

## Physicochemical properties of rice (*Oryza sativa* L.) flour and starch of two Indonesian rice varieties differing in amylose content

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### Abstract

Physicochemical properties of rice flour and rice starch differing in amylose content were evaluated in Setra Ramos and Mentik Susu rice varieties. The objectives of this research was to determine the physical properties i.e. thermal, X-ray diffraction patterns and chemical properties i.e. moisture content, lipid content, protein content, total starch content, amylose content, FT-IR analysis, and <sup>13</sup>C NMR analysis of rice flour and rice starch of Setra Ramos and Mentik Susu. It was found that the rice starch of Setra Ramos had the highest To and Tp may be due to the higher of their amylose content whereas the rice flour of Mentik Susu had the highest Tc and ΔH. The rice starches of Setra Ramos and Mentik Susu had the higher moisture content than rice flours of Setra Ramos and Mentik Susu whereas the lipid content and protein content of rice starch of Setra Ramos were lower than its rice flour. The rice flour of Setra Ramos had the higher amylose content (23.69%) than rice flour of Mentik Susu. The spectra FT-IR for rice flour and rice starch of Setra Ramos and Mentik Susu showed a band at 400 and 700 cm<sup>-1</sup> to skeletal mode of amylose and amylopectin. The peak in 100 ppm of spectra <sup>13</sup>C NMR confirmed that the rice starches of Setra Ramos and Mentik Susu had A-type X-ray diffractions patterns.

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### Introduction

Rice (*Oryza sativa* L.) is a staple food for Indonesian people. In 2014 the per capita consumption of rice in Indonesia has reached 130 kg/capita/year. Although the per capita consumption of rice in Indonesia since 2012 to 2014 has decreased compare to 2011, the pattern of food consumption of Indonesian society is still dependent on rice. In Indonesia, rice is mostly consumed as cooked polished grains, whereas rice flour is used as an ingredient in many Indonesian cuisines. Starch is the largest component in rice grains and determines the quality of rice products (Hasjim *et al.*, 2013). Rice flour and rice starch have a unique characteristics. The physicochemical properties of rice affect the physical and chemical attributes of food during processing (Falade *et al.*, 2014). Therefore, it is necessary to understand the physicochemical properties of rice flour and rice starch.

Previous studies on the physical properties of rice flour and rice starch including on physical dimensions (Falade and Christopher, 2015), morphological properties (Ogawa *et al.*, 2003; Singh *et al.*, 2006; Cardoso *et al.*, 2007), damage grain and damage

starch (de la Hera *et al.*, 2013), degree of milling (Falade and Christopher, 2015), colour (Martinez *et al.*, 2014), gel textural (Kang *et al.*, 2003; Singh *et al.*, 2006); water and oil absorption capacity (Kadan *et al.*, 2008; de la Hera *et al.*, 2013); foam capacity and foam stability (Martinez *et al.*, 2014), swelling power and solubility (Wang *et al.*, 2002; Waterschoot *et al.*, 2014), pasting properties (Zhong *et al.*, 2009; Hasjim *et al.*, 2013), viscosity (Lawal *et al.*, 2011), thermal properties (Zhong *et al.*, 2009; Dhital *et al.*, 2015), crystallinity and recrystallinity (Baik *et al.*, 1997; Yu *et al.*, 2012). Chemical composition (Sagum and Arcot, 2000; Zhou *et al.*, 2002; Widowati *et al.*, 2006; Yu *et al.*, 2012; Lee *et al.*, 2013), mineral content (Lawal *et al.*, 2011), molecular weight distribution of amylose and amylopectin (Zhu *et al.*, 2011; Syahariza *et al.*, 2013), and amylopectin chain length distribution (Wang *et al.*, 2002; Benmoussa *et al.*, 2007; Chung *et al.*, 2011; Kowittaya and Lumdubwong, 2014) can influence the properties of rice starch.

Several famous varieties of rice in Indonesia market such as Pandan Wangi, Rojolele, and Mentik Wangi have been reported for amylose content. The amylose content of Pandan Wangi and Rojolele varieties were

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25.90% and 24.60%, respectively (Widowati *et al.*, 2006). No work on the physicochemical properties of rice flours and starches from Setra Ramos and Mentik Susu varieties has been reported. Therefore, this research was to determine the physicochemical properties of flours and starches of Setra Ramos and Mentik Susu varieties with differences in amylose content. The physicochemical properties include thermal, crystallinity, chemical composition, FT-IR analysis, and  $^{13}\text{C}$  NMR analysis.

## Materials and Methods

### Materials

Two different rice varieties were used in this study and were collected from local farm of Jogjakarta, Indonesia. The rice varieties were Setra Ramos (SR) and Mentik Susu (MS).

### Preparation for rice flour

Each of rice variety was ground with a mill and then passed through 100 mesh sieve. The rice flour were collected and stored in plastic bag at -4°C in a freezer until being analyzed. The rice flour then referred as rice flour Setra Ramos (RFSR) and rice flour Mentik Susu (RFMS).

### Preparation for rice starch

Starch was isolated from both rice flours by alkali extraction of the protein method (Sodhi and Singh, 2003) with slight modification. Rice flours (20 g, dry basis) was soaked in 200 ml 0.2% NaOH solution for 3 h and steeped at 20°C overnight. The steep liquor was drained off and the slurry was then diluted to the original volume with 0.2% NaOH solution. The process was repeated 4 times until the supernatant become clear and gives a negative reaction to the Biuret test for protein. The slurry was centrifuged at 3500 rpm and the starch was dried in cabinet drier at 50°C overnight. The starch was passed through 100 mesh sieve and stored in plastic bag at -4°C in a freezer until being analyzed. The rice starch then referred as rice starch Setra Ramos (RSSR) and rice starch Mentik Susu (RSMS).

### Thermal properties

Thermal properties of rice flour and starch were analyzed using a differential scanning calorimeter (DSC; type 4000, Perkin-Elmer Inc., USA). Rice flour or rice starch samples (40 mg, dry basis) pressed and placed in alluminium sample pans. Gelatinization was determined by heating starch in alluminium pan from 4°C to 450°C at a heating rate of 5°C/min. The onset (To), peak (Tp), and conclusion (Tc) of gelatinization

temperatures and the enthalphy of gelatinization ( $\Delta\text{H}$ ) were determined.

### X-ray diffraction analysis

The crystalline structure of rice flour and starch were analyzed using an X-ray diffractometer (XRD; type 6000, Shimadzu Inc., USA) at voltage of 40 kV and current of 30 mA Cu radiation. Diffractograms were obtained from 3° to 70° (2θ) at a scan rate 5°/min.

### Chemical composition analysis

The moisture, lipid, protein, amylose, and total starch content were analyzed according to AOAC method (1990) with modification. The results were reported on a dry basis. All the experiments were done in triplicate and results were presented as mean values.

### FT-IR analysis

Rice flour and rice starch were analyzed using a fourier transformed infrared spectroscopy (FT-IR; Prestige 21, Shimadzu Inc., USA). Rice flour or rice starch samples (2 mg, dry basis) was mixed with 200 mg of FT-IR grade potassium bromide (KBR) and pressed using a manual press for 20 min. The pellets were transferred into the FT-IR system. Each spectrum was recorded at a resolution of 4 cm<sup>-1</sup> in a range of 500-4000 cm<sup>-1</sup>.

### $^{13}\text{C}$ NMR analysis

Rice flour and rice starch were analyzed using a  $^{13}\text{C}$  nuclear magnetic resonance ( $^{13}\text{C}$  NMR; JEOL ECA-500, USA). Rice flour or rice starch sample (10 mg, dry basis) was dissolved in 0.6-0.7 ml dimethyl sulfoxide (DMSO). The mixture was transferred into  $^{13}\text{C}$  NMR system. The spectra recorded at 500 mHz at room temperature.

### Statistical analysis

The data were subjected to analysis of variance (ANOVA) and the significance of the difference among means was determined by Duncan's multiple range test ( $p < 0.05$ ) using SPSS software version 15.0 (SPSS Inc., Chicago, IL). Values expressed were means  $\pm$  SD.

## Results and Discussions

### Thermal properties of rice flour and rice starch

The RFSR, RFMS, RSSR and RSMS samples exhibited endotherms in gelatinization. The thermal properties of these samples are summarized in Table 1. It can be seen in Table 1 that all parameters of

Table 1.Thermal properties of RFSR, RFMS, RSSR and RSMS

Samples	To	Tp	Tc	Tc-To	$\Delta H$ (J/g)
RFSR	28.13 ± 0.89 <sup>a</sup>	59.61 ± 0.60 <sup>a</sup>	100.23 ± 0.25 <sup>b</sup>	72.10 ± 0.78 <sup>c</sup>	134.00 ± 0.03 <sup>c</sup>
RFMS	32.89 ± 0.68 <sup>b</sup>	65.38 ± 0.80 <sup>b</sup>	116.10 ± 0.15 <sup>d</sup>	83.20 ± 0.60 <sup>d</sup>	191.07 ± 0.84 <sup>d</sup>
RSSR	42.64 ± 0.59 <sup>d</sup>	74.12 ± 0.42 <sup>d</sup>	110.44 ± 0.52 <sup>c</sup>	67.80 ± 0.22 <sup>b</sup>	84.69 ± 0.35 <sup>b</sup>
RSMS	39.65 ± 0.50 <sup>c</sup>	66.68 ± 0.23 <sup>c</sup>	90.66 ± 0.29 <sup>a</sup>	51.01 ± 0.23 <sup>a</sup>	53.33 ± 0.73 <sup>a</sup>

Means not sharing a common letter in a column are significantly different at  $p < 0.05$

To: the onset of gelatinization temperature

Tp: the peak of gelatinization temperature

Tc: the conclusion of gelatinization temperature

Tc-To: the gelatinization temperature range

$\Delta H$ : the enthalpy of gelatinization

thermal properties differed significantly among samples. The RSSR had the highest of To ( $42.64^{\circ}\text{C}$ ) followed by RSMS ( $39.65^{\circ}\text{C}$ ), RFMS ( $32.89^{\circ}\text{C}$ ) and RFSR ( $28.13^{\circ}\text{C}$ ). RSSR had also the highest Tp ( $74.12^{\circ}\text{C}$ ) and differed significantly. The highest To and Tp for RSSR may be attributed to the higher content of amylose (23.69%). Amylose content, granular structures and lengths of the amylopectin could be affected the differences of To, Tp, Tc and  $\Delta H$  from different rice cultivars (Sodhi and Singh, 2003). In contrast, RFMS had the highest Tc ( $116.10^{\circ}\text{C}$ ), Tc-To ( $83.20^{\circ}\text{C}$ ) and  $\Delta H$  ( $191.07 \text{ J/g}$ ). These results were different with the report of Hasjim *et al.* (2013) who reported that the Tc rice flour ranged from  $72.3^{\circ}\text{C}$  to  $82.8^{\circ}\text{C}$ . Furthermore,  $\Delta H$  of the RFSR and RFMS were higher than RSSR and RSMS, which could be due to the presence of non-starch components in the rice flour. The protein, lipids and cell wall components could be influence the crystallinity of grains. These results were in accordance with the earlier report (Yu *et al.*, 2012).

#### X-ray diffraction patterns of rice flour and rice starch

The X-ray diffraction patterns of RFSR, RFMS, RSSR and RSMS are showed in Figure 1. The RFSR, RFMS, RSSR and RSMS had an A-type X-ray diffraction pattern with the diffraction at  $15^{\circ}$ , doublet  $17^{\circ}$  and  $18^{\circ}$ , and  $23^{\circ}$  ( $2\theta$ ). This type of X-ray diffractions patterns was similar to the previous study reported by Wani *et al.* (2012) and Yu *et al.* (2012). Furthermore, the doublet of  $17^{\circ}$  and  $18^{\circ}$  were clearly in RSSR and RSMS than in RFMS and RFSR.

It can be seen in Figure 1 the strongest peak in RFSR, RFMS, RSSR, and RSMS exhibited diffraction at  $15^{\circ}$  and  $18^{\circ}$  ( $2\theta$ ). The differences of amylose content between SR and MS do not resulted in the differences of diffractons patterns among the samples. This result was consistent with Zhu *et al.* (2011) who reported the low-amylase rice (16.1%) and intermediate-amylase rice (22.5%) had the same

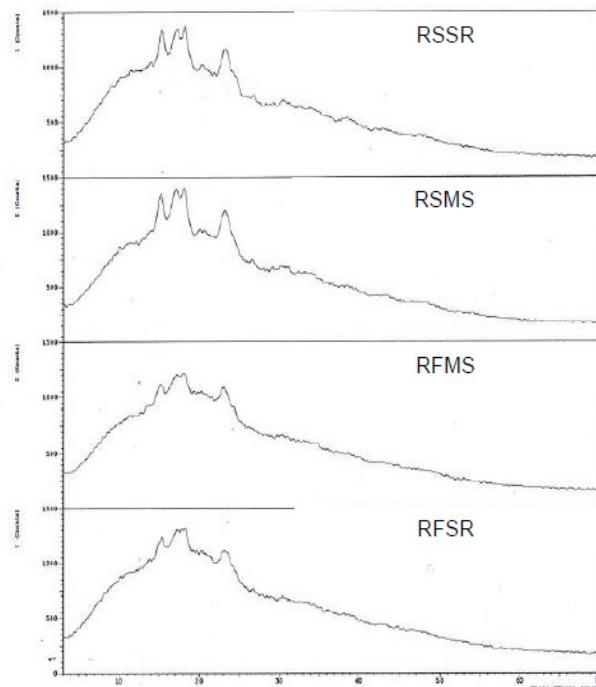


Figure 1. X-ray diffraction patterns of RFSR, RFMS, RSSR and RSMS

A-type X-ray diffraction patterns at  $15^{\circ}$ , doublet  $17^{\circ}$  and  $18^{\circ}$  and  $23^{\circ}$  ( $2\theta$ ).

#### Chemical composition of rice flour and rice starch

In general the moisture content of RFSR and RFMS was lower than RSSR and RSMS and differed significantly. Moisture content of RFMS and RFSR ranged from 10.02% to 10.24%, respectively whereas the moisture content of RSMS and RSSR ranged from 13.41% to 13.44%, respectively. The differences of moisture content between rice flours (RFSR and RFMS) and rice starch (RSMS and RSSR) could be due to the action of NaOH during the extraction process, which affected the internal modification of starch granules. The result of Cardoso *et al.* (2007) suggested that the rheological properties of starch

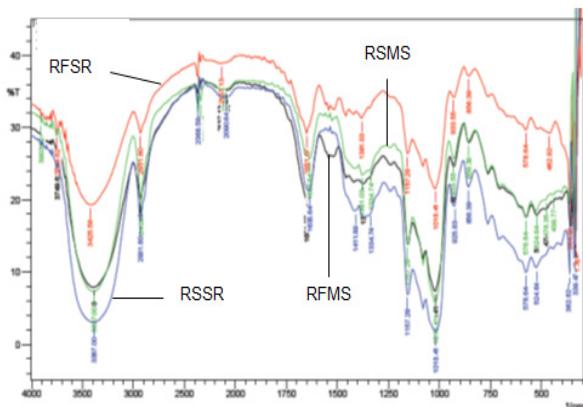


Figure 2. Spectra FT-IR of RFSR, RFMS, RSSR and RSMS

treated with NaOH solutions could be changed due to the dissociation of intermolecular hydrogen bonding in the amylopectin.

The RFSR, RFMS, RSSR and RSMS had the lipid content less than 1%. Furthermore, the protein content of RFSR and RFMS were higher than 6% whereas the protein content of RSSR and RSMS were lower than 1%. The 0.2% NaOH could produce starches with lower residual protein contents. These results were in accordance with the earlier reports (Lumdubwong and Seib, 2000; Cardoso *et al.*, 2007; Zhong *et al.*, 2009).

The total starch content of RFSR (94.32%) was higher than the total starch content of RFMS (86.97%) and differed significantly. The differences between the total starch content in both varieties could be affected by extraction process. Previously, the purity of rice starch reflected by the higher of total starch content and the lower of protein and lipid content (Lumdubwong and Seib, 2000). The higher of total starch content of RFSR was followed by the lower of protein and lipid content of this starch compare to RFMS.

The amylose content of RFSR was 23.69% whereas the amylose content of RFMS was 15.22%. The amylose content of RFSR was higher than RFMS and differed significantly. Previously, the rice has been classified based on their amylose content i.e. waxy rice (amylose content 1%), low-amylose rice (amylose content 16%), intermediate-amylose rice (amylose content 23%), and high-amylose rice (amylose content 55%) (Zhu *et al.*, 2011). Based on amylose content from the classification, SR variety in this research classified to intermediate-amylose rice (20-25%) whereas MS variety classified to low-amylose content (lower than 20%).

#### FT-IR analysis of rice flour and rice starch

The FT-IR spectra for RFSR, RFMS, RSSR and

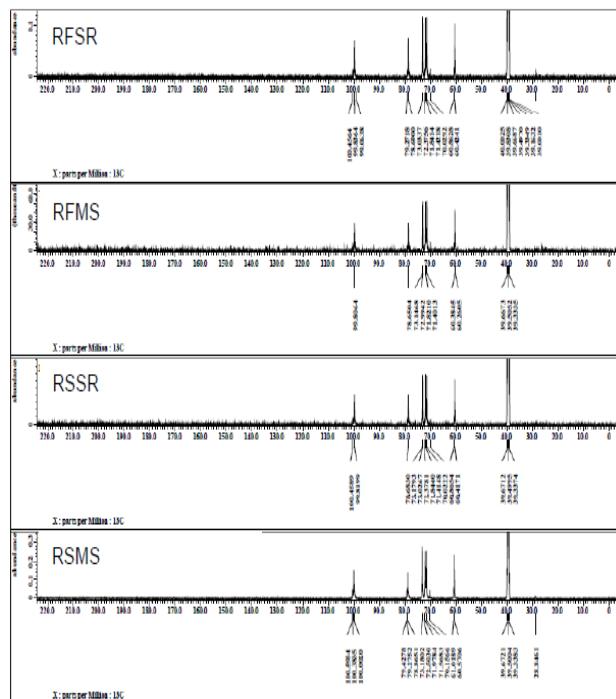


Figure 3. Spectra  $^{13}\text{C}$  NMR of RFSR, RFMS, RSSR and RSMS

RSMS are showed in Figure 2. It can be seen in Figure 2 that RFSR, RFMS, RSSR, and RSMS had the same patterns within a frequency band 400-4000  $\text{cm}^{-1}$ . All bands originated mainly from the vibrational modes of amylose and amylopectin. These results were in accordance with the earlier report (Flores-Morales *et al.*, 2012) who reported that the bands at 400 and 700  $\text{cm}^{-1}$  is associated to the skeletal modes of amylose and amylopectin. Infra-red spectra of RFSR, RFMS, RSSR and RSMS showed that similar functional groups were identified in rice flours (RFSR and RFMS) and rice starch (RSSR and RSMS). These functional groups include -C-H stretch and -OH group at 3749.62  $\text{cm}^{-1}$ , 3425.58  $\text{cm}^{-1}$  and 3387  $\text{cm}^{-1}$ ; -C=O group at 1157.29  $\text{cm}^{-1}$ ; -C-OH bending vibrations at 856.39  $\text{cm}^{-1}$  and 578.64  $\text{cm}^{-1}$  for RFSR and RFMS. In the other hand, the functional groups for RSSR and RSMS were -C-H stretch and -OH group at 3394.72-2924.09  $\text{cm}^{-1}$ ; amide and conjugated ketone at 1635.64  $\text{cm}^{-1}$ ; -C=O group at 1157.29  $\text{cm}^{-1}$ ; -C=O group, mostly aldehyde group at 1018.41  $\text{cm}^{-1}$ ; -C-OH bending vibrations at 856.39  $\text{cm}^{-1}$  and 578.64  $\text{cm}^{-1}$ . These results were in accordance with the earlier reports (Falade *et al.*, 2014; Falade and Christopher, 2015).

#### $^{13}\text{C}$ NMR analysis of rice flour and rice starch

The spectra  $^{13}\text{C}$  NMR for the RFSR, RFMS, RSSR and RSMS are showed in Figure 3. A carbon chemical shift for RFSR and RSSR has been identified in 99-100 ppm for C1, in 70-71 ppm and 78 ppm for

C2, C3, and C5, 78 ppm for C4, and in 61 ppm for C6. These results were similar with Flores-Morales *et al.* (2012) who reported that a carbon chemical shift for starch has been identified in 106-96 ppm for C1, in 70-73 ppm for C2, C3, C5, in 79-83 ppm for C4, and 59-62 ppm for C6.

It can be seen in Figure 3 that spectra from RSSR and RSMS had a peak in 100 ppm. The peak in 100 ppm showed that both rice starches had A-type X-ray diffraction patterns. This result confirmed with the result in Figure 1. Flores-Morales *et al.* (2012) reported that the peak in 100-102 ppm could be determined as the characterization of A-type X-ray diffraction patterns.

## Conclusion

The research indicated that the RFSR had the higher amylose compared to RFMS. RFSR and RFMS had the higher lipid content and protein content than RSSR and RSMS. RSSR had higher T<sub>0</sub> and T<sub>p</sub> may be due to higher amylose content than RSMS. RSSR and RSMS exhibited A-type X-ray diffraction patterns and supported by spectrum of <sup>13</sup>C NMR in 100 ppm. RFSR, RFMS, RSSR and RSMS had similar spectra patterns in FT-IR and <sup>13</sup>C NMR. The FT-IR analysis showed a characteristic band at 400 and 700 cm<sup>-1</sup> attributed to skeletal modes of amylose and amylopectin.

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