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Article · November 2022

DOI: 10.1016/j.focha.2022.100143

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Food Chemistry Advances 1 (2022) 100143

Contents lists available at ScienceDirect

Food Chemistry Advances

journal homepage: www.elsevier.com/locate/focha

Characterization of modified palmyrah tuber starch by pre-gelatinization, acid and dextrinization processes and its applicability

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ARTICLE INFO

Keywords:
Acid modified starch
Dextrinized starch
Native palmyrah tuber starch
Pregelatinized starch
Thickener

ABSTRACT

Palmyrah (*Borassus flabellifer* L) tuber is a cheap and rich source of starch. Modifications of native starches are carried out to provide starch products with specific properties. In the present study, three different starch modification techniques (pre-gelatinization, acid modification and dextrinization) were used to produce modified palmyrah tuber starch. The physicochemical and functional properties of the native and modified palmyrah tuber starch were evaluated and the applicability of the palmyrah modified starch as a thickner in instant soup also tested. The recovery yields of modified starches ranged between 75.90-91.96%. The dextrinized palmyrah starch showed significantly ($p<0.05$) the lowest swelling power and the highest average solubility. The gelatinization temperature (76.5-82.27 °C), amylose content (9.2-21.69%), amylopectin content (78.32-90.80%) varied among the native and modified starch. The particle sizes of the starch granules varied from 1.308-7.346 μm. The study revealed that the modification processes can greatly improve the physicochemical and functional characteristics of native starch. According to the sensory analysis of instant soup among trained panelists, high preference was observed for palmyrah pregelatinized starch than commercially available corn starch. Hence this study shows the feasibility of modified palmyrah starch as a thickening agent in instant soup.

1. Introduction

Palmyrah young tuber is a seasonal underutilized product of palmyrah palm (Thillainathan, 2019). The immature tuber is spindle-shaped and it includes 44 % organic matter, 50-55 % moisture, and 2% minerals. The main carbohydrate found in palmyrah tuber flour is starch (Thivya, 2018).

Starch is a polymer, chemically formed by long chains of sugar molecules that are connected together. Starch is rarely consumed in its native state and is widely employed in industry. Because they are unstable when exposed to changes in temperature, pH, and shear stresses, most native starches are limited in their direct application. Native starches are frequently changed to achieve specific qualities such as solubility, smoothness, adhesion, and temperature endurance in industrial operations. Several methods for producing modified starches with various properties and applications have been developed. All of these strategies change physicochemical qualities and structural attributes of starch in order to boost its value in the food and non-food industries. The industry of starch modification is always changing. Physical, chemical, and enzymatic approaches are used to modify starch (Krithika & Ratnamala, 2019). Annealing, heat/moisture, high-pressure, and osmotic pressure treatment, and pre-gelatinization are some of the physical modification methods for starch (Singh et al., 2016). Chemical modification involves the addition of functional groups to the starch molecule, resulting in a significant change in physicochemical properties (Krithika & Ratnamala, 2019).

Naguleswaran et al. (2010) had conducted researches on the extraction and characterization of native strach from Pamyrah dried seed shoot flour. The yield and purity of starch form Palmyrah dried shoot flour were 38.4% and 95.8%, respectively (Naguleswaran et al., 2010). But lack of researches were conducted on the modification of palmyrah native starch and its applicability. Palmyrah dried tuber flour was used as a thickner in trditional product like poridge and soup. But slight bitterness of the flour restricted its use as a thickner in various industrial products. Native starch extracted from palmyrah tuber can be modified with various techniques to achive specific qualities such as solubility and viscosity.

Hence this research was conducted to investigate the effects of three different modification methods which represent both physical and chemical modifications versus native starch on physicochemical and morphological properties. Pregelatinization, acid modification and dextrination were the modification techniques selected for this study based on cost effectiveness, availability of chemicals and ease of preparation. Findings of this study will be a backbone for various application of

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https://doi.org/10.1016/j.focha.2022.100143
Received 23 July 2022; Received in revised form 17 October 2022; Accepted 15 November 2022

modified starch in food industry. And the major intension of this study is to select best modification techique to use modified palmyrah tuber starch as a thickner in instant soup mix. This will optimize the underutilization of palmyrah tuber into a industrially viable product. This will help to empower the production of palmyrah tuber in Sri Lanka which directly influences the rural economy.

2. Materials and methods

2.1. Sample collection

Fresh matured Palmyrah tubers were collected from the local market in Jaffna, Sri Lanka.

2.2. Extraction of starch from palmyrah tuber

Palmyrah tuber was washed with distilled water to remove the contaminants, peeled and cut into approximately 1cm cubes. Then ground in high speed blender for 5 minutes with 1:4 weight/volume distilled water and filtered using double fold cotton cloth. Then water was added to residue 1:4 weight/volume and mixed well and filtered using double fold cotton cloth again. This procedure was repeated for 5 times. The filtrate was allowed to stand for 24 h for to settle down the starch and top liquid was decanted and discarded. After that distilled water was added to sediment and mixture was stirred again for 4 minutes. Then top liquid was decanted and discarded. This procedure was repeated until getting the pure starch. Finally, the starch was dried at 55 °C for overnight and ground, sieved, packed in air tight container (Hasmadi et al., 2021). This will be called as palmyrah native starch (PNS).

2.3. Preparation of palmyrah modified starch

2.3.1. Preparation of palmyrah pre-gelatinized starch (PPS)

Pre-gelatinized starch was prepared as mentioned by Tam et al. (2021) with some modification. The starch suspension was made (1:1 weight/volume) in a 250 mL beaker glass. The starch suspension was heated in water bath 63.0 °C for 5 min (Yousif et al., 2012). The paste from the process was dried in an oven at 40 °C for 24 h. Finally, starch was ground and kept in a bottle. The yield of pre-gelatinized starch from native starch was calculated (Jubril et al., 2012).

2.3.2. Preparation of palmyrah acid modified starch (PAS)

Acid modified starch was prepared as mentioned by Yousif et al. (2012) with some modification. The 50mL of 0.1 mol L^{-1} HCl solution was added to a mixture of 100 g starch and 50 mL deionized water and mixed for 30 min. Then, the pH was adjusted to 7.0 with 1 mol L^{-1} NaOH. Neutralized starch was dried at temperature 40 °C for 24 h following washing three times and filtration with Filter Paper No1. Finally, starch was ground and kept in a bottle. The yield of acid modified starch from native starch was calculated (Babu et al., 2015)

2.3.3. Preparation of palmyrah dextrinized starch (PDS)

Dextrinized starch was prepared as mentioned in Yousif et al. (2012) with some modification. A weight of 75 g of starch was mixed thoroughly with 60 mL of 0.1 mol L^{-1} HCl, and then dried at 50 °C for 32 h. The dried starch was dissolved in 75 mL deionized water and pH was adjusted to 7.0 by adding 1 mol L^{-1} NaOH. The starch was dried in an oven at 40 °C for 24 h. Finally, starch was ground and kept in a bottle. The yield of dextrinized starch from native starch was calculated.

2.4. Analysis of proximate composition

Proximate analysis (Moisture, Protein, Ash, fat, crude fibre and carbohydrate content of starch samples) was done by AOAC, 2000 method.

2.5. Determination of amylose and amylopectin

Amylose and amylopectin of the starch sample was determined according the method described by Ishiwu et al. (2017).

2.6. Determination of granular morphology

About 100 mg of starch sample was dispersed in 9.9 mL of distilled water and held for 15 min at room temperature with constant stirring (Onitilo et al., 2007). The two drops of starch solution were stained with one drop of 0.05% iodine solution (0.05 g of iodine was dissolved in 100 mL of 1% KI solution) and a thin smear was prepared on a glass slide with a cover slip. Microscopic measurement of starch granules was done using light microscope under the magnification power of 400x (Wijesinghe et al., 2015).

2.7. Analysis of particle size

Particle size analysis of starch was analysis according to the procedure described by Lu et al. (2018) using NANO DS duel light scattering Particle size analyzer (Nano DS Sn 110). The concentration of starch suspension tested was diluted with distilled water to around 1mg mL^{-1}. The samples were measured at a scattering angle of 90 °. The wavelength of the laser used was 660 nm.

2.8. Analysis of functional properties

Water absorption capacity and oil absorption capacity of starch samples were measured according to the method described by Babu & Parimalavalli (2014). Swelling power and solubility were determined according to the method described by Oktaviana & Saepudin (2021). Gelatinization temperature was determined according the method described by Gunorubon & Kekpugile (2012). Paste clarity of starch samples was determined according to the method described by Heebthong et al. (2016). Synersis of starch sample was determined according to the procedure described by Mtunguja et al. (2016). Viscosity of starch sample was determined according to the procedure described by Oktaviana & Saepudin (2021)

2.9. Preparation of vegetable soup mixture

The instant vegetable soup mix was prepared according to the procedure described by Ranaweera KKDS (2014) with some modification. A vegetable soup mixture was prepared with steamed and dehydrated dhal (33.33%), blanched and dehydrated carrot powder (20%), leeks (11.47%), tomatoes (6.67%) and garlic (0.8%), salt (4.67%), starch (10%), white pepper (2.67%), cinnamon and curry leaves (0.2 % each). Approximately 7.5 g of powder was reconstituted with 50 mL of boiling water, and simmered for 5 minutes.

2.9.1. Sensory analysis of soup mixture with incorporated starch sample

PNS, PPS, PAS, PDS and CS (commercially available starch) were coded as 726, 352, 576, 893, and 291 respectively. The sensory evaluation was conducted with 15 trained panelists in Palmyrah Research Institute with nine-point hedonic test to measure the sensory properties of appearance, colour, aroma, texture (thickness), taste, after taste and overall acceptability. Fried man test in Minitab 17 software was used to analyze the sensory data.

2.9.2. Statistical analysis

Fried man test in Minitab 17 software was used to analyze the sensory data. One way ANOVA in Minitab 17 was used to analyze other results

Table 1
Proximate composition analysis of palmyrah native and modified starch.

Composition (%)	PNS	PPS	PAS	PDS
Recovery Yield (From Native starch)	-	80.01 ± 0.33^{b}	91.96 ± 0.44^{a}	75.90 ± 1.37^{c}
Moisture	10.36 ± 0.22^{b}	11.36 ± 0.09^{a}	10.42 ± 0.03^{b}	10.46 ± 0.17^{b}
Ash	0.07 ± 0.00^{b}	0.07 ± 0.00^{b}	0.07 ± 0.00^{b}	0.08 ± 0.00^{a}
Fat	0.14 ± 0.00^{a}	0.12 ± 0.00^{c}	0.13 ± 0.00^{b}	0.13 ± 0.01^{b}
Crude fiber	0.34 ± 0.01^{a}	0.25 ± 0.01^{b}	0.23 ± 0.01^{bc}	0.19 ± 0.01^{c}
Protein	0.53 ± 0.12^{a}	0.26 ± 0.00^{ab}	0.18 ± 0.00^{b}	0.22 ± 0.06^{b}
Total carbohydrate	88.08 ± 0.09^{ab}	87.81 ± 0.28^{b}	88.43 ± 0.05^{ab}	88.70 ± 0.13^{a}
Amylose	18.77 ± 0.06^{c}	21.69 ± 0.34^{a}	20.42 ± 0.42^{b}	9.2 ± 0.29^{d}
Amylopectin	81.23 ± 0.06^{b}	78.32 ± 0.34^{d}	79.58 ± 0.42^{c}	90.80 ± 0.29^{a}

Values followed by the same letters in the same row are not significantly different ($p \leq 0.05$); PNS: Palmyrah native starch, PPS: Palmyrah pre-gelatinized starch, PAS: Palmyrah acid modified starch, PDS: Palmyrah dextrinized starch.

3. Results and discussion

3.1. Preparation of modified starch

Table 1 shows the yield of modified starch from palmyrah native starch (PNS). There was a significant difference between the yield percentage of modified starch from PNS ($p<0.05$). The recovery yield of PPS from PNS was 80.01% which was lower than pre-gelatinized starch prepared from sweet potato starch which was 82.22% (Jubril et al, 2012). The recovery yield of PAS was 91.96% where the recovery yield of acid-modified sweet potato starch was 99.30% and 93.71% with 1% and 5% hydrochloric acid respectively. The recovery yield of PDS was 75.90% and it is lower than PPS and PAS. Industries are focused on high recovery yield of modified starch from native starch. Therefore, PAS is more suitable than PPS and PDS.

3.2. Analysis of proximate composition

Table 1 shows the proximate composition of native and modified starch. The starch must have low moisture content in order for the items to have a long shelf life. (Akubor &Igba, 2019). The industrial maximum allowable limit for moisture in starch was 15% (Benesi et al., 2005). Initial moisture content of all starch samples was below the industrial recommended value.

There was a significant difference in ash content among the PNS and modified palmyrah tuber starch. There was no significance difference among PNS, PPS and PAS in ash content. Yousife et al. (2012) reported, dextrinized corn starch had high ash content (0.90 ± 0.01%) similar to PDS.

The PNS had high crude fat % than modified palmyrah tuber starch. Because the lipids were found in the starch granules as an amylose-lipid inclusion complex. With polar fatty compounds, such as higher fatty acids, the linear fraction of starch molecules (amylose) forms helical clathrates. The amylose-lipid complexes were insoluble, but they dissociate when heated in water over a certain temperature (Swinckels, 2007). Chemical modification techniques of starch reduced the fat % due to the various degradation (Lawal, 2004).

There was a significant difference in crude fiber content among the PNS and modified palmyrah tuber starch. When considering the native and pre-gelatinized starch of unripen banana fruit, native banana starch (2.43 %) had high crude fiber % than pre-gelatinized banana starch (2.35 %) (Akubor & Igba, 2019). Chemical modification techniques of starch reduced the crude fiber % due to the various degradation (Lawal, 2004). The crude fiber content of PNS was higher than other native starch of tubers such as *Manihot esculenta C* (0.28 ± 0.01 %) (Pérez et al., 2005).

The crude protein content of PNS (0.53 ± 0.12^{a} %) was higher than PPS (0.26 ± 0.00^{ab} %), PAS (0.18 ± 0.00^{b} %) and PDS (0.22 ± 0.06^{b} %). But crude protein content of PNS was lower than (2.5 %) reported in the extraction of starch from Palmyrah dried seed flour by Naguleswaran et al. (2010). Native potato starch (0.55^{a} %) had high crude protein content than pre-gelatinized (0.42^{b} %), acid-modified (0.33^{d} %) and dextrinized potato starch (0.35%) (Gadalla et al., 2014). The crude protein content of pre-gelatinized starch was reduced through leaching (Akubor & Igba, 2019) and due to protein denaturation. Heating of protein above 50 °C, causes breaking of non-covalent bonds such as hydrogen, hydrophobic, and hydrostatic bonds that are involved in secondary and tertiary structure. Acid modification reduces the crude protein content through precipitation (Falade et al. 2019).

There was a significant difference in total carbohydrate content among PNS and modified palmyrah tuber starch. Total carbohydrate content was higher for PDS (88.70 ± 0.13^{a} %) than PNS (88.08 ± 0.09^{ab} %), PPS (87.81 ± 0.28^{b} %) and PAS (88.43 ± 0.05^{ab} %). This showed the level of purity of the modified starch compared to the native starch.

3.3. Determination of Amylose and amylopectin

The ratio of amylose to amylopectin was typically consistent for a given starch species. The amylose percentage of maize and sorghum starch was substantially higher (about 28%) than tuber and root starches (potato, tapioca, arrowroot), which contains about 20% amylose (Swinckels, 2007). According to Table 1, there was a significant difference in % amylose among the PNS and modified palmyrah tuber starch. Amylose content was high in PPS (21.69 ± 0.34%). The disruption of starch granule could be the reason for these changes. The increase in amylose content is also found in pre-gelatinized yam, corn, and rice starch (Tam et al., 2021). Acid attacks the amorphous regions of dextrinized starch and break down its glycosidic linkages (Akin et al., 2014). Amylose % decreased with the steeping time and concentration of acid. Hence, PAS had high amylose percentage than PDS. PDS had the highest amylopectin content than native and other modified starch (PPS, PAS). The three major reactions taking place during dextrinization are glycosidic bond cleavage, glycosidic bond formation, and repolymerization (Maria et al., 2017). This may cause the increase in amylopectin content.

3.4. Determination of granular morphology

The granular shape, starch molecular orientation (polarised light), and long-range amylose distribution, as well as content, were all studied using light microscopy with Iodine staining (Blennow et al., 2003).The morphology of PNS, PPS, PAS and PDS granules were showed in Fig. 1. PNS and modified palmyrah tuber starch granules exhibited spherical, oval, round and irregular globular shape. Native and modified starch samples were not had a definite shape. PNS and PAS had small, medium and large granules. PPS and PDS had medium and large granules. Potato starch granules exhibited oval and spherical shape (Swinckels, 2007). Abiodun et al. (2018) reported that pre-gelatinized and native trifoliate yam starches were similar in granular morphology but acid-treated starches have different shape (Figs. 2 and 3).

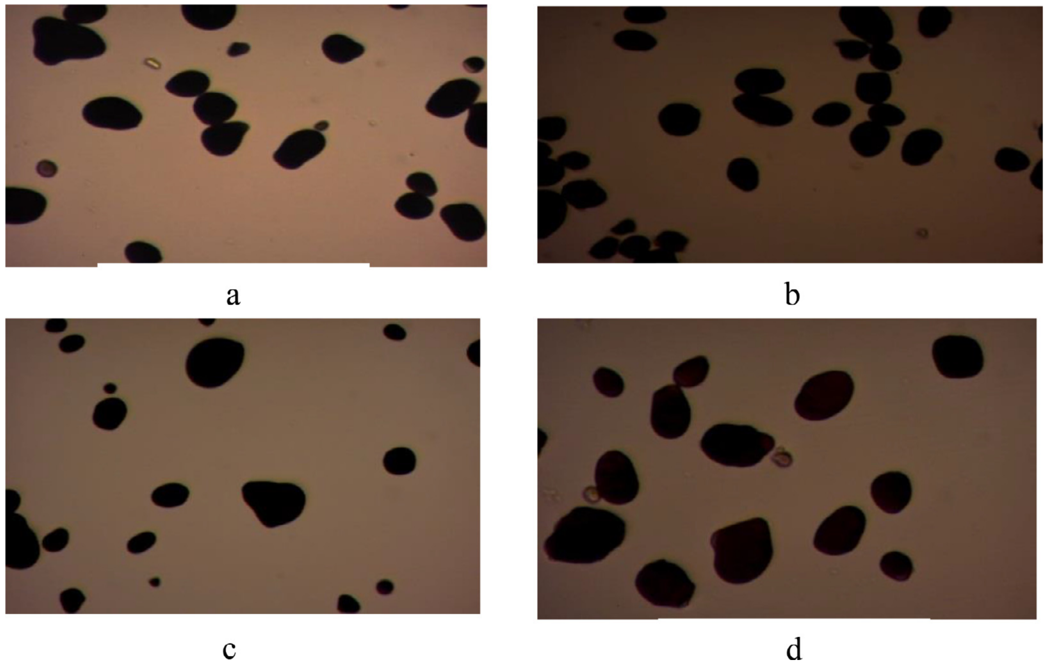

Fig. 1. The images of light microscopic views (400x); a: Palmyrah native starch, b: Palmyrah pre-gelatinized starch, c: Palmyrah acid modified starch, d: Palmyrah dextrinized starch.

Table 2
Particle analysis of palmyrah native and modified starch.

Sample	D10 (nm)	D50 (nm)	D90 (nm)	Average particle size(nm)
PNS	3473.1	5953.1	10251.8	6584.1
PPS	3298.66	5008.5	7573.3	5352.5
PAS	1290.7	1307.7	1324.7	1307.7
PDS	5664.8	7143.7	8992.5	7345.8

PNS: Palmyrah native starch, PPS: Palmyrah pre-gelatinized starch, PAS: Palmyrah acid modified starch, PDS: Palmyrah dextrinized starch.
D10 - The size point below which 10% of the material was contained.
D50 - The size point below which 50% of the material was contained.
D90 – The size point below which, 90% of the total volume of material in the sample was contained.

3.5. Analysis of particle size

Starches were classified into four categories based on the size of individual granules: Large-above 25 µm, medium – from 10 to 25 µm, small – from 5 to 10 µm, and very small – below 5 µm. (Molenda et al., 2006). According to the present study PNS, PPS and PDS had small granules but PAS (1.31 µm) had granules below the range of small granule and categorized as very small.

According to Table 2 & Fig. 2, Particle size of the PAS was very lower than PNS, PPS and PDS. Glycosidic linkages in the amorphous part of the granule were broken by acid treatment of granular starch and the crystalline section was not affected. The degree of acid hydrolysis can regulate the particle size of the products (Lu et al., 2018). Pre-gelatinization and acid modification process decreased the particle size of the PNS. Meanwhile dextrinization process increased the particle size of the PNS.

3.6. Analysis of functional properties

According to Table 3, There was a significant difference in % WAC among the PNS and modified palmyrah tuber starch. WAC % of PPS (115.40 ± 1.12^{a} %) and PDS (96.97 ± 5.39^{b} %), were higher than PAS (60.53 ± 1.20^{c} %) and PNS (45.66 ± 0.055^{d} %). Similarly, Kotanc and Akta(2006), also reported that pre-gelatinization and dextrinization process increased the both water holding capacity of wheat and corn starches than native starches. There was a significant difference in % OAC among the PNS and modified palmyrah tuber starch. There was no significance difference between PNS and PPS, PAS and PDS.

Modification process for palmyrah native starch increased the solubility percentage. The results of solubility of PNS and modified palmyrah tuber starch of present study was similar to the solubility of native and modified potato starch (Gadallah et al., 2014). Solubility of dextrinized potato starch was 52.38%, which was higher than native (13.87 %), pre-gelatinized (12.49 %) and acid-thinned (12.52%) at 90 °C.

There were no significance differences between PPS (4.10 ± 0.09^{a}) and PAS (4.20 ± 0.04^{a}) in swelling power and it is higher than PNS (3.47 ± 0.02^{b}) and PDS (2.72 ± 0.03^{c}). PPS had high swelling power due to the disruption inside starch granule and rearrangement of starch granule which enable the release of granule content (Tam et al., 2021).

The percentage of syneresis, which indicates the release of water from the starch gel during the freeze-thaw cycle process, was used to represent the freeze-thaw stability of starch. High synersis was observed in PDS (65.99 ± 0.04^{a} %). Yousif et al. (2012) have also reported that dextrinized potato starch has higher syneresis followed by native, pre-gelatinized and acid-thinned potato starches. PDS was not suitable for the development of products that require conservation at low temperature, such as sauces and baked goods.

The percentage of transmittance (T %) was used to determine the clarity of the starch solution. Starch for thickening fruit pie filling should be transparency; however, starch for spoonable salad dressing should be opaque. The clarity of starch granules varies significantly depending on the source of the starch and can be changed chemically. One of the most important properties of starch paste is its clarity. PDS (95.06 ± 0.22^{a} %) had the highest transmittance compared to others.

The temperature at which starch gelatinizes into a totally translucent gel was known as the gelatinization temperature. More heat is required to break it the stronger the intermolecular connection between the starch molecules, thus the gelatinization temperature is increased (Gunorubon & Kekpugile, 2012). PPS (75.5 °C$\pm1.00^{bc}$) and PAS (72.5 °C$\pm1.00^{c}$) had low gelatinization temperature compared to native

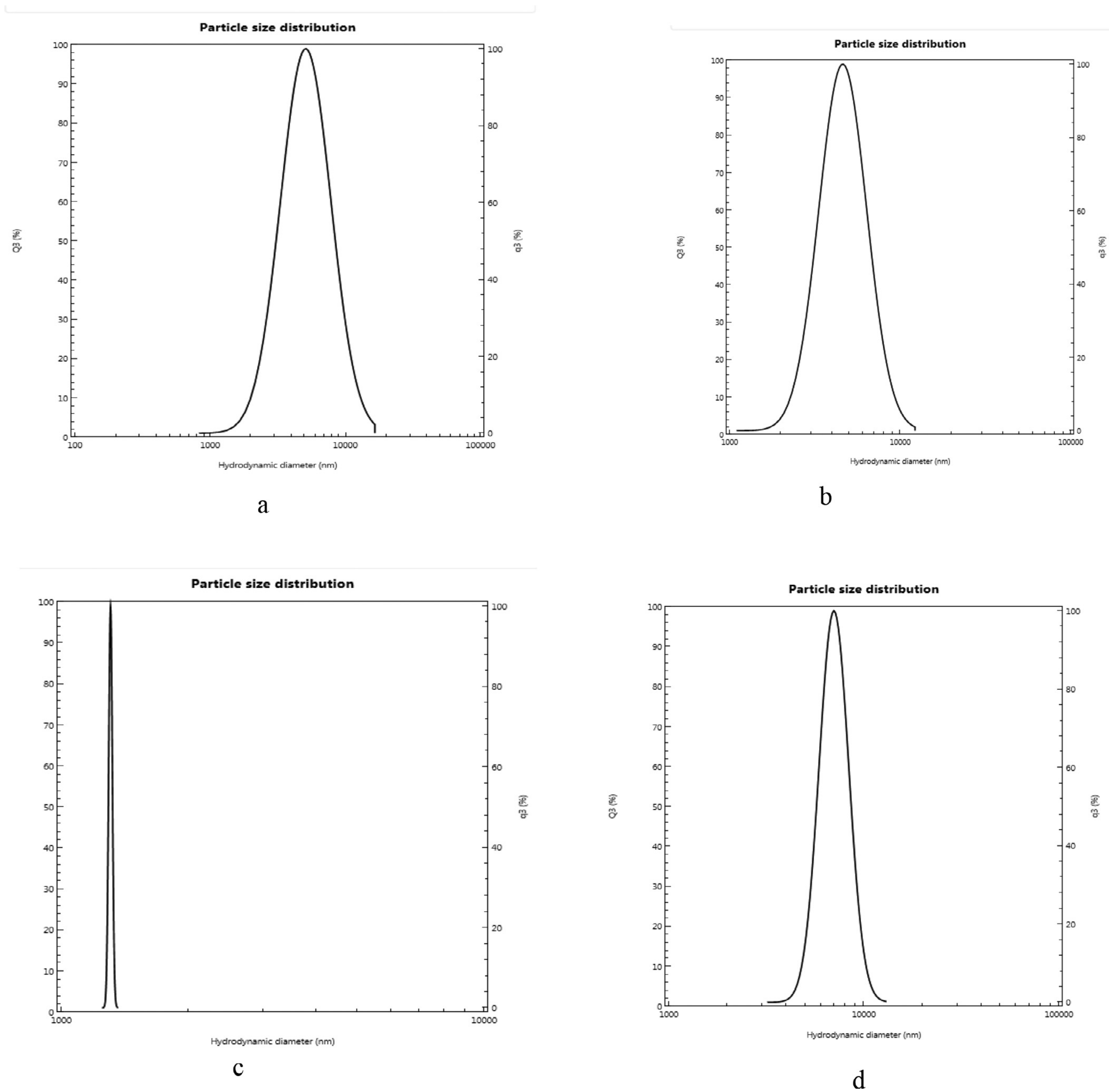

Fig. 2. Appearance of native and modified Palmyrah tuber starch; a: native starch, b: pregelatinized starch, c: Acid modified starch, d: dextrinized starch.

Table 3
Analysis of functional properties of palmyrah native and modified starch.

Sample	WAC %	OAC %	Average Swelling Power %	Average Solubility %	Transmittance	Viscosity ($mm^2\ s^{-1}$)	Gelatinization Temperature (°C)	Synersis %
PNS	45.66±0.06^{d}	81.30±0.45^{b}	3.47±0.02^{b}	0.92±0.02^{b}	48.01±4.63^{b}	82.4±0.1^{b}	76.5± 1.50^{ab}	38.97±2.70^{b}
PPS	115.40±1.12^{a}	86.33±1.32^{b}	4.10±0.09^{a}	1.27±0.02^{b}	28.34±1.57^{c}	85.2±1.13^{a}	75.5±1.00^{bc}	24.83±1.97^{c}
PAS	60.53±1.20^{c}	94.63±3.39^{a}	4.20±0.04^{a}	1.63±0.22^{b}	44.20±1.19^{b}	84.4±0.1^{a}	72.5±1.00^{c}	36.76±0.00^{b}
PDS	96.97±5.39^{b}	96.24±2.14^{a}	2.72±0.03^{c}	9.67±0.55^{a}	95.06±0.22^{a}	4.0±0.1^{c}	82.27±2.25^{a}	65.99±0.04^{a}

Values followed by the same letters in the same column are not significantly different ($p \leq 0.05$); PNS: Palmyrah native starch, PPS: Palmyrah pre-gelatinized starch, PAS: Palmyrah acid modified starch, PDS: Palmyrah dextrinized starch.

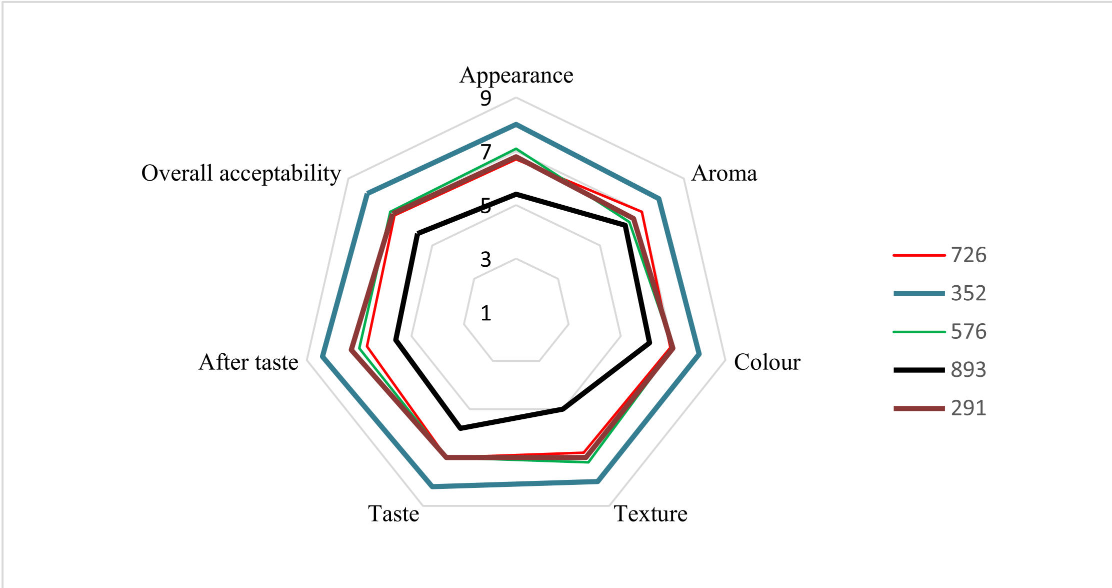

Fig. 3. Radar diagram for median score of sensory.
(726: Palmyrah native starch, 352: Palmyrah pregelatinized starch, 576: Palmyrah acid modified starch, 893: Palmyrah dextrinized starch, 291: Commercially available corn starch).

starch. Omoregie (2020) reported that pre-gelatinized starch exhibit reduced gelatinization temperature and time. Decrease in gelatinization temperature was attributed to depolymerization of the starch molecules, resulting in a weakened granule organization (Yousif et al., 2012). Time taken for gelatinization increased to PDS compared to other starch. As a result gelatinization temperature also increased for PDS.

There was a significant difference in viscosity among the PNS and modified palmyrah tuber starch. The viscosity was high for PPS (85.2 ± 1.13^{a} mm^2 s^{-1}) and PAS (84.4 ± 0.1^{a} mm^2 s^{-1}). But dextrinization process decreased the viscosity of PNS. Viscosity also increased with the increase in amylose of content of starch. Xie et al. (2009) also reported that higher amylose content of starch resulted in a higher apparent viscosity over the same shear rate range.

3.7. Sensory evaluation of soup mix

According to the scores for sensory evaluation (Fig. 3) sensory attributes appearance, aroma, colour, texture, taste after taste and overall acceptability. PPS incorporated soup mix sample had high score and PDS incorporated soup mix had lower sensory score. There were no significant differences between PNS, PAS and CS incorporated sample sensory score in Tukey comparison.

4. Conclusion

The study revealed that the modification processes can greatly improve the physicochemical and functional characteristics of native starch and the feasibility of application of modified palmyrah starch as a thickening agent. The crude protein, lipid and fiber contents of modified starches significantly decreased compared to native starch. The viscosity of starches and the textural properties of starch gels strongly correlated with the amylose percentage. The water and oil absorption capacities of palmyrah starches were significantly increased after the modification processes. The dextrinized palmyrah starch showed significantly ($p<0.05$) the lowest swelling power and the highest average solubility. The pre-gelatinized palmyrah starch showed a significantly decreased level of syneresis. According to the sensory analysis as a thickner in instant soup, high preference was observed for palmyrah pregelatinized starch compared to commercially available corn starch.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

N. Sobini: Conceptualization, Writing – original draft, Project administration. **S. Darsiga:** Methodology, Data curation. **T.C. Kananke:** Supervision. **S. Srivijeindran:** Supervision.

Data availability

Data will be made available on request.

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