ABSTRACT

The diversification of food is the solution of rice imports elevation, rice consumption dependence, as well as lack of protein calories for baby in Indonesia. Analogue rice from local resources such as various starches, both from tubers and nuts. Analogue rice is produced from flour with the addition of water or other nutritional components. To improve consumer appeal, the natural dyes addition also can increase nutrition product. This research aimed to analyze the effect of pigment sources and formulation of tapioca and soybean flour. The result showed that protein increased from 4.54% to 5.30% and iron (Fe) increased from 2.58 to 14.54 ppm. The best analog rice was produced from T1B2 (85% tapioca : 15% soybean flour x red spinach) which had moisture content (4.96%), fat (3.23%), protein (5.70%), absorption capacity (175.89%), color intensity (L = 52.40; a+ = 1.50; b+ = 10.10), antioxidant activity (88.55%), total pigment (4.86 mg L−1), and iron (20.11%). The organoleptic score was taste (3.17 = quite like), texture (3.30 = quite like), shape (3.03 = quite like), and preference (3.23 = quite like).

Keywords: green spinach; iron; protein; red spinach

INTRODUCTION

Rice is the highest source of carbohydrates that mostly consume of Indonesian population which affect to the rice dependence. Indonesia is well known as an agricultural country, but still imports rice from other countries to meet the needs. Prevention to reduce the quantity of import and dependency can be solve by diversifying food consumption. Food diversification, namely analogue rice from various raw materials can be made from flour with the addition of water (Budijanto and Yuliyanti, 2012).

The raw material, which has the high potential, is tapioca flour, which made from cassava. Tapioca flour has high carbohydrate and calorie content, therefore it can be used as an alternative carbohydrate source. It also contains starch, namely amylose and amylopectin, which gelatinize easily in the analogue rice producing process. The previous research on the analogue rice produced using tapioca flour resulted starch granules, had moisture 10.41% – 13.08% and water absorption capacity 36.98% – 64.32%, but nutritional value wasn’t analyzed (Yudanti, et al., 2015). The substitute material used is soybean flour which aims to increase the protein content of analogue rice. It is commonly used as a major component in the producing of high protein foods (Manley, 2000). According to Cahyadi (2007) soybean flour had a high protein, about 34.8% that had functional properties included water and fat binding, emulsifying and thickness. The resulting analog rice is added with natural dyes to increase consumer appeal, iron and antioxidant content. The natural dyes used are spinach and red spinach extracts which contain very high iron and antioxidants. The purpose of this study was to determine the effect of pigment sources and the proportion of tapioca and soybean flour to the physico-chemical and organoleptic properties of analogue rice.
METHODS

The nested design with 2 factors with 3 replication was applied. The first factor was pigment sources with 3 levels (without pigment, green spinach, and red spinach). The second factor was the proportion of tapioca and soybean flour with 4 levels composed: 85%:15%; 80%:20%; 75%:25%; 70%:30%. Therefore it produced 12 combination of treatments. This research consisted of 3 main steps, it started with natural dyes extraction from spinach, followed by analogue rice production and analysis of parameters. The tapioca and soybean flour were analyzed the water content, protein, and fat properties (AOAC, 2005). The spinach (green and red) were analyzed the water content, antioxidant, pH, total dissolved solid (TDS), and pigment total. While the Analogue rice were identified the water content, fat, protein, color intensity ($L^*$, $a^*$, and $b^*$), absorption capacity, total pigment, antioxidant, iron, and organoleptic. This research conducted in the Food Science and Technology Laboratory (UMM), Chemistry Laboratory (UMM), and Food Quality and Safety Laboratory, Brawijaya University Malang.

Material

The raw material spinach obtained from the agricultural land of Faculty of Agriculture-Animal Science, University of Muhammadiyah Malang (UMM), which were harvested by farmers at the age of 25–30 days. The soybean flour was produced in Banguntapan, Kabupaten Bantul, di Yogyakarta. The additional material for analysis was aquades, KCl, Na-asetat, methanol, HCl 37%, DPPH (2,2–diphenyl–1-pycrilhidrazine), ethanol 96%, petroleum benzene, Na$_2$SO$_4$– HgO, H$_2$SO$_4$, NaOH, HCl 0,02 N.

Tool

The equipment that used was analytical scales (GR-200), desiccator, fume hood, kjedhal flask, oven, color reader (CR 10 Conica Minotta), pH meter (type 875), hand-refractometer, and spectrophotometer (Shimadzu UV-1800). The tools included satin fabrics, steamers, extruders, cabinet dryers, and juicers.

RESULT AND DISCUSSIONS

Raw Material Analysis

Table 1 showed that the analysis results were different from the literature. This is due to the variety, climate, soil fertility, and the maturity harvesting period. The water content in raw materials was higher than literature, it could be caused by the process and method analysis used, the ratio of water and materials, temperature, and storage process after harvesting (Pratiwi et al., 2008).

| Parameter       | Tapioca | Soybean |
|-----------------|---------|---------|
| Water content (%) | 11.23   | 10.92$^a$ | 6.23   | 4.87$^b$ |
| Protein (%)     | 0.26    | 0.19$^a$ | 36.71  | 34.39$^b$ |
| Fat (%)         | 0.1     | 0.02$^a$ | 22.49  | 25.53$^b$ |

Note: (a) Astawan (2009); (b) Widodo (2001)

The green and red spinach extract were analyzed the water content, antioxidant activity, pH value, total dissolved solids and total pigment (Table 2). The result showed that water content of green (88.73 %) and red (85.48 %) was lower than literature. The pH value of red spinach extract was lower (2.22) than green spinach (6.7), but it still in acid condition. This is because red spinach contains anthocyanin pigments, that more stable in acidic conditions
(Siskawardani et al., 2017). This is in accordance to Sa’ati, et al., (2016), in the extraction process anthocyanin was more stable under acidic conditions, therefore acid distilled water solvent was used. The total dissolved solids (TDS) in the green spinach extract were lower (2.2 °Brix) than red spinach (3.2 °Brix). The total dissolved solids indicates the amount of dissolved solids, which is related to the pigment content in a high solvent medium.

The value of antioxidant activity in red spinach was (75.52%) higher than green spinach (67.02%). Green spinach contains chlorophyll and red spinach contains anthocyanin, both were flavonoid compound that has ability as an antioxidant. According to Tina, et al., (2015), chlorophyll had potential as antioxidant, which could prevent excessive oxidation in the body. Chlorophyll was also rich in superoxide dismutase enzymes, with function as powerful antioxidants in neutralizing free radical activity. It was consistent with Raghvendra et al. (2011) anthocyanin is flavonoid compounds that had antioxidant properties.

| Table 2. Spinach extract composition |
|-------------------------------------|
| Parameter                        | Green Result | Literature  | Red Result | Literature |
| Water Content (%)                | 88.73        | 91.6        | 85.48      | 88.5       |
| Antioxidant Activity (%)         | 67.02        | 54.21       | 75.52      | 68.11      |
| pH                               | 6.7          | 5.5         | 3.53       | 2.22a      |
| TDS (°Brix)                      | 2.2          | 2.4         | 3.2        | 3.1b       |
| Total Pigment (mg L⁻¹)           | 7.41         | 6.06d       | 41.58      | 76.96c     |

Note: (a) Saati et al. (2014); (b) Saati (2012); (c) Adam (2015); (d) Rahmi (2017).

ANALOGUE RICE PROPERTIES

Water Content

The analysis of variance showed that pigment sources and the proportion of tapioca and soybean flour had very significant effect on the water content of analogue rice (Table 3). This due to the both green and red spinach have fiber content 2.7% and water content around 85%–90%, which can affect the water content value. The water content due to pigment sources also caused by TDS, the lower TDS resulted higher moisture content (Saati, 2011).

The addition of soybean flour in higher amount produced higher water content. It also has a very high protein content that will be denatured in analogue rice producing process especially in steaming and drying. Therefore, the denatured proteins will decrease the water absorb ability due to the formation of a strong matrix (Winarno, 2004).

Fat Content

The result of analysis of variance indicated that pigment sources and proportion of tapioca flour and soybean flour was significantly affect to fat content. The higher proportion of soybean flour added resulted to the higher fat content of analogue rice (Table 3). This is due to the fat content in soybean flour (22.49 %) was higher compared to tapioca flour (0.6%). The fat content of soybeans is higher than that of tapioca flour by 20.6% (Ministry of Health of the Republic of Indonesia, 2014). In addition, high levels of fat are also caused by the lack of fiber content, thus there is not complete absorption of fat by fiber (Verma and Banerjee 2010).

Protein Content

There was significantly effect of pigment sources and proportion of tapioca flour and soybean flour on the protein content. The results indicated that high proportion of soybean flour added in the producing of analog rice increased protein content (Table 3). This is due to soybean flour contains no less than 50% protein and its very good source of isoflavones.
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(Heinernen, 2003). It is also well known to be rich in nutrients that contain eight amino acids, which are essential and needed by the body. Red spinach also gave the higher increasing protein compared to the green spinach. Green spinach has a protein content 2.86 g per 100 g (Fordham and Hadley, 2003), while red spinach is 62.26 g kg$^{-1}$ (Sarker and Oba, 2019).

| Table 3. Analogue rice properties |
|----------------------------------|
| Treatment | Pigment Sources | Proportion (Tapioca: Soybean) | Water Content (%) | Fat (%) | Protein (%) | Absorption Capacity (%) | Fe (ppm) |
|----------|-----------------|-----------------------------|-----------------|--------|-------------|------------------------|---------|
| T1B0     | Control         | 85% : 15%                  | 2.58$^{ab}$     | 3.83$^{bc}$ | 4.94$^a$   | 171$^f$              | 17.53$^a$ |
| T2B0     |                 | 80% : 20%                  | 3.09$^c$        | 4.38$^{bc}$ | 6.82$^e$   | 160$^{ef}$           | 17.09$^a$ |
| T3B0     |                 | 75% : 25%                  | 2.56$^a$        | 2.69$^{ab}$ | 8.34$^g$   | 144$^{cd}$           | 21.19$^{bc}$ |
| T4B0     |                 | 70% : 30%                  | 3.38$^{cd}$     | 4.18$^{bc}$ | 9.66$^a$   | 124.1$^{ab}$         | 20.75$^{bc}$ |
| T1B1     | Green Spinach   | 85% : 15%                  | 3.73$^{de}$     | 2.23$^a$   | 5.28$^b$   | 165.1$^{ef}$         | 25.95$^d$ |
| T2B1     |                 | 80% : 20%                  | 4.25$^i$        | 2.89$^{ab}$ | 6.30$^d$   | 144.3$^{cd}$         | 26.71$^e$ |
| T3B1     |                 | 75% : 25%                  | 4.59$^g$        | 3.19$^{ab}$ | 8.52$^h$   | 137$^c$              | 33.16$^f$ |
| T4B1     |                 | 70% : 30%                  | 4.96$^{gh}$     | 5.87$^d$   | 9.47$^i$   | 1164$^a$             | 35.29$^a$ |
| T1B2     | Red Spinach     | 85% : 15%                  | 4.96$^{ph}$     | 3.23$^{ab}$ | 5.70$^c$   | 175.9$^f$            | 20.11$^b$ |
| T2B2     |                 | 80% : 20%                  | 4.61$^{gh}$     | 5.86$^d$   | 7.04$^f$   | 158.4$^e$            | 20.40$^{bc}$ |
| T3B2     |                 | 75% : 25%                  | 4.59$^{gh}$     | 4.59$^{cd}$ | 8.29$^g$   | 144.4$^{cd}$         | 24.42$^c$ |
| T4B2     |                 | 70% : 30%                  | 5.07$^h$        | 4.38$^{bc}$ | 10.24$^k$  | 118.9$^{ab}$         | 27.40$^a$ |

Note: The value followed the same letter in same column indicated not significant different.

Absorption Capacity

There was significant effect of pigment sources and the proportion of tapioca and soybean flour on the absorption capacity. In this case, higher tapioca flour added produced higher absorption capacity of analog rice (Table 3). Tapioca flour has a high amylose content of 30%, which able to increase absorption capacity. Water absorption is influenced by the high starch composition found in raw material. The nature of amylose, which is easily soluble in water will increase absorption capacity. In addition, water absorption is also influenced by the moisture content of analog rice. The lower water content, the higher absorption capacity of the analog rice. This was in accordance to Awuchi et al. (2019) that absorption capacity was inversely proportional to water content, the lower water content of the food, will absorb water highly.

Iron (Fe) Content

Pigment sources and the proportion of tapioca and soybean flour significantly affected iron content. The iron content in green spinach extract was higher than red spinach pigment extract. According to Bandini and Aziz (2001), that the iron content in green spinach is higher than red spinach. The high content of soybean flour that was added increased the iron content in analog rice. This is caused by soybean flour as raw materials have good mineral sources. Soybeans have a lot of good mineral content, such as Fe that is quite high, compared to other minerals. Ibrahim et al. (2008) stated that soybeans had good potential as a mineral source, and the most important mineral was Fe, it was about 9.04 – 13.32 mg per 100 g.

Antioxidant Activity

Separately, the pigment sources was significant affected the antioxidant (Table 4.). Sunarni (2007) confirmed that natural antioxidants mostly produced from the extraction of natural ingredients in plants. Natural antioxidants spread in several parts of the plant, such as wood, bark, roots, leaves, fruit, flowers, seeds and pollen. The value of antioxidant activity in analog rice was largely contributed by red spinach (85.21 %) which contained vitamin C and anthocyanin (Blando et al., 2018).
Table 4. The antioxidant activity and enhancement based on pigment sources

| Treatment               | Antioxidant Activity (%) | Enhancement (%) |
|-------------------------|--------------------------|-----------------|
| B0 (Control)            | 82.61<sup>a</sup>        | 1.94            |
| B1 (Green spinach)      | 84.55<sup>ab</sup>       | 2.60            |
| B2 (Red spinach)        | 85.21<sup>b</sup>        | 2.60            |

Note: The value followed the same letter in same column indicated not significant different.

**Total Pigment**

Based on the analysis of variance, it detected that pigment sources and the proportion of tapioca and soybean flour gave a very significant effect on the total pigment. The red spinach gave higher value of total pigment than green spinach (Table 5). The chlorophyll pigments from green spinach extract and anthocyanin pigments from red spinach extract were contributed, thus resulting value was significant difference. The fewer tapioca and higher soybean flour resulted lower total chlorophyll. The addition of soybean flour that has yellow color, thus affecting the pigment content especially chlorophyll. Siskawardani et al. (2017) identified that anthocyanin stability was influenced by environmental temperature (heating process), the increasing temperature caused the breakdown of anthocyanin molecule. The formation of chalcone is the first step of anthocyanin degradation. Heating formed carbinol from anthocyanin degradation and colorless derivatives, causing a decrease in the value of color retention.

Table 5. Total pigment of analogue rice

| Treatment   | Pigment Sources       | Proportion (Tapioca: Soybean) | Total Pigment (%) |
|-------------|-----------------------|-------------------------------|-------------------|
| T1B1        | Green                 | 85% : 15%                     | 5.55<sup>bc</sup> |
| T2B1        | Spinach               | 80% : 20%                     | 3.69<sup>ab</sup> |
| T3B1        | Spinach               | 75% : 25%                     | 2.90<sup>ab</sup> |
| T4B1        |                       | 70% : 30%                     | 1.91<sup>a</sup>  |
| T1B2        | Red                   | 85% : 15%                     | 22.76<sup>g</sup> |
| T2B2        | Spinach               | 80% : 20%                     | 17.30<sup>f</sup> |
| T3B2        | Spinach               | 75% : 25%                     | 12.44<sup>d</sup> |
| T4B2        |                       | 70% : 30%                     | 4.86<sup>bc</sup> |

Note: The value followed the same letter in same column indicated not significant different.

**Color Intensity**

The pigment sources and the proportion of tapioca and soybean flour was not significantly affect the lightness (L). However separately the pigment sources level significantly affect the lightness of analogue rice (Table 6). The values obtained was due to the proportion of adding tapioca and soybean flour only 5%, so resulting in the insignificant difference. The L value represents the reflected light that produces white achromatic, gray and black. It has range values from 0 to 100, the higher L value means the product has higher brightness level.

Table 6. Analogue rice lightness based on pigment sources

| Treatment   | Lightness |
|-------------|-----------|
| B0 (Control)| 57.2<sup>c</sup> |
| B1 (Green spinach) | 49.9<sup>a</sup> |
| B2 (Red spinach)       | 51.0<sup>ab</sup> |

Note: The value followed the same letter in same column indicated not significant different.

Table 7 showed that there was significant effect of pigment sources and the proportion of tapioca and soybean flour have on the redness and yellowish levels. The addition of green
spine was not negative (a-), this is due to the addition of soybean flour with yellowish color that impact the level of greenish (a-). The reddish value is contributed from red spinach pigment extract that is brown. This brownish due to the breakdown of anthocyanin molecule during long heating process. The yellowish level increased with the addition of soybean flour. This is due to the original color of the raw materials, which is bright and yellow.

Table 7. Analogue rice color properties

| Treatment | Pigment Sources | Proportion (Tapioca: Soybean) | Redness (a*) | Yellowness (b*) |
|-----------|-----------------|-------------------------------|--------------|-----------------|
| T1B0      | Control         | 85% : 15%                     | 3.5<sup>a</sup> | 13.7<sup>a</sup> |
| T2B0      | Control         | 80% : 20%                     | 3.5<sup>b</sup> | 14.3<sup>b</sup> |
| T3B0      | Control         | 75% : 25%                     | 3.5<sup>b</sup> | 14.3<sup>b</sup> |
| T4B0      | Control         | 70% : 30%                     | 3.8<sup>c</sup> | 14.9<sup>c</sup> |
| T1B1      | Green Spinach   | 85% : 15%                     | 0.3<sup>b</sup> | 10.7<sup>b</sup> |
| T2B1      | Green Spinach   | 80% : 20%                     | 0.1<sup>b</sup> | 11.3<sup>c</sup> |
| T3B1      | Green Spinach   | 75% : 25%                     | 0.6<sup>d</sup> | 11.0<sup>c</sup> |
| T4B1      | Green Spinach   | 70% : 30%                     | 0.3<sup>c</sup> | 12.6<sup>de</sup> |
| T1B2      | Red Spinach     | 85% : 15%                     | 1.5<sup>e</sup> | 10.1<sup>e</sup> |
| T2B2      | Red Spinach     | 80% : 20%                     | 1.5<sup>e</sup> | 10.8<sup>c</sup> |
| T3B2      | Red Spinach     | 75% : 25%                     | 1.5<sup>e</sup> | 12.3<sup>d</sup> |
| T4B2      | Red Spinach     | 70% : 30%                     | 2.4<sup>d</sup> | 12.5<sup>de</sup> |

Note: The value followed the same letter in the same column indicated not significant different.

**Organoleptic Test**

Organoleptic properties (taste, texture, shape and appearance) was significantly affected by pigment sources and the proportion of tapioca and soybean flour (Table 8). The panelists preferred the analogue rice taste without pigment addition. This is due to the pigment contributed to the slightly bitter taste in analogue rice. The texture and shape were affected by the addition of tapioca flour that able to increase the amylose content. Amylopectin addition resulted to softer, fluffier and tastier product (Suarni and Firmansyah, 2005). The preference was influenced by taste and texture score, it was subjective impression based on sensory sensitivity of panelist.

Table 8. Analogue rice organoleptic properties

| Treatment | Pigment Sources | Proportion (Tapioca: Soybean) | Taste | Texture | Shape | Preference |
|-----------|-----------------|-------------------------------|-------|---------|-------|------------|
| T1B0      | Control         | 85% : 15%                     | 2.53<sup>b</sup> | 2.80<sup>bc</sup> | 2.97<sup>de</sup> | 2.60<sup>b</sup> |
| T2B0      | Control         | 80% : 20%                     | 2.80<sup>cd</sup> | 3.13<sup>cd</sup> | 2.97<sup>de</sup> | 2.87<sup>cd</sup> |
| T3B0      | Control         | 75% : 25%                     | 2.60<sup>bc</sup> | 2.70<sup>b</sup> | 2.53<sup>bc</sup> | 2.67<sup>bc</sup> |
| T4B0      | Control         | 70% : 30%                     | 3.13<sup>de</sup> | 3.23<sup>d</sup> | 2.77<sup>de</sup> | 3.03<sup>de</sup> |
| T1B1      | Green Spinach   | 85% : 15%                     | 3.00<sup>de</sup> | 3.03<sup>cd</sup> | 3.41<sup>g</sup> | 2.73<sup>bc</sup> |
| T2B1      | Green Spinach   | 80% : 20%                     | 2.97<sup>de</sup> | 3.33<sup>d</sup> | 3.17<sup>fg</sup> | 2.87<sup>cd</sup> |
| T3B1      | Green Spinach   | 75% : 25%                     | 2.83<sup>cd</sup> | 2.87<sup>bc</sup> | 2.70<sup>cd</sup> | 2.93<sup>cd</sup> |
| T4B1      | Green Spinach   | 70% : 30%                     | 2.60<sup>bc</sup> | 2.77<sup>bc</sup> | 2.40<sup>bc</sup> | 2.70<sup>bc</sup> |
| T1B2      | Red Spinach     | 85% : 15%                     | 3.17<sup>e</sup> | 3.30<sup>d</sup> | 3.03<sup>ef</sup> | 3.23<sup>e</sup> |
| T2B2      | Red Spinach     | 80% : 20%                     | 2.53<sup>b</sup> | 2.80<sup>bc</sup> | 2.97<sup>de</sup> | 2.60<sup>b</sup> |
| T3B2      | Red Spinach     | 75% : 25%                     | 2.80<sup>cd</sup> | 3.13<sup>cd</sup> | 2.90<sup>de</sup> | 2.87<sup>cd</sup> |
| T4B2      | Red Spinach     | 70% : 30%                     | 2.60<sup>bc</sup> | 2.70<sup>b</sup> | 2.53<sup>bc</sup> | 2.67<sup>bc</sup> |

Note: The value followed the same letter in the same column indicated not significant different.
CONCLUSION

The pigment source and the proportion of flour has a very significant effect on water content, fat, protein, absorption capacity, iron, color intensity (a, b), total pigment, and organoleptic test of analogue rice. The best combination was T1B2 (85% tapioca: 15% soybean flour: red spinach) which has water content (4.96%), fat (3.23%), protein (5.70%), absorption capacity (175.89%), color intensity (L = 52.40; a+ = 1.50; b+ = 10.10), antioxidant activity (88.55%), total pigment (22.76 mg L$^{-1}$), and Fe (20.11 ppm). The organoleptic value was taste 3.17 (quite like), texture 3.30 (quite like), shape 3.03 (quite like), and preference 3.23 (quite like). The highest protein of analogue rice was T4B2 (70% tapioca: 30% soybean flour: red spinach) of 10.24%. The highest iron content was T4B1 (70% tapioca: 30% soybean flour: green spinach) was 35.29 ppm.

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