

## Research Article

### Modification of Porang (*Amorphophallus oncophyllus*) Flour by Acid and Thermal Process using Conventional Heating in Waterbath and Microwave Irradiation

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**Abstract:** Modified porang flour by lactic acid and conventional heating using waterbath (PAW) and microwave radiation (PAM) were determined its functional group using FTIR, the chemical structure were studied using <sup>1</sup>H-NMR, molecular weight were measured using HPLC-SEC and its microstructure were studied using SEM, particles size were measured using PSA and rheology characteristics were determined using Rheometer. The results showed that modified porang either PAW and PAM showed changes on functional group peaks compared to one of native porang flour included OH, CH<sub>3</sub>, C-C, C = O, C-O-C, mannose and glucose groups. <sup>1</sup>H-NMR spectra of modified porang flour (PAW and PAM) were similar mainly in proton of CH<sub>3</sub> and CH<sub>2</sub>, molecular weight were higher at PAW 2.0×10<sup>6</sup> and PAM 3.0×10<sup>6</sup>, while particles size of PAW and PAM were smaller. The microstructure of PAW and PAM were porous and spongy indicated that PAW and PAM had higher moisture accessibility on the amorph site. Storage modulus (G') dan loss modulus (G'') PAW and PAM decreasing with the increased temperature. Lower G' compared to G'' and modulus (G' and G'') did not showed crossing indicated that PAW and PAM have a stable visco-elasticity. It can be concluded that functional group peaks of modified porang flour (PAW and PAM) changed differently compared to native porang flour, <sup>1</sup>H-NMR spectra of modified porang flour were similar, with bigger molecular weight of PAM than PAW, the microstructure were porous and spongy and rheology characteristics indicated that an unchanged visco-elastic system by thermal treatments.

**Keywords:** Lactic acid, microwave, modified porang, porang flour, waterbath

## INTRODUCTION

Porang (*Amorphophallus oncophyllus*) is included in the tuber family of Araceae, one genus with konjac (*Amorphophallus konjac*) with major component of both plant are mannan and have composition variation between 8-10%. The other components also found were starch, protein, lipid and mineral. The mannan particles are distributed in whole tuber and encapsulated by protoplasm like a thin film and the space between mannan cell is parenchym. This parenchym tissue consist of thin wall cells containing starch granules. The konjac mannan cell filled with glucomannan polysaccharide covered by cell wall with size variations from 160 to 650 μm (Takigami *et al.*, 1997).

Konjac flour contain about 50-70% glucomannan, while konjac glucomannan extract could contain more than 90% as reported by Takigami (2000). According to Arifin (2001) porang flour contain glucomannan up to 64.98% in the form of polymer of D- mannose and D- glucose. Konjac flour also contain some impurities

which most are insoluble starch and cellulosa included protein and lipid and these impurities originated from "sack" that encapsulated konjac flour in the tuber (Tatirat *et al.*, 2013).

Konjac glucomannan were found in copolymer form of β-(1-4) D-glucose and D-mannose binding with molar ratio 1: 1.6 and low rate acetyl group (approximately 1 acetyl group in each 17 residue) at C-6 position (Maeda *et al.*, 1980; Katsuraya *et al.*, 2003). Gao and Zhang (2001a, 2001b) reported that as some hydroxyl group (-CH<sub>2</sub>OH) at C-6 position each unit was constitutionally very active, hence some chemical reactions could occurred at this position such as esterification, etherification and nitrition and according to Chen *et al.* (2005) graft Polymeration could also takes place in this site. The problem related to the thermal process of polysaccharide was a strong interchain interaction could occurred related to the extensive hydrogen bonding. This interaction produced polymer system specifically could go to thermal

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decomposition before reaching the melting point (Xiao *et al.*, 2001).

The drawback of porang (*Amorphophallus oncophyllus*) flour was low solubility in water (Irawan and Widjanarko, 2013), while the lack of konjac glucomannan included low solubility, low sol stability and low solution mobility (Pan *et al.*, 2013), beside porang flour could not formed colloid system with milk. As increasing a new source energy requirements, therefore the improvement of porang flour characteristics are very crucial. Pan *et al.* (2013) noted that physical, chemical and biological modification of konjac glucomannan had improved its functional properties.

One of the methods to disperse supramolecules physically had been developed i.e., by increasing the energy in polymer chain which was successfully to make the polysaccharide solution homogen in water as reported by Tao and Xu (2008). This method was using sonification,  $\gamma$  -irradiation, autoclave, pressure/heat and microwave.

The advantage of heating technique using microwave oven is time and energy efficiency and easy in application. Sample heating in microwave oven are due to the interaction of electromagnetic environment with chemical components insoluble. This interaction could rise a fast heat because of molecular friction and excitation. The utilization of microwave irradiation fasten the organic reaction to produce an efficient internal heating or volumetric heating in nucleus and directly combined with microwave energy and available cell i.e., solute, reagent or catalyst in reaction mixture (Zhang and Zhao, 2009).

Heat and radiation treatments could be combined with lactic acid to modify the physicochemical of a polysaccharide. Gong *et al.* (2006) had been successfully modified the physicochemical of maize flour using thermal treatment combined with lactic acid through lactic acid copolymerization graft reaction to starch. Therefore, the objective of this study was to investigate the modification of porang flour by conventional heating using waterbath and microwave radiation combined with lactic acid to obtain a better physicochemical quality modified porang flour.

## MATERIALS AND METHODS

**Sample preparation:** Porang flour were obtained from P.T. Perhutani Pare and 3 g of porang flour were added with 100 mL lactic acid solution in 250 mL Erlenmeyer flask and stirred thoroughly for 15 min at room temperature, then the mixture were heated in water bath at 90°C for 30 min (PAW) and radiated in microwave (Sharp R200J) for 10 min (PAM) respectively. Furthermore the porang flour solution were cooled down to room temperature before centrifuged for 10 min to separate undiluted components (Gong *et al.*, 2006; Xu *et al.*, 2009; Tatirat and Charoenrein, 2011; Yang *et al.*, 2013).

**FTIR analysis:** 0.01 g sample was homogenised with 0.01 g anhydrous KBr using a mortar and this mixture was vacuum hydraulic pressed at 1.2 psi before scanned in the absorption area at 600 up to 4000  $\text{cm}^{-1}$  (Widjanarko *et al.*, 2011).

**Nuclear Magnetic Resonance (NMR) measurement:** The NMR spectra were recorded by spectrophotometer JEOL JNM-A500 FT-NMR (500 MHz) at 25°C, using chloroform as diluent and tetramethylsilane (TMS) as internal standard. Chemical shifts ( $\delta$ ) and coupling constants (J) reported as ppm and Hz in sequence (Enomoto-Rogers *et al.*, 2013).

**Molecular weight measurement (SEC-HPLD):** The molecular weight of porang flour and modified porang flour were measured by Size Exclusion Chromatography (SEC) method using HPLC Alliance 2695-Water, equipped with Refractometry Index Detector and BioBasic SEC-1000, 300 mm $\times$ 7, 8 mm column, 5  $\mu\text{m}$  (Thermo Scientific). The mobile phase were Dapar sodium phosphate 0,1 M pH 7, 2 with a flow rate of 0.5 mL/min. Dextran (Sigma) dengan MW 150.000, 410.000 dan 670.000 Dalton (Da) were used as standard (Pranamuda *et al.*, 2012).

**Particle size measurement:** Particle Size Instrument (Malvern ZEN3690, Malvern), equipped with 10 mW HeeNe laser at 633 nm was used for measuring the particle size and the measurements were carried out at 25°C and 90° scattering angle which will give the particle diameter distribution estimation (Riou *et al.*, 2002; Wang *et al.*, 2005).

**Morphology structure determination by Scanning Electron Microscope (SEM):** Morphology structure of porang flour surface were determined using Scanning Electron Microscope (SEM). The samples were coated with gold in vacuum condition and the determination were carried out at magnification of 2000-8000 times (Wang *et al.*, 2011).

**Rheology test:** The AR-G2 Rotary Rheometer equipped with cone plate geometry diameter 40 mm (cone angle 4°, gap 108 micro m) was used for rheology test at varied temperature in linear viscoelastic regime. The samples surface were covered with silicon oil to prevent from drying. Temperature sweep test was done by gel heating from 75 $\pm$ 1°C with 1.0°C/min increment using 1% strain. Data  $G'$  and  $G''$  were recorded (He *et al.*, 2012).

## RESULTS AND DISCUSSION

**Functional group of modified porang flour:** FTIR spectra of either porang flour and modified porang flour are presented in Table 1 and Fig. 1 and IR spectra of

Table 1: FTIR spectra of porang flour and modified porang flour

Porang flour without treatment (P)			Porang flour treated with lactic acid and heated using waterbath. (PAW)			Porang flour treated with lactic acid and irradiated in microwave (PAM)		
Broad spectrum (cm <sup>-1</sup> )	Group	Type of vibration	Broad spectrum (cm <sup>-1</sup> )	Group	Type of vibration	Broad spectrum (cm <sup>-1</sup> )	Group	Type of vibration
806.19	CH	Bending				1045.35	C-O-C	Bending
896.84	CH	Bending				1091.53	C-H	Bending
1027.99	C-O-C	Stretching	1047.27	C-O-C	Bending	1130.21	C-C	Stretching
1054.99	C-H	Bending	1091.53	C-H	Bending	1234.35	C-O-C	Stretching
1120.56	C-C	Stretching	1130.21	C-C	Stretching			
1149.50	C-O-C	Stretching	1234.36	C-O-C	Stretching			
1248.79	C-O-C	Stretching						
1379.01	C-H	Bending	1375.15	C-H	Bending	1377.08	C-H	Bending
1448.44	CH <sub>3</sub>	Bending	1456.16	CH <sub>3</sub>	Bending	1456.16	CH <sub>3</sub>	Bending
1639.38	C=O	Stretching	1639.38	C=O	Stretching	1645.12	C=O	Stretching
1724.24	C=O	Stretching	1725.17	C=O	Stretching	1726.17	C=O	Stretching
2156.27	Double bond	Stretching	2048.26	Double bond	Stretching	2046.33	Double bond	Stretching
2927.74	CH	Stretching	2638.44	CH	Stretching	2636.51	CH	Stretching
			2557.43	CH	Stretching	2553.58	CH	Stretching
3390.63	OH	Stretching	3402.20	OH	Stretching	3427.27	OH	Stretching

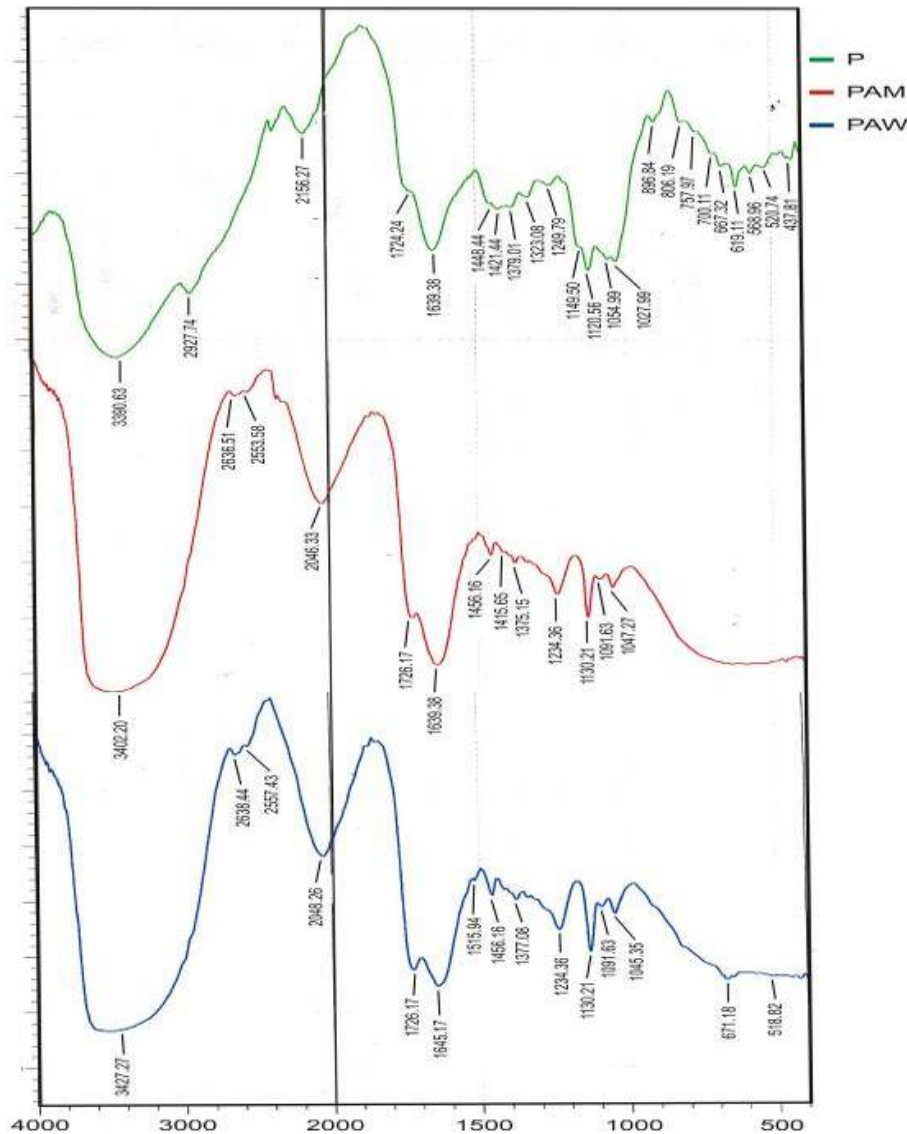


Fig. 1: FTIR spectra of porang flour and modified porang flour

porang flour (P), lactic acid-waterbath modified porang flour (PAW) and lactic acid-microwave radiation modified porang flour (PAM) as broad bands were in the range of 4000-500  $\text{cm}^{-1}$ .

The band peak of porang flour (P) was 3390.63  $\text{cm}^{-1}$  which indicated the presence of hydrogen bond i.e., O-H group from glucomannan of porang flour, while band of 2927.74  $\text{cm}^{-1}$  indicated C-H bond from methyl group and asymmetric stretching vibration was observed at 2156.74  $\text{cm}^{-1}$  from cumulative double bond. C=O group from acetyl was observed at 1724.24  $\text{cm}^{-1}$  and 1639.38 was retention of C-O which related to hydroxyl. The infra spectra bended of CH<sub>3</sub>, CH, C-O-C, C-C were at 1448.44  $\text{cm}^{-1}$ , 1379.01  $\text{cm}^{-1}$ , 1249.79  $\text{cm}^{-1}$  and 1149.50  $\text{cm}^{-1}$ , 1120.56  $\text{cm}^{-1}$  respectively and stretching broad spectra at 1027.99  $\text{cm}^{-1}$  for C-O-C, 896.84  $\text{cm}^{-1}$  and 806.19  $\text{cm}^{-1}$  for mannose.

The FTIR spectra of porang flour (P) in this study were similar with glucomannan konjac as reported by Huang *et al.* (2010) and Xia *et al.* (2010) in the range of 4000-500  $\text{cm}^{-1}$ . While Xia *et al.* (2010), Meng *et al.* (2014) and Jin *et al.* (2014) observed that stretching vibration of O-H group of carboxylate were in the range of 3600-3300  $\text{cm}^{-1}$ . According to Huang *et al.* (2010), Ha *et al.* (2011), Meng *et al.* (2014) and Jin *et al.* (2014) the stretching broad of C-H group at about 2900 was methyl group of glucomannan. Pan *et al.* (2013) noted that asymmetric stretching broad vibration of cumulative double bond indicated by peak at 2150  $\text{cm}^{-1}$ . Xu *et al.* (2008) and Liu *et al.* (2009) also reported that stretching broad vibration of C-O of acetyl group was at 1736  $\text{cm}^{-1}$  and Jin *et al.* (2014) observed that stretching vibration of C-O related to hydroxyl group was at 1638  $\text{cm}^{-1}$ , while CH<sub>3</sub> bending was observed at 1447  $\text{cm}^{-1}$  (Pivsa-Art *et al.*, 2014).

Furthermore, Chua *et al.* (2012) noted broad spectrum at 1370  $\text{cm}^{-1}$  was for CH bending and Xu *et al.* (2009) found the stretching broad of C-O bending was at 1250  $\text{cm}^{-1}$ . Broad spectrum of 1150  $\text{cm}^{-1}$  was for C-O-C as reported by Chua *et al.* (2012) and Yu *et al.* (2007) observed the stretching broad spectrum of C-O-C was at 1150  $\text{cm}^{-1}$  while stretching broad spectrum of 1027  $\text{cm}^{-1}$  was for C-O-C as noted by Widjanarko *et al.* (2011). According to Huang *et al.* (2010) broad spectra of mannose were at 876  $\text{cm}^{-1}$  and 808  $\text{cm}^{-1}$ , while Meng *et al.* (2014) observed broad spectra of mannose at 871  $\text{cm}^{-1}$  and 804  $\text{cm}^{-1}$ .

The modification treatments of PAW and PAM porang flour resulted a OH group stronger spectra indicated by sharper tip of peak and according to Huang *et al.* (2010) this condition showed an hydrogen intermolecular bond of native porang flour which have a partial breakage. The increased of hydroxyl group in glucomannan konjac indicated bigger opportunity to form hydrogen intermolecular bond (Pan *et al.*, 2011).

Methyl group of porang flour (P) which found at spectra band of 2927.74  $\text{cm}^{-1}$ , shifted into two peaks for

PAW and PAM namely 2638.44  $\text{cm}^{-1}$  and 2557.43  $\text{cm}^{-1}$  also at 2635  $\text{cm}^{-1}$  and 2553.58  $\text{cm}^{-1}$  each in its sequence. It is possible due to methyl group could react in specific way with lactic acid.

The stronger C-C bonding at PAW (1130.21  $\text{cm}^{-1}$ ) and PAM (1130.21  $\text{cm}^{-1}$ ), according to Xu *et al.* (2009) are possibly due to specific interaction between lactic acid and glucomannan of porang flour. Peak intensity of C-O increased from 1724.24  $\text{cm}^{-1}$  (P) to 1725.17  $\text{cm}^{-1}$  (PAW) and 1726.17  $\text{cm}^{-1}$  (PAM) and this condition according to Gårdebjer *et al.* (2015) indicated modification had occurred on porang flour i.e., by addition number of C=O group from lactic acid. While Gong *et al.* (2006) noted that stretching absorption of carbonyl from lactic acid was at 1749  $\text{cm}^{-1}$  with a strong peak indicated occurrence of graft copolymer of maize starch and lactic acid.

Peak of CH<sub>3</sub> on porang flour (P) increased from 1448.44  $\text{cm}^{-1}$  to 1456.16  $\text{cm}^{-1}$  (PAW) and 1456.16  $\text{cm}^{-1}$  (PAM) indicated the addition of CH<sub>3</sub> group from lactic acid to glucomannan molecule from porang flour. While peak increasing of C-O-C on porang (P) flour from 1027.99  $\text{cm}^{-1}$  to 1047.27  $\text{cm}^{-1}$  (PAW) and 1045.35  $\text{cm}^{-1}$  (PAM) and also C-O-C on porang (P) flour from 1149.50 to 1234.36  $\text{cm}^{-1}$  (PAW) and 1234.35  $\text{cm}^{-1}$  (PAM) also indicated addition of C-O-C group from lactic acid to glucomannan molecules of porang flour.

The abundance amount of very active hydroxyl group (-CH<sub>2</sub>OH) at C-6 position for each unit of glucomannan component resulted some chemical reactions occurred at this position such as esterification, etherification, nitration and branch polymerization (Xu *et al.*, 2009). Unfortunately broad spectra of ~806 and ~896 at PAW dan PAM samples were not appeared and this is condition according Xu *et al.* (2009) are possibly due to deformation of mannose molecule present in porang flour.

**Modified porang structure:** Two similar signal were observed as showed by the NMR spectra as presented in Fig. 2, although there were no differences on signals of either modified porang using waterbath (PAW) and microwave irradiation (PAM). PAM resulted a signal at 1.3 ppm with integral values of 3.011 and 2.999 indicated the proton from CH<sub>3</sub>, while PAW at same signal with integral value of 3.090 and 3.081 indicated proton from CH<sub>3</sub> as well. However, at signal of 4.3 ppm either of PAM and PAW indicated proton of CH<sub>2</sub> group although there is a possibility also indicated proton from mannose and glucose at same signal. The <sup>1</sup>H-NMR spectra of porang flour and modified porang flour either PAW and PAM are presented in Fig. 2.

Pandey *et al.* (2008) noted that strong signal at 1.52 ppm observed as methyl group, while Choi *et al.* (2013)

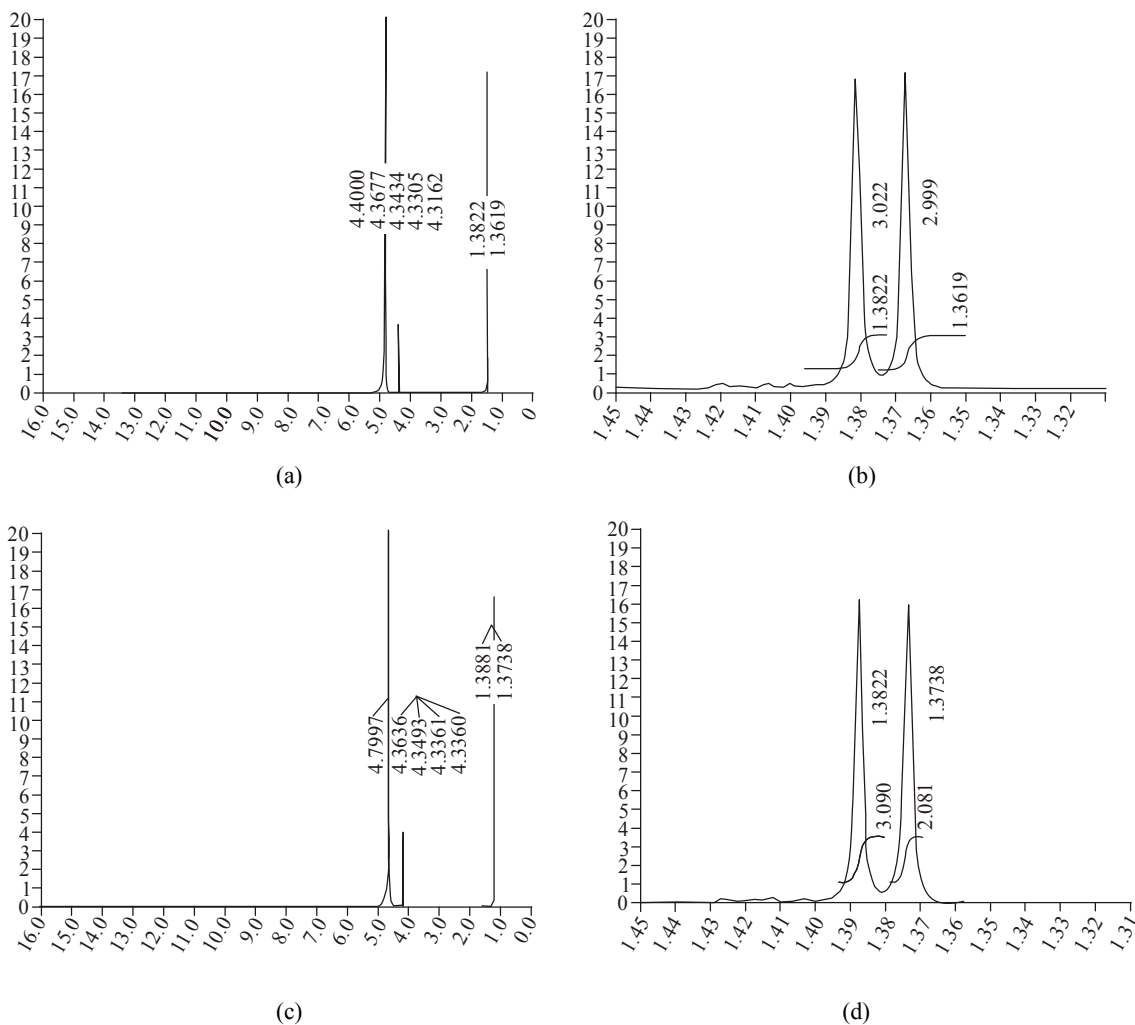


Fig. 2: <sup>1</sup>H NMR spectra of modified porang flour

Table 2: Molecular weights of porang flour and modified porang flour (PAW and PAM)

Sample	Molecular weight (Dalton)
Lactic Acid-Waterbath (PAW) modified porang flour.	$2.0 \times 10^6$
Lactic Acid-Microwave (PAM) modified porang flour.	$3.0 \times 10^6$
Porang flour	$< 1.0 \times 10^5$

observed proton of methyl from polyactic at 1.55 ppm and proton of CH<sub>3</sub> was found by Pivsa-Art *et al.* (2014) at 1.50 ppm. An *et al.* (2009) observed proton of mannose at 4.29 ppm and of glucose at 4.27 ppm.

**Molecular weight of modified porang flour:**

Molecular weights of modified porang flour PAW and PAM are presented in Table 2 and Fig. 3 and it was found that molecular weight of modified porang flour (PAW) was  $2.0 \times 10^6$  while molecular weight of PAM was  $3.0 \times 10^6$ . Li and Xie (2003) reported that molecular weight of glucomannan were in the range of  $1.033 \times 10^6$ - $1.088 \times 10^6$  and Xu *et al.* (2008) noted molecular weight

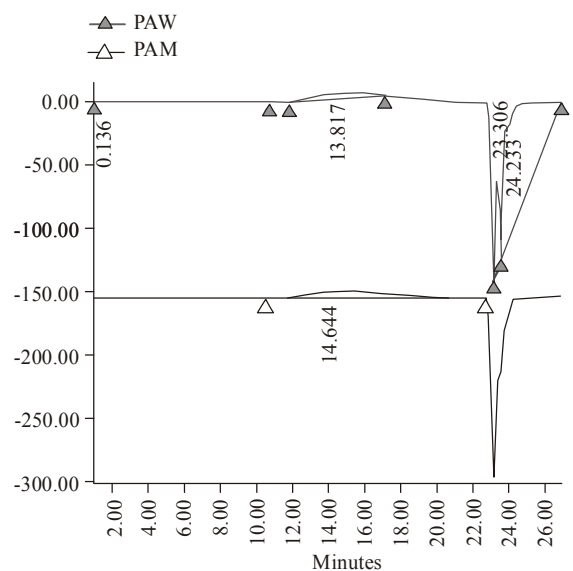


Fig. 3: HPL-SEC spectra of porang flour and modified porang flour

Table 3: Particles size distribution of native and modified porang flour ( $\mu\text{m}$ )

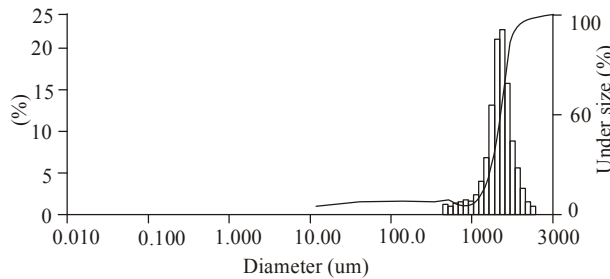
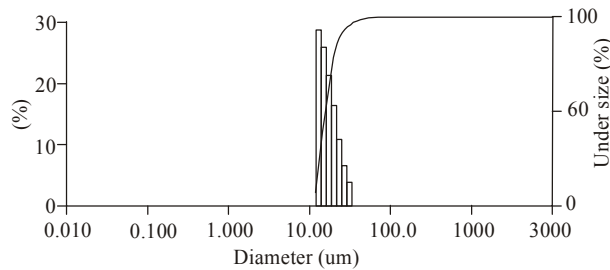
Treatments	Means	
	Smallest	Biggest
Native porang flour (P)	8.4902	831.2676
PAM	0.1434	39.3229
PAW	0.1164	20.5980

of their sample was  $4.81 \times 10^5$ . Furthermore, Wang *et al.* (2012) noted molecular weight of glucomannan was  $1.34 \times 10^6$ , while Luo *et al.* (2012) reported molecular weight of their sample was  $1.07 \times 10^6$ . This condition indicated a reaction between lactic acid and glucomannan component of porang flour.

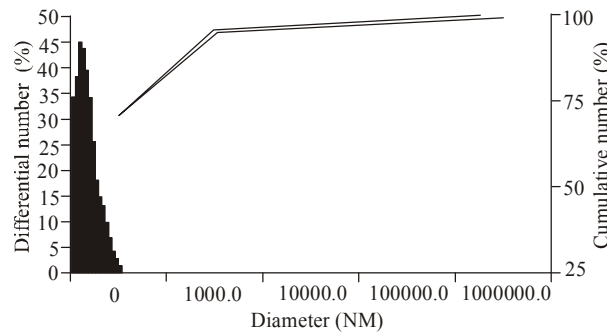
**Particles size of modified porang flour:** The results of particle size measurement are presented in Table 3 and Fig. 4 and heating treatment in waterbath and microwave resulted reduction of particle size compared to native porang flour particle size. Particle size of native porang flour was  $8.4902 \mu\text{m}$  became  $0.1434 \mu\text{m}$  for particles of microwave irradiation modified porang

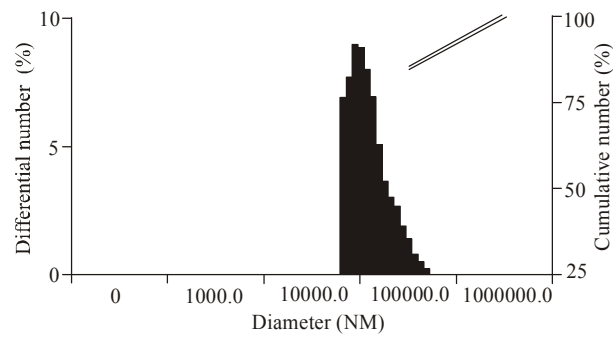
flour (PAM) and  $0.1164 \mu\text{m}$  for particles of waterbath heating modified porang flour (PAM). While distribution of biggest particle size of native porang flour was  $831.2676 \mu\text{m}$  and reduced to  $39.3229 \mu\text{m}$  (PAM) and  $20.5980 \mu\text{m}$  (PAW). According to Tatirat and Charoenrein (2011) and Tatirat *et al.* (2012) particle size of Konjac flour were around  $285.31 \mu\text{m}$  and  $112.4 \mu\text{m}$ . These results are possibly due to heating porang flour in acid condition at  $90^\circ\text{C}$  which caused breakage of particles and microwave irradiation could separate the lamella from porang flour granula, release of polymers as consequence of tissue changes. Kratchanova *et al.* (2004), reported that microwave treatment could modified capiler porosity of plant tissue, intermolecular friction which resulted from microwave heating resulting in internal cell pressure caused cell rupture hence cell components were flown out.

**Microstructure of modified porang flour:** Figure 5 showed the results of surface microstructure of porang flour (P) using Low SEM and a crystal like structure was observed while modified porang flour in waterbath

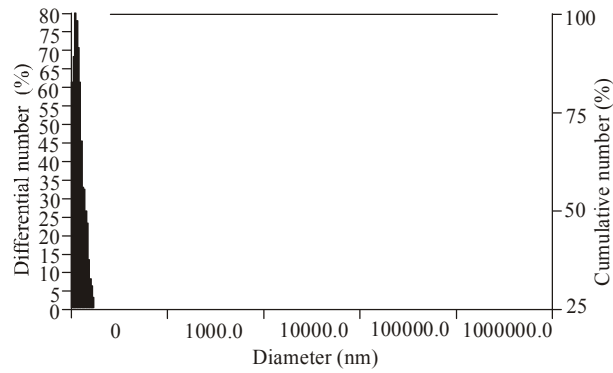


(a)





(b)



(c)

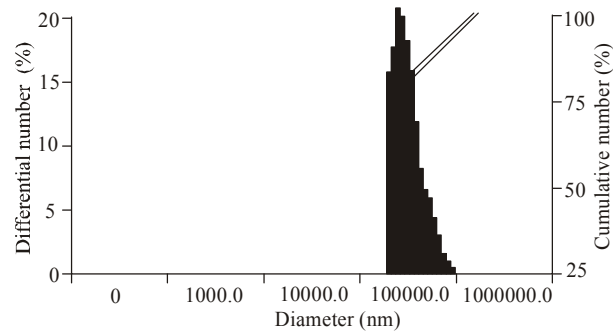
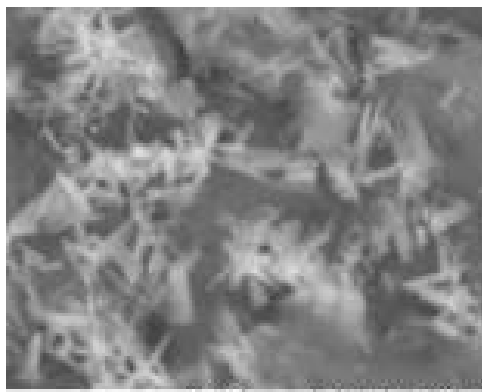
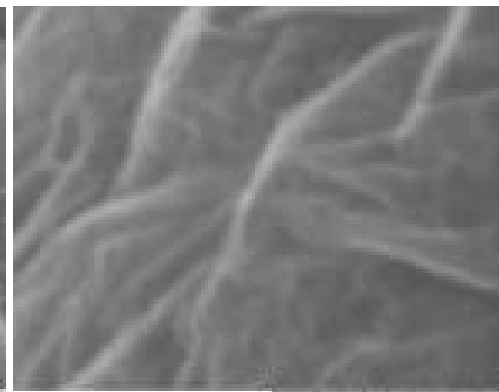


Fig. 4: Particles size distribution of native and modified porang flour; (a): Particles size distribution of native porang flour; (b): Particles size distribution of modified porang flour by heating in waterbath (PAW); (c): Particles size distribution of modified porang flour by microwave irradiation (PAM)



(a)



(b)

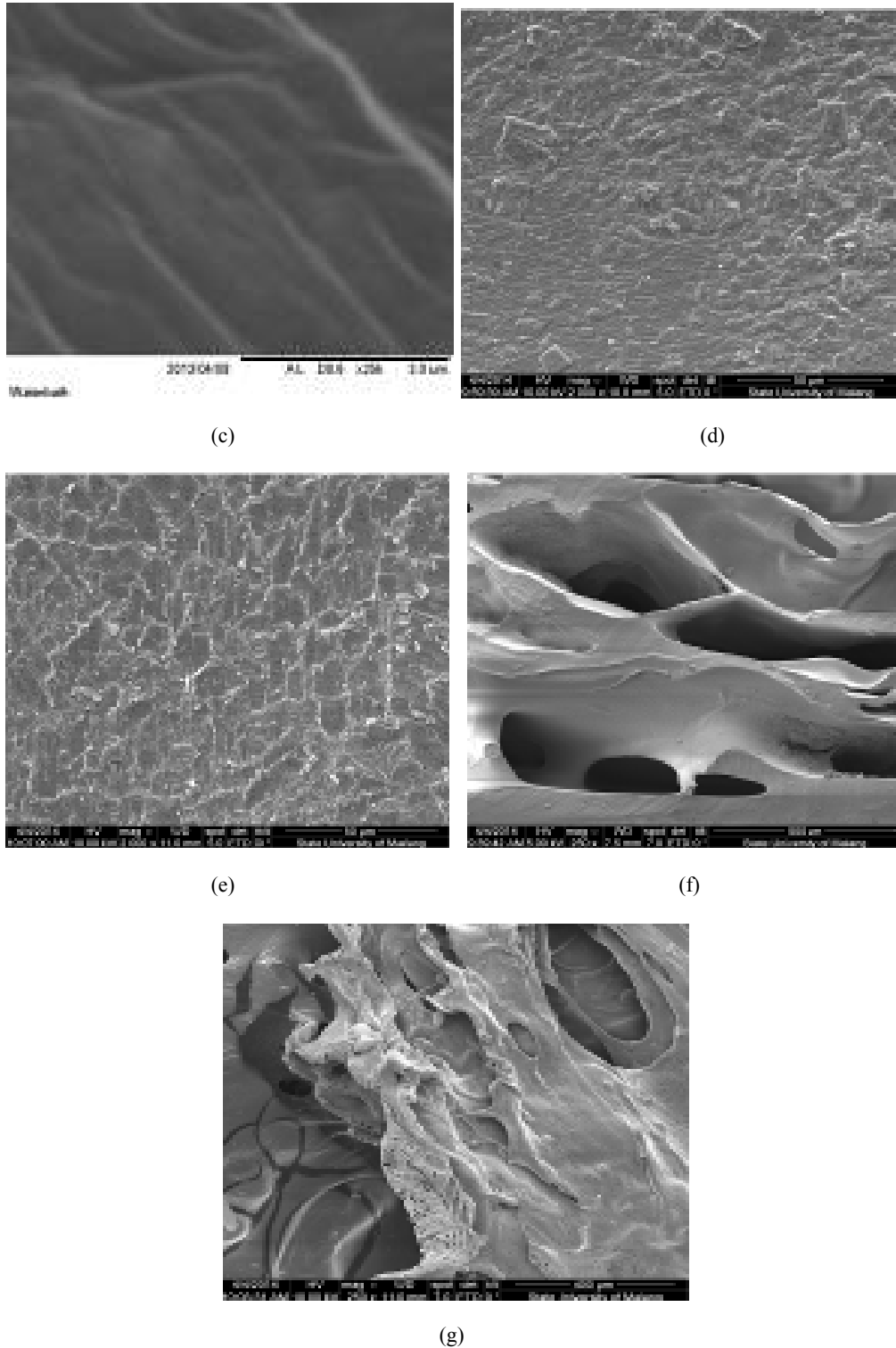


Fig. 5: Surface microstructure of porang flour and modified porang flour (PAW and PAM); (a): P (8000x); (b): PAW (25000x) Low SEM; (c): PAM (25000x) Low SEM; (d): PAW (25000x) High SEM; (e): PAM (25000x) High SEM; (f): PAW (25000x) cross section High SEM; (g): PAM (25000x) cross section High SEM

(PAW) and microwave irradiation (PAM) also using Low SEM showed a smooth and uniform surface structure. However if High SEM used surface structure of either PAW and PAM were rather rough, non-porous

and uniform. Cross section microstructure with High SEM showed PAW surface structure were more porous and spongy compared to PAM. According to Charoenrein *et al.* (2011) and Wen *et al.* (2009)



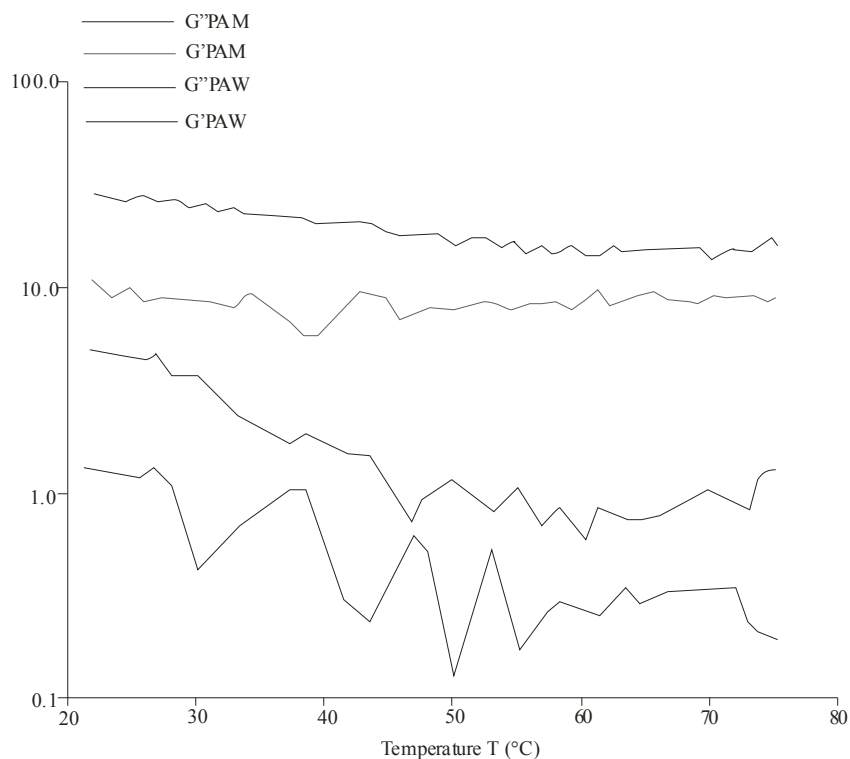


Fig. 6: Rheology of modified porang flour

reported that glucomannan have moisture accessibility to higher amorph site. The smooth surface structure of either PAW and PAM indicated a homogen mixture formation between lactic acid and porang flour where interfacial interaction were occurred properly between lactic acid and porang flour.

**Rheology of modified porang flour:** The effect of temperature on visco-elasticity traits of modified porang flour with lactic acid and heating in waterbath (PAW) and microwave irradiation (PAM) were investigated using dynamic temperature oscillatory test as presented in Fig. 6. Storage modulus ( $G'$ ) and loss modulus ( $G''$ ) of PAW and PAM observed decreasing with the increased of temperature. This condition possibly in relation to increase activity of molecular chain and weakness of moisture interaction with porang flour hence a decrease of intermolecular hydrogen bond which support the flow capacity of solution as also reported by Du *et al.* (2012), Luo *et al.* (2013) and Jin *et al.* (2014).

It is interesting to note that storage modulus ( $G'$ ) of PAW and PAM were much lower compared to loss modulus ( $G''$ ) either at low or high temperatures. Crossing of modulus ( $G'$  dan  $G''$ ) from PAW and PAM did not occur indicated that as noted by Wang *et al.* (2012) either PAW and PAM had an unchange visco-elastis system and according to Jin *et al.* (2014) had a thermo-irreversible gel characteristic. Furthermore, during heating gel formation of PAW and PAM did not happen and this condition according to Chen *et al.*

(2011) indicated there was an acetyl group play an important role on gelation characteristic of PAW and PAM. Hydrogen bond and hydrophobic interaction both play an important role in network formation, although hydrophobic interaction had a bigger effect on konjac glucomannan gelation characteristic. Therefore, either hydrogen bond and hydrophobic interaction could act as molecular strength in konjac glucomannan gelation formation (Chen *et al.*, 2011).

## CONCLUSION

It can be concluded that functional group peaks of modified porang flour (PAW and PAM) changed differently compared to native porang flour included OH, CH<sub>3</sub>, C-C, C = O, C-O-C groups as well as glucose and mannose groups. H<sup>1</sup>- NMR spectra of modified porang flour (PAW and PAM) were similar to the proton original of CH<sub>3</sub> and CH<sub>2</sub>, with bigger molecular weight i.e., PAW was  $2.0 \times 10^6$  and PAM was  $3.0 \times 10^6$  and smaller in particles size. The PAW and PAM surface microstructure were observed porous and spongy and rheology characteristics indicated that PAW and PAM had an unchanged visco-elastic system by thermal treatments.

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