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Effect of sago and tapioca starches on the physicochemical and textural properties of expanded rice product coloured with red beetroot (*Beta vulgaris*) powder

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Abstract

Colour plays an important role in food especially in increasing the aesthetic value of food products. However, the increasing awareness on health implications of synthetic colourants has led to increased market for natural colourants. In this study, the retention of colourant from red beetroot powder in extruded rice flour containing 20% sago or tapioca starch was investigated. The moisture content of the rice flour-starch blends and 100% rice flour (control) was adjusted to 10% and the samples were extruded at 80ºC - 160ºC, with 120 rpm screw speed and 40 rpm feeder speed. The expansion, density, water absorption index (WAI), water solubility index (WSI), hardness, crispness, colour, and betanin content of the extrudates were measured. The results showed that rice flour-sago starch extrudates (RSE) and rice flour-tapioca starch extrudate (RTE) had better expansion compared to the control (100% rice flour) extrudate. The expansion of RTE was not significantly different from that of RSE and no significant difference was found in the densities of these two extrudates. Presence of sago or tapioca starch decreased the WAI and increased the WSI of the extrudates. The WAI of RSE, however, did not differ significantly from that of RTE. The hardness (18.37 kg) and crispness (126.55 kg.sec) of the control extrudate were higher than that of the RSE (16.97 kg, 110.07 kg.sec) and RTE (14.84 kg, 92.77 kg.sec). There was no significant difference between the redness values of the extrudates. However, retention of betanin in the extrudates was highest in RTE (36.06% retention), followed by RSE (34.14%) and lowest in the control extrudate (27.82%). Addition of tapioca starch or sago starch can help to improve the physical and textural properties of betanin coloured rice extrudates with tapioca starch giving higher betanin retention.

Introduction

Food colourant is an important ingredient often used in food production to make food appear appetizing and appealing to consumers. The various commercially available food colourants mostly consist of synthetic chemicals that can affect child behaviour, cause allergies, asthma, and carcinogenic effects (Amchova *et al*., 2015). This has led to a number of synthetic colourants being banned due to their toxicity. Recently, there is an increasing interest in the food industry to replace synthetic colourants with natural colourants (Rodriguez-Amaya, 2016). Natural colourants are pigments that are present in leaves, fruits, vegetables, flowers, insects, bacteria and fungi. These pigments include chlorophylls, carotenoids, anthocyanins and betalains (Delgado-Vargas *et al*., 2000). In addition to improving the aesthetic value of food, natural colourants are also known to have antioxidative benefits (Martins *et al*., 2016).

Red beetroot (*Beta vulgaris*) is the edible root from a beet plant that belongs to the Chenopodiaceae family. This vegetable contains a lot of beneficial nutrients including minerals and antioxidants (Janiszewska, 2014). Betalains, which are a group of water-soluble pigments of a bright red colour, can be extracted from red beetroot. Betalains from red beetroot can be divided into two groups: betacyanins (red pigments) and betaxanthins (yellow pigments). Betanin is the most common betacyanin present in red beetroot (Stinzing and Carle, 2004). The European Union as well as the United States Food and Drug Administration (FDA) have approved betanin from red beetroot (E162) as a natural food colourant (Martins *et al*., 2016). Aside from having

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a pleasing colour, betanin also has high antioxidative properties that can improve the nutritional quality of food (Moreno *et al*., 2008). However, in accordance with other natural colourants, betanin is sensitive towards temperature, light and oxygen (Herbach *et al*., 2006).

Extrusion cooking is a fast cooking method that involves high temperatures, pressure and shear stress that happens concurrently. Starch degradation during extrusion cooking is mainly due to mechanical energy and thermal energy instead of gelatinization, which involves thermal energy and moisture (Li *et al*., 2014). The combination of shear stress and heat during extrusion cooking changes the starch granules from a solid state to viscous melt as the starch degrades. The difference in pressure between the viscous melt (moisture vaporization) and the atmospheric pressure causes the bubbles inside the melt to grow upon exiting the extruder, and this produces expanded product (Moraru and Kokini, 2003).

The blending of tapioca starch with other starches has commonly been used in expanded products due to its high expansion (Fiorda *et al*., 2015; Akonor *et al*., 2016). Sun and Yoo (2015) found that a blend of tapioca and rice starches was able to improve the physical properties of rice starch based expanded products. Other than tapioca starch, sago starch has also been conventionally used to make expanded products (Cheow *et al*., 2004; Tongdang *et al*., 2008).

Although studies on extrusion cooking of rice flour with red beetroot powder, cactus pear pulp and amaranth seeds have been conducted by Durge *et al*. (2013), El-Samahy *et al*. (2007), and Ilo *et al*. (1999), respectively, pigment retention after extrusion cooking was not measured in these studies. In addition, very limited studies on the behaviour of sago starch during extrusion cooking have been conducted. Therefore, the objectives of this study were to determine the effect of sago and tapioca starches on the physical and textural properties of RSE and RTE coloured with red beetroot powder, and to determine betanin retention in the products.

Materials and methods

Materials

Beras Nasional rice was purchased from Padiberas Nasional Berhad, Selangor D.E., Malaysia. Sago and tapioca starches were procured from a bakery in Selangor D.E., Malaysia. Red beetroot was obtained from a fresh market in Selangor D.E., Malaysia.

Rice flour preparation

The rice was ground (Good and Well grinder, Malaysia) and the flour was sieved (200 µm mesh size) using a sieve shaker (Haver EML Digital Plus, Germany).

Production of beetroot powder

Beetroot powder was produced according to the method described by Muhammad *et al*. (2015). Red beetroots were washed, drained, peeled and juiced using a Santos juice extractor (Vaulx-en-Velin, France). The juice was collected, mixed with 30% maltodextrin and spray dried using a pilot scale spray drier (Niro, Germany) with an inlet temperature of 150ºC, outlet temperature of 75ºC and a 10 mL/min feed rate. The beetroot powder was collected and stored in aluminium laminated polyethylene (ALP) pouches at 4ºC until further usage.

Extrusion feed preparation

Three types of extrusion feeds were prepared by adding 8 g of red beetroot powder to 100 g of rice flour (control), rice flour-sago starch (80:20) blend and rice flour-tapioca starch (80:20) blends. The feed moisture was adjusted by adding distilled water until a moisture content of 10% was achieved and the feed was pre-conditioned overnight at 4ºC. The three types of feeds will be referred to as rice, rice-sago and rice-tapioca feeds from here on.

Total starch, amylose and amylopectin contents determination

Total starch of each feed was determined by the Total Starch Assay Procedure from Megazyme International Ireland Limited (2005). The feed was treated with thermostable α-amylase and amyloglucosidase. The solution was then transferred into a 100 mL volumetric flask and diluted with distilled water to mark before its absorbance was determined at 510 nm using a Perkin Elmer Lamda 35 UV-Vis spectrophotometer. Amylose was determined using an iodine-binding procedure using a FIAstar 5000 analyser unit (FOSS Co., Höganäs, Sweden) as explained by Abdul Manan (2015). Amylopectin content was determined by calculating the difference between total starch and amylose content measured.

Pasting viscosity measurement

Pasting viscosity of each feed mixture was determined using a rapid visco analyser (RVA, Newport Scietific Instrument and Engineering, Australia) to predict the transformation of the starch in the feed during the extrusion process. The moisture of each feed was adjusted to 14% by adding distilled water and for the first 2 min, the feed slurry was held at 50ºC, before being heated to 95ºC at the 8th min.

The temperature was held constant at 95ºC for 4 min and after that the feed paste was cooled to 50ºC for 8 min.

Extrusion process

The extrusion process was conducted using a single screw Brabender extruder (KE19 Duisburg, Germany). A screw compression ratio of 2:1 (Figure 1) and a 4 mm in diameter round die were used during the extrusion process. A feed rate of 40 rpm, a screw speed of 120 rpm and a cutter speed of 115 rpm were employed throughout the extrusion. The temperature of the first, second, third and fourth extruder barrels were held constant at 80ºC, 100ºC, 120ºC and 160ºC, respectively. The extrusion feed was placed in the feeder and the extrudates were collected and dried in an oven at 30ºC overnight.

Figure 1: Extrusion screw with a compression of 2:1 used in this study.

Expansion measurement

The expansion of each type of extrudate was calculated in accordance to Ding *et al*. (2006). The average diameter of 10 randomly selected extrudates was measured using a calliper. The expansion of the extrudate was calculated as the ratio between the average diameter of the extrudate and the diameter of the die opening.

Bulk density determination

The bulk density of the extrudates was determined using a solid displacement method as described by Joardder *et al*. (2015). Rapeseeds with diameters ranging from 1.5 to 2.0 mm were used as the displacement medium. The extrudates were placed in a 100 ml glass vial and the empty space was filled with rapeseeds until the 100 ml mark was reached with gentle tapping. The bulk density of the extrudates was calculated as the mass of extrudates per unit volume (g/cm3).

Water absorption index (WAI) and water solubility index (WSI) measurements

WAI and WSI of the extrudates were determined by using the AACC method 56 – 20 (AACC, 2000).

Texture analysis

Texture analysis of the extrudates was performed with a TA.XT Plus Texture Analyser (Stable Micro System, UK) and an Ottawa Cell. The force in compression mode was recorded in addition to the hardness and crispness values.

Colour analysis

The colour of the extrudates was measured using a CR-400 Chroma Meter (Konica Minolta, Inc., Tokyo, Japan). The hue angle (Hº) and chroma value (C) of the extrudates were calculated as described by Cai and Corke (1999).

Extraction and quantification of betanin

Betanin was extracted from each type of extrudate according to Camire *et al*. (2007). Ground extrudate (2 g) was placed in a centrifuge tube filled with 9 mL of distilled water and 3 mL of methanol. The mixture was then stirred (20 min), sonicated (30 min) and centrifuged at 3500 g^{-1} (10 min). The extract (supernatant) was filtered through a 0.45 µm nylon filter before analysis. Betanin was also extracted from the feed in a similar manner but with distilled water.

Betanin was subsequently quantified using a Perkin Elmer Lamda 35 UV-Vis spectrophotometer at a wavelength of 538 nm (Herbach *et al*., 2007). Betanin content was calculated using the following equation:

$$BC\left(^{mg}/_{kg}\right) = \frac{(A * DF * MW * 1000)}{\varepsilon * L}$$

BC = betanin content,
A = absorbance at 538 nm corrected by the absorbance at 600 nm,
DF = dilution factor,
MW = molecular weight of betanin (MW = 550 g/mol),
ε = molar extinction coefficient of betanin (ε = 60 000 L/mol.cm), and
L = path length of the cuvette (ml).

Betanin contents of each feed and the corresponding extrudate were quantified, and the retention of betanin in the extrudate was calculated.

Statistical analysis

The data obtained were analysed for statistical significance by analysis of variance (ANOVA). Significant difference was determined at $p<0.05$ using the Tukey's test. Pearson correlation was also performed with SPSS 16.0 (SPSS Inc., Chicago, Ill., U.S.A.).

Results and discussion

Total starch, amylose and amylopectin contents of extrusion feeds

Total starch, amylose and amylopectin contents of the rice, rice-sago and rice-tapioca extrusion feeds are shown in Table 1. The three types of feeds did not differ in their total starch contents and they ranged from 78.3 to 86.9 g/100 g of feed. The amylose content of the rice-sago feed was higher than that of the rice-tapioca feed which was followed by that of the rice feed. The amylopectin content, however, was the highest in the rice-tapioca feed followed by that in the rice-sago feed and rice feed.

Table 1: Total starch, amylose and amylopectin contents of rice, rice-sago (80:20) and rice-tapioca (80:20) feeds.

Parameters	Rice	Rice-sago (80:20)	Rice-tapioca (80:20)
Total starch (g/100g)	78.33 ± 0.66[a]	83.89 ± 4.03[a]	86.91 ± 3.73[a]
Amylose content (g/100g)	13.84 ± 0.07[a]	17.19 ± 0.61[c]	16.46 ± 0.20[b]
Amylopectin content (g/100g)	64.49 ± 0.07[a]	66.70 ± 0.25[b]	70.45 ± 0.20[c]

Values are means ± standard deviations of three replicates. Means with different letters in each row are significantly different ($p<0.05$).

Pasting viscosities of extrusion feeds

Figure 2 shows that all three feeds would start to gelatinize at around 86.2 – 88.4ºC. The hot paste viscosity of the rice feed was higher than that of the rice-sago feed which was followed by that of the rice-tapioca feed. Their hot paste viscosities, however, were much lower than their cold paste viscosities which followed a similar trend. It was observed that pasting viscosity of the feed was inversely related to its amylopectin content (Table 1).

Expansion of the extrudates

The addition of sago and tapioca starches significantly ($p<0.05$) increased the expansion of the extrudates compared to the control (100% rice) sample (Table 2). There was no significant difference between RSE and RTE expansion; however, RTE had a higher expansion value (2.15) than RSE (2.12). During extrusion, the starch in the feed forms a fluid melt and contains bubbles from the water vapour. The fluid melt then forms cell wall around the air bubbles and allows them to hold their shape. Upon exiting the extruder, the bubbles expand due to the pressure difference and cause expansion in the extrudates.

According to Ding *et al*. (2005), the expansion of the extrudates was influenced by the elasticity and the viscosity of the melt. The rice (control), rice-sago and rice-tapioca feeds differed in their amylopectin content (Table 1). The rice-tapioca feed contained the highest amylopectin content followed by the rice-sago feed and 100% rice feed. Amylopectin is highly branched and has a flexible structure resulting in a greater elasticity of the melt. Unlike amylose, amylopectin polymers do not hydrogen bond easily, thus forming low viscosity melt. This is supported by the lower pasting viscosities of rice-tapioca and rice-sago feeds than that of 100% rice feed as shown in Figure 2. Therefore, RTE and RSE had a higher expansion than that of the rice extrudate due to greater elasticity and lower viscosity of theie fluid melts that favour air bubble growth in both extrudates. A similar observation was made by Tongdang *et al*. (2008).

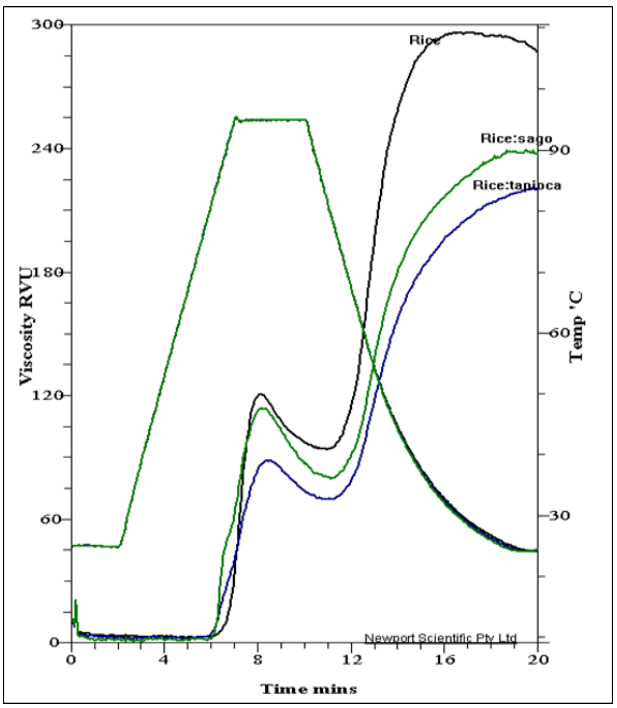

Figure 2: Pasting viscosities of rice, rice-sago (80:20), rice-tapioca (80:20) extrusion feeds measured using a Rapid Viscosity Analyzer (Newport Scientific, Australia).

Bulk density of the extrudates

The rice extrudate (control) had the highest density (0.30 g/cm^3), followed by RSE (0.24 g/cm^3) and RTE (0.21 g/cm^3). However, there were no significant differences in the densities of all the extrudates. Density of the extrudates would be negatively correlated with their expansion as highly expanded extrudates contain bigger air bubbles.

WAI and WSI of the extrudates

The WAI measures the volume occupied by the extrudate after swelling in excess water. This is an important parameter if the extrudate is to be consumed as breakfast cereal with milk. High WAI of starch usually is indicative of a high degree of starch damage due to starch gelatinization and fragmentation (Yağci and Göğüş, 2011). However, during low moisture extrusion cooking, starch melting dominates over starch gelatinization and results in low WAI value due to starch dextrinization (Ding *et al*., 2006). Table 2 shows that the WAI of RSE and RTE were lower than that of the control extrudate suggesting that higher levels of starch damage and dextrinization have occurred during the extrusion of RSE and RTE. The rice-sago and rice-tapioca feeds contained higher amylopectin (Table 1) than the control feed. Branched amylopectin as compared to linear amylose is more susceptible to damage by shear degradation, leading to lower WAI.

WSI measures the amount of soluble components released from the extrudates and is indicative of degradation of their molecular components after the extrusion process. WSI of the extrudates should thus be inversely proportional to their WAI. Table 2, however, shows only WSI of RSE to be higher than that of the control extrudate. Although the WSI of RTE was higher than that of the control extrudate, they did not differ significantly from WSI of RSE.

Table 2: Physical and textural properties of rice extrudate (control), rice-sago extrudate (RSE), and rice-tapioca extrudate (RTE).

Parameters	Rice Extrudate Control	RSE	RTE
Expansion	2.01 ± 0.07 ^{a}	2.12 ± 0.08 ^{b}	2.15 ± 0.10 ^{b}
Density (g/cm^3)	0.30 ± 0.12 ^{a}	0.24 ± 0.07 ^{a}	0.21 ± 0.06 ^{a}
WAI (g/g)	5.75 ± 0.12 ^{a}	4.46 ± 0.03 ^{b}	4.83 ± 0.10 ^{b}
WSI (%)	18.36 ± 0.31 ^{a}	20.23 ± 0.04 ^{b}	18.79 ± 0.16 ^{a}
Hardness (kg)	18.37 ± 0.91 ^{a}	16.97 ± 0.95 ^{ab}	14.84 ± 0.89 ^{b}
Crispness (kg.sec)	126.55 ± 3.76 ^{a}	110.07 ± 8.64 ^{b}	92.77 ± 5.20 ^{c}

Values are means ± standard deviations of three replicates. Means with different letters in each row are significantly different ($p<0.05$).

Key: WAI – Water absorption index, WSI – Water solubility index

Textural properties of the extrudates

RTE had the lowest hardness value (14.84 kg) and crispness value (92.77 kg.sec) compared to RSE and the control extrudate (Table 2). According to Paula and Conti-Silva (2014), the crispness of extruded snacks analysed using Kramer shear probe was negatively correlated to their sensory properties. Hence, extrudates with low crispness values will be more acceptable to consumers. As mentioned earlier, the melted rice-tapioca feed had the greatest elasticity and lowest viscosity due to the high amylopectin content of the feed. In addition to that, the rice-tapioca feed had the lowest pasting viscosity upon gelatinization (Figure 2). High elasticity and low viscosity of the melt favour the growth of air bubbles thus produce soft extrudates with thinner cell walls upon exiting the die opening due to the pressure difference between the air inside the melt and the atmosphere (Ding *et al*., 2005). There extrudates have lower hardness and crispness values as shown in Table 2.

Colour of the extrudates

Figure 3 shows the three types of expanded rice products coloured with red beetroot powder. Although there were no significant differences in the redness of the three types of extrudates, the yellowness (b*) and the hue angle (H^o) of the extrudates were significantly different from each other. The b* value and H^o of the control extrudate were higher than that of the other two extrudates (Table 3). This indicated degradation of betanin was higher in the control extrudate compared to that in RSE and RTE. As reported by Herbach *et al*. (2004), the amount of neobetanin (yellow pigment) increased more than double the initial amount after subjecting betanin to thermal treatment. The luminosity (L) or brightness of the control extrudate was also higher than that of the other two extrudates.

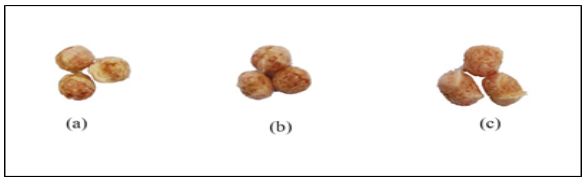

Figure 3: Expanded rice products coloured with red beetroot powder. (a) rice extrudate, (b) rice-sago extrudate, and (c) rice-tapioca extrudate.

Table 3: Colour properties, betanin content and betanin retention of rice extrudate (control), rice-sago extrudate (RSE), and rice-tapioca extrudate (RTE).

Parameters	Rice Extrudate	RSE	RTE
L*	62.21 ± 0.01 [a]	60.82 ± 0.38 [b]	60.79 ± 0.32 [b]
a*	14.46 ± 0.04 [a]	14.42 ± 0.07 [a]	14.49 ± 0.21 [a]
b*	15.38 ± 0.21 [a]	14.19 ± 0.10 [c]	14.60 ± 0.04 [b]
Hue angle (Hº)	46.70 ± 0.13 [a]	44.56 ± 0.12 [c]	45.26 ± 0.09 [b]
Chroma (C)	20.55 ± 0.05 [a]	20.23 ± 0.11 [a]	21.13 ± 0.30 [b]
Betanin content (mg/kg)	16.33 ± 0.23 [a]	18.12 ± 0.01 [b]	18.16 ± 0.05 [c]
Betanin retention (%)	27.82 ± 0.41 [a]	34.14 ± 0.09 [b]	36.06 ± 0.05 [c]

Values are means ± standard deviations of three replicates. Means with different letters in each row are significantly different ($p<0.05$).

Key: L*- Luminosity, a* - redness value, b* - yellowness value

Betanin content and retention in the extrudates

Betanin contents of the extrudates ranged from 16.33 mg/kg to 18.12 mg/kg and the retention was between 27.82% and 36.06%, with RTE having the highest value while the control extrudate having the lowest value. The betanin retention of the control extrudate was lower compared to the other two extrudates as feeds containing higher amylopectin would take shorter time to pass through the extruder than those with lower amylopectin levels. The shorter feed passage time would reduce its contact time at high temperature and, therefore, lower the degradation of betanin in the feed and its extrudates as was observed.

Table 4 shows that betanin retention was negatively correlated to the luminosity (L*), yellowness (b*) and hue angle (Hº) of the extrudates, r = -0.847, r = -0.842, and r = -0.846, respectively.

Table 4: Correlation between colour values and betanin retention of the extrudates.

Parameters	L*	a*	b*	Hue angle	Chroma	Betanin retention
L*	1	-0.248	0.787	0.844	-0.295	-0.847
a*		1	0.207	0.130	0.627	-0.102
b*			1	0.963	0.195	-0.842
Hue angle				1	0.148	-0.846
Chroma					1	0.357
Betanin retention						1

Correlation coefficients obtained close to 1.000 and -1.000 indicate good positive and negative correlations, respectively.

Key: L*- Luminosity, a* - redness value, b* - yellowness value

Conclusion

Substitution of 20% rice flour with sago starch or tapioca starch during the extrusion process significantly improved the physical and textural properties of the rice extrudates. The retention of betanin, however, was higher in the rice-tapioca extrudate than in the rice-sago extrudate.

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