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SUCCINYLATION OF POTATO (*Ipomoea batatas*) STARCH: EFFECT OF VARYING ETHANOL CONCENTRATION ON PASTING, STRUCTURAL, MORPHOLOGICAL, AND FUNCTIONAL PROPERTIES

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ABSTRACT

The shortcomings arising from usage of native starches in various industrial processes have demanded the need for modification in order to enhance its functionality. This study investigated the impact of ethanol concentration on pasting, structural, morphological, and functional properties of succinylated potato starch. Some granules of the potato starch sample also appeared to be multilobed and fractured. The Fourier transform infrared spectroscopy (FT-IR) study revealed the presence of carbonyl group (1747 cm^{-1}) in the starch chains as an additional functional group after succinylation. The statistical analysis of the study data revealed a significant ($p < 0.05$) decrease in protein, fat, fibre, ash, and reduction in moisture contents after modification. Analysis with visco-analyzer showed reduction in pasting parameters after modification. Modification of native potato starch with ethanol improved the functionality of the starch by imparting some additional physicochemical and functional properties. These improved properties upon modification, therefore, suggest some potential favorable qualities for special applications.

Keywords: Potato starch; succinic anhydride; ethanol treatment; functional properties.

INTRODUCTION

Like cellulose and chitin, starch is a polysaccharide carbohydrate with wide distribution in natural products (Mohamed, 2021). It is one of the most abundant polysaccharides, with distinct physicochemical properties. Due to its inexpensive price, it is frequently employed as a raw material in industries, renewability and biodegradability (Zhang *et al.*, 2017). As a natural polymer, the industry has made extensive use of native starch as packaging material and as a major raw material in the production of food products such as bakery products, pasta, tortillas, and snacks. Moreover, due to its functionalities, the usage of starch has been extended to the production of dairy products such as yogurt and dessert (Agama-Acevedo *et al.*, 2019; Magallanes-Cruz *et al.*, 2017). However, native starch has shown several disadvantages in its usage. These shortcomings include instability of paste under shearing, poor process ability, poor paste clarity, retrogradation, acid or freezing, and its solubility in common organic solvents, which have continued to limit its wide applications, especially in food industries (Zhang *et al.*, 2017). Hence, the need for modification to enhance functional properties. A number of studies have investigated the role of alcohol-mediated modification of native starches (Sun *et al.*, 2021; Kaveh *et al.*, 2019). Reports of one of these studies showed the preservation of the integrity of starch granules after alcohol-assisted treatment (Sun *et al.*, 2021). In this investigation, the influence of diverse alcoholic-alkaline treatments on the properties of tapioca starch was reported. Kaveh *et al.* (2019) reported enhancement in the functional properties, as well as other characteristics of the modified starch. The mucoadhesion of the tapioca starch was said to improve with increased alkali portion, while a negative association with increase ethanol content was observed. Zhang *et al.* (2017) also reported a marked improvement in the swelling properties of starch granules upon ethanol-heating treatment. According to their report, increase in heating temperature positively influenced the production of the non-crystalline starch granules. Acid hydrolysis of sorghum starch in the presence of

ethanol has reportedly showed a solubility as high as 58.4% and swelling power as low as 11 g/g (Singh *et al*., 2021). According to Kaur *et al*. (2011), alcoholic-alkaline treatment of sago and corn starches at 35 °C and constant ethanol concentration resulted in improved properties of the starch. Tao *et al*. (2021) also reported the role of alcoholic-alkaline treatment in inducing substantial changes in the physicochemical properties of waxy rice starch. According to the study of Chen *et al*. (2019), NaOH/urea aqueous system possibly altered the solubility factor of maize starch. In this study, reduction in the degree of crystallinity and the glass transition temperature were also reported.

Zhang *et al*. (2012) in their study described a method for preparing gelatinized starches. Starch granules were treated using aqueous ethanol at different ratios of starch to water to ethanol, followed by using a rotary evaporator to heat for the removal of ethanol. They obtained optimum gelatinization at starch/water/ethanol ratio of 1:3:6 for normal maize, potato and tapioca starches, while starch/water/ethanol ratios of 1:2.5:6.5 and 1:20:0 were found most suitable for waxy maize and high amylose maize starches, respectively. Sun *et al*. (2014) opined that the increased sugar alcohols content at 95 °C significantly decreased the swelling power of wheat starch.

However, there is currently a dirt of information on the use of intermediate concentration of alcohol to investigate the functional properties of starch. Thus, the purpose of this investigation was to ascertain the different concentrations of ethanol, a monohydric alcohol, on the structural, pasting, morphological and functional properties of succinylated potato starch.

MATERIALS AND METHODS

Materials

Tubers of potato (*Ipomoea batatas*) were bought in a food market located in Ibadan, Oyo State, Nigeria. The tubers were thoroughly washed with water and then carefully selected to ensure that only healthy samples, that is tubers without any indication of spoilage were used. The reagents used in this study were all analytical grade.

Isolation and purification of starch

The potato starch was isolated according to Awokoya (2011) with slight modification as illustrated in Figure 1. Potato tubers weighing 49.5 kg were peeled by hand, water-washed, and grated. In order to separate the pulp's starch from the grated pulp, the mixture was then suspended in 7 L of distilled water for 5 hours. To preserve the fiber, a sieve was used to separate the pulp in suspension through a muslin cloth. The starch-adhesive fiber residue was rewashed once more, and then it was thrown away. To allow for sedimentation, the obtained starch was left for 12 hours. The starch was produced after the decanted supernatant was washed twice after removal with 7 L of distilled water to get rid of the proteins and fiber (which was purified by re-suspension in distilled water). After another 12 hours of sedimentation, the starch was decanted. In an airtight plastic bag, starch was put and left at room temperature (approximately 27 °C) lasted for approximately 48 hours before being milled into a powder form.

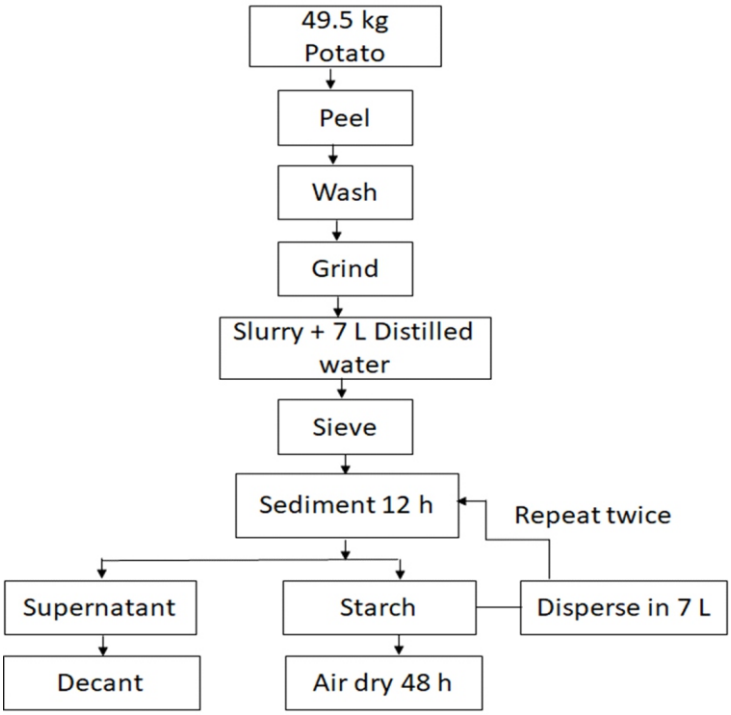

Figure 1: Schematic diagram for extraction of potato starch.

Preparation of starch succinate

Starch succinate was prepared as described by Sathe and Salunkhe (1981) with slight modification. Starch (100 g) was weighed into a 300 mL distilled water and magnetically stirred at 200 rpm for 1 h. With the use of 1 M NaOH, pH of the slurry was adjusted to 9.0. The suspended starch was treated with 5 g succinic anhydride at a controlled pH range of 8.0 – 9.0 over a period of 2 h to obtain starch succinate. The succinylated starch was designated as SCCS. The pH obtained slurry was next brought down to 4.0 using 0.5 M hydrochloric acid. The slurry was filtered and given six rounds of distilled water washing. The leftover modified starch was allowed to air dry for 36 h.

Preparation of ethanol induced succinylated starch (EISS)

Ethanol induced succinylated starches were prepared by treatments with aqueous ethanol at a high temperature (40 °C) according to the previous procedure (Zhang *et al.*, 2017), with a few changes. Fifteen grams (15 g) of SCCS starch was added to 400 mL different concentrations of ethanol solution [5 to 60% (v/v)] in a three necked round bottom flask equipped with moto-driven stirrer and heated for 2 h in a water bath set at 60 °C. One drop of 0.1 M H_2SO_4 solution was added to the suspension to act as catalyst. The suspensions were then left at room temperature for 60 min. The suspensions of starch were filtered. With 1 L of ethanol, the residue was once more washed three times. The finished starch was applied to a paper filter sheet, allowed to air dry for eight hours at room temperature, was sieved (through mesh size: 150 μm), and then was put in airtight plastic containers for storage. The ethanol induced succinylated starches (EISS) were designated as EISS-5, EISS-10, EISS- 20, EISS-40, and EISS-60. Native and untreated potato starch was used as control (NTS).

Functional and Physicochemical Properties of Starch

Proximate analysis

The method described by Standard Association of Official Analytical Chemistry (AOAC) (1996) was chosen for the estimation of the moisture content, carbohydrate, crude protein, total ash, crude fibre and crude fat. Percentage (%) carbohydrate was calculated according to Equation 1.

[100 – (% moisture + % protein + % fat + % crude fibre + % ash)] 1

Cold-water solubility

To assess the cold-water solubility (S_C) of both native and modified starches, Zhang *et al.* (2017)'s method was used with a minor modification. During a 20-minute period, one gram (1 g) of potato starch was consistently magnetically stirred at a speed of 200 rpm in 100 mL of distilled water. Using a table-top centrifuge, the suspension was spun at 6000 rpm for 10 min. The leftover material was baked to a consistent weight at 110 °C, and the supernatant was then decanted. The S_c of the starch was estimated according to Equation 2:

$$S_C = \frac{m_2}{m_1} \times 100\% \qquad 2$$

where m_1 and m_2 represent the total dry weight of the sample and the supernatant, respectively.

Effect of ethanol concentration on gelation

The procedure described by Coffman & Garcia [1977] was adopted to determine the least gelation concentration. In separate test tubes, 5 mL of distilled water was used to scatter samples of starch ranging in weight percentage from 2 to 18% w/v. The Whirlmix mixer (Cenco Instrumenten, Breda, The Netherlands) was used to thoroughly mix the starch dispersions for 10 minutes. After that, the material was heated for 1 h at 80 °C in a water bath. For 2 h, the samples were rapidly chilled in cold water. The least gelation concentration (LGC) was found to be the lowest concentration when the substance from the inverted test tube did not drop or slip.

Paste clarity and swelling power

The procedure outlined was used to measure the clarity of the paste by Rafiq *et al.* (2016) with slight modification. A 1% [w/v] starch slurry was made with distilled water and cooked for 30 min. while being constantly stirred in a boiling water bath. The slurry was chilled for 1 h at ambient temperature (around 27 °C). By employing a UV-vis spectrophotometer (Shimadzu UV-Vis-1800 Spectrophotometer, Canby, Oregon, USA) at a wavelength of 650 nm and a distilled water blank, the clarity was assessed.

Weighing 0.1 g of dry starch into a clean, dried test tube allowed us to measure the starch's ability to swell. The test tube's contents were mixed with a Whirlmix mixer for 50 seconds after 10 mL was infused with distilled water. Afterward, the test tube was heated in a thermostatic water bath for 30 minutes at 50, 60, and 70 °C while using fresh 0.1 g dry starch at each temperature (GFL, Burgwedel, Germany). This was followed by cooling to room temperature (~27 ℃) and centrifugation at 6000 rpm for 10 min. The supernatant was carefully removed and the test tube with its content was weighed. The swelling power was determined according to Equation 3.

$$Swelling\ of\ starch = \frac{W_2 - W_1}{Weight\ of\ starch\ (g)} \qquad 3$$

where W_1 represents the weight of test tube + dry starch and W_2 represents the test tube + residue and the water it retained.

Pasting characteristics

Olatunde *et al.* (2017)'s method was slightly modified to ascertain the pasting characteristics of NTS (control) and EISS samples using a Rapid Visco Analyzer (RVA Super4 model, Perten Instruments, Kurva, Sweden). The exact sample weight and adequate water volume required for the test was first estimated by determining the moisture content of the starch sample. Accurately weighed amount (3 g) of starch (NTS or EISS) was placed in a RVA aluminum test canister and 25 ± 0.1 mL of distilled water was added. At a constant shear rate, an automatic heating and cooling cycle was used. The temperature of the starch sample was held at 50 °C for one minute during this procedure. This was followed by heating to 95 ℃ within 3 min. After another 2 min of maintaining the temperature at 95℃, the sample was cooled to 50 ℃ within 3 min and kept at 50 ℃ for 2 min. Both numerical and graphical readings were shown on the monitor. Variables like the pasting temperature, breakdown point, peak viscosity, final and setback viscosities were recorded. The viscosities were estimated in centipoises.

Emulsifying properties

The method of Chen *et al.* (2019) was employed in determining the emulsion stability (ESI) and emulsifying activity (EAI) of the EISS samples. An 8.0 mL of the 2.0 mg/mL EISS sample solution and 2.0 mL of soybean oil were combined in 15 mL centrifuge tubes to create the starch emulsions. The mixture was homogenized

for 5 min at 6000 rpm in a Whirlmix mixer. A pipette was used to remove two 50 µL cuvettes of the emulsion at varying periods as follows: the first aliquot was taken immediately after homogenization, and the second aliquot was taken after the emulsion had been left undisturbed at about 10 min. The 0.1% sodium lauryl sulfate (SLS) solution in 4.95 mL was used to immediately dilute each sample. A cuvette with a 1 cm path length and 2.0 mL of the resultant solution were employed to test the absorbance at 500 nm using a UV-vis spectrophotometer (Shimadzu UV-Vis-1800 Spectrophotometer, Canby, Oregon, USA). Equations 4 and 5 were used to estimate the ESI and EAI, respectively:

$$EAI\left(\frac{m^2}{g}\right) = \frac{2 \times 2.303}{C \times (1-\emptyset) \times L \times 10^4} \times A_0 \times D \qquad 4$$

$$ESI(\%) = \frac{A_{10}}{A_0} \times 100 \qquad 5$$

where A_0 and A_{10} are the absorbances (measured at 500 nm) at time 0 and after 10 min, respectively. In the experiment, C stands for the oil-volume fraction of the emulsion (0.2 v/v), Ø for the oil weight/unit volume (g/mL), L for the optical route, and D for the dilution (100 in the experiment)

Determination of the blue value (BV) and colour of EISS – iodine complex

The BVs of NTS and each EISS sample were determined by UV-vis spectrophotometry according to the method of Morrison & Lainglet (1983), with slight modifications as described by Klucinec & Thompson (1998) and Gilbert & Spragg (1964). Dry starch (40 mg) was weighed and dispersed in 10 mL dimethyl sulfoxide (DMSO) containing 10% of 6 M urea. Aliquot 1.0 mL of each sample was placed in a 100 mL volumetric flask, to which 95 mL of distilled water and 2 mL of an aqueous I_2 - KI solution was added (the latter solution was prepared with 200 mg of I_2 and 2 g of KI in 100 mL of distilled water). The mixtures were stirred thoroughly and left for 20 min for full development of colour. The absorbance reading was measured at 620 nm. Visual monitoring of the colour of the ethanol induced succinylated starch – iodine complex was also performed. The same procedure was repeated for the blank (starch was absent in the blank). Measurements were obtained in triplicates and the BV was estimated using Equation 6 as described by Gilbert and Spragg, 1964.

$$Blue\ value = \frac{Absorbance\ at\ 620\ nm \times 4}{Concentration\ (\frac{mg}{dl})} \qquad 6$$

Microscopic appearance and granule size

To determine the shape of the starch grains, scanning electron microscopy (SEM) and light microscopy (LM) were also utilized. Utilizing a double-layer mechanical stage and an 18-megapixel digital camera, typical optical microscopy was used to obtain light micrographs of the starch samples (OMAX trinocular light microscope A35140U, China). Analysis of the starch granules size was performed as described by Awokoya *et al.* (2020). Samples were viewed through a coverslip under 40 × objectives. A small layer of starch grains was dusted onto a carbon sticker to prepare the sample for SEM. It was subsequently given a 30-min gold coating using a sputter coater (Balzers Union, FL-9496). Images were captured using a VegaTescan equipped with an EDS and a SEM INCAPentaFET 3. (Oxford ISIS, JSM-7900F).

FTIR spectroscopy of NTS and EISS samples

A SHIMADZU Spectrum FTIR-8400S spectrometer with an AutoIMAGE System was used to obtain the FTIR spectra of the NTS control and EISS samples. After synthesizing the starch sample with KBr, pellets were produced. In the frequency range of 4000 - 400 cm^{-1}, attenuated total reflectance spectroscopy was carried out on the pellets.

Statistical analysis

Using SPSS, statistical analyses of the study data were conducted, and the results were presented as the mean value and standard deviation. Using a statistical software suite written in the R programming language, data were subjected to analysis of variance (ANOVA) (R Core Team). Using a significance test threshold of 5% ($p < 0.05$), Duncan's multiple range test was also utilized to distinguish the significant means from the ANOVA.

RESULTS AND DISCUSSION

Characterization of ethanol induced succinylated starch

Scanning electron microscopy

The SEM micrographs of NTS and different EISS granules are shown in Fig. 2. The surface of native potato starch was principally smooth and oval in shape without cracks. This morphology is in agreement with the reports of Awokoya *et al.,* 2020; Cira-Chávez *et al*., 2021; Zhang *et al*., 2020. Also, the granules of EISS-5 did not show any crack, which was evidence of low-concentration ethanol treatment. However, with increasing ethanol concentration, EISS-10 and EISS-20 granules showed some indentations in their appearances, with EISS-20 more indented compared to EISS-10. This result is in agreement with the report of Gonzalez *et al.* (2018) for rice bean starch extracted with ethanol. Furthermore, with increase in ethanol concentration from 20 to 60%, the surface structure became elongated and multi-lobed. At these concentrations, the starches appeared to show apparent changes in granular size and shape. These changes could be attributed to slight granular contusion during the modification process. This report finds similarity to the observation of Thuy *et al.* (2020) who ascribed the increased granular size of acid-alcohol treated Jackfruit seed starch to swelling of granules during modification.

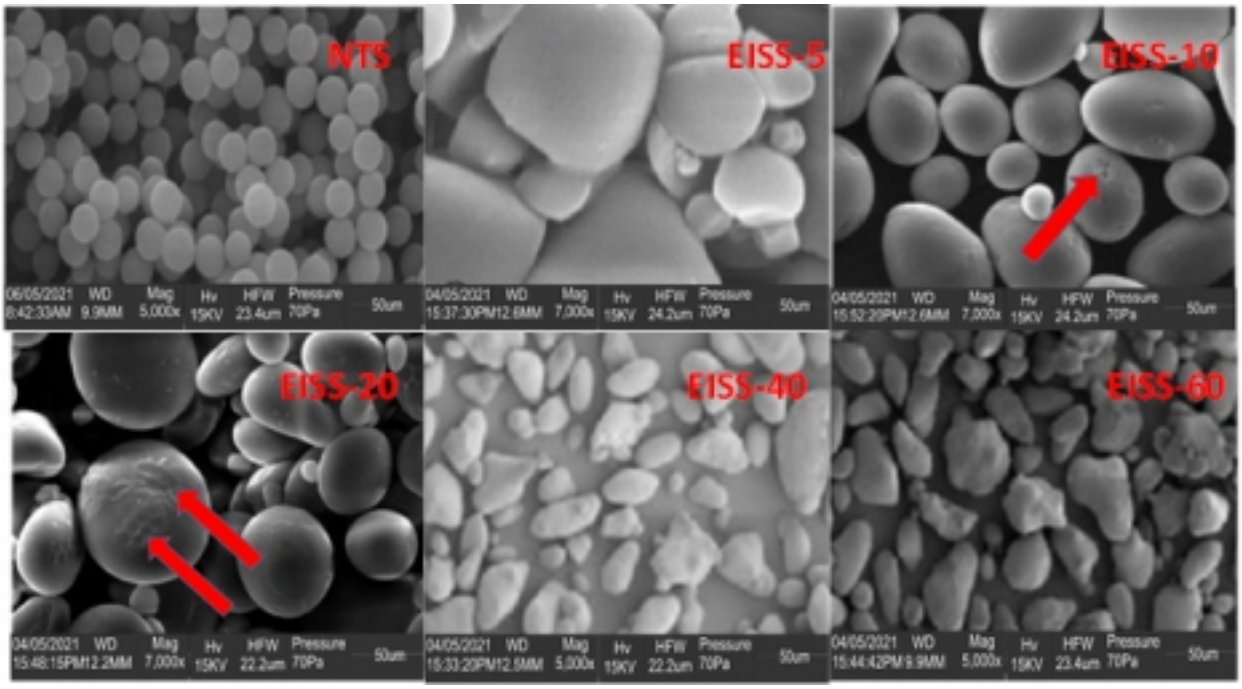

Figure 2: SEM micrographs of native and different EISSs.

Light microscopy

The micrographs observed via light microscopy are presented in Figure 3. From the micrographs, the control native sample showed an oval or kidney structure. The granule surface is relatively smooth and free from pores, crannies or fractures. There were no apparent changes on the surfaces of EISS-5, EISS-10 and EISS-20 treated starch granules, except that the agglomerates were observed in granule surfaces, which was in agreement with previous work (Buffo *et al.,* 2002). For EISS-40 and EISS-60 granules, a very different morphologies were observed. It seemed that starch granules appeared fractured, which contributed to the rough surface, compact, smaller size and alveolate shape of both EISS-40 and EISS-60 granules, corroborating the results obtained by SEM. In addition, the changes in size, shape and surface wrinkling/roughness patterns of the granules at these concentrations were most likely caused by the expansion of the granule under the influence of ethanol.

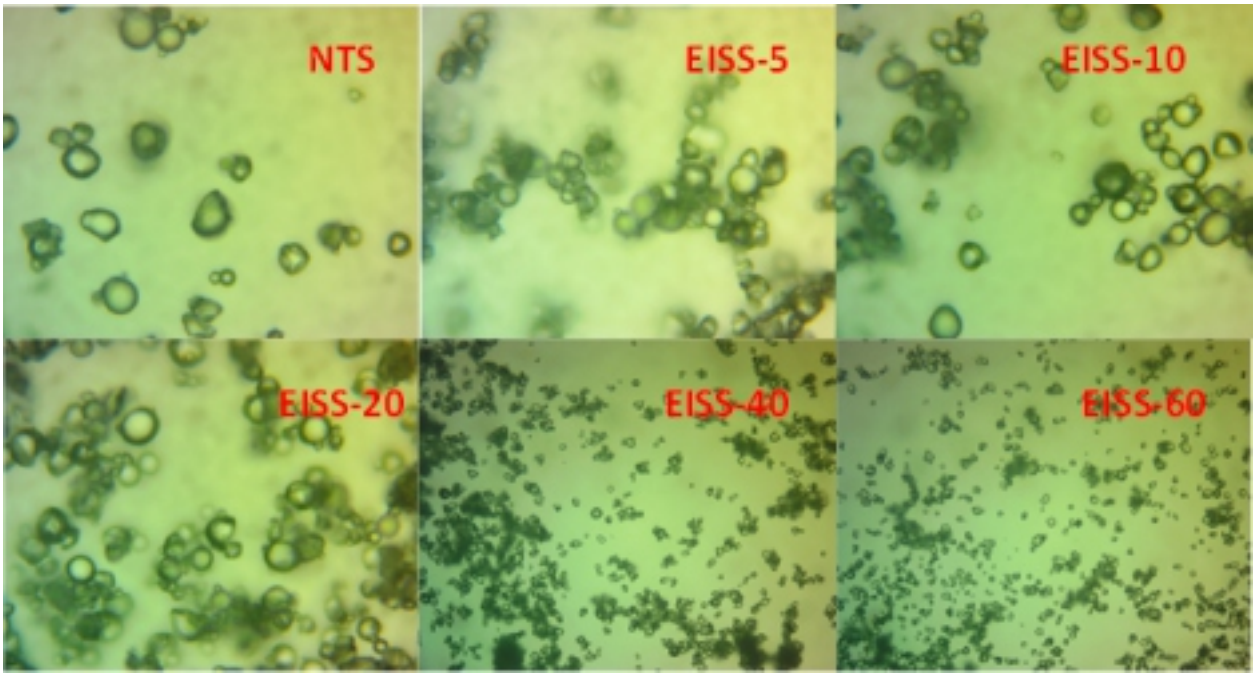

Figure 3: Standard optical light micrographs of native and different EISSs at 40.

FTIR spectroscopy

Figure 4 shows the FTIR spectra of NTS and EISSs. The strong and broad band observed in the range of 3600-3100 cm^{-1} could be ascribed to the stretching vibration frequency of the –OH group in glucose residues of the starch molecule, and the extent of inter- and intra-molecular hydrogen bond formation is indicated by the peak width. All starch samples revealed a distinct band around 1028 cm^{-1}, which could be attributed to the angular deformation of C-O-H and C-C bond, or skeletal vibration of α-(1→4) glycosidic linkage (Shi *et al.,* 2018; Awokoya *et al.,* 2020). The band between 1180 and 1068 cm^{-1} has been attributed to the presence of amylopectin within the starch granules. The band between 1643 and 1630 cm^{-1} could be due to vibrations of the water molecules absorbed in the noncrytalline region of the starch (Kizil *et al*., 2002). Also, the band at 538 cm^{-1} in all the samples could be ascribed to the skeletal vibrational mode of the pyranose ring (Kizil *et al*., 2002). Compared to native starch, the new absorption band at 1747 cm^{-1} in all the EISS samples confirmed the presence of carbonyl vibration of an ester group. This new absorption band confirms the formation of succinylated starch products during the esterification process (Chi *et al.,* 2008).

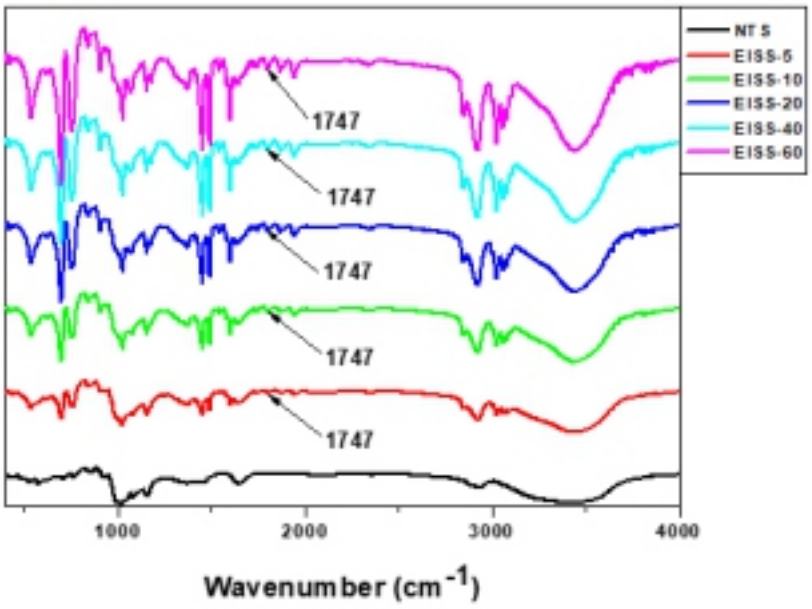

Figure 4: FTIR spectra of native and different EISSs.

Proximate analysis of NTS and EISS samples

The results of proximate analysis of NTS and EISS samples of *Ipomoea batatas* are as shown in Table 1. The ash content of native starch was 0.86% and in the range of 0.25 – 0.67% for EISS samples. With an increase in concentration of ethanol, ash content in EISS samples decreased from 0.67(EISS-5) to 0.25(EISS-60). This suggests the leashing in weight of product at the expense of minerals originally in the native starch. Similar results were reported for buckwheat and maize starches in a study conducted by Goel *et al.* (2020) and Awolu *et al.* (2020). The moisture contents of the EISS samples increased significantly ($p < 0.05$) compared to that of native starch. The amount of moisture in the starches ranged between 13.07 to 15.41%. It is worth noting that the moisture content of EISS-40 and EISS-60 increased more significantly, possibly due to the hydrophilic synergistic power of ethanol. This is in conformity with the report of Sahnoun *et al.* (2016) in their evaluation of enzymatically hydrolysed, acetylated and dually modified corn starch. There were reductions in fibre, fat and protein contents of EISS samples in comparison to native starch, although the values were not significantly different. The obtained values for percentage protein, fat, fibre and ash for all the samples were less than 1%. These results demonstrated high level of purity of the starch fractions (Lawal *et al.,* 2004).

Table 1: Proximate analysis of NTS and EISS samples.

Starch	Parameter (%)						
	Protein	Fat	Fibre	Ash	Moisture	Carbohydr.	Dry matter
NTS	0.69±0.02^{a}	0.31±0.01^{a}	0.72±0.01^{a}	0.86±0.02^{d}	13.07±0.01^{a}	84.35±0.02^{a}	86.93±0.05^{a}
EISS-5	0.47±0.02^{a}	0.23±0.01^{a}	0.59±0.02^{a}	0.67±0.03^{a}	13.69±0.03^{f}	84.35±0.07^{d}	86.31±0.04^{a}
EISS-10	0.29±0.01^{a}	0.17±0.03^{a}	0.42±0.01^{a}	0.51±0.01^{a}	14.21±0.02^{b}	84.40±0.04^{b}	85.79±0.01^{a}
EISS-20	0.21±0.01^{a}	0.14±0.01^{a}	0.35±0.02^{a}	0.43±0.01^{a}	14.67±0.01^{c}	84.20±0.03^{b}	85.33±0.01^{a}
EISS-40	0.15±0.02^{a}	0.11±0.03^{a}	0.28±0.01^{a}	0.31±0.21^{a}	15.05±0.02^{e}	84.10±0.14^{f}	84.95±0.03^{a}
EISS-60	0.08±0.02^{a}	0.05±0.01^{a}	0.13±0.01^{a}	0.25±0.16^{a}	15.41±0.02^{e}	84.08±0.04^{b}	84.59±0.02^{a}

All values are expressed as mean ± standard deviations (n=3). Each column with different superscripts (a, b, c, d, e, f) indicate significant differences between samples, ($P < 0.05$).. NTS: Native potato starch; EISS: Ethanol induced succinylated starch at different concentrations; EISS-5, EISS-10, EISS-20, EISS-40, and EISS-60; Carbohydr: Carbohydrate.

Cold-water solubility

Cold water-solubility, S_c is a measured of un-sedimented soluble molecules and highly swollen granules, when subjected to moderate centrifugation (Rajagopalan and Seib, 1992). The cold water solubilities of native and EISSs are shown in Fig. 5. The results showed increase in S_c with increasing ethanol concentration. The S_c of potato native starch prior to modification was only 0.4%. However, higher solubility values of 1.3, 3.5, 4.8, 6.1, and 7.7% for EISS-5, EISS-10, EISS-20, EISS-40 and EISS-60, respectively were recorded after modification. The consequential increase in S_c values of starch following modification could be due to the presence of more hydrophilic free and active hydroxyl groups, induced by increment in ethanol concentration. The ethanol must have induced a weakening effect on the intermolecular binding forces of the starch, consequently facilitating the leaching of granular particles of the starch. Amylose content, as well as crystalline structure of starch has been reported to have influences on its cold water solubility (Jivan *et al.,* 2014). According to Kim (1987), more water is generally absorbed by starch particles with low crystallinity in comparison with those with high crystallinity. In this work, low crystallinity was observed in the EISSs. Therefore, their high cold-water solubility could be attributed to the interruption of the samples crystalline structures, as a result of the alcohol-induced treatment which permitted greater interactions between the starches chains and water molecules. Hu *et al.* (2016) also reported similar observation in their investigation of using octenyl succinic anhydride modified starch.

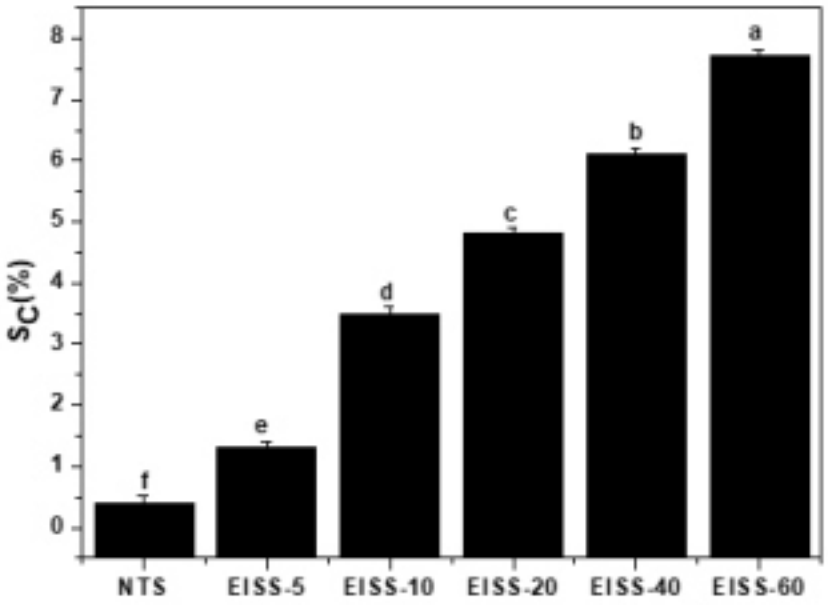

Figure 5: Cold-water solubility of native starch and EISSs. The different lowercase letters (a–f) marked above the bars indicate statistical differences ($p < 0.05$).

Gelation properties

The tendency of starch to form gel is a beneficial quality in food industries. This parameter is determined by measuring the Least Gelation Concentration (LGC) of the starch. In this study, the LGC of NTS, EISS-5, EISS-10, EISS-20, EISS-40, and EISS-60, were found to be 10, 8, 8, 6, 2, and 2, % (w/v), respectively (Table 2). The result obtained revealed significant reduction in LGC after modification. Generally, the lower the LGC of a starch, the better its gelation property. Hence, native potato starch with LGC of 10%, will require high starch concentration to form a gel, whereas both EISS-40 and EISS-60 which formed gels at a very low concentration of 2% will need small amount of starch to form gels. The gel strengths of the EISS samples may be attributed to the rigidity provided by disruption of its crystalline nature, especially, interruption in the amylopectin architecture aided amylopectin retrogradation. In earlier studies, Awokoya *et al.* (2020) and Lawal *et al.* (2004) also reported reduction in LGC following modification.

Table 2: Gelation characteristics of NTS and EISS samples (on dry basis).

Concentration (% w/v)	Starch sample					
	NTS	**EISS-5**	**EISS-10**	**EISS-20**	**EISS-40**	**EISS-60**
2	-Liquid	-Liquid	-Liquid	-Viscous	+Gel	+Gel
4	-Liquid	-Viscous	-Viscous	-Viscous	+Gel	+Gel
6	-Viscous	-Viscous	-Viscous	+Gel	+Firm gel	+Firm gel
8	-Viscous	+Gel	+Gel	+Gel	+Firm gel	+Firm gel
10	+Gel	+Gel	+Gel	+Firm gel	+Firm gel	+Firm gel
12	+Gel	+Firm gel	+Firm gel	+Firm gel	+Firm gel	+Firm gel
14	+Firm gel	+Firm gel	+Firm gel	+Firm gel	+Firm gel	+Firm gel
16	+Firm gel	+Firm gel	+Firm gel	+Firm gel	+Firm gel	+Firm gel
18	+Firm gel	+Firm gel	+Firm gel	+Firm gel	+Firm gel	+Firm gel
LGC[a]	10	8	8	6	2	2

[a]Least gelation concentration.

Clarity and swelling power of starch

Paste clarity is considered as a crucial functional property of starch in the production of some food products. Here, the assessment of paste clarity (measured as light transmittance at 650 nm) of potato starch gels was observed to gradually decrease after modification (Figure 6). This may be traceable to the formation of gels from the joining of hydroxyls groups between starch chains and ethanol through hydrogen bonds, a mechanism known as retrogradation. Also, a higher transmittance value (86.9%) was observed for native potato starch, indicating the highest transmittance value, when compared to modified starch pastes. This result found merit in the report of Jacobson *et al.* (1997), who attributed their observed reduced starch paste transmittance to a number of factors, which include granular swelling, leaching of amylose and amylopectin chains, and amylose and amylopectin chain lengths. Among the modified starches, the highest opaque value (lowest transmittance value) was observed in EISS-60 (31.5%), whilst the lowest was observed in EISS-5 (48.7%). These values are consistence with those reported for potato starch by Awokoya *et al.*, (2020). The modified starches gave lower transmittance values in relation to the native starch. The swelling powers of native and EISS samples at 50 ℃, 60 ℃, and 70 ℃ are summarized in Table 3. The EISSs exhibited lower swelling power in all cases when compared to NTS. It was also observed that the swelling power has an inverse relationship with temperature, decreasing from 2.91 to 2.22, 1.97 to 1.42, and 1.39 to 0.61 g/g for 50 ℃, 60 ℃, and 70 ℃, respectively. EISS-60 had the least swelling power at all temperatures, suggesting the presence of strong forces that binds the granules, consequently enabling them to resist swelling. Ealier study (John *et al.*, 2002), attributed the reduction in swelling power of modified starches to increase in high proportion of soluble dextrin of both small and medium chain lengths in starch granules

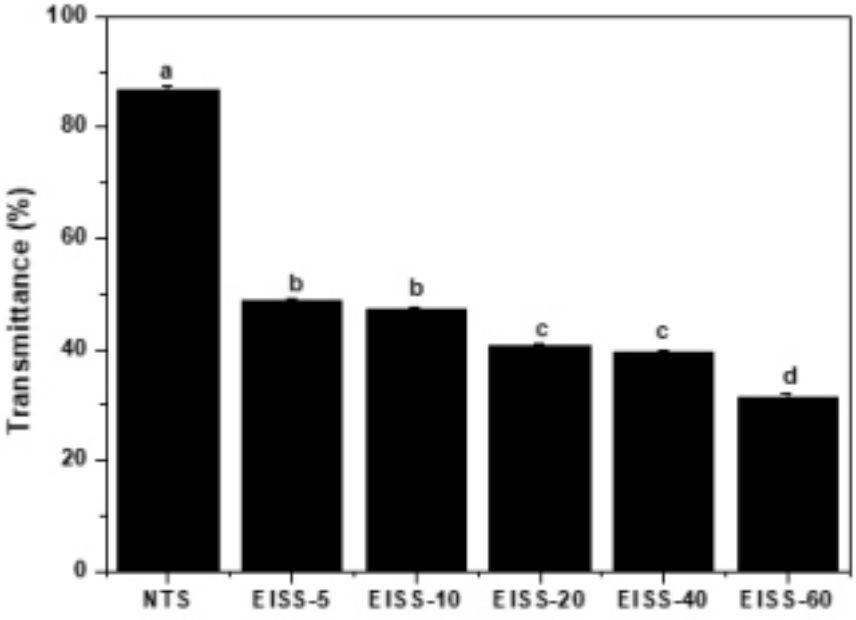

Figure 6: Paste clarity of native starch and EISSs. The different lowercase letters (a–d) marked above the bars indicate statistical differences ($p < 0.05$).

Table 3: Swelling power of NTS and EISS samples.

Starch	Swelling power		
	50 °C	60 °C	70 °C
NTS	2.91±0.03^{c}	1.97±0.03^{c}	1.39±0.03^{e}
EISS-5	2.78±0.02^{a}	1.87±0.01^{d}	1.28±0.02^{a}
EISS-10	2.76±0.01^{a}	1.83±0.03^{a}	1.18±0.01^{a}
EISS-20	2.56±0.01^{a}	1.62±0.01^{a}	1.11±0.02^{f}
EISS-40	2.34±0.02^{b}	1.51±0.03^{a}	1.06±0.01^{a}
EISS-60	2.22±0.02^{b}	1.42±0.01^{a}	0.61±0.01^{a}

All values are expressed as mean ± standard deviations (n=3). Each column with different superscripts (a, b, c, d, e, f) indicate significant differences between samples, ($P < 0.05$). NTS: Native potato starch; EISS: Ethanol induced succinylated starch at different concentrations; EISS-5, EISS-10, EISS-20, EISS-40, and EISS-60.

Pasting properties

The pasting parameters of the native potato and modified starches are shown in Table 4. Concentration of ethanol at varying degrees affected the pasting properties of the potato starch. In addition, as the concentration of the ethanol was increased, a progressive decrease in trough, peak and final viscosities of the potato starch was observed. This decline in peak viscosity could be due to the reduction in swelling power of the starch after modification. However, the breakdown value was largest at 10% alcohol concentration, indicating the ease of retrogradation of potato starch at that alcohol concentration above others (Sun *et al*., 2021). Also, the EISS-60 sample displayed the lowest setback value but highest pasting temperature. Notably, the range of the pasting temperatures of all the samples was from 72.6 to 81.4 °C. These values are quite high when compared with those reported for other tuber and root starches (Hoover, 2001). This could be ascribed to the existence of a strong bonding forces within the interior of the starch granule. In addition, the increment in pasting temperature of the starch after modification gives credence to the notion that modified starches exhibited greater resistance towards swelling and rupture.

Table 4: Pasting properties of NTS and EISS samples.

Parameter	Starch sample					
	NTS	**EISS-5**	**EISS-10**	**EISS-20**	**EISS-40**	**EISS-60**
Peak viscosity (cP)	8080.2	7977.8	7583.5	6600.6	5766.8	4499.7
Trough viscosity (cP)	4846	3972.3	2433.7	2010.3	1999.6	1409
Breakdown viscosity (cP)	234.2	4005.5	5149.8	4590.3	3767.2	3090.7
Final viscosity (cP)	11288	9889.9	7799.8	7739.8	7006.1	5558.1
Setback viscosity (cP)	6442	5917.6	5366.1	5729.5	5006.5	4149.1
Pasting temperature (°C)	72.6	74.9	76.5	77.7	78.9	81.4

Emulsifying properties

EAI and ESI are indexes used in expressing the emulsifying properties of emulsifiers at the water-oil interface. In this study, the emulsifying properties were conducted to observe the coarse emulsion separation by monitoring the visible boundary formation. As presented in Table 5, the EAI of the starches ranged between 0.84 and 2.42 m^2/g, indicating a significant increase ($P < 0.05$) of the EAI after modification. Additionally, with increasing ethanol concentration from 5 to 60%, the EAI of the EISS samples increased at first, and then decreased significantly ($P < 0.05$). For example, EISS-20 exhibited the highest EAI (2.42 m^2/g), while that of EISS-40 and EISS-60 were 1.99 and 1.69 m^2/g, respectively. The result of a previous study showed the direct dependence of the ability of an emulsion on protein (as a result of rapid adsorption, unfolding and reorientation of the protein) at the oil-water interface (Carvalho *et al.,* 2006). In addition, for EISS-40 and EISS-60 samples, multi-lobed aggregates could be said to have accounted for the lower EAI values compared to EISS-20. A similar observation was reported by Feng *et al.* (2021), for whey proteins isolates. Also, the ESI of native starch was 58.90%, while that of EISS increased to 73.47, 79.76, 96.61, 81.16, and 80.56% for EISS-5, EISS-10, EISS-20, EISS-40 and EISS-60, respectively. Among all the samples analysed, the emulsifying stability of EISS-20 was found to be significantly higher which is consistent with the results obtained for emulsifying activity.

Table 5: EAI and ESI of NTS and EISS samples.

Samples	$EAI\ (\frac{m^2}{g})$	*ESI* (%)
NTS	0.84 ± 0.49^{c}	58.90 ± 1.41^{b}
EISS-5	1.04 ± 0.21^{b}	73.47 ± 2.13^{b}
EISS-10	1.24 ± 1.30^{b}	79.76 ± 1.71^{a}
EISS-20	2.42 ± 0.23^{a}	96.61 ± 2.41^{a}
EISS-40	1.99 ± 0.33^{a}	81.16 ± 1.21^{b}
EISS-60	1.69 ± 0.18^{b}	80.56 ± 2.01^{b}

All values are expressed as mean ± standard deviations (n=3). Each column with different superscripts (a, b, c) indicate significant differences between samples, ($P < 0.05$).. NTS: Native potato starch; EISS: Ethanol induced succinylated starch at different concentrations; EISS-5, EISS-10, EISS-20, EISS-40, and EISS-60.

Iodine staining

The blue value and the colour of starch-iodine complex are shown in Table 6. A general reduction in blue value was noticed with increase in ethanol concentration. This suggests a gradual flexibility of amylose as the ethanol concentration increased. When starch is derivatized, it tends to have the ability to take on different conformations within the granules, and since starch retrogradation is a process mainly associated with amylose molecule, the effective changes must have been those related to amylose units. The obtained values indicated that the screw-shaped architecture or arrangement of amylose in solution is responsible for heterogeneous formation with iodine, and was progressively damaged with increase in ethanol concentration (Nwokocha and Ogunmola, 2014).

Table 6: Blue value and EISS – iodine complex colour.

Samples	Blue value	Starch – iodine colour
NTS	0.772 ± 0.04^{a}	Blue black
EISS-5	0.751 ± 0.13^{b}	Blue black
EISS-10	0.736 ± 0.07^{a}	Blue black
EISS-20	0.716 ± 0.11^{a}	Blue black
EISS-40	0.680 ± 0.09^{a}	Blue black
EISS-60	0.676 ± 0.02^{a}	Blue black

All values are expressed as mean ± standard deviations (n=3). Each column with different superscripts (a, b,) indicate significant differences between samples, ($P < 0.05$). NTS: Native potato starch; EISS: Ethanol induced succinylated starch at different concentrations; EISS-5, EISS-10, EISS-20, EISS-40, and EISS-60.

CONCLUSION

Native potato starch was slurried in succinic anhydride solution treated with different concentration of ethanol. The resulting starches were investigated by optical light microscopy, SEM, and FT-IR. With modification of the starch, obvious structural changes were observed, and this was made evident by the appearance of FTIR absorption band at 1747 cm^{-1}.

The setback, peak, final, trough viscosities, swelling power, paste clarity, and blue value of the starches were directly correlated with each other and were significantly decreased following modification, while the cold-water solubility, pasting temperature and emulsion stability were increased.

The results obtained go with existing knowledge of the functional characteristics of starch in intermediate alcohol concentrations. It therefore concluded that ethanol induced succinylated starch has a potential application as an effective disintegrant in directly compressed tablets.

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