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Effect of hydrothermal modifications on functional, pasting and structural properties of false banana (*Ensete ventricosum*) starch

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Abstract

Starch extracted from e*nsete* (*Ensete ventricosum*, Musaceae) also called false banana, was modified by hydrothermal methods of annealing (ANN) and heat moisture treatment (HMT) processes. The effects of treatments on functional, pasting properties, morphology and diffraction pattern of the starch were studied. Swelling power and solubility changed significantly ($p<0.05$) with modification. Water absorption (89.3-152.4%) and oil absorption (105.0-161.3%) capacities increased significantly ($p<0.05$) with ANN and HMT. Alkaline water retention decreased with ANN but increased significantly ($p<0.05$) with HMT. Hydrothermal modifications led to reduction in least gelation capacity of *ensete* starch. In terms of the pasting properties studied, the hydrothermal modifications imparted improved gel strength, increased paste stability, reduced retrogradation tendency and slowed staling rate on *ensete* starch. Scanning electron micrographs depicted fairly angular and elliptical shapes with diverse sizes for the starch granules. Clustering of granules, mucilage formation, fissures and surface indentation which were gaining prominence with increasing moisture level and temperature of treatment were the hallmarks of modified samples. Native and modified *ensete* starches showed similar type-B diffraction pattern with maximum peak range of 19.8-20.0^{o} (2Ɵ). Findings of this work showed that hydrothermally modified *ensete* starches possess excellent value-added potentials for utilization in pharmaceuticals and food applications.

Keywords: *Ensete*; heat-moisture treatment; Annealing; gel strength; paste stability; retrogradation

Introduction

Ensete (*Ensete ventricosum*, Musaceae), popularly referred to as false banana because of the semblance of its tree to banana tree, is an indigenous plant to Ethiopia where its derived products serve as foods for about 10 million people in the Southern and Western parts of the country [1]. Though, *ensete* does not bear banana fruits, its corms and pseudo stems are large reservoir of starch and are used in the production of two highly important local foods, *kocho* and *bulla* [2].

Kocho is prepared from the pulp of the pseudo stem and corm, while *bulla*, a desiccated juice, is obtained from the pulp [1]. According to Gebre-Mariam and Schmidt [2], these foods are prepared by cutting and crushing the pseudo stem and corm and collecting the exudate, which is a starch containing liquid. The wet starch (*bulla*) is collected and the remaining fibrous material is what is referred to as *kocho*. They both represent high energy foods, with low fat and fibre content. *Bulla* is the most preferred and highly appreciated because of its lower fibre content and whiter colour [1]. *Bulla* is used for making porridge and *atmit* (a hot syrup), whereas *kocho* is used in the preparation of bread [2].

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Besides being an excellent source of starch, the plant is reported to be well adapted to varying weather conditions. It is drought resistant and could also be grown on highlands because of its ability to withstand low temperatures, unlike cassava [1]. It was also reported to give high yield per hectare compare to other crops, available all year round, and have high starch and energy contents [1]. These are important potentials that could promote the diversification of its cultivation, make it an attractive industrial crop and eventually enhance the economy and livelihood of the rural populace. *Ensete* starch contains minimal amount of impurities like proteins and lipids [2] and would therefore require minimal or no purification for industrial application.

Starch is found in abundant quantity in plant where it is stored as carbohydrate and it is the major source of energy for man [3]. The composition of starch makes it a versatile food ingredient possessing attributes for innumerable industrial applications [4]. Its array of functional properties makes it the most commonly used hydrocolloid for various industrial applications.

Amylose (linear polymer) and amylopectin (branched polymer) are the major constituents of starch. The shape of granules, especially the appearance of concentric layers appears to be varied by the amylose content [5]. The relative content of the two polysaccharides in starch of a particular crop or origin confer functionality on that starch.

However, despite the enormous use of starch in food and non-food applications, starch in its natural form (native starch) has shortcomings which limit its industrial uses. Native starch retrogrades easily when cooked and therefore cannot withstand high temperature of processing, its paste has gelling tendency and easily undergo syneresis [6]. It is therefore, modified to correct some or all of these shortcomings, enhance its properties and meet today's processing demand due to recent technological advancement [4].

Native starch is modified by physical, chemical and biotechnological means. The choice of starch modification method is often dictated by its industrial end use. Physically modified starches are preferred in the food and pharmaceutical industries because of their relative safety and therefore would not require huge cost of safety studies needed to certify them by regulatory bodies. Hydrothermal modification is the most common physical method often use for food application. There are two popular types of hydrothermal modification: HMT and ANN. Hydrothermal modification was reported to effect favourable changes on the properties of starch for various specific industrial applications [7-9].

Utilization of *ensete* starch is mostly limited to its use in the production of two Ethiopian local foods, *bulla* and *kocho*. Properties of native *ensete* starch have been reported [2, 10]. The hydrothermal modification of the starch could add scope to its use and enhance its applications in food and pharmaceutical industries. Therefore, the objective of this study was to determine the effect of HMT and ANN on some properties of *ensete* starch.

Materials and methods

Materials

Ensete (*E. ventricosum*) starch packaged by mimibaltina, Addis-Ababa, Ethiopia was supplied by Knel Trading Import and Export, South Africa. Sodium hydrogen bicarbonate sol (code number: 36486-1L) was supplied by Sigma-Aldrich Chemical, Saint Louis, USA.

Starch Preparation

Native starch

The starch was sieved with 150 micron sieve size to remove impurities and dried in a forced air oven at 48 ^{o}C for 24 h to reduce its moisture content from 20% to 12%.

Annealing

The method described by Jacobs and Delcour [11] was used for the preparation of annealed starch. Starch was added to distilled water in the ratio 1:2 (w/v) in a container. The container was sealed and heated at 50 ^{o}C for 24 h in a water bath. The slurry was allowed to pass through a filter paper (Whatman No.1). The residue was oven dried at 35 ^{o}C for 24 h, cooled and packed in an airtight bag Ziploc bag for further analyses.

Heat moisture treatment

Heat moisture treatment (HMT) was carried out according to the method of Li et al., [12]. Previously determined moisture content of native *ensete* starch was raised to 20, 25 and 30% through dispersion in distilled water and subsequently heated at 110 ^{o}C in hot air oven for 16 h. The treated samples were cooled and packed in Ziploc bag for further analyses.

Functional Properties of Native and Modified *Ensete* Starches

Swelling power and solubility

Starch dispersion (500 mg of starch in 20 mL distilled water) was heated for 30 min in a thermostated water bath with shaker at 60 ^{o}C. The slurry was centrifuged (after rapid cooling) at 1900 x g for 15 min using Avanti® J-26XPI super speed centrifuge (Beckman Coulter, USA). Supernatant was carefully transferred into evaporating dish, while the weight of residue was noted for swelling power determination.

$$\text{Swelling power (SP)} = \frac{C_2 - C_1}{X} \qquad 1$$

Where, X is the weight of starch, C_1 is the weight of centrifuge tube and starch slurry, and C_2 is the weight of centrifuge tube and residue after decanting.

The evaporating dish containing the supernatant was weighed and dried at 110 ^{o}C for 20 min. Starch solubilized in water was the remnant after drying the supernatant. Solubility was expressed as gram/100 g of starch [9].

Water absorption capacity

The method of Beuchat et al. [13] was employed for the determination of water absorption capacity **(**WAC). Ten milliliter distilled water was poured into a conical centrifuge tube containing 1 g of starch sample. The suspension was left at room temperature for 1 h and then centrifuged at 200 x g for 30 min using Avanti® J-26XPI super speed centrifuge (Beckman Coulter, USA). The residue was weighed with the tube after draining the supernatant. Water absorption was expressed as change in weight based on percentage of the original sample weight.

Oil absorption capacity

The oil absorption capacity of the ensete starch sample was determined using the method described by Falade and Okafor [14]. Sun flower vegetable oil (10 mL) of 0.87 g/cm^3 density was poured into a weighed centrifuge tube containing 1 g of starch sample and properly mixed using stainless steel spatula. The suspension collected and then was centrifuged at 350 x g speed for 15 min with Avanti® J-26XPI super speed centrifuge (Beckman Coulter, USA). The supernatant was carefully decanted and the weight of the tube and its content were noted. The oil absorption capacity is the increase in weight expressed as percentage of the sample.

Least gelation concentration

The least gelation concentration of starch was determined using the procedure as described by Sathe and Salunkhe [15]. Starch sample from 2-20% at 2% stepwise increase was dispersed in 5 mL distilled water in a separate test tube and heated in a water bath at 80 ^{o}C for 1 h. Then, rapidly cooled under tap water and stored at 4 ^{o}C for 2 h. Least gelation concentration was the concentration when the sample from the inverted tube did not slip.

Alkaline water retention

The weight of test tube containing 1.0 g starch sample was assumed to be Y_1. 5.0 mL of 0.1 M NaHCO3 was added into the test tube, mixed in a vortex mixer for 30 s and left at room temperature for 20 min. The mixture was centrifuged at 200 rpm and the supernatant decanted at an angle of 10-15 ^{o} to the horizontal [16]. Weight of the test tube and the residue after draining was taken as Y_2.

Alkaline water retention (AWR) (g/g) of sample $= Y_2 - Y_1$. 2

Pasting Properties

The pasting properties of starch samples were determined using a Rapid Visco Analyser (RVA model 4500, Perten instruments, Australia). The pasting properties were conducted on 14 % moisture basis. Starch sample (3.42 g) was dispersed in distilled water (25.08 g) and stirred in an RVA container. The slurry was subjected to programmed heating and cooling cycle under constant strain as described by Yadav et al. [9]. The idle temperature (peak temperature) was 91 ^{o}C.

Microscopic Studies

The granule morphology of *ensete* starch samples was examined with a scanning electron microscope (EVO/LS15, ZEISS International, Germany). Starch granule (thin layer) was gold coated with an ion sputtering device (EIKO IB-3 ion coater, Eiko Engineering Company, Hitachinaka, Japan) and examined under the microscope. The sizes of the starch granules were measured with the aid of image analysis software (AnalySIS, Soft Imaging System, Germany).

X-ray Diffraction (XRD)

The continuous X-ray diffraction patterns of starch samples were obtained with a powder X-ray diffractometer (Empyrean, PANanalytical, Almelo, the Netherlands) coupled with a sample changer and image plate detector. The scanning region of diffraction were registered at Bragg angle (2Ө) 3^o to 40^o with generator settings of 40 KV and 40 mA. Scan step time was 8.255 s.

Data Analysis

Replicated data were analysed using SPSS 15.0 (SPSS Inc. USA) and the means were reported. Differences among means were separated using Duncan Multiple Range Test (DMRT).

Results and Discussion

Functional Properties

Swelling capacity and solubility

As shown in Table 1, swelling capacity and solubility of starch decreased significantly ($p < 0.05$) with hydrothermal modifications. Increase in moisture content caused significant ($p < 0.05$) decrease in swelling capacity and solubility of HMT starch samples. The trends observed have been reported by other authors for annealed and HMT starches from other sources [16, 17, 9]. The decrease in swelling capacity and solubility for annealed and with increase in moisture content in HMT treated starches was reported by Adebowale et al. [17] to be as a result of structural re-orientation inside the granules occasioned by the treatments. The effects indicated existence of strong binding forces within the crystallites including strong interaction of the micellar networks and amylopectin molecular structure [18, 19]. The mobility of starch chains in the amorphous region at high moisture content of treatments which facilitated interactions between starch chains in crystalline regions was reported by Hoover and Vasanthan [7] to be a factor in the resulting strong binding forces.

Water absorption capacity

Water absorption capacity (WAC) of the starches is presented in Table 1. Hydrothermal treatments significantly ($p < 0.05$) increased WAC. HMT enhanced hydrophilic tendency of *ensete* starch. Knowledge of WAC of a material will guide the processor on the amount of water that should be added during processing involving the material for specific application. Higher WAC is beneficial in such food products as thickeners and confectioneries. Therefore, hydrothermally modified starch will find important applications in such products.

Oil absorption capacity

Oil absorption capacity significantly ($p < 0.05$) increased from 105.67% in native *ensete* starch to 161.33% in heat moisture treated starch at 25% moisture level (Table 1). These results are similar to the findings of Olayinka et al. [16]. Abraham [20] reported that the lipophilic covering formed on the surface of the granule as a result of the treatments might be responsible for the increase in the lipophilic tendency. Oil absorption capacity is a desirable characteristic in products where emulsifying capacity is of great importance. Examples include mayonnaise and batter in frying products.

Alkaline water retention (AWR)

Alkaline water retention is an important functional property of starch that will guide its use and processing under alkaline aqueous conditions [21]. Alkaline water retention of the *ensete* starches are shown in Table 1. The effect of ANN on native starch which caused reduction in the AWR was similar to the observation of Falade and Ayetigbo [21] on some yam cultivars. However, similar to the reports of Olayinka et al. [16], Adebowale et al. [17] and Yadav et al. [9], AWR increased significantly ($p < 0.05$) with HMT at 30% moisture inclusion. This result showed that HMT enhanced AWR of *ensete* starch and would therefore encourage its use in processing under alkaline aqueous condition.

Least gelation concentration

Ensete starch samples showed excellent gelation characteristics (Table 2). Least gelation concentration of 4% for native *ensete* starch recorded in this study was lower than the values reported for banana (6%), plantain (8%) white sorghum starch (18%) African yam bean (8%) and Indian water chestnut (34%) [9, 16, 17, 22]. Hydrothermal modifications enhanced gelation ability of *ensete* starch. Annealed and HMT starches formed soft gel at 2% concentration. Least concentration for firm gel formation was reducing progressively from 8% for AES and H20ES as moisture level in the modified samples increased. This was in agreement with the observation of Adebowale et al. [17] on hydrothermally modified African yam bean. The exceptional gel forming ability of native and hydrothermally modified *ensete* starches at very low concentration would make them better choices in development or formulation of products where strong gelation capability is required.

Pasting Properties

Pasting properties are important quality indices in predicting the functional behavior of starch and their applicability to food systems. Pasting properties of the studied *ensete* starches are shown in Table 3 while the pasting patterns are shown in Fig. 1. Pasting temperature, which initially decreased with hydrothermal treatments, was later increasing with moisture level in HMT samples. The increase in pasting temperature with increase in moisture level buttresses the report of Eliasson [23] on the existence of increasingly strong interactive forces within the starch granules which necessitated more thermal energy before breakdown of granule structure and formation of paste occurred. Peak viscosity is the maximum swelling attained by the granules during heating of starch before the eventual disintegration [24]. Peak viscosity (PV) of the starches reduced with increased moisture inclusion. This decrease in PV was reported by Hofstee [25] as cited by Gebre-Mariam and Schmidt [2] to be due to increased presence of water which lubricates the swollen granules. The lubrication favours the formation of crystallites in the amorphous region and this confers extra strength on the granule structure [17]. This was buttressed by the report of Hoover [26] that the lower the peak viscosity, the stronger the cohesive forces within the granules. The time requires to attain peak viscosity is known as peak time. It is a measure of the resistance of starch granules to disintegrate during heat processing. Peak time of starches in this study was decreasing with the level of moisture inclusion in the starch samples. The trends observed for peak temperature, peak viscosity and peak time with levels of moisture inclusion showed that hydrothermal treatments enhanced the formation of crystallites which caused additional energy to be expended before breakdown of granule and formation of paste could occur [27]. Similar trends were reported by Olayinka et al. [16], Adebowale et al. [17] and Yadav et al. [9] for hydrothermally treated white sorghum starch, African yam bean and Indian water chestnut, respectively. Breakdown viscosity (BV) is a measure of disintegration of starch granules through the action of heat and shear force. Breakdown viscosity value gives the fragility of starch [28]. High BV value indicates that the starch granules are fragile and will be disintegrated with minimal heat and shear force. Hydrothermal modifications led to reduction in the BV values of the *ensete* starch samples. The reduction was more pronounced in HMT starches and was reducing progressively with increasing level of moisture inclusion. This indicates an increased resistance to the effect of heat and shear force and therefore, better stability of the starch pastes. Setback viscosity (SBV) measures the ease of retrogradation of starch. Retrogradation tendency increases with increasing SBV value. The increasing SBV value with hydrothermal modifications implied increased rate of staling during cooling. The staling rate increased with increasing rate of moisture inclusion in HMT samples. This showed that native *ensete* starch will be a better candidate in confectionery and bakery [3]. The impact of hydrothermal modifications on *ensete* starch is due to the reorganization of the components of the starch granules resulting in the formation of crystallites in the amorphous region which strengthened the molecular chains and limit the absorption of water inside starch matrices [12].

Microscopic Studies

Electron micrographs of the *ensete* starches are shown in Fig. 2. Fairly angular and elliptical appearance of the starch observed in this study is in agreement with the report of Gebre-Mariam and Schmidt [2]. Fissures and surface indentation observed in modified starches which were becoming more pronounced with the increase in moisture level have been reported to as a result of exposure of starch to excessive moisture for long time [17]. Clustering of granules and formation mucilage which were gaining prominence with increased moisture level were observed in heat-moisture treated starches. They were reported by Yeh et al. [29] to be due to the effect of heat treatment in excess moisture which resulted in the gelatinization of starch. It is important to point out that there was no clustering in the annealed sample. This could be due to the fact that the temperature of treatment (50 ^{o}C) was below the gelatinization temperature of *ensete* starch. Similar to the observation of Abraham [20] and Adebowale et al. [17], hydrothermal modifications did not affect the morphology of starch granules. This indicated that the action of HMT was basically peripheral causing agglomeration of granules. This confirmed the findings of Li et al. [12] that the formation of crystallites limits the absorption of water inside the micelles [30].

X-ray Diffraction of *Ensete* Starches

The X-ray diffraction patterns of native and modified *ensete* starches are shown in Fig. 3. Native *ensete* starch showed maximum peak at 19.84^{o} 2Ɵ with relative intensity of 21.75%. The maximum peak value is different from 17^{o} at 2Ɵ reported by Gebre-Mariam and Schmidt [2] in their study on *ensete* starch. However, the crystals in the two studies reflected similar B-type diffraction patterns like potato starch crystals. Hydrothermal treatments did not affect X-ray diffraction patterns. Prominent peaks for hydrothermally modified samples ranged from 19.760^{o} to 20.035^{o}.

Conclusions

This study is an exposition of potential applications of native and hydrothermally modified *ensete* starch in food and pharmaceutical industries. Hydrothermal modification of *ensete* starch through the application of ANN and HMT processes led to favourable changes in functional and pasting properties of the starch. The changes were due to the effects of modifications on starch granules leading to their reorganization at molecular levels and subsequent formation of strong interactive forces which held the granules firmly. The changes in the properties include decreasing swelling power and solubility, increasing water and oil absorption capacities, gel formation at very low concentration, increasing peak temperature, peak viscosity, peak time and setback viscosity, and decreasing breakdown viscosity. These changes implied better paste stability, excellent gelling strength, utilization in alkaline medium, good emulsifying capability, low retrogradation tendency and reduced staling rate. These are excellent characteristics that would enhance its utilization as binder, thickener, stabilizer, and emulsifier with application in such food products as sauce, mayonnaise, bakery and confectionery among others.

The authors declare no conflict of interest.

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Figure captions

Fig. 1 RVA pasting patterns of native and modified *ensete* starches (Images were obtained and processed in Paint (Windows® 7 accessory) environment for clarity). A: native *ensete* starch; B: annealed *ensete* starch; C: HMT *ensete* starch at 20% moisture inclusion; D: HMT *ensete* starch at 25% moisture inclusion; E: HMT *ensete* starch at 30% moisture inclusion.

Fig. 2 Scanning electron micrographs of native and modified starches. a: native *ensete* starch; b: annealed *ensete* starch; c: HMT *ensete* starch at 20% moisture inclusion; d: HMT *ensete* starch at 25% moisture inclusion; e: HMT *ensete* starch at 30% moisture inclusion.

Fig. 3 X-ray diffraction pattern of native and modified starches (Images were obtained and processed in Paint (Windows® 7 accessory) environment for clarity). NES: native *ensete* starch; AES: annealed *ensete* starch; H20ES: HMT *ensete* starch at 20% moisture inclusion; H25ES: HMT *ensete* starch at 25% moisture inclusion; H30ES: HMT *ensete* starch at 30% moisture inclusion.

Table 1 Functional properties of native and modified *ensete* starch

Sample	Swelling power (g/g)	Solubility (g/100g)	WAC (%)	OAC (%)	AWR (g/g)
NES	1.94^{c}	1.07^{ab}	89.33^{a}	105.67^{a}	1.11^{abc}
AES	1.75^{bc}	0.6^{a}	104.00^{a}	114.33^{ab}	0.78^{a}
H20ES	3.20^{e}	4.33^{c}	120.33^{bc}	135.67^{cd}	-
H25ES	2.27^{d}	0.93^{ab}	123.67^{c}	161.33^{e}	-
H30ES	1.83^{bc}	1.13^{ab}	152.40^{d}	150.07^{de}	1.32^{bc}

Data reported are means of triplicate. Means in the same columns followed by different letter(s) superscript are significantly different at 5% confident level ($p<0.05$).

NES: native *ensete* starch; AES: annealed *ensete* starch; H20ES: HMT *ensete* starch at 20% moisture inclusion; H25ES: HMT *ensete* starch at 25% moisture inclusion; H30ES: HMT *ensete* starch at 30% moisture inclusion; WAC: water absorption capacity; OAC: oil absorption capacity; AWR: alkaline water retention.

Table 2 Least gelation capability of native and hydrothermal modified ensete starch

Sample	**State**	**Concentration (%)**									
		2	4	6	8	10	12	14	16	18	20
NES	Gelation	Nil	Gel	Gel	Gel	Gel	Gel	Gel	Gel	Gel	Gel
	Appearance	Viscous	Soft	Soft	Soft	Firm	Firm	Firm	Very firm	Very firm	Very firm
AES	Gelation	Gel	Gel	Gel	Gel	Gel	Gel	Gel	Gel	Gel	Gel
	Appearance	Soft	Soft	Soft	Firm	Firm	Very firm	Very firm	Very firm	Very firm	Very firm
H20ES	Gelation	Gel	Gel	Gel	Gel	Gel	Gel	Gel	Gel	Gel	Gel
	Appearance	Soft	Soft	Soft	Firm	Firm	Very firm	Very firm	Very firm	Very firm	Very firm
H25ES	Gelation	Gel	Gel	Gel	Gel	Gel	Gel	Gel	Gel	Gel	Gel
	Appearance	Soft	Soft	Firm	Firm	Very firm	Very firm	Very firm	Very firm	Very firm	Very firm
H30ES	Gelation	Gel	Gel	Gel	Gel	Gel	Gel	Gel	Gel	Gel	Gel
	Appearance	Soft	Firm	Firm	Very firm	Very firm	Very firm	Very firm	Very firm	Very firm	Very firm

NES: native *ensete* starch; AES: annealed *ensete* starch; H20ES: HMT *ensete* starch at 20% moisture inclusion; H25ES: HMT *ensete* starch at 25% moisture inclusion; H30ES: HMT *ensete* starch at 30% moisture inclusion.

Table 3 Pasting properties of native and modified *ensete* starch

Sample	Pasting temperature (ºC)	PV (cP)	Peak time (min)	Trough (cP)	FV (cP)	BV (cP)	SBV (cP)
NES	71.45	7544	4.40	3887	4408	3657	521
AES	70.00	6190	4.40	3923	5012	2267	1089
H20ES	69.15	4748	4.60	3299	5101	1449	1802
H25ES	71.40	4075	4.80	3477	5454	598	1977
H30ES	75.75	3089	6.00	2950	4544	139	1594

Not replicated

NES: native *ensete* starch; AES: annealed *ensete* starch; H20ES: HMT *ensete* starch at 20% moisture inclusion; H25ES: HMT *ensete* starch at 25% moisture inclusion; H30ES: HMT *ensete* starch at 30% moisture inclusion; PV: peak viscosity; FV: final viscosity; BV; breakdown viscosity; SBV: setback viscosity.

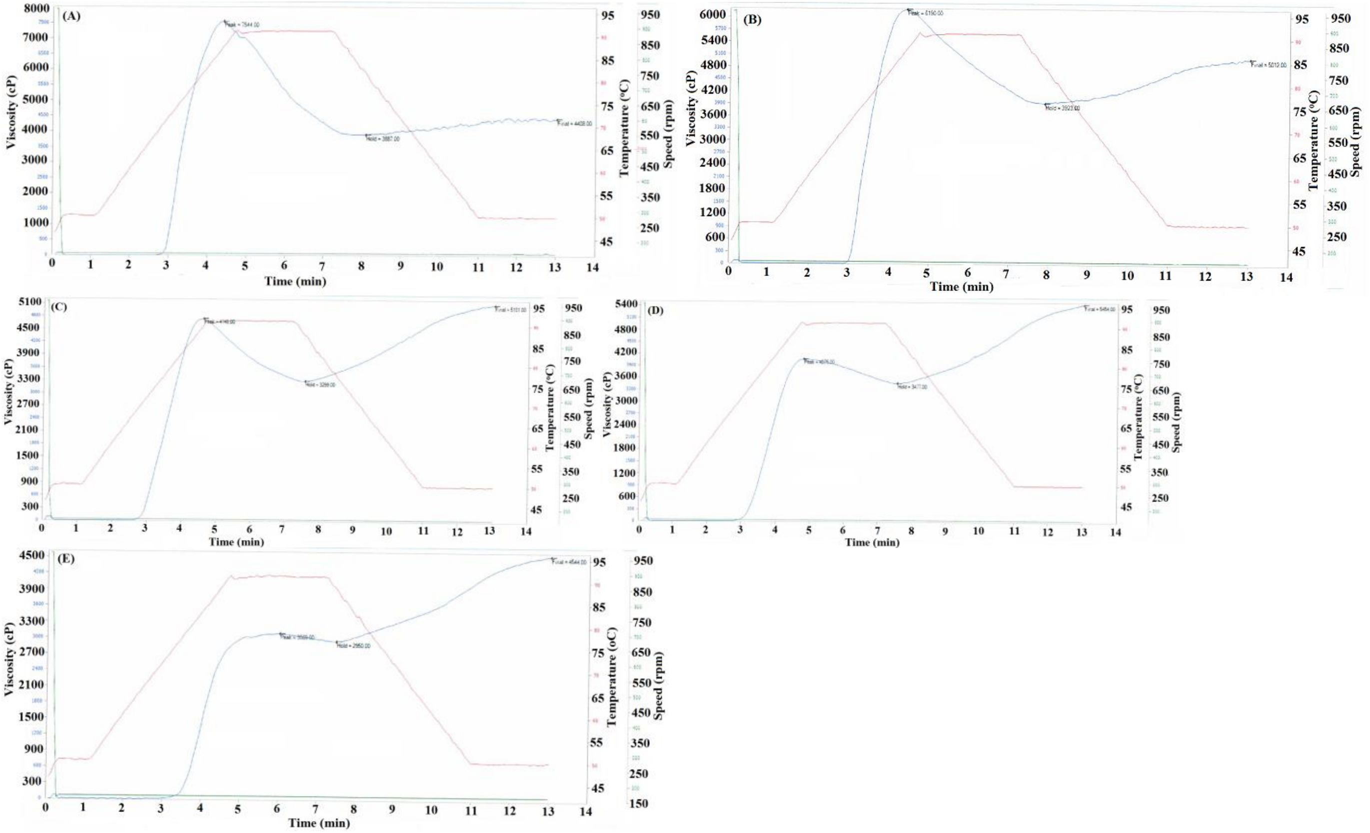
(A)
Peak = 7544.00
Hold = 3887.00
Final = 4408.00
(B)
Peak = 6190.00
Hold = 3923.00
Final = 5012.00
(C)
Final = 5101.00
(D)
Hold = 3477.00
(E)
Hold = 2950.00
Final = 4544.00
Viscosity (cP)
Temperature (°C)
Temperature (oC)
Speed (rpm)
Time (min)
8000
7000
6000
5000
4000
3000
2000
1000
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
95
85
75
65
55
45
950
850
750
650
550
450
350
250
150

Fig. 1

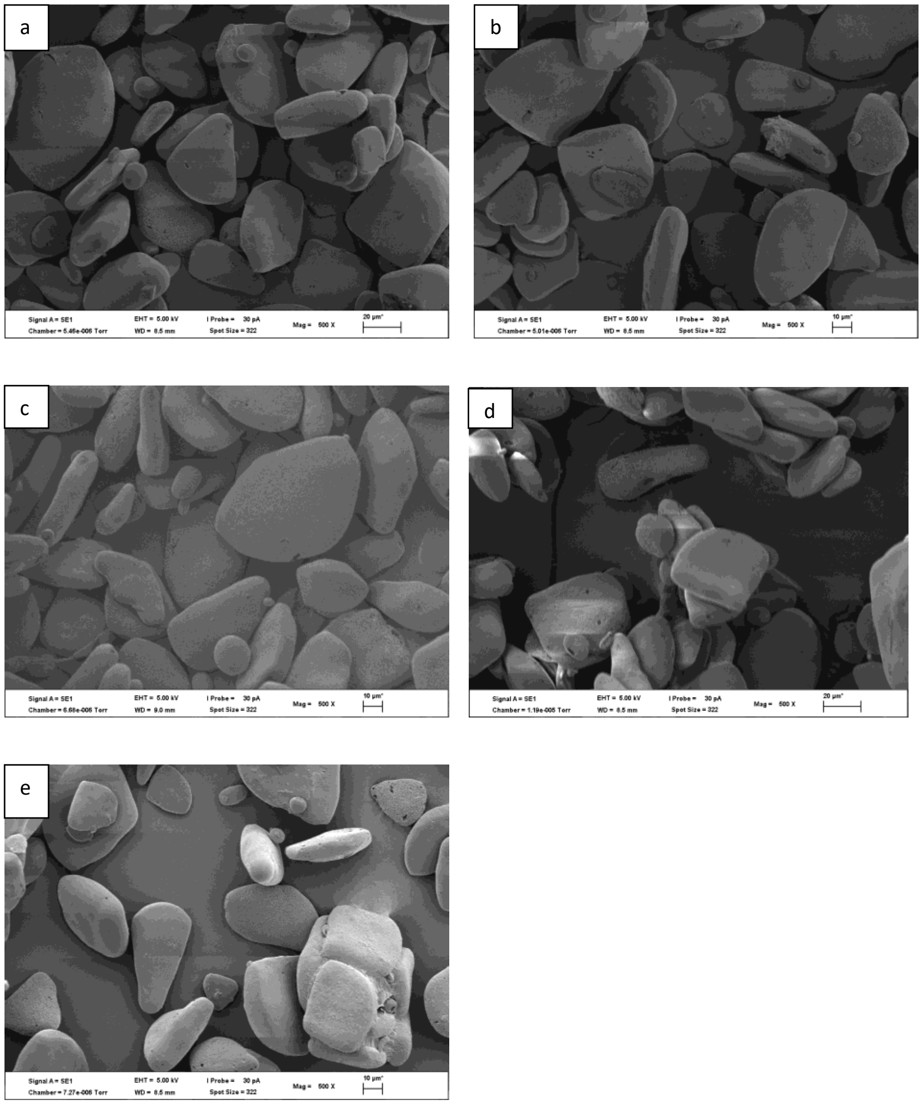
a
Signal A = SE1
Chamber = 5.46e-006 Torr
EHT = 5.00 kV
WD = 8.5 mm
I Probe = 30 pA
Spot Size = 322
Mag = 500 X
20 µm*
b
Signal A = SE1
Chamber = 5.01e-006 Torr
EHT = 5.00 kV
WD = 8.5 mm
I Probe = 30 pA
Spot Size = 322
Mag = 500 X
10 µm*
c
Signal A = SE1
Chamber = 6.68e-006 Torr
EHT = 5.00 kV
WD = 9.0 mm
I Probe = 30 pA
Spot Size = 322
Mag = 500 X
10 µm*
d
Signal A = SE1
Chamber = 1.19e-005 Torr
EHT = 5.00 kV
WD = 8.5 mm
I Probe = 30 pA
Spot Size = 322
Mag = 500 X
20 µm*
e
Signal A = SE1
Chamber = 7.27e-006 Torr
EHT = 5.00 kV
WD = 8.5 mm
I Probe = 30 pA
Spot Size = 322
Mag = 500 X
10 µm*

Fig. 2

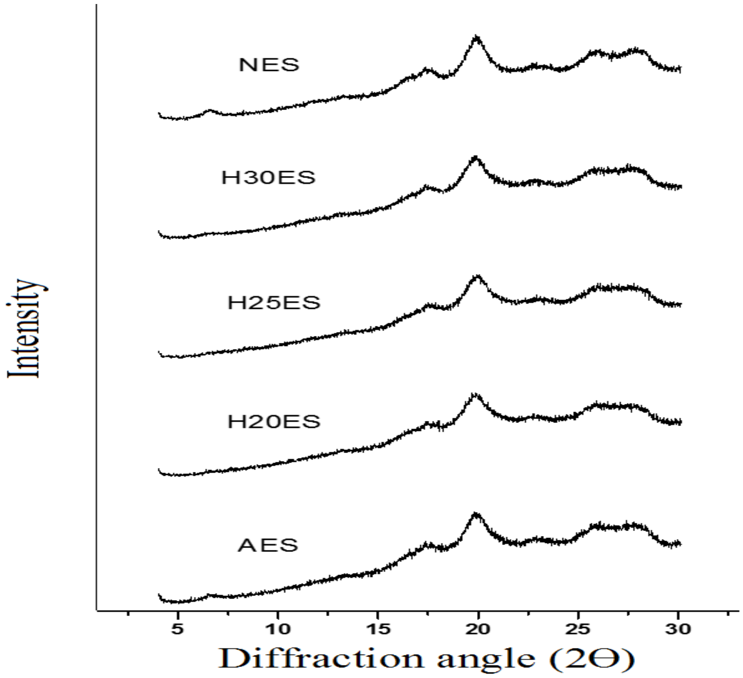
NES
H30ES
H25ES
H20ES
AES
Intensity
5
10
15
20
25
30
Diffraction angle (2Θ)

Fig. 3