Synthesis and Characterization of Dual Properties Coagulant from Natural Precursors for Raw Water Treatment

Syazwani Mohd Asharuddin[1], Norzila Othman[1*], Nur Shaylinda Mohd Zin[1], Mohd Fadhil Md. Din[2], Vicky Kumar[1]

[1]Faculty of Civil Engineering and Built Environment,
 Universiti Tun Hussein Onn Malaysia, 86400 Parit Raja, Johor, MALAYSIA

[2]Faculty of Civil Engineering,
 Universiti Teknologi Malaysia, 81310 Skudai, Johor, MALAYSIA

*Corresponding Author

Abstract: A novel coagulant with dual properties was produced by manipulating the chemical characteristics of natural precursors. In the present study, cassava peel starch (CPS) was isolated from agriculture waste and lemongrass extract was procured using soxhlet extraction using ethanol. A preliminary study consisting of chemical analysis and antimicrobial test was done onto the lemongrass extract to ensure the presence of the active group responsible for antimicrobial effect. The modified cassava peel starch (MCPS) was synthesized using novel acid-alkali grafting method to incorporate active compounds from lemongrass extract onto the starch backbone. The modified starch was subjected to Scanning Electron Microscopy coupled with Electron Dispersive X-Ray Spectrometry (SEM-EDX) for surface morphological and elementral distribution analysis. The molecular composition analysis was accomplished using Fourier Transform Infrared Spectrophotometry (FTIR) while the efficacy of the modification process was further analyze using proton Nuclear Magnetic Resonance Spectroscopy (1H NMR). The SEM-EDX and FTIR results revealed impressive changes in the physical surface morphology, elemental distribution and chemical structure of the starch post-modification. The results also indicated successful integration of lemongrass active groups onto the starch molecules which provides promising potential of the modified starch towards proposed application for water clarification and disinfection.

Keywords: Natural coagulants, flocculant, water disinfectant, water treatment, starch, modified starch

1. Introduction

Starch is one of the most abundant biological macromolecules on earth and can be find in numerous sources ranging from rice, wheat, tubers, fruits and vegetables. In the crude form, the skeletal framework of starch consists of a mixture of two polymers of anhydroglucose units, amylopectin and amylose. Amylopectin is a highly branched polymer of a-D-glucopyranosyl residues linked together by 1-4 linkages with 1-6 bonds at the branch points which these molecules compose the major component of most starches and plays an essential role in defining the characteristics of the starch. Whereby, amylose is a linear polymer of 1-4 linked a-D-glucopyranosyl units with low molecular weight, which makes up 25% of the starch [1].

In current years, starch-based flocculants have become more prominent in the field of water treatment due to their significant characteristics of widespread availability, low cost, environmental friendliness, and biodegradability.

*Corresponding author: norzila@uthm.edu.my
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Moreover, owing to the attractive molecular composition of starch that contains large numbers of hydroxyl groups on its saccharide ring, starch can be easily modified chemically to satisfy various applications by the introduction of different functional groups onto its backbone [2,3]. A wide spectrum of modification techniques have been tested on starches from numerous sources and these starches were investigated for their flocculating abilities [1].

Dimethylamine-based, polyacrylamide-based and acrylamide-based graft copolymerisation is among the various modification techniques that most broadly studied in persistence to enhance the performance of starch to be potential coagulants. Unfortunately, along with the growing concern on human health effect, despite to their superior effect on water clarification, the technique has been blasted with critics due to the potential toxicity of the acrylamides. WHO [4] reported that following ingestion, acrylamide is readily absorb from the gastrointestinal tract and widely distributed in body fluids. It has the potential to cross the placenta, affect germ cells as well as impairs reproductive function. Additionally, human exposure via the consumption of drinking water treated with synthetic polyelectrolytes grafted with acrylamide revealed the development of widespread central and peripheral system dysfunction [5] The European standard only permits a maximal concentration of 0.1 mg/L for acrylamide and 0.5 mg/L for polyacrylamide with an acrylamide concentration of 0.02%. Polymer restrictions have also been regulated because of their toxicity effects on the environment [6]. Several studies exposed the severe toxic effects of cationic polyacrylamide to aquatic life as the polymer tends to sorb on fish gills and causing gill lesions and increase frequency of necrotic cells [7]. Besides, polyacrylic acids, polystyrene sulfonic acids, and polyacrylamide are synthetized from nonrenewable oil-based materials and are considered hardly biodegradable [8].

Emerging concern associated with environmental drawbacks brought by the synthetic polyelectrolytes therefore have led to a significant research body aimed to replace the toxic monomers with natural and sustainable alternatives, which would eliminate the risks related to the presence of toxic monomers or impurities. There are possibilities of reducing exposure to the toxic chemical monomers carried by the treated water by using lower dosage of the chemical coagulants in combination with an alternative flocculant such as modified starch using natural monomers.

Hence, to the best of our knowledge, no studies have tested the modification process of starch incorporated with natural precursors such as lemongrass extract. Lemongrass extracts contains active ingredients like myrcene, citronellal, citronellol and geraniol [9]. In recent years, antibacterial property found in the extract of lemongrass has been elucidated from various researches with promising result. α-citral (geranial) and β-citral (neral) are among the major bioactive compound that contributing to the antimicrobial activity of the extracts. These components possess the ability to inhibit the growth of both Gram positive and Gram negative bacteria [10].

Therefore, the present research discovers the potential usage of starch isolated from waste materials discarded from agricultural industries. New coagulant modification approach by avoiding toxic chemical monomers was proposed using the principle of acid-alkali crosslinking method by incorporating lemongrass extract intended to produce a flocculant with enhanced capability to aid flocculation and disinfection of *Escherichia coli* (*E.coli*) in the water.

2. Materials and Method

2.1 Isolation of Starch

Isolation of cassava peel starch (CPS) was performed according to the method described by Benesi [11] and Fakir et al. [12] with slight adjustment where the starch was isolated from cassava peels instead of cassava flesh. The cassava peels were procured from a local cassava chips factory. Good quality peels were selected and cleaned with tap water to remove any surface impurities. The outermost layers were scrapped off manually. 100g peel then suspended in 200 ml distilled water and pulverized using domestic blender for 5 minutes. Subsequently, 2000 mL of distilled water was added into the suspension and stirred for 2 minutes. Then the suspension was filtered using doubled fold muslin cloth and the filtrate was allowed to stand for 2 hours for sedimentation. Then the sediment was collected and sun dried for at least 24 hours to remove the moisture content. Dried CPS was kept in air tight glass container for further experimental use.

2.2 Preparation of Lemongrass Extract

Lemongrass stalks were purchased from local markets around Parit Raja, Johor, Malaysia. 500g of lemongrass stalks were fragmented into 10mm long pieces and dried in oven at 60°C for 72 hours to remove the moisture content. The dried samples then were crushed using domestic blender to increase the surface area. The samples were stored in air-tight bottle for usage in subsequent extraction process [13,14].

15g of crushed lemongrass samples were added into extraction thimbles. 200ml of solvents (95% ethanol and 95% methanol) were added into different round bottom flasks. Extraction using the soxhlet apparatus was done for 7 hours at 70°C. After the extraction completed, the extracts were distilled off at 65°C using rotary evaporator. 10 mL of the final extracts were diluted into several concentrations (20% - 100%) using sterilized distilled water for antimicrobial test [15, 16]. Remaining extracts were stored in air-tight amber glass bottles at 4°C for further analysis using FTIR and GC-MS.

2.3 Modification of Starch Using Lemongrass Extract

Native CPS (25 g, dry basis) was suspended in 100 mL of 100% v/v ethanolic extract of lemongrass and stirred at 150 rpm for 1 hour at 25 °C, then the reaction was started by adding 1 mL of concentrated (36% by weight) hydrochloric acid (HCl) and allowed to proceed at 25°C for 24 hours with continuous stirring at 150 rpm. The reaction was stopped by adding 14 mL of 1 M sodium bicarbonate (NaHCO3). The solution was cooled in ice-bath for 5 min, and then centrifuged at 4000 rpm for 5 min. Subsequently, the precipitate was sun dried until it reached constant weight. Modified cassava peel starch (MCPS) were finally stored as solid powders to achieve long-term shelf stability.

2.4 Physico-Chemical Study of CPS and MCPS

The surface morphology and chemical composition were observed through SEM-EDX (JEOL JSM-6380LA) spectroscopy. The surface chemical functional groups of both CPS and MCPS were characterized by Fourier transform infrared (FTIR, Perkin Elmer Spectrum 100) spectroscopy with the measured wavenumber from 400 to 4000 cm-1 and 1H nuclear magnetic resonance (1H NMR, Bruker AVANCE Model DRX-500) spectroscopy using D_2O as solvent. The X-ray patterns of the starches were obtained using an XRD-6100 diffractometer (Shimazu Co., Kyoto, Japan) within scanning region of the two-theta angle being from 4° to 37°.

3. Results and Discussion

3.1 Characterization of Lemongrass Extract

Characterization study was carried out by FTIR analysis to know about the complex formation and interaction of major functional groups involved in the modification of the CPS. These results helped in confirming the presence of important active functional groups that make up the antimicrobial compound in lemongrass extract.

As presented in Fig. 1, the adsorption band structures observed between 3150 and 3000 cm-1 are almost exclusively indicative of unsaturation (C = C- H) and/or aromatic rings and their presence in the IR spectrograph obtained is thus indicative of the presence of aromatic compounds [17]. The strong methylene/methyl band (1446.5 cm-1) and a weak methyl band (1384.8 cm-1), plus a band at 740 cm-1 (methylene rocking vibration) is indicative of a long-chain linear aliphatic structure. The saturated hydrocarbon C- H stretching absorptions all occur below 3000 cm-1. Carbonyl compounds are often the strongest band in the spectrum and lie between 1700 - 1500 cm-1 its exact position being dependent upon its immediate substituent. For a double-bonded functionality, conjugation plays an important role in the observed carbonyl frequency. This includes connection to an aromatic ring or conjugation to a C = C or another C = O [17]. According to Natrajan et al. [18], the peak in the range of 1700 - 1500 cm-1 indicating the presence of citral, an antimicrobial compound in lemongrass oil. The FTIR result obtained in the present study is also comparable to pure geraniol compound which showed similar presence of functional groups including OH- CH_3-, C=C, -C = CH- and C-O-H [19]. Geraniol is a form of citral, a constituent of lemon grass and lemongrass essential oil (up to 85% of volatile oil content) have been found to exhibit a potent antibacterial action against various pathogens including *Escherichia coli* and *Salmonella enterica* [9].
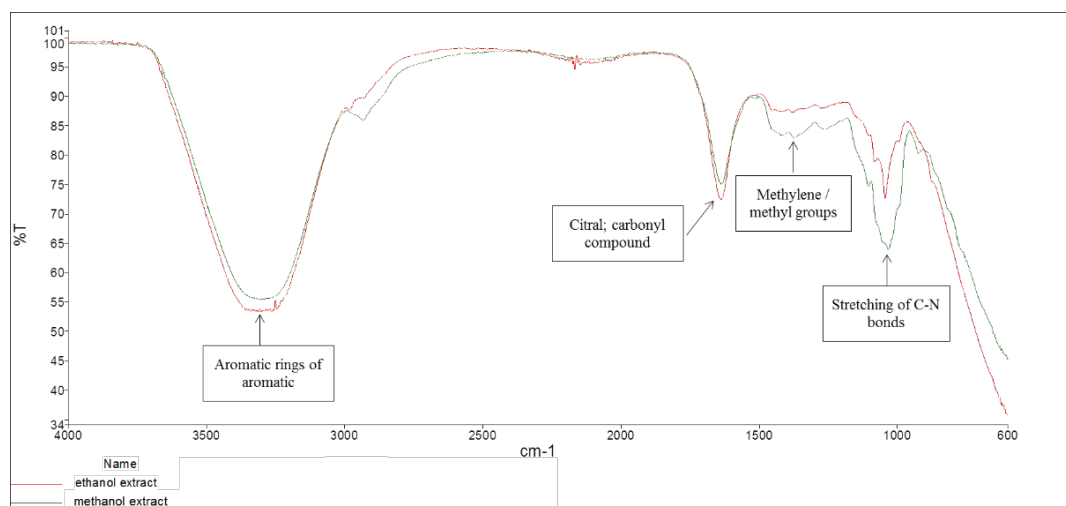

Fig. 1 - FTIR spectrum of ethanolic and methanolic extract of lemongrass

GC-MS analyses were performed with the aim to compare the compositions of the ethanolic and methanolic extract of lemongrass. It was observed that citral is the major compound found in both lemongrass extracts of ethanol and methanol as the solvents (Fig. 2 and Fig. 3). However, the intensity is significantly higher for ethanolic extract.

Citral, an acyclic monoterpene aldehyde is the key constituent to evaluate the quality of lemongrass extracts [20, 21]. Citral is a racemic mixture of two isomers, geranial (α-citral) and neral (β-citral) and commonly used as a raw material for the perfumery, cosmetics and pharmaceutical industries including the synthesis of vitamin A [9].

According to a review on antibacterial activity of lemongrass extracts written by Mosquera et al. [22], a number of components have been identified in the essential oil extract of the plant. Although there is variability in the percentages found, there is consistency in its major components, including citral. Additionally, Negrelle & Gomes [23] have pointed out that irrespective of the lemongrass origin, the essential oils of *Cymbopogon citratus* are mainly composed of citral (30 to 93.74%).10 Tests have been carried out to identify the metabolites responsible for the antibacterial activity. These activities are shown in two of the three main components of oil, identified through chromatography and mass spectrometry methods. While the components (neral) α-citral and (geranial) β-citral cause individually antibacterial action on gram-negative and gram-positive strains, while the third component, myrcene on its own shows no antibacterial activity.
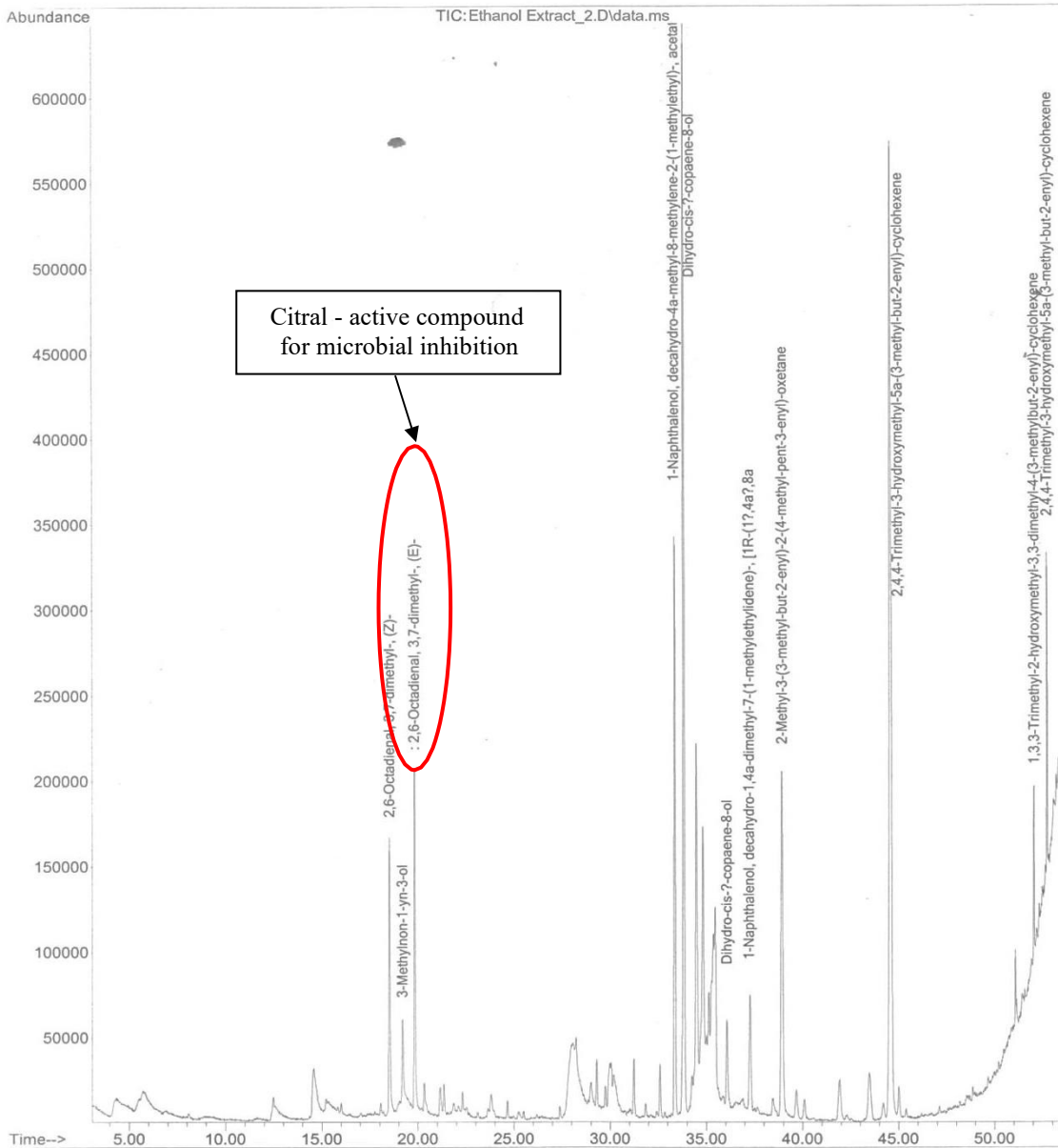

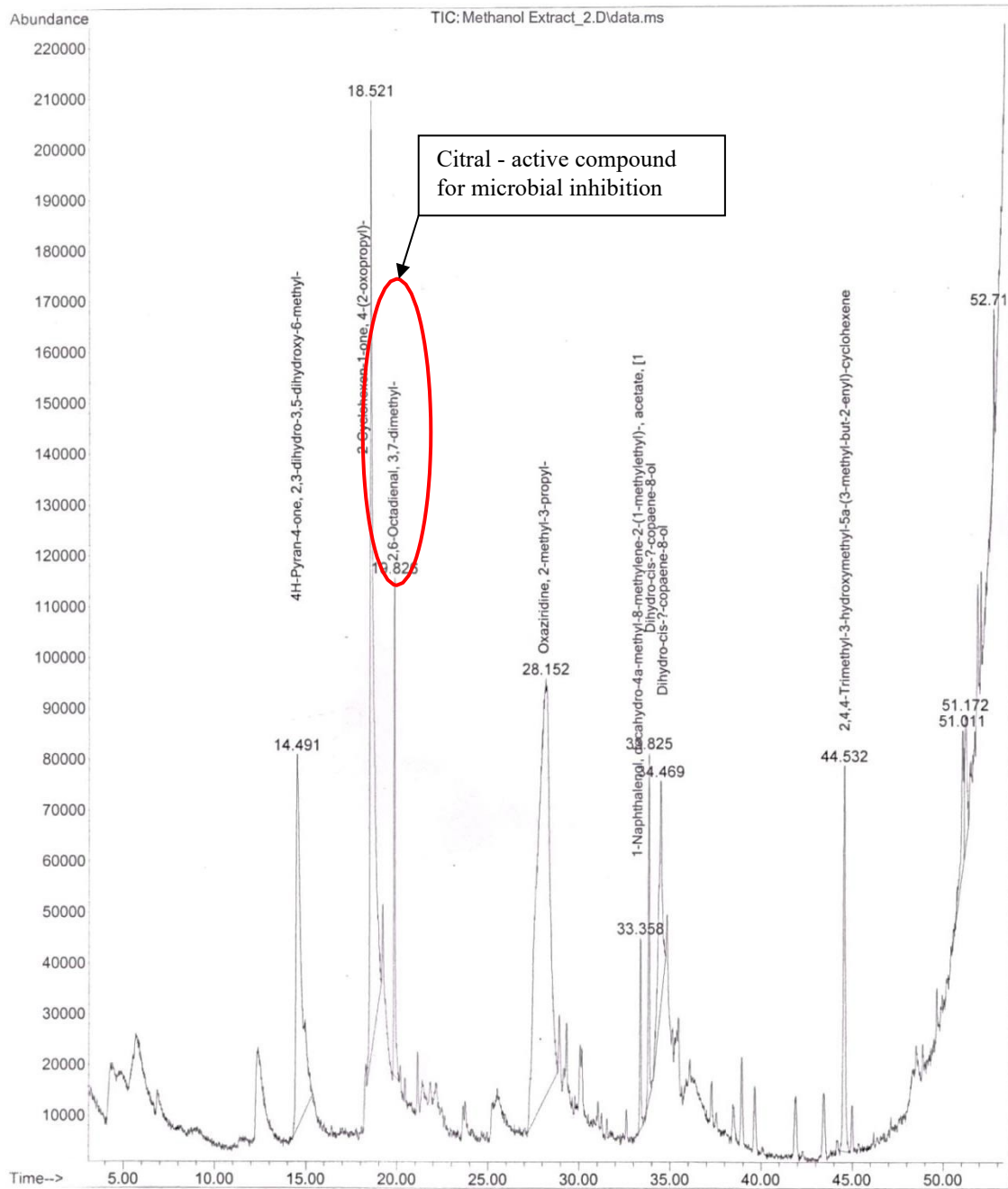**Fig. 2 - GC-MS spectrum of ethanolic extract of lemongrass**

Fig. 3 - GC-MS spectrum of methanolic extract of lemongrass

3.2 Antimicrobial test of lemongrass extract against *E.coli*

Results of the antimicrobial activity of lemongrass extracts portrayed the growth inhibition effects were detected using Kirby-Bauer method as presented in Fig. 4. The extracts were tested on gram negative bacteria - *E.coli*. Based on the zone of inhibition as tabulated in Table 1, the ethanol extract indicates better antimicrobial activity compared to methanol extract with highest diameter of inhibition zone recorded at 27 mm for 100% extract concentration which is comparable to the diameter of inhibition zone recorded for ampicillin antibiotic (28 mm).

The comparative effects of different concentration of lemongrass extracts and the standard antibiotic disc on the target organism are demonstrable indications of the extract as an antibacterial agent. The results obtained were comparable to other antimicrobial studies described by several literatures [24]-[26]. Similar antibiotic susceptibility pattern also had been reported by Onawunmi & Ongulana [27] and the authors suggested that the test organisms particularly gram negative were found to be more susceptible to lemongrass extracts than standard antibiotic.
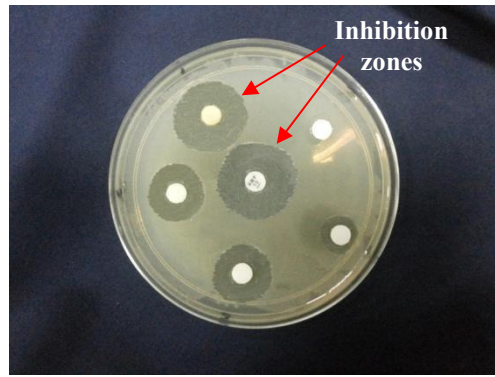
Fig. 4 - Antimicrobial test using Kirby-Bauer method

Table 1 - Inhibition zone diameter of lemongrass extracts against *E.coli*

Test organism	Extract concentration (%)	Zone of inhibition (mm)			
		Ethanol extract	Methanol extract	Ampicilin antibiotic (positive control)	Blank disk (negative control)
E.coli	20%	11	7		
	40%	15	13		
	60%	20	15	28	--
	80%	22	18		
	100%	26	21		

3.3 Characterization of Native and Modified Starch

As presented in Fig. 5, CPS presented smooth and non-porous surface with starch granules were appeared in irregular spherical in shape, truncated and bowl-shaped topography structure. Some ridges were also identified that appear to mark sites where granules were in close contact with one another. These observations were in parallel with morphological studies of native cassava starch conducted by Charoenkul et al. [28] and Xia et al. [29]. The microscopic analysis of MCPS showed small pores were detected in minimal amount and the starch granule surface appeared to be rough and wave-like creases were spotted as reported by Palacios-Fonseca et al. [30].

Generally, the starch granules sizes of the CPS and MCPS were observed in the range of 6 - 13 μm. These sizes were comparable to those reported by Charoenkul et al. [28] (4-20 μm), Niba et al. [31] (9-20 μm), Sriroth et al. [32] (8 - 22 μm),), Ji et al. [33] (10 - 20 μm) and Monroy et al. [34] (6-12 μm). According to Lindeboom et al. [35], starch granules can be into four size classes: large (greater than 25 μm), average (10 to 25 μm), small (5 to 10 μm), and very small (less than 5 μm). The granules in the storage roots were observed to be larger in diameter than in the fibrous root. The granule size increased from the outermost layer towards the innermost layer of the storage roots. The size of starch granules plays an important role in the modification of the microstructure due to a capacity for trapping kinetic energy [36].
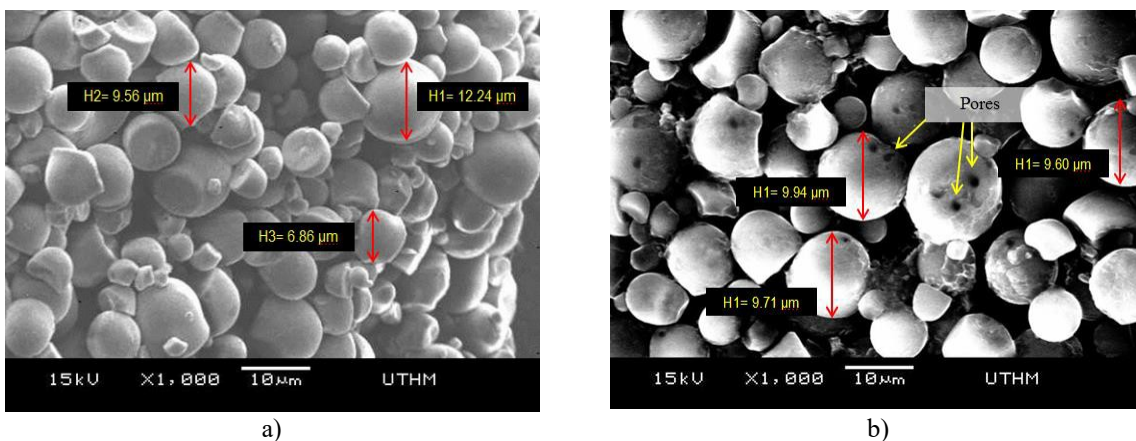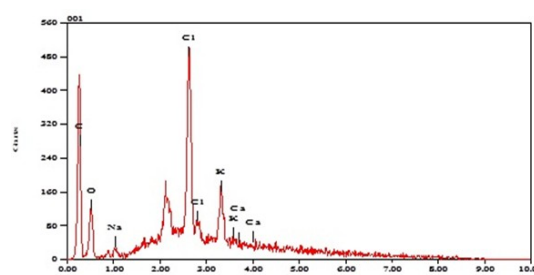

a)

b)

Fig. 5 - SEM micrograph of (a) native CPS at X1000 (b) MCPS at X1000

The differences in the elemental contents between native CPS and MCPS samples were observed. The EDX analysis done on the native CPS only identifies the presence of prominent C and O peaks which confirm the supposition that the starch granules were purely made up from carbohydrate monomers (Fig. 6). However, apparently, the modification of CPS using lemongrass extract and hydrochloric acid had imposed the surface elemental characteristic of the MCPS that increasing the possibility of antimicrobial ability and retaining metallic ions in different parts of the coagulant which is supported by the findings obtained from EDX analysis as presented in Fig. 7. Elements of Na, K and Ca were found in very minimal percentage in the native CPS, whereas a significant percentage of these elements were detected in the modified samples with significant addition of Cl element. The appearance on those elements in the MCPS indicates the occurrence of modification and proved that the modifying agents, lemongrass extract and HCl were grafted onto the cassava peel starch.
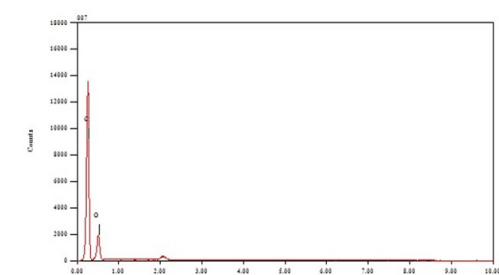
Element	(keV)	mass%	Error%	At%	Compound	mass%	Cation	K
C K	0.277	76.04	0.15	80.87				117.4442
O K	0.525	23.96	1.11	19.13				24.6908
Total		100.00		100.00				

Element	(keV)	mass%	Error%	At%	Compound	mass%	Cation	K
H K								
C K	0.277	49.79	0.13	70.28				37.9293
O K	0.525	10.46	0.28	11.08				17.1366
Na K	1.041	0.56	0.15	0.41				1.6760
Cl K	2.621	26.99	0.26	12.90				95.6591
K K	3.312	11.90	0.46	5.09				38.6482
Ca K	3.690	0.30	0.60	0.13				1.0256
Total		100.00		100.00				

Fig. 6 - EDX spectrum of CPS   **Fig. 7 - EDX spectrum of MCPS**

The molecular level changes of the starch resulted from the modification process were evaluated using FTIR and the spectra are showed in Fig. 8. According to Kacurakova et al. [37], FT-IR spectra within the range of 1200 ± 800 cm^{-1} region indicating the main polysaccharides present in the complicated systems of polysaccharide mixtures. The overall shape of a polysaccharide spectrum is determined by the backbone polysaccharide composition but can also be strongly influenced by the side chain constituents. The broad band observed between 3500 - 3200 cm^{-1} may be assigned to O-H groups of free hydroxyl groups and bonded O-H group in polymeric compounds such as alcohols, phenols and carboxylic acids presented in pectin, cellulose and lignin on the CPS. The peak at wavenumber between 3000 - 2850 cm^{-1} may indicate the stretch of symmetric or asymmetric CH, CH$_2$, of aliphatic acids. Furthermore, the characteristic peak appeared between 1750 - 1680 cm^{-1} are demonstrating the stretching vibration of C=O bond of carboxyl groups. Whereas the peaks observed between 1375 - 1300 cm^{-1} may reflect the stretching vibration of ionic carboxylic groups of pectin. While the deep peaks between 1300 - 1000 cm^{-1} indicate the C-O stretching of -C-O-OH side group and C-O-C gycosidic bond vibration. The results were in agreement with FTIR analysis done onto native starch by other studies as summarized in Table 2.

Distinctive changes were found where the peak intensity for MCPS at 1016 cm^{-1} was increased significantly. Compared to the peaks for native CPS within the wavenumber of 1400 - 1200 cm^{-1}, the peaks for MCPS were observed to be reduced and shifted towards 1500 cm^{-1}. This region was characteristic of aliphatic amines. Similarly, prominent broad peak between 3750 - 3200 cm^{-1} which assigned to O-H group of free hydroxyl groups and bonded O-H group in polymeric compound was also observed to be shifted after modification process took place from 3263 cm^{-1} to 3436 cm^{-1} [37].

Furthermore, the characteristic peak appeared between 1750 - 1680 cm^{-1} are demonstrating the stretching vibration of C=O bond of carboxyl groups. Whereas the peaks observed between 1375 - 1300 cm^{-1} may reflect the stretching vibration of ionic carboxylic groups of pectin. While the deep peaks between 1300 - 1000 cm^{-1} indicate the C-O stretching of -C-O-OH side group and C-O-C gycosidic bond vibration [38].

It was clearly observed from the FTIR spectrum that carboxyl and hydroxyl groups were present in abundant since cassava peel starch is principally composed of glucose monomers. These groups that constituted in biopolymers may function as proton donors; hence depronomated hydroxyl and carboxyl groups may be involved in the formation of floc complex when the starch is applied together with primary coagulant. The primary coagulant may initially forms micro flocs which gradually grow to become macro flocs when the MCPS is added. Moreover, many observations made on bioflocculants suggest that carboxyl, hydroxyl, carbonyl as well as amino (amine and amide) groups were the preferred groups contributing towards the flocculating ability of the biomaterial [39]. The functional groups plays the key role for the flocculation process via two methods that are hydrophilicity characteristics which are utilized to extend the polymer chain; and the functional groups extent the gap between particles to effectively adsorbs the particles [40].
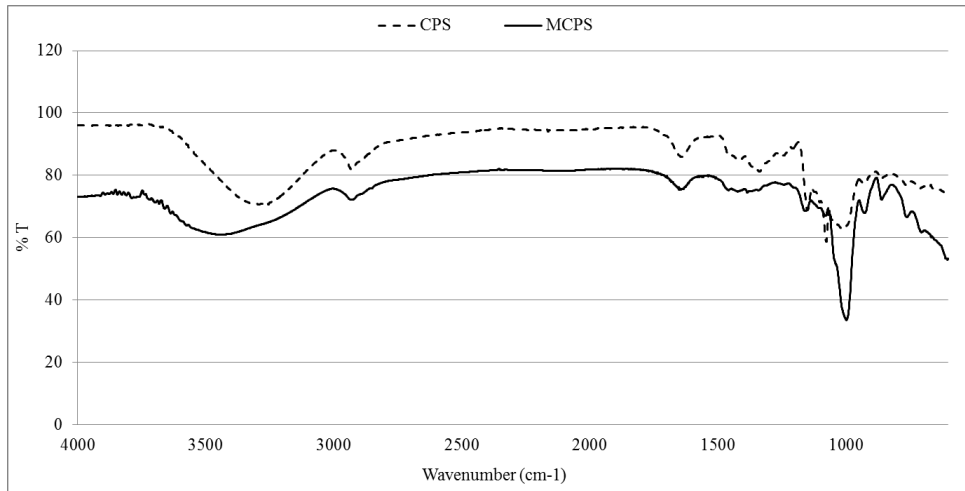
Fig. 8 - FTIR spectrum of CPS and MCPS

Table 2 - Summary of functional groups identified in native starches

Identified groups	Wavelength range (cm^{-1})		
	CPS (present study)	Cassava starch (Lomelí-Ramírez et al. [41]).	Tapioca starch (Sacithraa et. al. [42])
O-H groups of free hydroxyl groups and bonded O-H	3500 - 3200	3300	4000 - 2500
Symmetric or assymetric stretching of CH, CH$_2$ of aliphatic acids	3000 - 2850	2926	3000 - 2850
Stretching vibration of C=O bond of carboxyl groups	1750 - 1680	1715	2000 - 1500
C-O stretching of COOH	1300 - 1000	1160	1242 - 1016

The crystallinity of starch is related to amylopectin linear chains which may give different X-ray diffraction patterns depending on starch source [43]. As can be seen in Fig. 9, both CPS and MCPS exhibited same typical A-type crystalline pattern with strong reflections at about 2θ 16° and 24° and unresolved doublet at 2θ 18° and 19°. Similar crystalline pattern of starch were also reported by Shariffa et al. [44]; Mohd et al. [45]; Gao et al. [46]. This result supported the hypothesis chemical modifications had little effects on the crystalline structure, but rather concentrated primarily in the amorphous lamellae of starch granules [47].
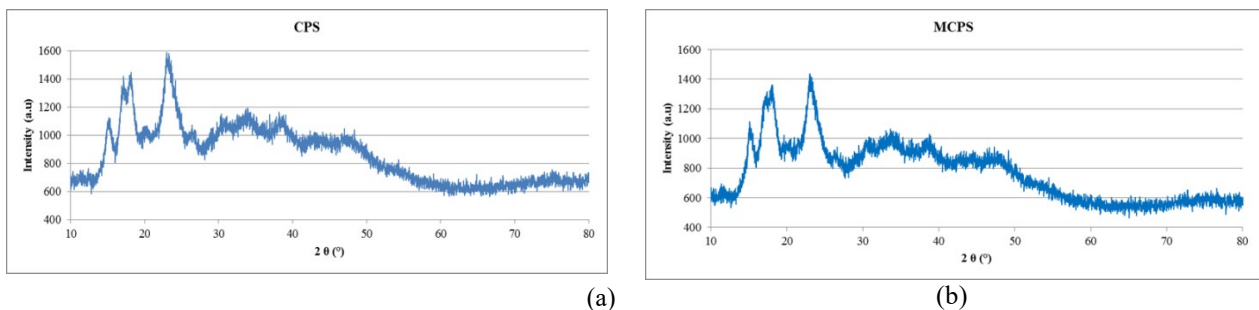

(a)   (b)

Fig. 9 - XRD spectra of (a) native CPS and (b) MCPS

According to Harry et al. [48], the crystalline pattern of starch can be classified into four types, A, B, C and V type. Type A, B and C are the crystal structures of natural starches whereas the V type are complexes formed by amylose and lipids. A-form starch gives stronger diffraction around 2θ 15°, 17°, 18° and 23° with absence of peak in the range, 5° to 6°. B-form possesses strongest diffraction at 17° and other smaller peaks at 20°, 22° and 24°. C pattern is a mixture of A and B. The present X-Ray diffraction studies also proved that modified CPS suffered change in crystallinity. Reduction

of intensities was observed after the modification process which suggesting change in crystallinity of the starch after the treatment. Such lowered reflection of intensities could most likely be attributed to the weakened or partial destruction of inter and intra molecular hydrogen bonds in amylopectin [46]. Gao et al. [46] also suggested that partial destruction of crystal structure of starch could allow more hydroxyl groups to react with other species in aqueous solution. The starch crystallinity can be affected by by four factors including crystal size, the number of crystalline region affected by crystalline content and length of chain, the orientation of double helices within the crystalline area and the extent of interaction between the double helices [45, 46]. Tukomane et al. [46] also reported that crystalline region is an ordered arrangement of double helical amylopectin structures which reduces with increase in the amylose and is increased by hydrolysis of starch.

4. Summary

The integration of active groups from lemongrass extract onto cassava peel starch was successfully accomplished. The observation showed the modified starch undergone minor changes in the physical characteristics by the signs of wave-like creases on the surface and slightly shifted crystalline structure. Moreover, the chemical characteristics of the modified starch were observed to be significantly altered following the modification treatment. Therefore, the results from the present study provide promising potential of manipulating natural precursors as alternative for starch modification in order to enhance the starch properties as flocculant and disinfectant for water treatment.

Acknowledgement

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