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Impact of heating temperatures on the properties of instant cassava flour

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Abstract: Native cassava flour can be modified to be instant flour by heating the cassava flour in ethanol solution. The impact of heating temperatures of 60, 80, and 100 °C (coded as ICF-60, ICF-80, and ICF-100) on the properties of instant cassava flour (ICF), including colour, morphological, and thermal properties, water absorption, and solubility indexes and pasting behaviour, were investigated. Results showed that ICF produced at higher temperatures exhibited lower lightness, higher redness, and yellowness values. ICF-60 and ICF-80 still displayed the granular forms and birefringence properties of native starches, while granules of ICF-100 were broken and partially lost their birefringence properties. Results of X-ray diffraction (XRD) technique and differential scanning calorimetry (DSC) analysis suggested that the amylopectin double helixes of crystalline regions within the structure of ICF orientated to more perfect conformation before they were disrupted at the highest heating temperature (100 °C). During hydration, the starch granules of ICF-60 and ICF-80 absorbed water into their granules; meanwhile, ICF-100 entrapped water within the matrix formed by the entanglements of ICF-100 particles. Results of pasting behaviour analysis indicated that ICF-60 and ICF-80 showed better thermal stability while ICF-100 exhibited the highest cold viscosity.

Keywords: flour modification; impact of heating in ethanol; ethanol; structural-physicochemical-morphological properties

Cassava (*Manihot utilissima*) is known as an important world commodity since it is used as a major ingredient in many industries such as food, pharmacy, textile, and others. The total world production of cassava in 2016 reaches 277 million tons which is contributed by the main cassava producer countries, including Nigeria, Thailand, Brazil, and Indonesia (FAO 2016). For ingredients of the food industry, cassava is simply processed to be cassava flour by the drying process, or it can be extracted to obtain cassava starch. Both, cassava flour and cassava starch, are important ingredients to prepare starch-based instant food products.

Instant flour can be prepared by several pregelatinisation techniques such as extrusion (Akande et al. 2017), spray drying (Eastman and Moore 1984), high hydrostatic pressure (Liu et al. 2016), drum drying (Wadchararat et al. 2006; Li et al. 2014). Among those methods, drum drying is the most widely implemented in the food industry since it is quite efficient in which the cooking and drying process can be done with one

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equipment (Berk 2018). Preparation of instant flour can also be carried out by adopting the process of cold water swelling starch. In this method, flour is cooked with gelatinisation inducer and preventer agents in certain proportions and temperature (Eastman and Moore 1984; Zhang et al. 2012; Dries et al. 2014). The advantage of this method is that it can be applied on a lab scale. Therefore, it is suitable for those who still develop an instant product on a small scale. Moreover, heating flour in ethanol is considered to be the simplest method to produce instant flour. The protocol only requires ethanol as the converting reactant, which evaporates from the product during the dehydration process, thus leaving little or no ethanol residue in the final product (Zhang et al. 2012; Dries et al. 2014).

The characteristics of instant flour must be identified prior to its implementation for developing instant food. For example, instant sorghum flour quickly disperses in water, creating a soft texture for non-gluten bakery (Schober et al. 2005). Pregelatinised rice flour can improve the elasticity of non-gluten dough and acts as a binder that holds water and gas in the matrix of non-gluten dough (Carrillo-Navas et al. 2016). Thus, the main objective of this study was to investigate the impact of heating temperatures on the properties of instant cassava flour (ICF).

MATERIAL AND METHODS

Material. Cassava tubers were obtained from the local market at Subang, West Java, Indonesia. Cassava flour was prepared by following the method of Lu et al. (2020) with modifications. It was prepared by peeling, shredding, drying, and crushing the fresh cassava root tubers and was then passed through a 60 mesh screen (laboratory test Sieve D-42757; Retsch Gmbh, German). Analytical grade ethanol was purchased from Merck (Germany).

Sample preparation. ICF was prepared by the method of Dries et al. (2014) with some modifications. Cassava flour (15 g) was dispersed with 45 ml of ethanol (50% v/v) in Schott bottles (Schott Duran, Germany). It was then heated at different temperatures of 60, 80, and 100 °C (coded as ICF-60, ICF-80, and ICF-100) for 30 min [water bath shaker type 1086; Gesellschaft für Labortechnik (GFL), Germany] and cooled at room temperature for 3 h. The sediment was separated from the supernatant by vacuum filtration (vacuum pump type 840.3FT.18; Labofort KnF, Germany), washed with absolute ethanol three times, and finally dried in an oven at 55 °C for 12 h (UM500; Memmert, Germany). The dried sample was ground (grinder HR2115; Philips, Indonesia) and sieved with a sieve with an aperture size of 150 μm (laboratory test sieve D-42757; Retsch Gmbh, Germany). All treatments were carried out in triplicate.

Colour properties. A 3NH colourimeter (Shenzhen ThreeNH Technology Co., Ltd., China) was employed to determine the colour parameter of samples. The CIELAB colour parameters including L^*, a^*, and b^* were obtained with the light source of D65 and observation angle of 10°. The L^* represents the lightness of the colour (L^* = 0 denotes black and L^* = 100 denotes white), a^* indicates the colour level between red and green in which tendency to the red colour is presented in positive value (max. value is +100) and to green colour is a negative value (min. value is –80), b^* is the extent of yellowness or blueness in which the tendency to yellow colour is indicated by positive value (max. value is +70) and to blue colour is a negative value (min. value is –50) (Hunter 1975).

Morphological properties of dried ICF. The morphological properties of dry samples were observed by a scanning electron microscope (JEOL JSM IT300; JEOL, Japan). The sample was mounted on a metal stub and coated with gold. Then, an accelerating voltage of 2 kV was used during observation.

Morphological properties of hydrated ICF. A light microscope (BX41; Olympus, Japan) was employed to observe the morphology and birefringence properties of hydrated samples. The sample (10 mg) was dispersed with 5 ml of water in a test tube (Iwaki-Pyrex, Indonesia), and then it was shaken with a vortex mixer for 1 min (VM-300; Gemmy, Taiwan). One drop of sample solution was placed on a microscope objective glass then it was closed by its cover. The morphology and birefringence properties of the hydrated samples were observed by using normal and polarised modes, respectively.

The crystalline structure of ICF. The crystalline structure of ICF was assayed by using X-ray diffraction (XRD) technique (SmartLab; Rigaku, Japan) using a method of Nakorn et al. (2009) with modification. The diffractogram of the sample was reported in the 2θ range (range of 5 to 28). The crystallinity of the sample was calculated by the Equation (1).

$$Relative\ crystallinity\ (\%) = \frac{Area\ of\ crystalline\ peaks}{Total\ area\ of\ diffractogram} \times 100\% \quad (1)$$

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Thermal properties. A differential scanning calorimeter (DSC) (DSC Star® System; Mettler-Toledo GmbH, Switzerland) was used to determine the thermal properties of the sample. Prior to analysis, the instrument was calibrated using indium, and an empty aluminium pan (aluminum crucible standard; Mettler-Toledo GmbH, Switzerland) was used as a reference during analysis (Lai 2001). A sample of about 5 mg on a dry weight basis was placed in an aluminium pan. Distillate water was added; thus, the sample moisture content is 70% (w/w). The aluminium pan was sealed, weighed (analytical balance MS104TS/00; Mettler-Toledo GmbH, Switzerland), and equilibrated for at least an hour at room temperature. The sample was scanned from a temperature of 25 °C to 140 °C with a heating rate of 5 °C min^{-1} (DSC Star System; Mettler-Toledo GmbH, Switzerland). The thermal properties of the sample were recorded, including onset temperature (*To*), peak temperature (*Tp*), conclusion temperature (*Tc*) and transition enthalpy (ΔH).

Pasting properties. The pasting properties of samples were determined by using Rapid Visco Analyser (RVA--TecMaster; PerkinElmer, USA) following the method of Lai (2001) with slight modifications. Sample suspension (12% w/v) was made by mixing 3 g of sample with 25 ml of water in an aluminium pan (standard sample cans for TecMaster RVA; PerkinElmer, USA). The analysis was carried out by the following sequence: equilibration at 35 °C for 2 min, heating until 95 °C with the heating speed of 11.8 °C min^{-1}, holding for 2.5 min, then cooling until 35 °C with cooling speed of 11.8 °C min^{-1} (RVA-TecMaster; PerkinElmer, USA). The pasting curve was plotted between viscosity in the rapid visco analyser (RVA) unit (arbitrary unit) versus heating and cooling time. Pasting parameters including peak viscosity, trough viscosity, breakdown viscosity, final viscosity, setback viscosity, and pasting temperature were reported.

Statistical analysis. Statistical analysis was performed using SPSS 17.0, in which the ANOVA procedure was employed. Duncan's post-hoc test was used to verify the significant differences between the mean values ($P < 0.05$).

RESULTS AND DISCUSSION

Colour properties. The three-colour parameters, L^*, a^*, and b^*, of native cassava flour and ICF are presented in Table 1. The ICF-60 and ICF-80 are brighter in colour, as indicated by the higher L^*, lower a^*, and b^* than the untreated sample. This could be due to the colour component of cassava flour i.e. carotenoids leached out from the flour into ethanol solution during heating treatment (Palermo et al. 2014). ICF-100 exhibited the darkest colour among the samples. This might be because the component of cassava flour, particularly starch, had undergone gelatinisation during heating at 100 °C. The native crystalline structure of starch components, i.e. amylopectin, disrupted to become an amorphous structure above the gelatinisation temperature of starch (Huang et al. 2014). Starch with a higher proportion of crystalline structure exhibits a brighter colour than those with a higher proportion of amorphous structure (Hunger 1999).

Morphological properties of dried ICF. Figure 1 illustrates the morphological properties of dried native cassava flour and ICF. The image indicates that the major component of untreated cassava flour is starch granules, while other cassava flour components, i.e. fat, protein, and fibre, are minor (Lu et al. 2020). The cassava starch granules showed round shapes with some truncated and smoothed surface granules (Figure 1A). Most starch granules of ICF-60 and ICF-80 still displayed intact granules, but some granules showed partial melting and indenting (arrows in Figures 1B and 1C). Almost all starch granules of ICF-100 showed destroyed debris shapes-like particles. The native cassava starch is fragile and easy to be disrupted during heat treatments (Rolland-Sabaté et al. 2012) due to its lack of amylose-tie chains.

Table 1. Colour parameters of untreated cassava flour and instant cassava flour (ICF) from heating temperatures of 60, 80, and 100 °C (ICF-60, ICF-80, and ICF-100), respectively (mean ± SD; $n = 5$)

Sample	L^*	a^*	b^*
Untreated	86.858 ± 0.004^{b}	1.655 ± 0.012^{c}	10.097 ± 0.003^{c}
ICF-60	87.277 ± 0.003^{d}	0.919 ± 0.012^{a}	7.930 ± 0.010^{b}
ICF-80	86.968 ± 0.003^{c}	1.035 ± 0.006^{b}	7.592 ± 0.004^{a}
ICF-100	77.591 ± 0.004^{a}	3.484 ± 0.009^{d}	13.918 ± 0.014^{d}

$^{a-d}$Different letters within columns indicate statistically significant differences ($P < 0.05$); SD – standard deviation

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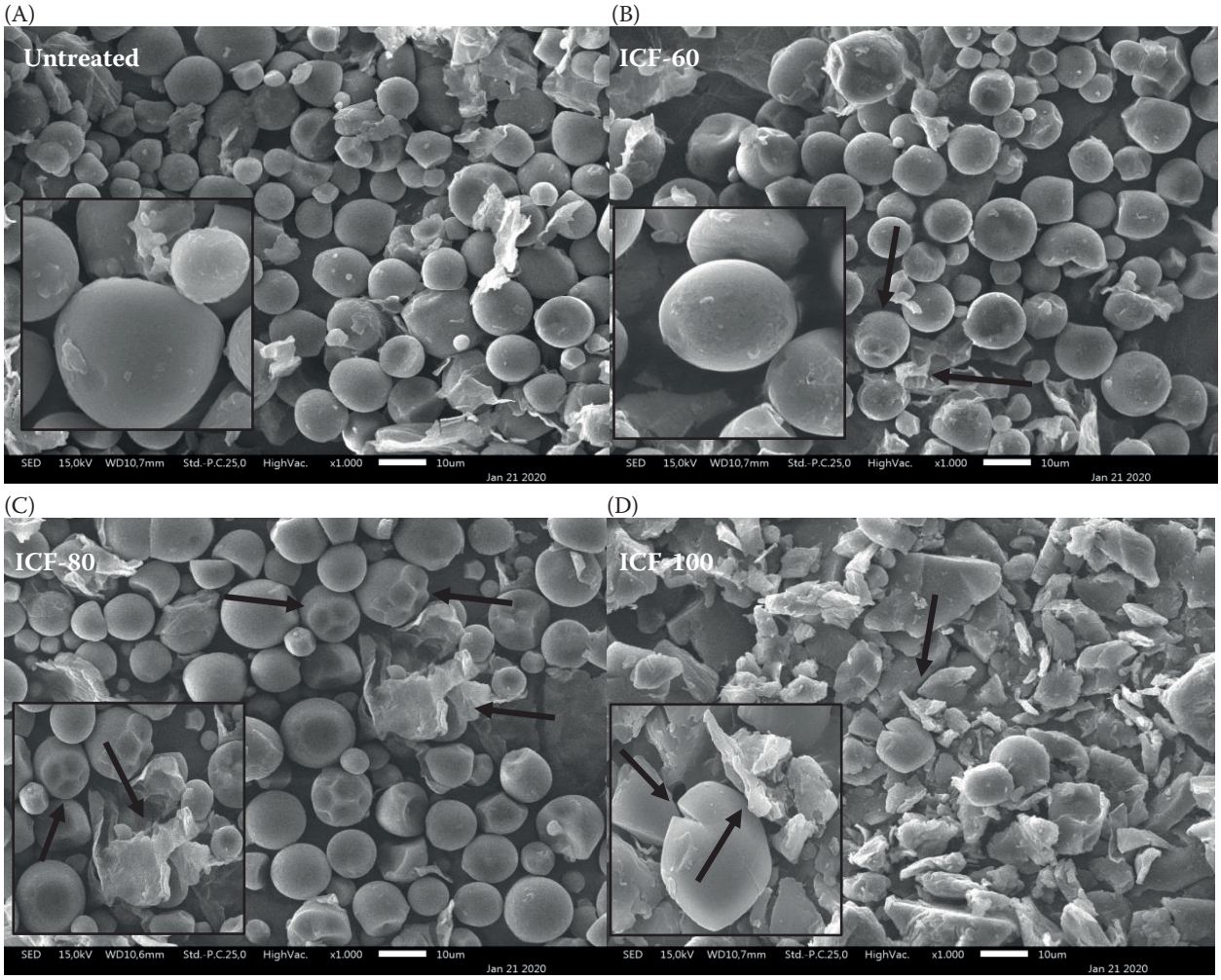

Figure 1. Scanning electron micrograph of (A) untreated cassava flour and instant cassava flour (ICF) from heating temperatures of (B) 60 °C (ICF-60), (C) 80 °C (ICF-80) and (D) 100 °C (ICF-100), respectively

Morphological properties of hydrated ICF. The mi-crographs of hydrated native cassava flour and ICF are presented in Figure 2. The native cassava flour displayed starch granules which showed oval in shape with distinct Maltese cross characteristics (arrows in Figure 2Ab). The birefringence properties of ICF-60 and ICF-80 were still obvious (arrows in Figures 2Bb and 2Cb), indicating that the crystalline region within the starch granules was not destroyed during the heating treatment at temperatures of 60 °C and 80 °C. Some granules of ICF-100 lost their Maltese cross properties (arrows in Figure 2Db), suggesting that disruption on the crystalline part of the starch granules has occurred at 100 °C. In the system of heating ethanol treatment, the amount of free water required for the gelatinisation process is limited; therefore, the gelatinisation temperature of starch granules in this system is higher than that in the aqueous system (Vermeylen et al. 2006). Moreover, the complex of amylose-ethanol may have occurred at the periphery of starch granules during the heating in ethanol treatment (Zhang et al. 2012). The complex enhances the strength of starch granules from disruptions during heat treatment (Dries et al. 2014; Sarifudin et al. 2019).

The crystalline structure of ICF. The XRD graph and crystallinity profiles of native cassava flour and ICF are presented in Figure 3 and Table 2, respectively. The major peaks of A-type starch at 2θ of 15, 17, 18.1, and 23.3° (Singh et al. 2006) was observed at the diffractogram of the untreated sample. The diffractograms of ICF-60 and ICF-80 are identical to the diffractogram of the untreated sample. Moreover, the crystallinity of ICF-60 and ICF-80 is similar to that of the untreated sample (Table 2). These results suggested that the crystalline structure of native starch was not disrupted during heating in ethanol at temperatures

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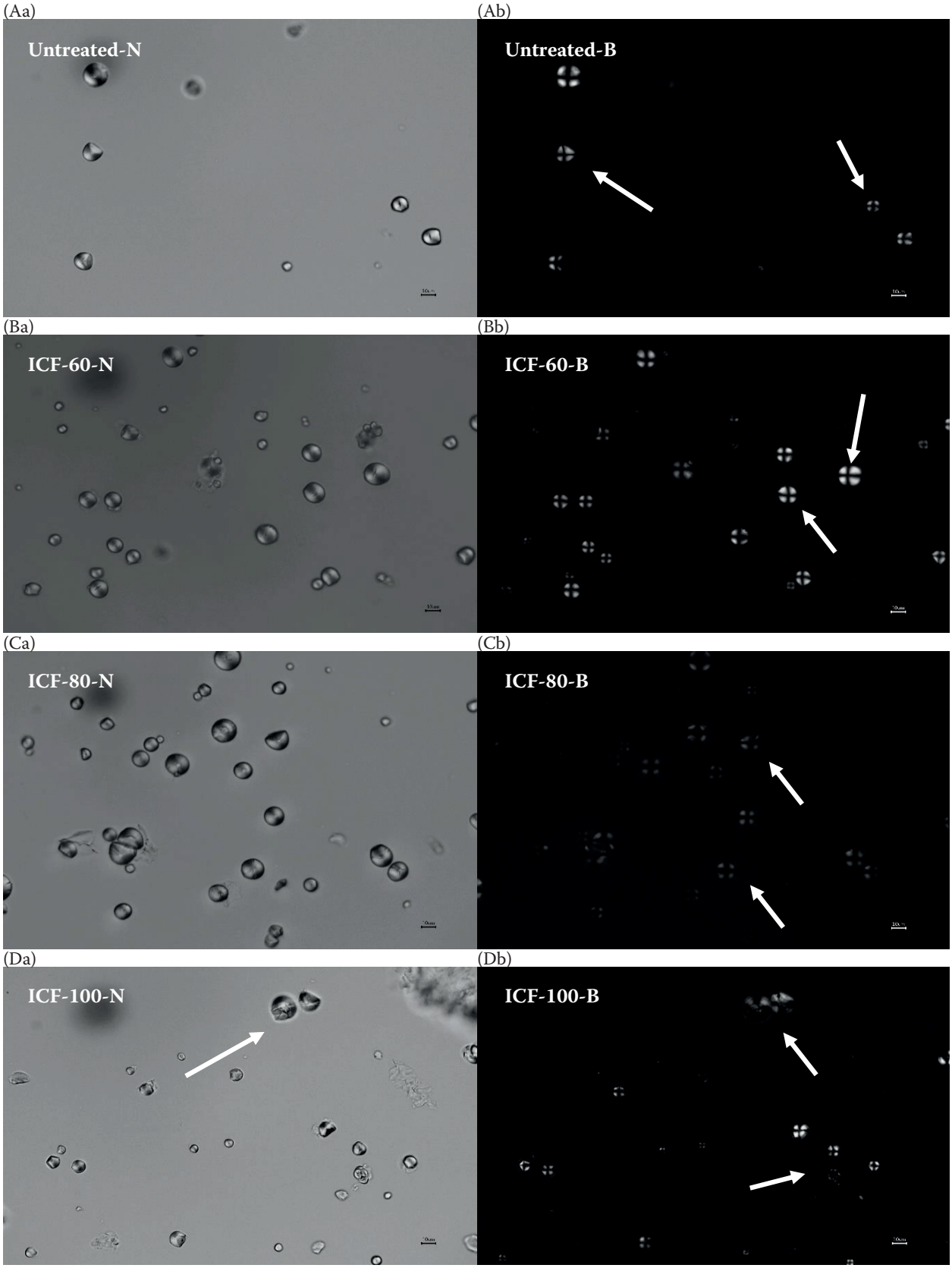

Figure 2. Micrograph of (A) untreated cassava flour and instant cassava flour (ICF) from heating temperatures of (B) 60 °C (ICF-60), (C) 80 °C (ICF-80) and (D) 100 °C (ICF-100) observed in normal (N) and birefringence (B) modes, respectively

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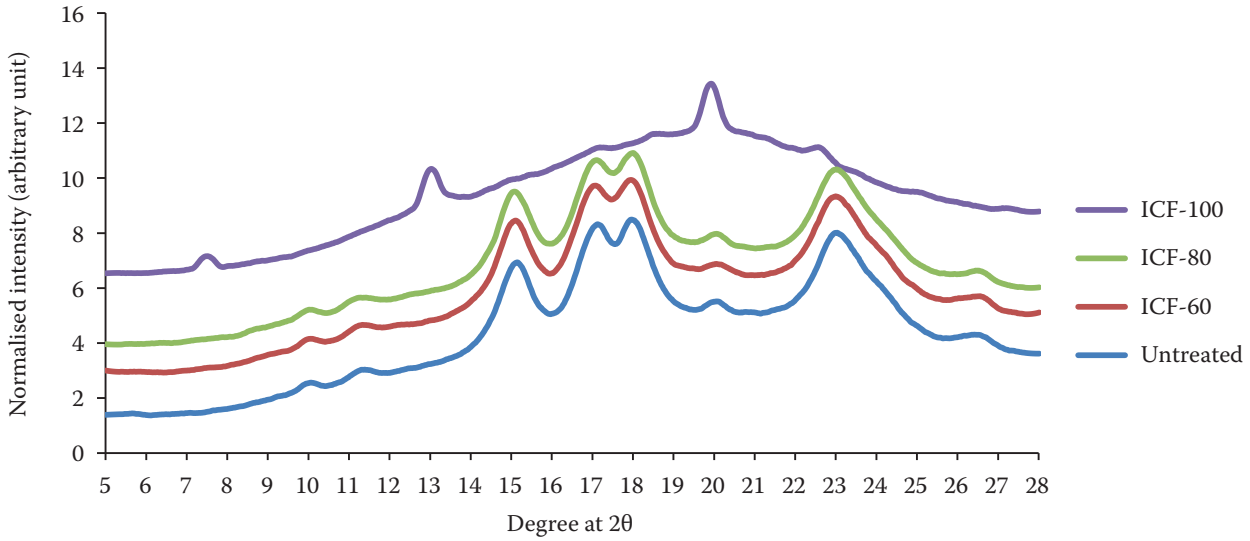

Figure 3. X-ray diffraction (XRD) profile of untreated cassava flour and instant cassava flour (ICF) from heating temperatures of 60, 80, and 100 °C (ICF-60, ICF-80, and ICF-100), respectively

of 60 and 80 °C. The identity peaks of native cassava starch were not shown in the diffractogram of ICF-100; instead, the identity peaks of V-type crystalline structure identified at 2θ of 7.8, 13.6, and 20.9° (Le Bail et al. 1995) were observed (Figure 3). The V-crystalline structure is known to be water-soluble (French and Murphy 1977); therefore, the presence of this structure will enhance the solubility of the ICF.

Table 2. Crystallinity of untreated cassava flour and instant cassava flour (ICF) from heating temperatures of 60, 80, and 100 °C (ICF-60, ICF-80, and ICF-100), respectively

Sample	Crystallinity (%)
Untreated	32.1
ICF-60	32.1
ICF-80	32.2
ICF-100	10.2

Thermal properties. The result of the thermal analysis of native cassava flour and ICF is presented in Table 3. The onset, peak, and end-set temperatures of ICF were higher than that of the untreated sample (Table 3). Moreover, the enthalpy of ICF was higher than that of the untreated sample, then the enthalpy of ICF-80 and ICF-100 gradually decreased. Sarifudin et al. (2019) reported that in heating starch in a low-water-content system, the thermal forces pushed the crystalline lamellae to realign toward a more perfect configuration. The strength of crystalline lamellae to stand toward thermal forces reaches its limit until a certain temperature depending on the starch origin and the heating systems (Perry and Donald 2000; Vermeylen et al. 2006; Dries et al. 2014; Dries et al. 2016; Sarifudin et al. 2019) in which in this experiment was 60 °C. Beyond this temperature, the crystalline lamellae start to disorder gradually, as indicated by the decrease of enthalpy (Table 3). Heating starch in ethanol at above 100 °C leads to complete annihilation of crystalline lamellae, which

Table 3. Thermal properties of untreated cassava flour and instant cassava flour (ICF) from heating temperatures of 60, 80, and 100 °C (ICF-60, ICF-80, and ICF-100), respectively (mean ± SD; n = 2)

Sample	Onset	Peak	Endset	Enthalpy
	(°C)			(J g^{-1})
Untreated	68.91 ± 0.07^{a}	74.08 ± 0.00^{a}	79.88 ± 0.01^{a}	8.55 ± 0.25^{b}
ICF-60	70.71 ± 0.06^{b}	74.75 ± 0.24^{b}	80.14 ± 0.14^{a}	10.13 ± 0.38^{c}
ICF-80	71.02 ± 0.04^{c}	74.84 ± 0.12^{b}	79.96 ± 0.06^{a}	8.94 ± 0.30^{b}
ICF-100	73.22 ± 0.05^{d}	76.46 ± 0.06^{c}	80.08 ± 0.11^{a}	1.60 ± 0.08^{a}

$^{a–d}$Different letters within columns indicate statistically significant differences (P < 0.05); SD – standard deviation

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Table 4. Pasting profile of untreated cassava flour and instant cassava flour (ICF) from heating temperatures of 60, 80, and 100 °C (ICF-60, ICF-80, and ICF-100), respectively (mean ± SD; n = 2)

Sample	Peak viscosity	Trough viscosity	Breakdown viscosity	Final viscosity	Setback viscosity	Pasting temperature
	(RVU)					(°C)
Untreated	4 015.5 ± 65.8^{a*}	2 182.5 ± 33.2^{b}	1 833.0 ± 98.9^{b}	3 468.0 ± 18.4^{a}	1 285.5 ± 51.6^{a}	77.8 ± 0.6^{b}
ICF-60	5 416.0 ± 42.4^{b}	2 749.5 ± 10.6^{c}	2 666.5 ± 31.8^{c}	4 334.0 ± 130.1^{b}	1 584.5 ± 119.5^{a}	77.8 ± 0.5^{b}
ICF-80	5 328.5 ± 140.7^{b}	1 706.0 ± 247.5^{a}	3 622.5 ± 106.8^{d}	5 188.5 ± 3.5^{c}	3 482.5 ± 251.0^{b}	79.4 ± 0.5^{c}
ICF-100	3 857.0 ± 127.3^{a}	2 386.0 ± 168.3^{bc}	1 471.0 ± 41.0^{a}	5 534.5 ± 116.7^{d}	3 148.5 ± 51.6^{b}	42.5 ± 0.0^{a}

$^{a–d}$Different letters within columns indicate statistically significant differences (P < 0.05); SD – standard deviation; RVU – relative value units

is indicated by the disappearance of melting endotherm peak (Rajagopalan and Seib 1992).

Pasting properties. The pasting profiles of native cassava flour and ICF are presented in Table 4. The peak temperature and peak viscosity of the untreated sample were 77.8 °C and 4 015.5 relative value units (RVU), respectively. Surprisingly, the peak viscosities of ICF-60 and ICF-80 were higher than that of the untreated sample, which was 5 416 RVU and 5 328.5 RVU for ICF-60 and ICF-80, respectively. Peak viscosity is a measure of the thickening power of starch representing the strength of crystalline lamellae during heating (Zhu et al. 2019). These results suggested that the alignment of crystalline lamellae of ICF-60 and ICF-80 was stronger than that of the untreated sample. This was confirmed by the result of thermal analysis (Table 3), in which the enthalpy of ICF-60 and ICF-80 was higher than that of the untreated sample. The double helixes of crystalline lamellae aligned toward a more perfect configuration induced by thermal forces during heating starch in ethanol (Sarifudin et al. 2019). The pasting temperature and peak viscosity of ICF-100 were lower than those of the untreated samples, indicating that most of the ICF-100 granules were already broken (Figure 1D).

The breakdown viscosity of ICF-100 is significantly smaller than that of the untreated sample (Table 4). Since breakdown viscosity refers to the difference between the peak and trough viscosity, therefore, it reflects the thermal stability of the starch slurry. The low breakdown viscosity value of ICF-100 indicated that ICF-100 is less thermal-stabile than the untreated sample (Zhu et al. 2019). Moreover, the setback viscosities of all ICF samples were higher than that of the untreated samples (Table 4). These results indicated that the starch slurries of ICF were easier to undergo retrogradation (Zhu et al. 2019). At low temperature, the solubilised starch polymers and insoluble fragments of the slurry of ICF-100 were easier to entangle together, forming the three-dimensional structure of retrograded starch (arrow in Figure 2Da) (Thomas and Atwell, 1999) as indicated by the highest final viscosity (Table 4).

CONCLUSION

Heating temperatures indeed impacted the properties of ICF. Heating at 60 °C and 80 °C produced ICF that exhibited colour and starch granule morphology similar to that of the untreated flour, but it showed higher thermal resistance. Heating at 100 °C produces ICF that displayed broken starch granules. Suspension of ICF-100 was susceptible to retrogradation and provided the highest final viscosity.

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