

Composition, microstructure, and physicochemical properties of starches from Indonesian cowpea (*Vigna unguiculata*) varieties

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Abstract

Starch is the most abundant carbohydrate in the pulse seeds. Pulse starches are increasingly concerned in the potential to incorporate into food products due to high amylose and resistant starch content. In this research, starches from five varieties of Indonesian cowpea (*Vigna unguiculata*) were evaluated for their composition, microstructure, and physicochemical properties. The yield of starch (on the whole seed basis) ranged between 17.78% (KT4) and 22.93% (KT8), and amylose content (on dry basis) ranged between 39.09% (KT4) and 42.78% (KT7). Granules of cowpea starch were oval to spherical shape with a smooth surface and the mean granule diameter (Z average) ranged between 7.91 μm (KT5) and 15.51 μm (KT4). The X-ray diffraction patterns of cowpea starches were C_A-type crystallinities as showed strong peaks at 15°, 17°, and 23° (2 θ), and an extra peak at 18° (2 θ). Thermal properties, pasting properties, Water and Oil Holding Capacity (WHC and OHC) of cowpea starches differed significantly, whereas, FT-IR spectra showed a similarity of characteristic bands.

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Keywords

Cowpea

Starch

Microstructure

Physicochemical properties

Introduction

Cowpeas (*Vigna unguiculata* (L.) Walp.), a native pulse of West Africa, are one of the most important food pulse crops, which has been grown in the semi-arid tropics covering Asia, Africa, Southern Europe, Central and South America (Akibode and Maredia, 2011). It has a number of commonly used names, e.g. southern pea, black-eyed pea, crowder pea (Huang *et al.*, 2007), niebe, wake, ewa (in West Africa) (Ashogbon and Akintayo, 2013), and kacang tunggak (in Indonesian language). Cowpea is cultivated for its immature pods and mature seeds and is consumed extensively in Africa and, to a lesser extent, in Asia (Prinyawiwatkul *et al.*, 1996).

An estimation of 14.5 million hectares land is planted with cowpea each year in the worldwide. According to FAO Statistics in 2013 (FAO, 2013) total world production of dried cowpeas was 6.2 million metric tons which Africa was responsible for 94%. In West Africa, cowpea seeds are consumed as boiled seeds or in combination with other foods (like plantain, maize, and rice), akara (fried cowpea paste), and moinmoin (steamed cowpea paste) (Ashogbon and Akintayo, 2013). Meanwhile, in Indonesia, cowpea is cultivated as a minor pulse in many provinces and whole dried cowpea seeds

are consumed as soup ingredients and traditional snacks. This leads to limit the utilization of cowpea, therefore, it is not as popular as soybeans, red beans, and mung beans.

Cowpea seeds contain about 53-66% carbohydrate and most of which is found in the form of starch (Huang *et al.*, 2007; Hoover *et al.*, 2010; Ashogbon and Akintayo, 2013). Starches are macromolecules synthesized by plant tissues and have various application in both food and non-food products. The composition, molecular structure, properties, and modification of various pulse starches have been reviewed by Hoover *et al.* (2010). Previous studies of the cowpea starches had been focused primarily on the structure and physicochemical properties, mostly from Africa region, and no more than two cultivars (Chung *et al.*, 1998; Huang *et al.*, 2007; Adebooye and Singh, 2008; Hoover *et al.*, 2010; Ashogbon and Akintayo, 2013). However, there is a lack of information on the structure and physicochemical properties of cowpea starches among different cowpea cultivars, especially from South-East Asia region.

The composition of cowpea seed, particularly protein and starch content, varies considerably according to cultivar and the origin of the seed (Taiwo, 1998). Therefore, a greater understanding

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on the physicochemical properties of cowpea starch among different cowpea cultivars, which is a major component, is crucial in providing the scientific basis for new food product development, particularly in Indonesia. Cowpea starch could be useful in the manufacture of thickeners, gelling agent, extenders and textural modifiers in food formulation (Adebooye and Singh, 2008), and also to incorporate into food products due to their high amylose content, rapid retrogradation, and resistance to enzyme hydrolysis. In the present study, starches from five different varieties of cowpea grown in Indonesia were isolated and characterized on their composition, microstructure, and physicochemical properties.

Materials and Methods

Materials

Cowpea (*Vigna unguiculata*) varieties (KT4, KT5, KT7, and KT8) were obtained from the Indonesian Research Center for Legumes and Tubers, Malang, East Java, Indonesia, and local variety (KTL) from the local farmer in Yogyakarta, Indonesia. The five cowpea varieties were selected based on the highest productivity and nutritional composition among others.

Starch isolation

Starch was isolated with wet milling method according to Huang *et al.* (2007) with slight modifications. Cowpea seeds were split by grinder then were steeped in distilled water (ratio of seed: distilled water = 1:3) at 4°C for 24 h. The steep water was decanted, and the softened pulses were ground with a blender for 3 min in distilled water (4°C) at high speed then the slurry was filtered through filtered clothes. The residual pulps were ground again for 3 min in the blender with distilled water (4°C), then filtered. The suspensions from two sieving were mixed and allowed to settle overnight at 4°C. The supernatant was drained off. The starch sediment was redissolved in 0.05 M NaOH and kept at 4°C for overnight then neutralized with 2 M HCl to pH 6 at 4°C for overnight. Thereafter, the starch sediment was rinsed with distilled water and allowed to settle at 4°C for overnight until the settled starch gave a firm and dense deposit on the bottom. The sediment was recovered and dried at 40°C for overnight, ground into powder using a blender then sieved through 100 mesh and kept in a sealed container at 4°C until further used.

Determination of chemical composition

Chemical composition of starches were

analyzed for their moisture (Method No. 925.09), crude protein (Nx6.25) (Method No. 920.87), total lipid (Method No. 920.39) with petroleum ether as a solvent, and ash content (Method No. 923.03) following the standard method of analysis (AOAC, 2005). The starch content was determined using acid hydrolysis method and the amylose content was analyzed according to the method of Juliano (Perez and Juliano, 1978; Mohammadkhani *et al.*, 1999).

Determination of granule morphology and size distribution

Granule morphology of starch was determined by scanning electron microscopy (SEM) (FEI, InspectTM S50, USA). The starch sample was mounted on a sample holder (± 10 mm, aluminium stub) using double-sided adhesive tape and then coated with gold-palladium (60:40). An accelerating potential of 10 kV was used during micrography. The size distribution of starch granules were measured using a Malvern dynamic light scattering particle size analyzer (Zetasizer, Malvern Instruments Ltd., UK). Water was used as a dispersing medium for all starches. The refractive index values used for water and starch were 1.33 and 1.50, respectively.

Determination of Oil and Water Holding Capacity

Oil and Water Holding Capacity (OHC and WHC) of starches were determined using a method of Larrauri *et al.* (1996). Ten milliliters of commercial olive oil or distilled water were added to 100 mg of the starches, stirred at 1100 rpm and left at room temperature for 1 h. After centrifugation at 3000 g for 10 min, the residue was weighed. OHC and WHC were calculated as g oil and g water per g of dry sample, respectively.

X-ray diffraction

X-ray diffraction analysis was conducted using an X-ray diffractometer (Shimadzu XRD-6000, Shimadzu Corp., Japan) operated at 40 kV and 30 mA. Diffractograms were obtained from 3° to 40° (2 θ) at a scanning speed of 5°/min.

Determination of thermal properties

The thermal properties of starches were conducted using a differential scanning calorimeter (DSC TA-60WS, Shimadzu, Japan). Starch sample (3.0 mg) was weighed into a standard aluminium hermetic pan and then added with 10 μ l of distilled water using micropipette (ratio of starch: distilled water = 1:3). Samples were hermetically sealed and allowed to stand for 90 min before heating in DSC. Another hermetic pan which contains alumina powder (6.0

mg) was used as a reference and placed next to sample pan in DSC chamber. Sample and reference pan were heated at the rate of 10°C/min from 30°C to 130°C. Onset temperature (T_o), peak temperature (T_p), and enthalpy of gelatinization (ΔH) were calculated using TA-60WS software associated with the DSC.

Determination of pasting properties

Pasting properties of starches were determined using a Rapid Visco Analyzer (RVA-S4, Newport Scientific, Australia) equipped with a ThermoLine software program for Windows 3 (TCW3). Starch sample (3.5 g) was weighed into the sample container and then added with 25 g of distilled water. Starch slurries were held at 50°C for 1 min, heated to 95°C at a rate of 6°C/min, held at 95°C for 5 min, cooled to 50°C at a rate of 6°C/min, and held at 50°C for 2 min. The speed was 960 rpm for the first 10 s, then 160 rpm for the remainder of the experiment. Pasting temperature, peak viscosity, breakdown, setback, and final viscosity were obtained from the viscogram.

Fourier transform infra-red (FT-IR) spectroscopy

Two milligrams of the starches were homogeneously dispersed in 200 mg of KBr (pellet procedure). KBr-pelletized starch samples were analyzed using IR Prestige-21 (Shimadzu Co., Japan) spectrometer in the range of 4000-400 cm^{-1} at 25°C.

Statistical analysis

All the experiments were performed in triplicate, and experimental data also were analyzed using analysis of variance and expressed as a mean value \pm standard deviation. A Duncan's multiple range test was conducted to assess significant differences among experimental mean values ($p < 0.05$). All statistical computations and analyses were conducted using SPSS version 16.0 for Windows.

Results and Discussion

Chemical composition of cowpea starches

The yield of the cowpea starches (on the whole seed basis) was in the range of 17.78-22.93% (Table 1), which had similarity with the earlier publication for other pulses (Hoover *et al.*, 2010), but higher than those reported for other cowpea varieties (Adebooye and Singh, 2008). Meanwhile, Ashogbon and Akintayo (2013) found a higher yield of cowpea starches (38%-40%) compared to Indonesian cowpea.

The purity of the cowpea starches was judged on the basis of the low ash, total lipid, and the nitrogen content. The ash, total lipid, and nitrogen content of cowpea starches differed significantly, within the

range reported for starches from other legumes and cowpea varieties (Huang *et al.*, 2007; Hoover *et al.*, 2010; Ashogbon and Akintayo, 2013; Maaran *et al.*, 2014). Based on the nitrogen content, all Indonesian cowpea starches have high purity because of less than 0.6% protein (Tester *et al.*, 2004). The amylose content of the cowpea starches was in the range of 39.09% (KT4) and 42.78% (KT7), and differed significantly. These values were within the range reported for starches from the other pulses such as kidney bean, mung bean, and pigeon pea (Hoover *et al.*, 2010), and Indian cowpea varieties (Adebooye and Singh, 2008), but higher than those reported for starches from Chinese cowpea varieties (Huang *et al.*, 2007) and Nigerian cowpea varieties (Ashogbon and Akintayo, 2013).

Pulse starches are characterized by high amylose content that influences on functional and physicochemical properties, including pasting, gelatinization, retrogradation, and swelling behaviour of the starches (Blazek and Copeland, 2008). The difference of the yield and the composition of cowpea starches from Indonesia and other cowpea varieties could be contributed to a different method for starch isolation, the genotype of cowpea varieties, and the environmental factors that affect the physiological state of the seeds.

Granule morphology and size distribution of cowpea starches

Scanning Electron Microscopy (SEM) was used in further observation the submicroscopic shape and surface characteristics of the cowpea starch granule. The shape of the cowpea starch granules showed the presence of large oval to spherical starch granule (Figure 1) and the surface appeared to be smooth, which had similarity with previous reports (Adebooye and Singh, 2008; Hoover *et al.*, 2010; Ashogbon and Akintayo, 2013).

The five cowpea starches from different varieties differed significantly in their granule size and mean diameter (Z average). The granule size distribution of cowpea starches were unimodal, which similar to the previous report (Huang *et al.*, 2007). The Z average of cowpea starches differed significantly and ranged between $7.91 \pm 0.76 \mu\text{m}$ (KT5) and $15.51 \pm 3.07 \mu\text{m}$ (KT4) (Table 1). This result was similar to previous studies reported by Rao and Okechuckwu (1997) (ranged of 3-64 μm and mean 18.7 μm) and Huang *et al.* (2007) (mean diameter 15.5 μm). Starch granule size may affect on physicochemical properties, such as gelatinization and pasting, enzyme susceptibility, crystallinity, and solubility (Lindeboom *et al.*, 2004). Larger granules tend to swell more on cooking and

Table 1. Yield, chemical composition, size granule, Oil and water holding capacity of cowpea starches

| Characteristics | Cowpea varieties | | | | |
|--------------------|-------------------------|--------------------------|-------------------------|-------------------------|--------------------------|
| | KT4 | KT5 | KT7 | KT8 | KTL |
| Yield (%) | 17.78±0.29 ^c | 20.87±0.36 ^b | 21.90±0.81 ^a | 22.93±1.60 ^a | 20.16±1.48 ^b |
| Moisture (% db) | 11.43±0.92 ^a | 11.76±0.36 ^a | 11.01±0.21 ^a | 12.04±0.04 ^a | 12.13±0.13 ^a |
| Ash (% db) | 0.17±0.01 ^a | 0.14±0.01 ^b | 0.10±0.03 ^c | 0.11±0.01 ^c | 0.15±0.00 ^{ab} |
| Total lipid (% db) | 0.12±0.02 ^b | 0.11±0.01 ^b | 0.05±0.01 ^c | 0.05±0.02 ^c | 0.19±0.01 ^a |
| Protein (% db) | 0.35±0.00 ^b | 0.14±0.00 ^d | 0.14±0.00 ^d | 0.34±0.00 ^b | 0.49±0.07 ^a |
| Starch (% db) | 87.13±0.30 ^b | 87.60±0.16 ^{ab} | 87.94±0.38 ^a | 87.89±0.20 ^a | 87.60±0.02 ^{ab} |
| Amylose (% db) | 39.09±1.23 ^b | 39.76±1.52 ^b | 42.78±1.41 ^a | 39.86±0.72 ^b | 39.67±1.13 ^b |
| Z-average (µm) | 15.51±3.07 ^a | 7.91±0.76 ^c | 10.55±0.56 ^b | 10.30±3.39 ^b | 11.94±2.53 ^b |
| OHC (g/g) | 0.50±0.02 ^b | 0.47±0.06 ^b | 0.51±0.06 ^b | 0.57±0.07 ^{ab} | 0.63±0.06 ^a |
| WHC (g/g) | 0.64±0.06 ^c | 0.69±0.03 ^{bc} | 0.73±0.06 ^{bc} | 0.78±0.05 ^b | 0.99±0.06 ^a |

^{a,b,c} Different superscripts within a row indicate significant differences among varieties ($p < 0.05$).

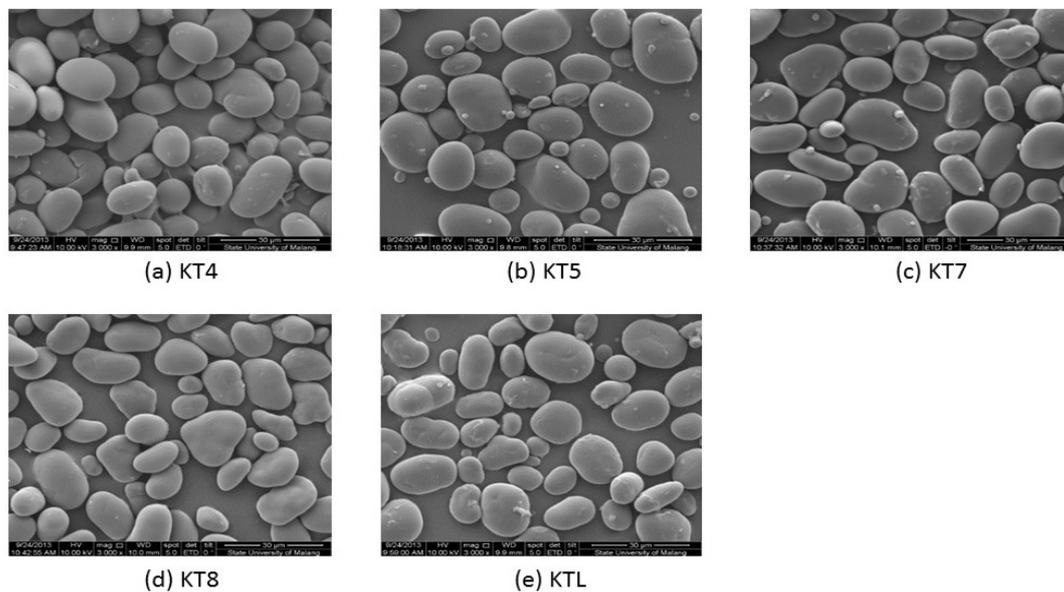


Figure 1. Scanning electron micrographs of cowpea starches

consequently granule size is an important factor that affects its physicochemical properties (Joshi *et al.*, 2013).

Oil and water holding capacity (OHC and WHC) of cowpea starches

WHC (0.64-0.99 g/g) and OHC (0.47-0.63 g/g) significantly differed amongst cowpea starches varieties (Table 1). KTL has the highest of WHC and OHC, whereas, KT4 has the lowest. Singh *et al.* (2004) reported that water bonding capacity (WBC) of chickpea starches from different cultivars ranged between 77.8% and 89.4%, meanwhile for faba bean, lentil, and field pea starches were 90, 98, and 82%, respectively (Hoover and Sosulski, 1986). WBC of starches may be influenced by the variation of granule

structure (Sandhu *et al.*, 2004) and the involvement of a larger proportion of the hydroxyl groups in the forming hydrogen and covalent bonds between starch chains than with water (Hoover and Sosulski, 1986). Regarding WHC and OHC, cowpea starches showed a similar pattern with raw chickpea flour (Aguilera *et al.*, 2011) but had an opposite pattern with raw cowpea flour (Avanza *et al.*, 2012). A high WHC of cowpea starches reveal to the potential of starches as a thickener and textural modifier agent in the food formulation, and due to their low OHC, these starches could be used as a potential ingredient in the fried product.

X-ray diffraction pattern of cowpea starches

There were four strong diffraction peaks at

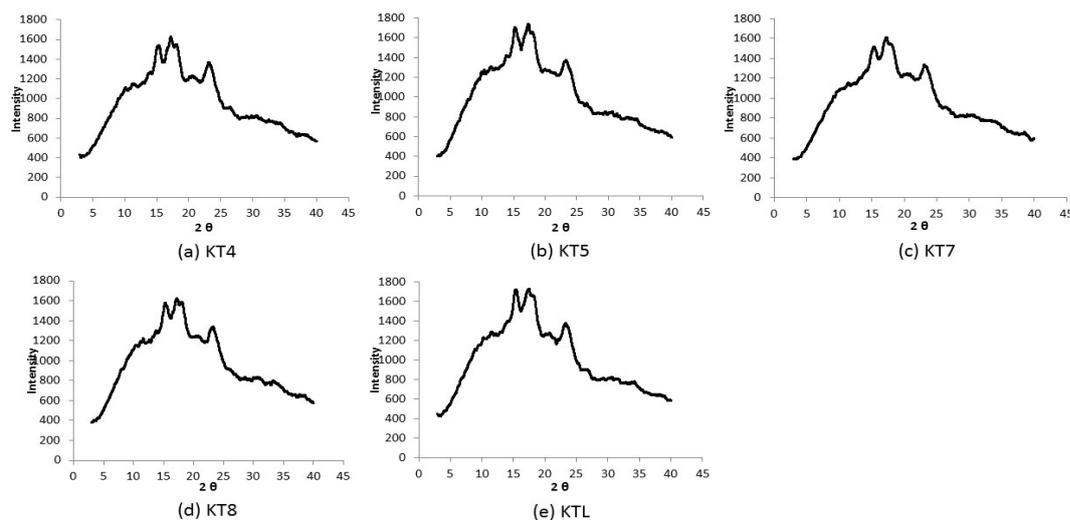


Figure 2. X-ray diffraction pattern of cowpea starches

15.3°, 17.4°, 18.2°, and 23.4° (2θ) in the diffraction spectra of the five cowpea starches (Figure 2). These diffractograms showed C-type starch crystallinity. With the exception of wrinkled pea starch, most pulse starches exhibited a C-type X-ray diffraction, which is intermediate between the A and B-types (Hoover *et al.*, 2010). Hizukuri (1985) postulated that the C-type starch can be further classified to C_A -type (closer to A-type), C-type and C_B -type (closer to B-type) according to the proportion of A- and B-type allomorphs. The XRD pattern of C-type starch gives singlet peaks at about 17° and 23° 2θ , and a few small peaks at around 5.6° and 15° 2θ . The XRD patterns of C_A - and C_B -type starches are similar to that of C-type, but there is a shoulder peak at about 18° 2θ and a strong singlet peak at 23° 2θ for C_A -type starch, and two shoulder peaks at about 22° and 24° 2θ for C_B -type starch (Cheetham and Tao, 1998; Cai and Wei, 2013; Cai *et al.*, 2014). According to Figure 2, all of Indonesian cowpea starches were C_A -type crystallinity, which are similar with previous reports (Huang *et al.*, 2007; Hoover *et al.*, 2010), as showed a strong peak at 23° 2θ and an extra peak at 18° 2θ .

Thermal properties of cowpea starches

DSC was used to study starch gelatinization which involved disruption of the native structure of the starch granule. The gelatinization temperature [(T_o -onset), (T_p -midpoint), (T_c -conclusion)], gelatinization temperature range (T_c-T_o), and enthalpy of gelatinization (ΔH) of cowpea starches differed significantly (Table 2). T_o represents melting of the weakest crystallites and in the range of 66.21°C–71.61°C, and T_p ranged between 76.19°C and 80.62°C. Gelatinization temperature range (T_c-T_o) represents variation in crystalline organization amongst granule

and in the range of 19.53°C–23.58°C. The higher T_o of KT5 and KTL indicated that their crystallites have a higher degree of stability and/or lesser amount of structural defect. The lower T_c-T_o of KTL indicated that its crystallites have a higher degree of crystalline perfection (describes the better alignment of double helices within the crystalline lamella) than those of the other starches. Lopez-Rubio *et al.* (2008) explained that the gelatinization temperature (T_o , T_p , and T_c) represent crystalline stability, whereas ΔH represents melting of amylopectin based crystals with potential contributions from both crystal packing and helix melting enthalpies. The gelatinization temperature (T_o , T_p , T_c) and gelatinization temperature range (T_c-T_o) of cowpea starches were higher than those earlier reported (Huang *et al.*, 2007).

The enthalpy of gelatinization (ΔH) represents the number of double helices that unravel and melt during gelatinization and was in the range of 8.07–9.79 J/g. The above values were lower than those reported for other cowpea starch varieties (Huang *et al.*, 2007). The enthalpy of gelatinization has been related to characteristics of the starch granule such as the degree of crystallinity and granule size (Bogacheva *et al.*, 2006), and it reflects the loss of double-helical order rather than the loss of crystallinity (Cooke and Gidley, 1992). Meanwhile, Tester (1997) has reported gelatinization properties, that greatly affected by the molecular structure of amylopectin, amylose/amylopectin ratio and the extent of lipid which form a complex with amylose chain. Thermal properties of legume starches have been influenced by the molecular architecture of the crystalline region, which corresponds to the distribution of amylopectin short chains (DP 6–11) and not by the proportion of crystalline region which

Table 2. Thermal and pasting properties of cowpea starches

| Properties | Cowpea varieties | | | | |
|---------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| | KT4 | KT5 | KT7 | KT8 | KTL |
| Thermal properties | | | | | |
| T_o (°C) | 66.21±0.01 ^c | 70.81±1.90 ^a | 69.21±1.57 ^b | 66.65±0.55 ^c | 71.61±1.08 ^a |
| T_p (°C) | 76.19±0.72 ^c | 80.62±1.82 ^a | 77.81±0.76 ^b | 78.24±0.64 ^b | 78.92±0.03 ^b |
| T_c (°C) | 87.92±0.29 ^b | 92.45±1.45 ^a | 89.58±0.09 ^b | 90.23±3.35 ^a | 91.14±0.05 ^a |
| $T_c - T_o$ (°C) | 21.71±0.30 ^a | 21.64±0.46 ^a | 20.37±1.48 ^b | 23.58±2.80 ^a | 19.53±1.03 ^b |
| ΔH (J/g) | 9.79±0.01 ^a | 8.07±1.53 ^b | 7.65±1.24 ^b | 9.64±0.48 ^a | 8.59±1.42 ^a |
| Pasting properties | | | | | |
| Pasting temperature (°C) | | | | | |
| | 76.30±0.28 ^e | 80.95±0.00 ^a | 80.53±0.04 ^b | 76.50±0.00 ^d | 78.13±0.04 ^c |
| Peak time (min) | | | | | |
| | 7.07±0.09 ^c | 7.84±0.05 ^b | 8.03±0.14 ^a | 7.80±0.28 ^b | 7.77±0.05 ^b |
| Peak viscosity (cP) | | | | | |
| | 1850.00±1.41 ^d | 1969.50±14.85 ^b | 2036.00±14.14 ^a | 1874.50±20.51 ^c | 1743.50±3.54 ^e |
| Through viscosity (cP) | | | | | |
| | 1365.00±66.47 ^b | 1335.00±5.66 ^b | 1440.50±44.55 ^a | 1275.50±37.48 ^c | 1363.50±43.13 ^b |
| Break down viscosity (cP) | | | | | |
| | 485.00±67.88 ^b | 634.50±9.19 ^a | 595.50±58.69 ^a | 599.00±16.97 ^a | 380.00±46.67 ^c |
| Final viscosity (cP) | | | | | |
| | 2526.00±49.50 ^a | 2416.00±18.38 ^c | 2364.50±19.09 ^d | 2457.50±10.61 ^b | 2521.50±10.61 ^a |
| Set-back viscosity (cP) | | | | | |
| | 1161.00±16.97 ^a | 1081.00±12.73 ^b | 924.00±25.46 ^c | 1182.00±26.87 ^a | 1158.00±32.53 ^a |

^{a,b,c} Different superscripts within a row indicate significant differences among varieties ($p < 0.05$).

corresponding to the amylose to amylopectin ratio (Noda *et al.*, 1996). A low T_o , T_p , T_c and ΔH reflect the presence of abundant amylopectin short chains.

Pasting properties of cowpea starches

Pasting refers specifically to change the starch upon further heating after gelatinization has occurred, including further swelling and leaching of polysaccharides from the starch granule, and increased viscosity which occurs with application of shear forces (Hoover *et al.*, 2010). The pasting temperature (PT), peak time (PTi), peak viscosity (PV), through viscosity (TV), breakdown viscosity (BV), final viscosity (FV), and setback viscosity (SV) of cowpea starches are shown in Table 2. Significant differences ($p < 0.05$) in pasting properties among different cowpea starches varieties were observed. Pasting temperature of cowpea starches ranged between 76.30°C-80.95°C, which were similar with earlier reports (Huang *et al.*, 2007; Adebooye and Singh, 2008). The high PT of KT5 indicates that this starch has a higher resistance to swelling and rupture due to the smallest of granule size, which is related to the higher of T_p from DSC.

PV is influenced by friction between swollen granules, amylose content, and relative crystallinity (Maaran *et al.*, 2014). Amongst starches, KT7 exhibited the highest of PV and the lowest of FV and

SV. The higher PV of KT7 is mainly due to the higher amylose content. The higher BV of KT5, KT8 and KT7 indicated less ability to resist heating and shear stress than other varieties. The higher of FV of KT4 and KTL indicated a higher ability of the starches to form a viscous gel, and more resistant to shear so this starches could form the more rigid gel.

SV measures recrystallization of gelatinized starch during the cooling period and reflects the interaction between leached amylose chains during the cooling cycle and presence of intact and/or fragmented granules embedded in the amylose network (Ambigaipalan *et al.*, 2011). The lower SV of KT7 reflected extensive granule disruption during the heating cycle, which has been shown in the highest of PV, so the resistance to the stirring action of the paddles during the cooling cycle would be minimum. The pasting properties of pulse starches were influenced by granule swelling, high amylose content, amylose leaching, relative crystallinity, and chain length of the starch component (Hoover *et al.*, 2010; Du *et al.*, 2014).

Fourier transform infra-red (FT-IR) spectra of cowpea starches

The characteristic bands of starch in FT-IR spectra can be divided into four main regions, as follows: below 800 cm^{-1} , 800-1500 cm^{-1} (the

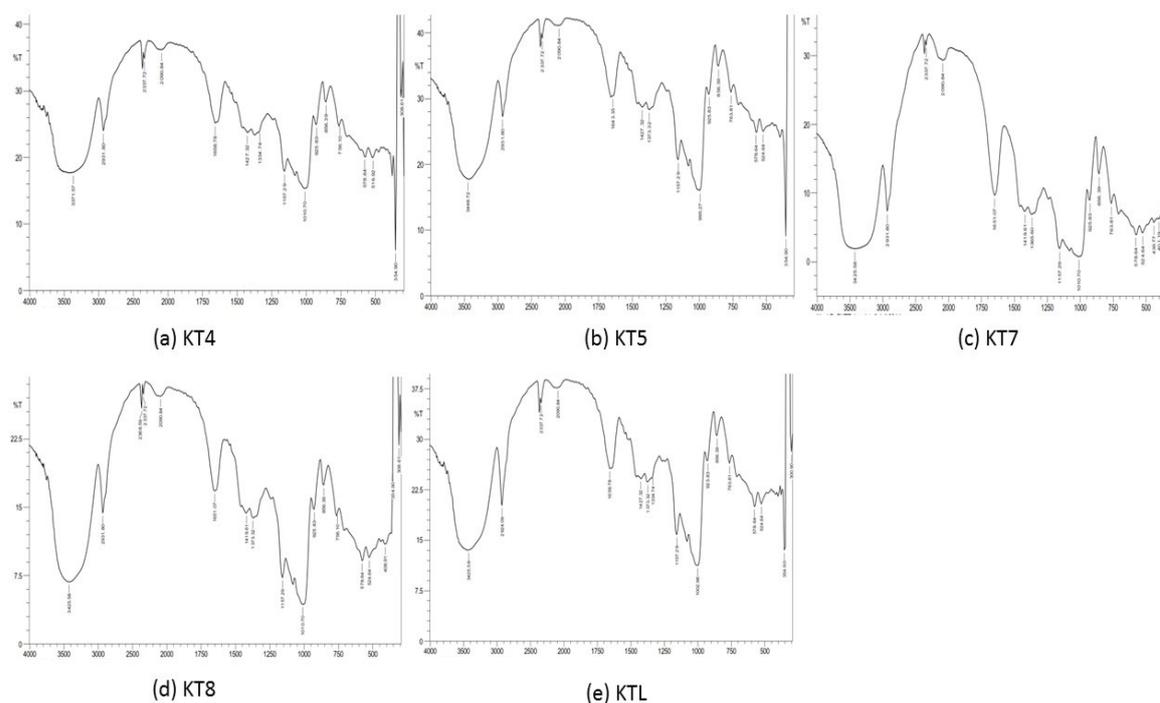


Figure 3. FT-IR spectra of cowpea starches

fingerprint region), 2800-3000 cm^{-1} (C-H stretching region), and 3000-3600 cm^{-1} (O-H stretching region) (Kizil *et al.*, 2002). All of cowpea starches showed a similar characteristic of bands (Figure 3). The band around 3425 cm^{-1} was attributed to hydroxyl groups stretching vibration which contained free hydroxyl groups and hydrogen bonded hydroxyl groups, especially the complex vibration stretches of intermolecular hydroxyl groups. The band at 2931 cm^{-1} was associated with $-\text{CH}_2$ stretching vibration with obscure from starch or lipid, or protein (Flores-Morales *et al.*, 2012). An intense band around 1651 cm^{-1} was attributed to the water adsorbed in the amorphous region of the starch and related to the crystallinity of starch (Kizil *et al.*, 2002). The weak bands at 1365 and 1427 cm^{-1} were attributed to $-\text{CH}_2$ twisting and bending which could be the identification of cowpea starches.

Three characteristic bands appeared at 925, 1010, and 1157 cm^{-1} were associated with C-O bond stretching (Khatoon *et al.*, 2009). The band near 925 cm^{-1} assigned to the C-O stretch of C-O-C in starch, and the bands near 1010 and 1157 cm^{-1} mainly attributed to anhydroglucose ring C-O stretch of C-O-H in starch. The band at 925 and 1010 cm^{-1} were associated with skeletal mode vibrations of α -1,4 glycosidic linkage (Joshi *et al.*, 2013), whereas, the band at 1157 cm^{-1} was associated with the ordered structures of starch (Guo *et al.*, 2014). It is interesting to note that the cowpea starches have the prominent band at 925 cm^{-1} , which are sensitive to water and

characteristic index of hydrophilicity of starches (Khatoon *et al.*, 2009).

Conclusion

The significant differences in composition, microstructure, and physicochemical properties were observed among the five cowpea (*Vigna unguiculata*) starches with respect to amylose content, mean granule diameter, thermal properties, pasting properties, WHC, and OHC. However, the shapes of starch granule, X-Ray diffraction pattern, and FT-IR spectra did not differ significantly amongst the starches. Striking differences in composition, morphological, and physicochemical properties of cowpea starches from different varieties revealed to its potential to be fit for various food formulations. Further research is also needed for the modification of cowpea starches by physical, chemical, enzymatic or combination of all so they can be used as a functional ingredient in various food formulations.

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