

Characterization of modified cassava flour (mocaf)-based biscuits substituted with soybean flour at varying concentrations and particle sizes

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Abstract

Mocaf can be used as an alternative raw material for making complementary food because it has high carbohydrate content. However, the protein content is low so that it is necessary to substitute other ingredients which have high protein content such as soybean. The objective of this study was to characterize the mocaf-based biscuits containing soybean flour at concentrations of 10%, 15%, and 20%, and particle sizes of 420, 250, and 177 μm . Pasting properties of composite flours were evaluated in terms of peak viscosity, breakdown viscosity, setback viscosity, final viscosity, and pasting temperatures, while physicochemical properties of mocaf-based biscuit and microstructures were investigated in terms of moisture, ash, protein, fat, carbohydrate, calorie contents, color, hardness, and fracturability. Higher concentrations of soybean flour were associated with increased ash, protein, and fat content, as well as hardness. Moreover, the hardness of biscuits varied significantly depending on the particle sizes of soybean flour. Finally, the highest protein contents were achieved using 20% soybean flour with a particle size of 420 μm .

1. Introduction

In general, complementary foods on the market are biscuits and instant porridge. Biscuits are small baked products made principally from flour, sugar, and fat (Manley, 1998) and have a long shelf life due to their low moisture contents. Although wheat flour is most commonly used for making biscuits, it is not produced in Indonesia, and alternative ingredients such as cassava flour are used to make biscuits as a complementary food. Modified cassava flour (mocaf) is a fermentation product of cassava; among many agricultural commodities produced in Indonesia, cassava production reached 19,053,748 tons in 2017 (BPS, 2018). The disadvantage of mocaf is its low protein content, at 1.77% (Afifah and Ratnawati, 2017), to meet the minimum protein content requirement of 6% in complementary foods (BSN, 2005). Alternative ingredients with high protein content and production in Indonesia are needed, one of them is soybean. Soybean production in Indonesia is relatively high, with an annual production of 538,253 tons in 2017 (BPS, 2018). Moreover, the protein content of soybean flour is 40.94% (Ratnawati *et al.*, 2019).

Particle size is considered one of the most important physical properties of powders because it affects flowability (Abu-Hardan and Hill, 2010). Specifically, small particles have higher cohesiveness, reflecting

greater contact area and stronger intermolecular forces between particles (Landillon *et al.*, 2007). Furthermore, dough rheology is influenced by particle sizes and their distributions (Moreira *et al.*, 2014; Ahmed *et al.*, 2016), and differences in particle sizes can be exploited to give different characteristics to food products, especially bakery products.

Previous studies have been reported on complementary foods based on wheat flour substituted with Dumbo catfish flour and soybean protein isolates (Mervina *et al.*, 2012); soybean flour, arrowroot starch, and sweet potato flour (Zulfa and Rustanti, 2013); arrowroot starch, soybean flour, and sweet potato flour (Aini and Wirawani, 2013). In other studies, non-wheat flour-based complementary foods have been made from maize, soybeans and moringa leaves (Odinakachukwu *et al.*, 2014); maize, millet and moringa leaves (Arise *et al.*, 2014); millet, sorghum, pumpkin and amaranth seed flour (Simwaka *et al.*, 2017). The use of mocaf as raw material for making complementary food is still rarely done, so this study is required to determine the characteristics of mocaf-based biscuits where soybean flours with varying particle sizes were used as substitutes at varying concentrations.

2. Materials and methods

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2.1 Materials

Mocaf was obtained from UKM Harapan Jaya, Subang, West Java, Indonesia. Soybean (*Glycine max*) was purchased from a local market at Subang. Soybeans were washed and soaked in water at 60°C–70°C for 3 hrs, then dehulled and dried at 50°C for 12 hrs. Particle sizes of soybean flours were reduced using a disk mill and a sieve until particle sizes of 420, 250, and 177 µm was achieved. Other ingredients included banana (*Musa acuminata*), egg yolk, powdered sugar, baking powder, unsalted butter, and lecithin.

2.2 Preparation of composite flour

Composite flour was made by weighing mocaf and soybean flour according to the composition in Table 1. After that, composite flour was mixed using a dry mill and then stored in polypropylene (PP) plastic bags for further analysis.

2.3 Preparation of mocaf-based biscuits

Biscuits were made in the Pilot Plant Bakery of the Research Center for Appropriate Technology, Indonesian Institute of Sciences, Subang, West Java, Indonesia. Biscuit formulations are shown in Table 1.

Unsalted butter, powdered sugar, egg yolk, and lecithin were mixed together using a high-speed mixer until they expanded. Banana puree and baking powder were then added and stirred to homogeneity using a low-speed mixer. Subsequently, soybean flour and mocaf were added and mixed by hand until a smooth dough was produced. Biscuit dough was sheeted to a final thickness of 7 mm and baked for 10 mins in an oven at 150°C. Biscuits were then inverted and baked for 20–30 min at 100°C. After cooling, the biscuits were placed in polypropylene (PP) plastic bags and were stored at ambient temperature for further analysis.

2.4 Pasting properties of composite flours

Pasting properties of composite flours (mocaf and soybean flours) were analyzed using a Rapid Visco

Analyzer (RVA-TecMaster, Macquarie Park, Australia). Suspensions of 3.5 g (14% wb) of flour in 25 g of distilled water were stirred at 50°C (160 rpm) for 1 mins, then heated from 50°C to 95°C for over 7.5 mins and maintained at 95°C for 5 mins. Suspensions were then cooled from 95°C to 50°C for over 7.5 mins and incubated at 50°C for 2 mins. Parameters were measured on the Visco-amylogram: peak viscosity (PV), breakdown viscosity (BV), final viscosity (FV), setback viscosity (SV) and pasting temperature (PT).

2.5 Evaluation of mocaf-based biscuits

Physicochemical analysis of samples was performed to determine proximate, calorie contents, color and textural properties. Proximate analysis was performed according to the Indonesian National Standard (BSN, 1992) procedures and included determinations of moisture, ash, and crude fat contents using Soxhlet extraction. Protein of biscuit was analyzed using a DuMaster protein analyzer (DuMaster D-480, Buchi, Switzerland). Total carbohydrate content was calculated by subtracting percent moisture, ash, protein, and fat contents from 100% (100-(% moisture + % ash + % protein + % fat)). Calorie content was calculated using the Atwater conversion factors for proteins (4 kcal/g), carbohydrates (4 kcal/g), and lipids (9 kcal/g), as reported by Osborne and Voogt (1978).

Color of biscuit was measured using a Chromameter (NH310, China). All determinations were performed in three replicates. Color characteristics were recorded as L* values of 0–100 representing dark to light, a* values representing degrees of redness to greenness, and b* values representing degrees of yellowness to blueness.

Textural properties was analyzed in terms of hardness and fracturability using a TA.XTPlus texture analyzer (Stable Micro System, Surrey, UK). A three-point bending rig (type HDP/3PB) was used to cut samples after placement on base beams that were 4 cm apart. Compression strengths was measured using the following conditions: test mode, compression; test speed,

Table 1. Formulations of mocaf-based biscuit doughs

Ingredients (%)	Samples									
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
Mocaf flour	50	40	35	30	40	35	30	40	35	30
Soybean flour	-	10	15	20	10	15	20	10	15	20
Egg yolk	10	10	10	10	10	10	10	10	10	10
Powder sugar	14	14	14	14	14	14	14	14	14	14
Banana puree	14	14	14	14	14	14	14	14	14	14
Unsalted butter	10	10	10	10	10	10	10	10	10	10
Lecithin	1	1	1	1	1	1	1	1	1	1
Baking powder	1	1	1	1	1	1	1	1	1	1

Samples B2–B4 were made using flour with a particle size of 420 µm, samples B5–B7 were made using flour with a particle size of 250 µm, and samples B8–B10 were made using flour with a particle size of 177 µm.

3 mm/s; target mode distance; distance, 4 mm. Peak forces (gf) and mean distances at breakage (mm) were recorded.

Microstructures of biscuit was analyzed using a Scanning Electron Microscope (SEM, Hitachi SU3500). Prior to SEM analysis, samples were placed on SEM holders and coated with gold under vacuum conditions. Sample images were taken at 2500 \times magnification with an accelerating voltage of 10 kV (Blaszczak *et al.*, 2004).

2.6 Statistical analysis

Data in tables are presented as averages from triplicate analysis. Significant differences in multiple comparisons were identified using analysis of variance (ANOVA), followed by Duncan tests for significance at 5%.

3. Results and discussion

3.1 Pasting properties

Pasting properties of mocaf and composite flours (mocaf-soybean flours) were determined using RVA (Table 2). In this study, mocaf (B1) had the highest peak, breakdown, and final viscosity, but the pasting temperature for mocaf was lower than that of composite flours containing soybean flour.

The peak viscosity of B1 was significantly different ($p < 0.05$) from that of composite flour. The peak viscosity of composite flour tended to decrease with increasing soybean flour concentration. It is due to the peak viscosity of soybean flour (19.17 cP) lower than mocaf (4,755 cP), so that the composite is made the peak viscosity will decrease (Afifah and Ratnawati, 2017; Ratnawati *et al.*, 2019). The addition of soybean flour in composite flours led to increased protein and fat contents. Accordingly, the protein and fat can inhibit interactions between starch granules and limit the swelling of starch, leading to changes in viscosity (Du *et al.*, 2013; Hamid *et al.*, 2015). The results in this study

similar with a previous study were conducted by Julianti *et al.* (2017), that showed the addition of soybean flour in composite flour consist of sweet potato flour and maize starch can decrease the peak viscosity of these blends. Furthermore, the addition of soybean flour with fine particles (177 μm) caused the greater peak viscosity than the addition of soybean flour with coarse particles (420 μm). This result in line with the previous study was conducted by Ahmed *et al.* (2015), the peak viscosity increased in very fine particles of water chestnut flour (1,172 to 1,218 BU).

According to Adebowale *et al.* (2008), high breakdown viscosity is associated with increased susceptibility of flour to withstand heating and shear stress during cooking. The breakdown viscosity of B1 differed significantly ($p < 0.05$) from those of B2, B3, B4, B7, and B10. The increasing level of soybean flour in composite flour can be decreased the breakdown viscosity. This relates to the fiber content of composite flour. Ratnawati *et al.* (2019) showed that the dietary fiber of composite flour substituted by 40% soybean flour (18.53%) higher than the dietary fiber of mocaf (9.58%). The hydrophilic group in the fiber will form hydrogen bonds with water thereby reducing the amount of water that can be absorbed by the starch granules (Julianti *et al.*, 2017).

The final viscosity of B1 was significantly different ($p < 0.05$) from other samples, it tended to decrease with increasing soybean flour addition. Similarly, smaller particle sizes of flours were associated with decreased final viscosity. The final viscosity was decreased due to the fat contained in soybean flour which can inhibit the swelling of the starch granules (Dautant *et al.*, 2007). The setback viscosity of B1 was not significantly different ($p > 0.05$) with B4 and B6 samples, but significantly different ($p < 0.05$) with other composite flours. The setback viscosity also decreased with the addition of soybean flour. In the previous study was conducted by Asante *et al.* (2013) water chestnut flour,

Table 2. Pasting profiles of composite flours (mocaf-soybean flour)

Pasting properties	Samples									
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
PV, cP	5035.17 ^a	2739.67 ^b	2020.83 ^b	1537.50 ^b	2943.33 ^b	2289.50 ^b	1795.50 ^b	2964.50 ^b	2403.33 ^b	1743.50 ^b
BV, cP	1425.00 ^a	481.17 ^b	209.00 ^b	80.83 ^b	792.17 ^{ab}	713.33 ^{ab}	513.00 ^b	946.33 ^{ab}	858.33 ^{ab}	571.00 ^b
FV, cP	4352.00 ^a	2749.83 ^b	2348.33 ^b	2016.33 ^b	2682.50 ^b	2152.50 ^b	1802.17 ^b	2558.17 ^b	2054.33 ^b	1671.33 ^b
SV, cP	741.83 ^a	491.33 ^b	536.50 ^b	559.67 ^{ab}	531.33 ^b	576.33 ^{ab}	519.67 ^b	540.00 ^b	509.33 ^b	498.83 ^b
PT, °C	72.18 ^a	72.57 ^{ab}	72.82 ^{ab}	73.49 ^b	72.72 ^{ab}	72.71 ^{ab}	73.10 ^{ab}	72.59 ^{ab}	72.69 ^{ab}	73.14 ^{ab}

B1, 100% mocaf; B2, mocaf + 10% soybean flour (420 μm); B3, mocaf + 15% soybean flour (420 μm); B4, mocaf + 20% soybean flour (420 μm); B5, mocaf + 10% soybean flour (250 μm); B6, mocaf + 15% soybean flour (250 μm); B7, mocaf + 20% soybean flour (250 μm); B8, mocaf + 10% soybean flour (177 μm); B9, mocaf + 15% soybean flour (177 μm); B10, mocaf + 20% soybean flour (177 μm). Values with differing superscript letters between columns differ significantly ($p < 0.05$) from each other.

Table 3. Physicochemical properties of mocaf-based biscuits

Parameter	Samples									
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
Moisture, % wb	4.65 ^{abc}	4.22 ^a	6.46 ^d	5.96 ^{bcd}	5.93 ^{bcd}	6.16 ^{cd}	4.98 ^{abcd}	4.47 ^{ab}	4.76 ^{abc}	5.54 ^{abcd}
Ash, % wb	1.52 ^a	1.97 ^c	2.07 ^d	2.32 ^e	1.89 ^{bc}	2.10 ^d	2.29 ^e	1.86 ^b	2.14 ^d	2.31 ^e
Protein, % wb	4.07 ^a	9.64 ^c	11.90 ^e	14.27 ^f	9.16 ^b	11.62 ^d	14.17 ^e	9.29 ^b	11.77 ^{de}	14.18 ^f
Fat, % wb	17.54 ^a	19.88 ^b	22.15 ^{cd}	24.20 ^f	21.24 ^{bc}	21.80 ^{bc}	23.28 ^{def}	21.66 ^{cd}	22.47 ^{cde}	23.98 ^{ef}
Carbohydrate, % wb	72.22 ^e	64.29 ^d	57.42 ^b	53.25 ^a	61.79 ^c	58.31 ^b	55.27 ^a	62.71 ^{cd}	58.86 ^b	53.99 ^a
Calorie, kcal/100 g	463.02 ^a	474.66 ^b	476.63 ^b	487.88 ^c	474.94 ^b	475.96 ^b	487.34 ^c	482.98 ^{bc}	484.77 ^{bc}	488.54 ^c
Color										
L*	49.88 ^{bc}	50.04 ^{bc}	45.46 ^a	48.02 ^b	51.35 ^{cd}	50.15 ^{bc}	48.97 ^b	55.08 ^f	53.62 ^{ef}	52.43 ^{de}
a*	3.64 ^a	5.25 ^c	3.93 ^a	5.26 ^c	5.29 ^c	5.45 ^c	5.73 ^c	4.59 ^b	5.54 ^c	5.31 ^c
b*	14.56 ^a	17.14 ^c	16.40 ^b	18.80 ^d	17.59 ^c	18.65 ^d	18.63 ^d	17.72 ^c	18.44 ^d	18.85 ^d
Hardness, gf	1242.13 ^a	3921.38 ^b	4141.14 ^{bc}	4995.42 ^{de}	3964.00 ^b	4158.62 ^{bc}	5690.87 ^c	4174.69 ^{bc}	4834.20 ^{cd}	5526.91 ^{de}
Fracturability, mm	22.04 ^c	22.14 ^c	20.68 ^{ab}	20.78 ^{abc}	20.61 ^a	21.08 ^{abcd}	21.66 ^d	20.64 ^a	21.29 ^{bcd}	21.36 ^{cd}

B1, 100% mocaf; B2, mocaf +10% soybean flour (420 µm); B3, mocaf + 15% soybean flour (420 µm); B4, mocaf + 20% soybean flour (420 µm); B5, mocaf + 10% soybean flour (250 µm); B6, mocaf + 15% soybean flour (250 µm); B7, mocaf + 20% soybean flour (250 µm); B8, mocaf + 10% soybean flour (177 µm); B9, mocaf + 15% soybean flour (177 µm); B10, mocaf + 20% soybean flour (177 µm). Values with different superscript letters between columns are significantly ($p < 0.05$) different from each other.

lower setback viscosity was related to high cooking quality in the finest fraction and the cooked starch did not retrograde or become stiff upon cooling.

There were no significant differences in pasting temperature of mocaf and composite flour (Table 2), except mocaf and B4 sample. The composite flour had higher pasting temperature than mocaf. These observations are similar to those reported by Ocheme *et al.* (2018), who showed that higher pasting temperatures with increasing groundnut protein concentrate (GPC) reflect higher water absorption capacity of the blends with higher GPC contents.

3.2 Physicochemical properties

In evaluations of physicochemical properties of biscuits (Table 3), moisture contents of samples ranged from 4.22% to 6.46%. The Indonesian National Standard (BSN, 2005) tolerates a maximum of 5% moisture in baby biscuits, and those made from the flour blends B1, B2, B7, B8, and B9 met this standard, whereas the other biscuit had higher water content.

The ash, protein, fat, and total calorie contents of the biscuit samples containing soybean flour were higher than those of the control. The ash content ranged between 1.52-2.32%, and was within the Indonesian National Standard (BSN, 2005) those maximum content of ash i.e 3.5%. This standard also regulates that the minimum content of protein in complementary food is 6%. The biscuits in this study with soybean flour addition have a protein content that is in accordance with the standard. The highest protein content was found in the B4 biscuit (14.27%), and the lowest protein content

was B1 biscuit (4.07%). Therefore, control biscuits made from mocaf not fulfill the Indonesian National Standards. The fat content of the present biscuits ranged from 17.54% to 24.20%, reflecting significant contributions of soybean flour to the fat content of biscuits. Soybean flour was known to have high-fat content i.e 25.01% (Ratnawati *et al.*, 2019). In this study, the biscuits produced not fulfilling Indonesian National Standards (BSN, 2005), it is due to the fat content exceeded 18%. According to the Indonesian National Standard (BSN, 2005), calorie content of biscuits is required to contain at least 4 kcal/g or 400 kcal/100 g. In this study, the calorie content of all samples were ranged 463.02-488.54 kcal/100 g, fulfilled the minimum energy content requirements.

Color parameters of food products are important because they affect consumer acceptance. The results in this study showed that the lightness values (L^*) of biscuits decreased with soybean flour contents (Table 3) and ranged between 45.46 and 55.08. Higher L^* values indicate a brighter appearance of biscuits. The soybean flour substitutions increased the protein content of the present biscuits and were negatively correlated with lightness, indicating major roles of Maillard reactions in color formation (Chevallier *et al.*, 2000). Laguna *et al.* (2011) suggested that proteins are subject to Maillard reactions when baked, leading to the development of brownish colors and decreased lightness values. The present color values followed a similar trend to that reported by Mieszkowska and Marzec (2016), who showed that the addition of chickpea flour to short-dough biscuits decreases L^* values from 80 to 77.9.

Higher concentrations of soybean flour were

associated with decreased redness values (a^* ; Table 3), and these were inversely proportional to yellowness values (b^*) of biscuits, which increased with concentrations of soybean flour, reflecting the yellowish color of soybean flour. Mieszkowska and Marzec (2016) similarly showed that the addition of chickpea flour to short-dough biscuits increases b^* values from 23.9 to 28.6.

Textural properties are important qualities of biscuit products as they influence consumer acceptance. In this study, the result showed that the addition of soybean flour increased the hardness of mocaf-based biscuits at all concentrations. Biscuit fracturability also tended to decrease with increasing soybean flour content. Mocaf biscuits had hardness values of 216~358% of the control (100% mocaf), indicating harder textures. Arun *et al.* (2015) previously identified dough components that affect the hardness of biscuits and showed interactions

between protein, fat, carbohydrates, and starch contents. Similarly, Mieszkowska and Marzec (2016) indicated that the addition of 20% chickpea flour increases the hardness values of biscuits from 24.7 to 35.2 N.

3.3 Microstructure

Microstructure analysis of biscuits using Scanning Electron Microscope (SEM) was shown in Figure 1. The images in Figure 1 indicate that soybean flour affects biscuit microstructures. In the biscuit control (B1), starch granules of mocaf were spread evenly and were round and large. In a study by Alcazar-Alay *et al.* (2015), the addition of cassava starch led to rounder granules with truncated shapes and larger size distributions (3–32 μm). Herein, soybean flour of various particle sizes (B2-B10) formed aggregates between starch granules, and the ensuing matrixes of starch, fat, and protein granules led to harder textures of biscuits. These observations reflect

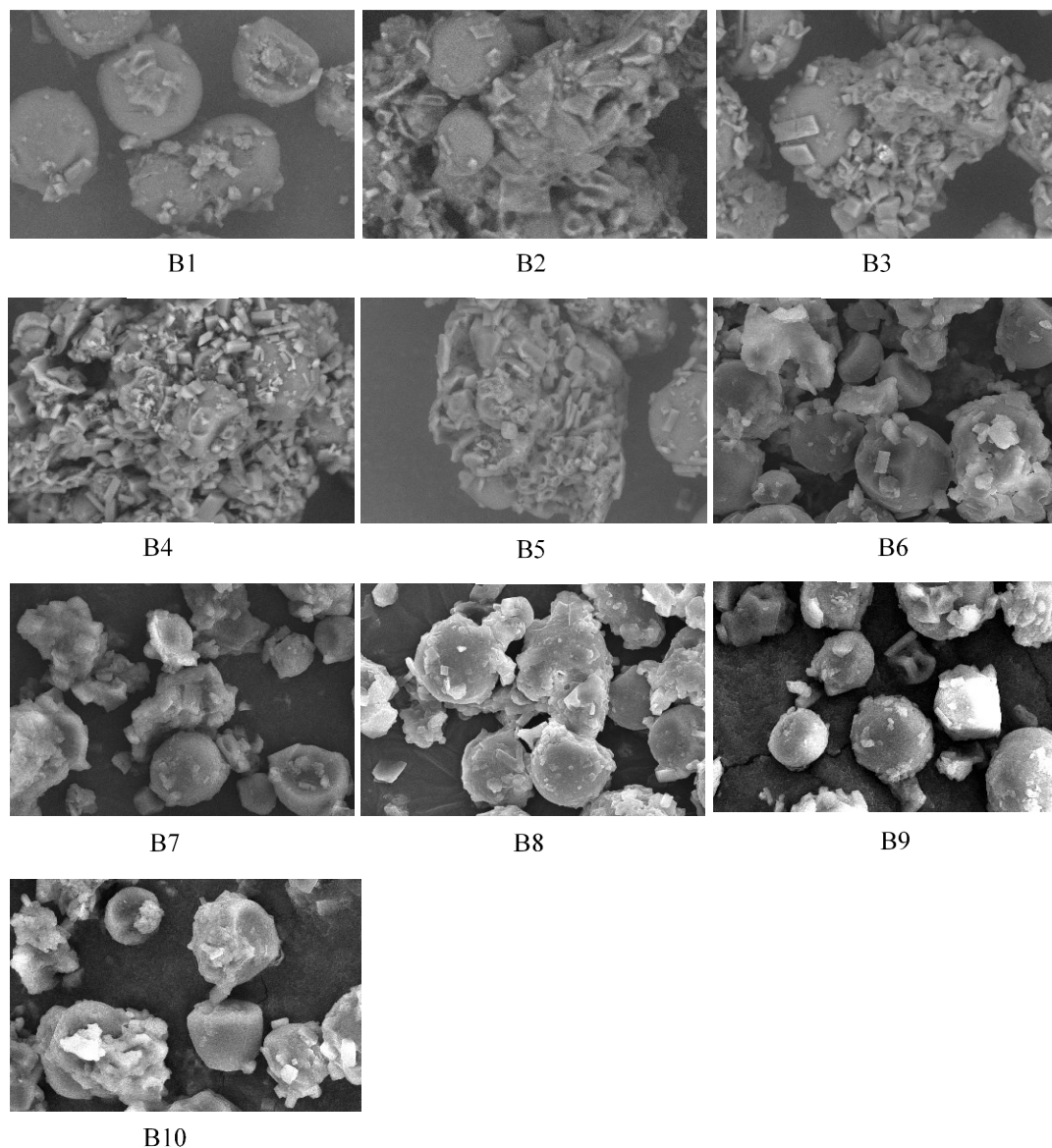


Figure 1. Microstructure images of mocaf-based biscuits. B1, 100% mocaf; B2, mocaf + 10% soybean flour (420 μm); B3, mocaf + 15% soybean flour (420 μm); B4, mocaf + 20% soybean flour (420 μm); B5, mocaf + 10% soybean flour (250 μm); B6, mocaf + 15% soybean flour (250 μm); B7, mocaf + 20% soybean flour (250 μm); B8, mocaf + 10% soybean flour (177 μm); B9, mocaf + 15% soybean flour (177 μm); B10, mocaf + 20% soybean flour (177 μm)

the inability of starch granules to absorb water after binding proteins and sugars, which cover the starch granules as shown in Figure 1. Chevallier *et al.* (2000) described biscuit structures as composite matrixes of sugar, protein aggregates, and lipids, in which intact or partially damaged starch granules are embedded. Starch granules in samples B2–B10 were also not intact, as can be seen in comparisons with the control (B1). Manley (1991) suggested that intact starch granules produce softer biscuits. Starch gelatinization also depends on the quantities and types of sugars in biscuit recipes (Filipev *et al.*, 2011).

4. Conclusion

The present analysis show that higher concentrations of soybean flour in flour composites are accompanied by increased ash, protein, and fat contents, and lead to increased hardness of the biscuits. Although particle sizes of soybean flour significantly affected the hardness, the highest protein contents were achieved with 20% soybean flour with a particle size of 420 µm.

Conflict of Interest

The authors declare no conflict of interest.

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