to calculate the swelling power as gram of sediment per gram of cassava starch. The soluble starch was the difference in the weight of the Petri dish after drying, thus used to calculate the percentage solubility as gram of soluble cassava starch per gram of cassava starch.

2.7 Determination of Paste Clarity and Freeze-Thaw Stability

The paste clarity was determined using the procedure of Craig et al. [17]. On dry weight basis, 1% aqueous dispersion of starch in distilled water was boiled at 100ºC for 30 min under constant stirring. The paste was cooled to 30ºC and the percentage transmittance was measured at 640 nm. The freeze-thaw stability was investigated using the method of Singh & Kaur [18]. Aqueous suspension of 5% w/v (dry weight basis) was prepared using distilled water. The suspension was heated at 95ºC for 30 min in a water bath and then cooled to below 25ºC with continuous stirring in order to prevent skin formation on the paste. The paste was then

subjected to a 5-cycle alternate freezing and thawing at 18 h and 3 h respectively. At the end of each cycle, the paste was centrifuged at 5,000 x g for 10 min and the amount of exudates calculated in percentage was determined and plotted against the number of freeze-thaw cycle.

2.8 Statistical Analyses

The data were subjected to analysis of variance (ANOVA) and the means were separated using Duncan's multiple range tests using SPSS statistical package version 17.

3. RESULTS AND DISCUSSION

The degree of acetylation increased as the volume of acetic anhydride increased (Fig. 1). This phenomenon may partly be due to the larger surface area for diffusion and absorption of the acetyl groups on the starch molecules. High concentration of acetic anhydride resulted in high molecular collision rate leading to greater availability of acetic anhydride molecules in the vicinity of starch [19]. Similar observations have been reported for corn starch [20]. The laboratory starch was processed via simple sedimentation process with purity level of 98.8% while the industrial starch was processed mechanically with purity level of 97.5%. The rasping processing of industrial starch led to higher reaction efficiency compared to the cassava TMS 30572 cultivar, which resulted in higher degree of acetylation when the same amount of acetic anhydride was added. Mechanical processing of industrial starch causes damage to the starch granule structure thus increasing the susceptibility to the chemical reaction than the undamaged starch granules [21].

3.1 Effect of Acetylation on Yield, Sedimentation and Bulk Density

As shown in Table 1, the yields of acetylated industrial starches (80-93%) were lower than TMS 30572 (96-98%) starches due to the high concentration of impurities (2.5%) in industrial starches which were removed during the acetylation. The highest yield was observed at acetylation of 2.99% and 3.98% for TMS 30572, and 3.60% for the industrial starch. The yields obtained were higher than the range of 82 - 88% reported earlier [19]. The sulphuric acid used as a catalyst has the tendency to cause extensive solubilisation of the crystalline structure during acetylation process and might have contributed to the observed higher yield.

The bulk density and sedimentation volume increased with increase in the degree of acetylation of the starches (Table 1). Narayana & Narasinga [11] reported similar result for winged beans flour. The sedimentation volume of the acetylated industrial starches was significantly higher than the native starches. Hence, acetylated starches will exhibit better cold water swelling than the native cassava starch.

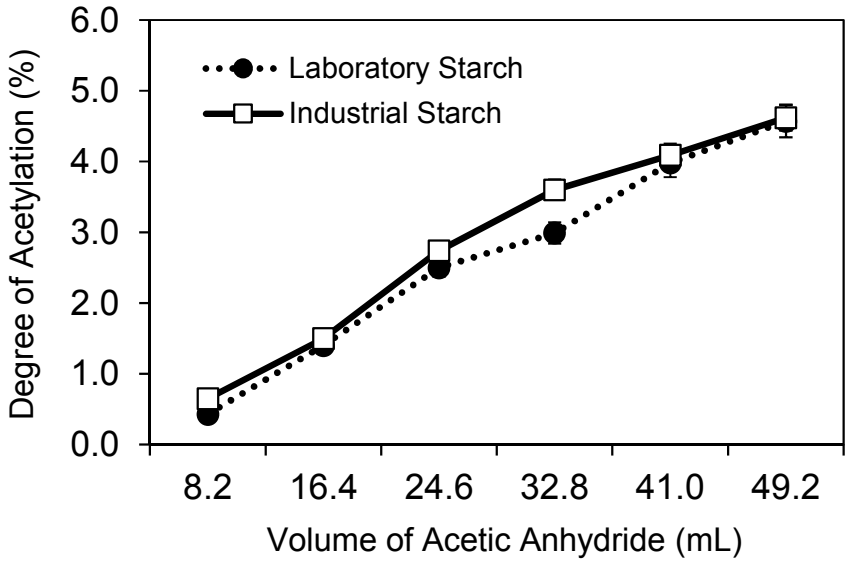

Fig. 1. Relationship between the volume of acetic anhydride and degree of acetylation of cassava starches

Table 1. Effect of acetylation on the yield, sedimentation and bulk density of cassava starches

Laboratory (TMS 30572) starch				Industrial starch			
DoA (%)	Yield (%)	Sedimentation (mL)	Bulk Density (g/mL)	DoA (%)	Yield (%)	Sedimentation (mL)	Bulk Density (g/mL)
0	98.70±0.04	6.00±0.82^{c}	0.81±0.01^{b}	0	92.00±1.21	4.50±0.16^{d}	0.71±0.03^{c}
0.43	96.00±0.08	7.00±0.41^{c}	0.81±0.01^{b}	0.65	80.00±1.70	6.50±0.82^{c}	0.72±0.01^{c}
1.4	96.00±0.05	7.50±0.94^{b}	0.83±0.02^{a}	1.5	88.00±0.80	7.00±0.16^{c}	0.78±0.04^{b}
2.5	96.00±0.05	7.50±0.84^{b}	0.83±0.02^{a}	2.74	90.00±0.80	7.50±0.14^{b}	0.82±0.02^{a}
2.99	98.00±0.08	7.50±0.62^{b}	0.83±0.05^{a}	3.6	93.00±1.40	7.50±0.08^{b}	0.82±0.05^{a}
3.98	98.00±0.08	7.90±0.82^{a}	0.84±0.05^{a}	4.09	90.00±0.90	7.70±0.05^{b}	0.83±0.01^{a}
4.57	97.00±0.05	8.00±0.62^{a}	0.84±0.02^{a}	4.62	90.00±0.80	8.10±0.05^{a}	0.84±0.03^{a}
APS	ND	8.00±0.47^{a}	0.78±0.02^{c}	APS	ND	8.00±0.04^{a}	0.78±0.02^{b}

Values are reported as Mean±SEM (n=3). Mean values with different letters in a column are significantly different (p≤0.05).DoA: Degree of acetylation; APS: acetylated potato starch; ND: not determined

3.2 Swelling Power, Solubility, Water and Oil Absorption Capacities

The swelling powers of the starches were investigated at temperature range of 50ºC – 90ºC, representing the pasting range of most starches. Native starch exhibited an increase in swelling power with increasing temperature as shown in Figs. 2A and 2B. Swelling power has been reported to correlate with solubility in different varieties of sweet potato starches [22]. Acetylation increased the swelling power at any given temperature perhaps due to the loosening of intra- and inter-molecular bonds in the starch molecules, which eventually increased the swelling capacities of the starches. However, there was no significant difference in the swelling power of the acetylated starch at temperature greater than 60ºC when the degree of acetylation was above 2.50% for TMS 30572 cultivar. Limited water available within the mixture at higher temperature may affect the swelling power as complete gelatinization and swelling do occur in excess water [21]. Solubility increased with increase in the degree of acetylation. There was a sudden rise in the solubility of the starch acetylated at 1.4% and 1.5% for TMS 30572 and industrial starches respectively at 60ºC. At temperatures >60°C, the solubility could not be measured as shown in Figs. 2C and 2D. This might be due to total percolation of water into the starch granules, resulting in limited amount of water available in the mixture since acetylation lowers the gelatinization temperature. Singh et al. [23] have reported that acetylation decreased the gelatinization temperature of acetylated sorghum starch.

The water and oil absorption capacities (WAC and OAC) of the starches are shown in Fig. 3. All the starches absorbed more oil than water when acetylated. Similar result has been reported for acetylated sweet potato starch [24]. The result showed that acetylation altered the starch polarity and leads to the introduction of hydrophobic group into the starch molecules.

3.3 Paste Viscosity and Clarity

The peak viscosity of APS was significantly higher than the cassava starches. As the temperature drops from 90°C to 70°C the viscosity remained fairly constant before it began to increase as the temperature falls below 70°C for both starches (Figs. 4A and B). The native starch showed higher paste viscosity than the acetylated starches when cooled to 30°C. The viscosity of cooked starch is important to the food industry. Both acetylated TMS 30572 cultivar and industrial starches showed lesser cooling viscosity suggesting reduced tendency towards retrogradation than the commercial acetylated potato starch (APS). Similar observation had been reported for sorghum starch where acetylation led to a decrease in the paste viscosity [23]. Substituent groups formed at lower temperature restricted the tendency of the starch molecules to realign after cooling, thus facilitating lower setback value and retrogradation for acetylated starches [25].

In attempt to determine the starch concentration that will match-up in hot paste viscosity to the acetylated potato starch, cassava starch at different concentration were acetylated at 2.5% and the hot paste viscosity determined at 90°C. At 5% starch concentration, the hot paste viscosity was lower than that of the acetylated potato starch at 5% concentration (Fig. 4C). However as the starch concentration increased, the hot paste viscosity also increased. The light transmittance values of the pastes increased

progressively with increase in the degree of acetylation for all the starches (Fig. 5). Increase in the degree of swelling and dispersion of the starch granules as well as reduced retrogradation tendency may also be responsible for the observed results [8,18].

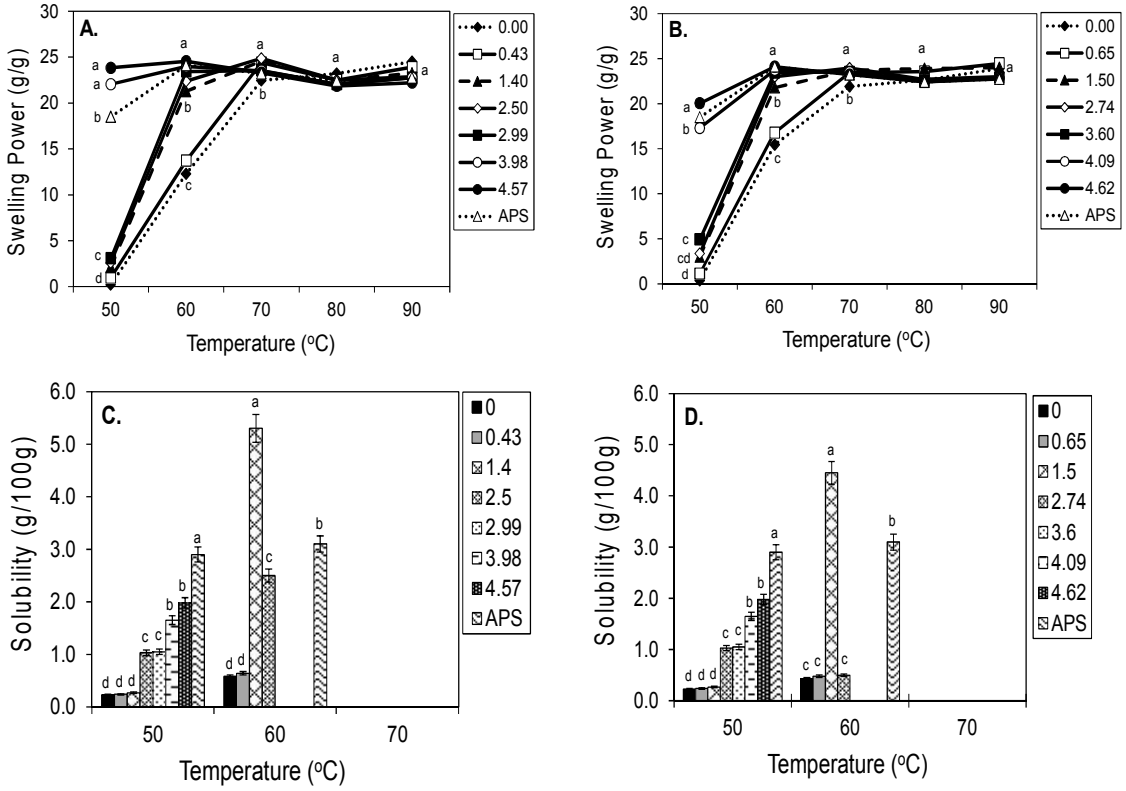

Fig. 2. Effect of temperature on the swelling power and solubility of cassava starches at different degrees of acetylation for TMS 30572 (A and C) and industrial starches (B and D). APS was used as control in the study. Values are reported as mean±SD, (n=3). Mean values with the same alphabet within a particular temperature are not significantly (p≤0.05) different according to Duncan multiple range test

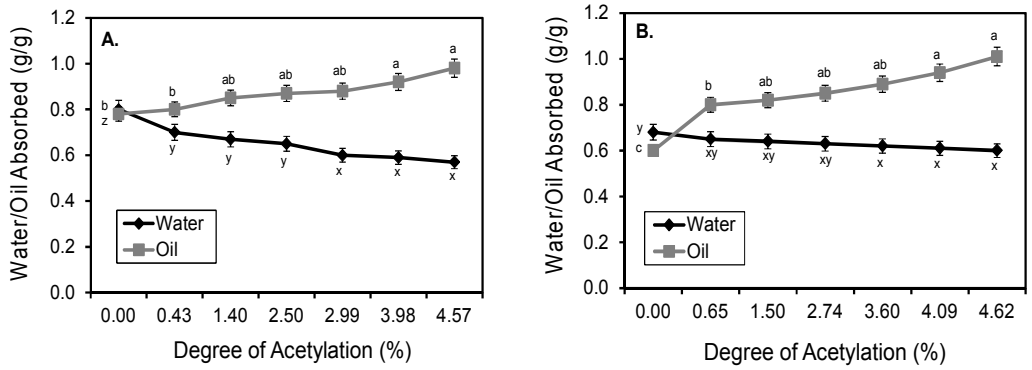

Fig. 3. Effect of acetylation on the water and oil absorption capacity of TMS 30572 (A) and industrial starches (B). Values are reported as mean±SEM, (n=3). Mean values with the same alphabet are not significantly (p≤0.05) different according to Duncan multiple range test

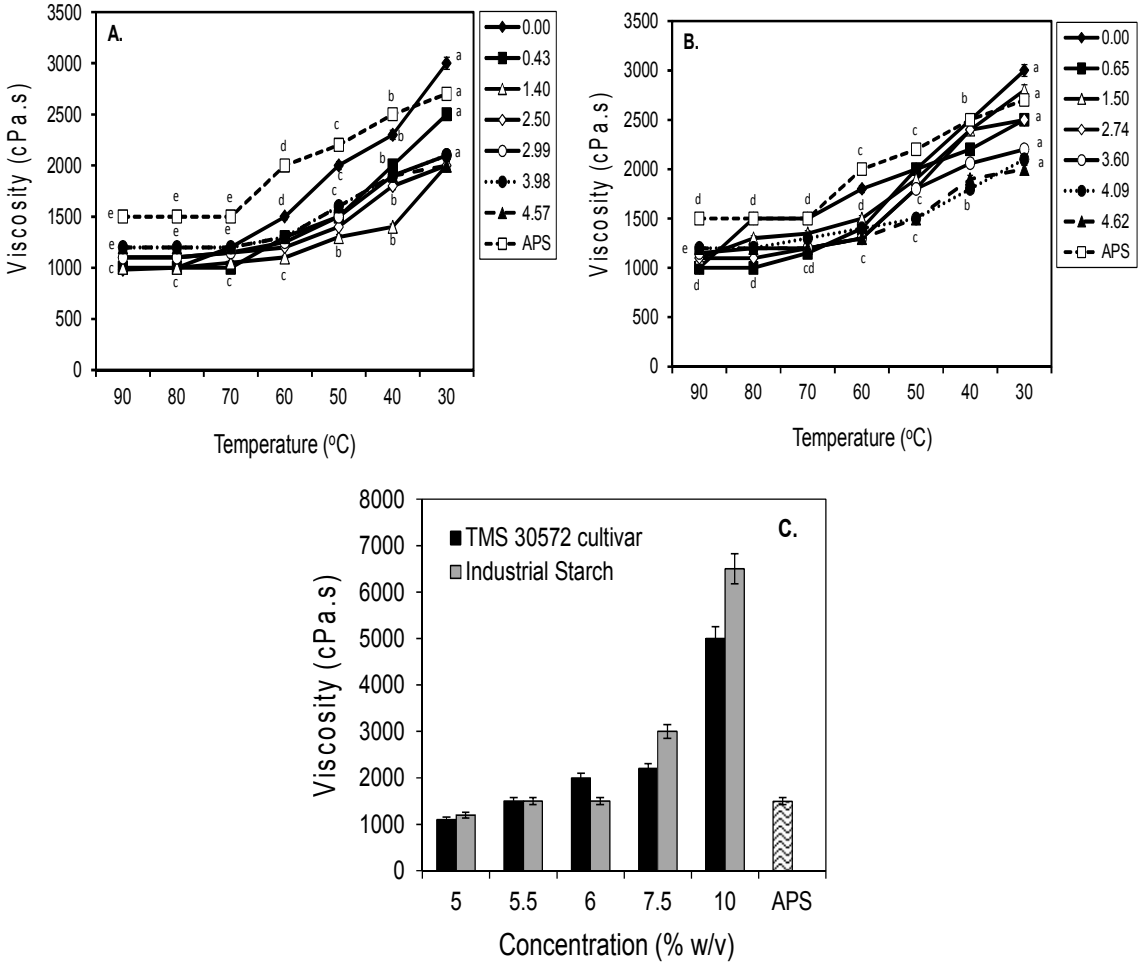

Fig. 4. Effect of temperature and starch concentration on paste viscosity in cassava starches (A) TMS 30572, (B) industrial starch at different degrees of acetylation, (C) effect of starch concentration on the paste viscosity of TMS 30572 starch acetylated at 2.5% and industrial starch acetylated at 2.74% compared with APS (acetylated potato starch) at 5% starch concentration. Values are reported as mean±SEM, (n=3). Mean values with the same alphabet across the temperature ranges are not significantly (p≤0.05) different according to Duncan multiple range test

3.4 Freeze-Thaw Stability

Pastes of roots and tubers starches are known to exhibit great stability under normal condition but cannot survive freeze-thaw cycles [26]. This poor freeze-thaw stability of native starch limits its application as a thickening agent in frozen foods [27]. Cassava starches exhibited unusual stability at higher degree of acetylation. TMS 30572 cultivar at acetylation level of 2.50% did not show any syneresis until the third freeze-thaw cycle when it exuded 13% water. At ≥3.98% degree of acetylation, there was no syneresis until the fourth freeze-thaw cycle with 16% exudates (Fig. 6). Waxy corn and Peruvian carrot starch gels exhibited similar freeze-thaw stability [27]. The percentage exudates decreased as the degree of acetylation increased. Jacobson et al. [28] have observed that in native pastes, the hydroxyl groups of starch gradually re-associate through hydrogen bonding and crystallize out of solution leading to syneresis when paste is allowed to cool or when refrigerated. Commercial acetylated potato starch exhibited the lowest stability with greater exudates due to syneresis when compared with all the acetylated cassava starches.

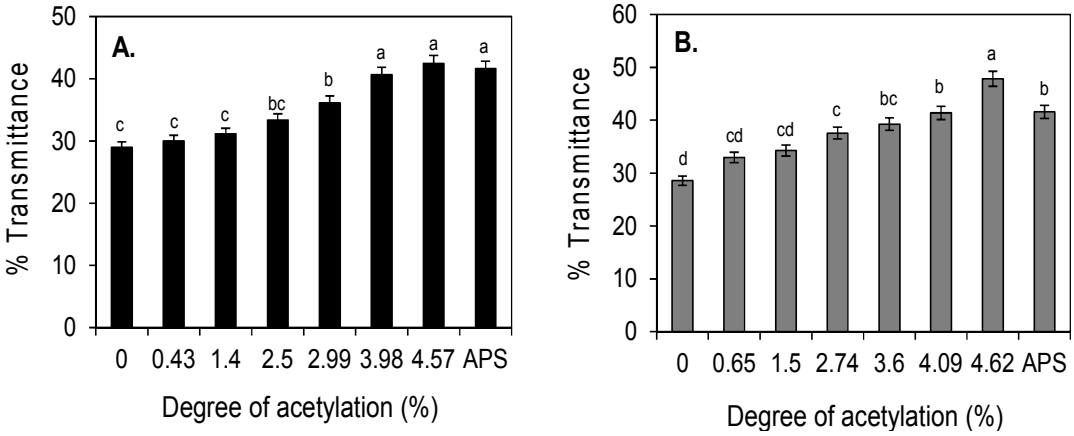

Fig. 5. Effect of acetylation on the paste clarity of laboratory (TMS 30572) starch (A) and industrial starch (B) measured as percentage transmittance. APS was used as control in the study. Values are reported as mean±SD, (n=3). Mean values with the same alphabet are not significantly (p≤0.05) different according to Duncan multiple range test

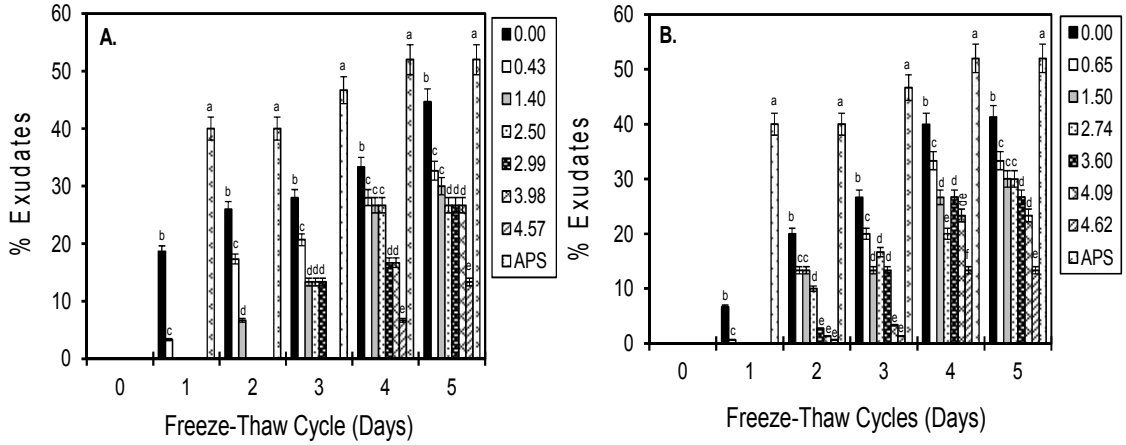

Fig. 6. Effect of acetylation on the freeze-thaw stability of laboratory (TMS 30572) starch (A) and industrial starch (B). APS was used as control in the study. Values are reported as mean±SD, (n=3). Mean values with the same alphabet at a particular day are not significantly (p≤0.05) different according to Duncan multiple range test.

4. CONCLUSION

Processing methods used for cassava starch isolation have influence on reaction efficiency of the final products. There was over 80% yield after acetylation, with improvement on the physical properties of native cassava starch. The functional properties such as swelling, solubility, viscosity, clarity, and freeze-thaw stability were also enhanced; making it a potential substitute to commercial APS in food system since acetylated starch has very low impact on the sensory properties of food. Also, due to increase in freeze-thaw stability, cassava starches with acetylation level ≥2.50% may serve as a good substitute for APS in frozen food industry.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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