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Low fat and high protein meat analogue of cowpea (*Vigna unguiculata*) with stabilizer cocoa pod husk extract (*Theobroma cacao* L.)

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Abstract

Meat analogue is a plant-based protein product with nutritional and sensory qualities similar to conventional meat. Meat analogue is usually made from wheat flour. People with gluten intolerance can not consume wheat flour. Gluten-free meat analogue is appropriate for such people. In this research, gluten-free meat analogue was made from cowpea flour and cocoa pod husk extract, in three different formulations. Cocoa pod husk extract serves as stabilizer and sticking agent. The formulation was based on the ratio of the main ingredients. The ratios were 98.5:1.5 (A), 99.0:1.0 (B), and 99.5:0.5 (C). The control meat analogue was made from cowpeas flour and commercial pectin with the ratio of 98.5: 1.5. The parameter of quality was the physicochemical aspect including moisture content, ash content, fat content, protein content, carbohydrate content, fibre content, amino acid quality, and *in vitro* protein digestibility. In addition, the microbiological, texture, and sensory analysis were also conducted. Meat analogue B had 41.56% water content, 4.71% ash content, 3.88% fat content, 19.16% protein content, 8.75% soluble fibre content, 10.21% insoluble fibre content, protein digestibility 65.94%, total plate count of 4.40×10^2 CFU/g and yeast and mould count of 2.27×10^2 CFU/g. It is considered the best formulation among the meat analogues.

1. Introduction

Meat analogue is made from non-animal protein and mimics red meat's appearance, taste, and texture (Mistry *et al.,* 2020). The composition of the ingredient can alter the taste, fatty acids, and saturated/unsaturated fat ratio of meat analogues (Bhat and Bhat, 2011). Meat analogues do not have animal fat or cholesterol, but they have a lot of healthy unsaturated fatty acids (Hoek *et al*., 2004).

Generally, meat analogues are made from gluten or soy protein. Gluten protein is used as binding agent and it can stimulate an immune reaction for gluten-intolerant people. The binding agent can be substituted by pectin (Nanta *et al*., 2021). Therefore, local cowpea with high protein content can be developed for the making of meat analogues. In addition, cocoa pod husk extract can be developed as a binding agent to substitute the commercial pectin.

Cowpea (*Vigna unguiculata*) can be used to make meat analogues because it contains essential nutrients and amino acids. In comparison to other legumes, such as chickpea, green gram and lupine, cowpea has a low-fat content (31–304 g/kg) (Gonçalves *et al*., 2016). An extract from the cocoa pod husk needs to be added to the cowpea flour in the making of meat analogue. Cocoa pod husk extract contains pectin that can alter the functional properties of food, such as viscosity, emulsion, gel, and improving the structure of foodstuffs (Fajri *et al*., 2018).

This research was conducted to produce meat analogue from cowpea with the addition of cocoa pod husk extract. This study aims to determine the best formulation of cowpea flour and cocoa pod husk extract as ingredient of meat analogue. The chemical, physical, microbiological, and organoleptic quality of meat analogue will be analysed.

2. Materials and methods

2.1 Materials

The primary ingredients for making meat analogue were cowpea and cacao pod husk. The other ingredients are mineral water, salt, onion powder, garlic powder, pepper mix, canola oil, vegan broth (nutmeg powder, carrots, celery, tomatoes, miso paste Enachan, Wan Ja Shan Organic Gluten Free Worcestershire Sauce, balsamic vinegar), baking powder, and commercial pectin.

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The materials for chemical and microbiological analysis were distilled water, hexane, Whatman filter paper no. 41 (GE Healthcare Life Sciences), H_2SO_4, NaOH, N catalyst, HCl, PP indicator, MR-BCG indicator, acetone, ethanol, NaCl, disposable Petri dish (Biologix), alcohol, Plate Count Agar medium (Oxoid CM0325), Potato Dextrose Agar medium (Oxoid CM0139), phosphate buffer, pepsin enzyme (CAS No.9001- 75-6, Sigma Aldrich), and pancreatic enzymes (CAS No.8049-47-6, Sigma Aldrich).

The equipment for analysis are texture Analyzer (Brookfield CT3), oven (Memmert UN 450), grinder (FOMAC), hot plate Magnetic Stirrer (Thermo Scientific Cimarec II), Fume hood (Biobase FH1000), Laminar Air Flow (ESCO Class II), shaker incubator (JSSI-300C), incubator (Memmert IN 450), autoclave (Hirayama Hiclave HVE-50), pH meter (EutechEcoscan), glassware (Herma), soxhlet, furnace (Thermolyne), Kjeldahl nitrogen analyzer (Buchi K-355, K-415, K-425), and Waters Acquity UPLC H-Class (Waters).

2.2 Cowpea flour making

Fresh cowpea was sorted, washed, then soaked for 14 hrs. Then it was steamed for 10 mins. After that, cowpea was dried using an oven at 55°C for 24 hrs. The dried cowpea was ground using a grinder and sieved through a 60 mesh. It is the making of cowpea flour as suggested by Larasati (2018).

2.3 Cacao pod husk extract making

Cacao pod husk were cut into small pieces and dried in an oven at 55°C for 24 hrs. The dried cocoa pod husk was ground and sieved through a 60 mesh. The extraction was made through mixing cocoa pod husk powder and distilled water with the ratio 1:12. After that, the mixture was heated at 80°C for 30 mins on a hot plate magnetic stirrer. Then it was filtered. The supernatant was evaporated through the heating at temperature of 90°C for 20 mins using a magnetic stirrer hot plate (Edahwati *et al*., 2013).

2.4 Meat analogue production

Meat analogue is made from cowpea flour, then garlic powder, onion powder, pepper, salt, canola oil, and mix with the vegan broth (Table 1). Meat analogue then steamed for 20 mins (Perera, 2011). In this research, 4 sample treatments were made and each treatment was replicated 3 times (completely randomized design).

2.5 Chemical analysis

The chemical analysis was performed for the cowpea flour, cacao pod husk extract and meat analogue. Chemical tests were conducted through measuring the moisture content with the gravimetric method (AOAC 952.08, 2016), ash content with the gravimetric method (AOAC 930.30, 2016), fat content with Soxhlet method (AOAC 948.15, 2016), protein content with Kjeldahl method (AOAC 992.23, 2016), and carbohydrate content with carbohydrate by difference (Kale *et al*., 2018). Pectin content were measured with gravimetric method, equivalent weight, methoxyl content, and galacturonic acid content with titration method (Ranganna, 2005).

Table 1. Formula of low fat and high protein meat analogue of cowpea (*Vigna unguiculata*) with stabilizer cocoa pod husk extract (*Theobroma cacao* L.)

Ingredients	Formulation			
	Control	A	B	C
Cowpea flour	98.5 g	98.5 g	99.0 g	99.5 g
Cocoa pod husk extract	-	1.5 g	1.0 g	0.5 g
Commercial pectin	1.5 g	-	-	-

2.5.1 Dietary fibre

To determine insoluble fibre, approximately 50 mL H_2SO_4 1.25% was added to fat-free sample in the Erlenmeyer. Then, it was heated for 30 mins and 50 mL of 3.25% NaOH was added to the sample and heated again for 30 mins. After that, the sample was filtered using Whatman paper No. 41 and washed with hot 1.25% H_2SO_4, hot water, and 96% ethanol. The filter paper was dried at 105°C, stored and heated to a constant weight.

To determine soluble fibre, 200 mL of warm 95% ethanol was added to the filtrate and allow it to precipitate for 1 hr. After that, the supernatant was filtered using Whatman paper 41 added with 0.25 g of celite. The next step was to wash the precipitate with ethanol 78%, ethanol 95%, and acetone. Then, it was dried at 105°C and weighed after cooling in a desiccator, incinerated at 550°C and weighed again until constant after cooling in a desiccator (Asp *et al*., 1983)

2.5.2 In vitro protein digestibility

Approximately 2 g of the meat analogue were incubated in 15 mL of 0.1 M HCl containing 1.5 mg pepsin and shaken for 1 hr at 30°C. Then, it was neutralized with 0.5 M NaOH and 4.0 mg pancreatin in 7.5 mL of phosphate buffer (0.2 M, pH 8.0). The mixture was shaken for 24 hrs at 30°C. The mixture was then filtered, and the residue was washed with distilled water and then air-dried. The dried residue was used to determine the protein determination with Kjeldahl procedure (Fasuan *et al*., 2018).

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2.5.3 Amino acid quality

Amino acid quality was analysed using chemical score methods. The essential amino acid content in the protein is determined using HPLC with C18 column at 49°C, gradient pump system, PDA detector, Accq. Tag Ultra, and distilled water as eluent (Waters, 2012). The amino acid content will be compared with the reference parameter-amino acid content in egg-from FAO. The lowest amino acid score will be divided by the reference parameter then multiplied by 100% to obtain the chemical score. The amino acid score with the lowest percentage is called the limiting amino acid (FAO, 2013).

2.6 Texture analysis

The texture analysis was employed to determine the hardness, gumminess, and chewiness of meat analogue using the Texture analyzer ProLite. The pretest speed was set at 5 mm/s, test speed at 0.5 mm/s, post-test speed at 5 mm/s, distance at 30%, trigger type force at 5 g (Hesyandi, 2018).

2.7 Microbiology analysis

2.7.1 Total plate count

Approximately 1 g of meat analogue of was diluted into 9 mL of sterile distilled water (10^{-1} dilution). Then, 1 mL of the 10^{-1} dilution was transferred to another dish (10^{-2} dilution) and the procedure was repeated for 10^{-5} dilutions. 1 mL of each dilution was put into the Petri dish and 15 mL of the plate count agar (PCA) was poured into each Petri dish. After that, the inoculum and the medium were carefully mixed by rotating the Petri dishes. After the medium hardens, it was placed in the incubator at 37°C for 48 hrs. The colonies formed were observed and counted to get the total plate count number (ISO, 2003 with modification).

2.7.2 Yeast and mould count

Approximately 1 g of meat analogue was diluted into 9 mL of sterile distilled water (10^{-1} dilution). Then, 1 mL of the 10^{-1} dilution was transferred to another dish (10^{-2} dilution) and the procedure was repeated for 10^{-5} dilutions. The next step was to put 0.1 mL of each dilution on pre-poured solidified potato dextrose agar (PDA) plates and to incubate 48 hrs at 37°C (FDA, 2017)

2.8 Sensory analysis

The sensory properties are evaluated using hedonic test of thirty untrained panellists-students of Universitas Atma Jaya Yogyakarta. Evaluation of the sensory properties includes colour, texture, taste, aroma, and overall value. The hedonic scaling ranges from 1 (lowest) to 4 (highest) (Tjokrokusumo *et al*., 2019).

2.9 Data analysis

The data were analysed using SPSS version 15. The analysis includes ANOVA and Duncan's Multiple Range Test (DMRT) with a 95% confidence level.

3. Results and discussion

3.1 Chemical properties of cowpea flour and cacao pod husk extract

The proximate content of cowpea flour is lower (Table 2) than the findings in the literature (Alayande *et al*., 2012) and it is likely attributed to the soil of cowpea. Khalid and Elhardallou (2016) used white cowpeas from Sudan and Sreerama *et al*. (2012) used cowpeas from India. This research used brown cowpeas from Yogyakarta. Cowpea flour had a lower ash content, protein, and carbohydrate compared to jack bean flour (Olalekan and Bosede, 2010). In addition, cowpea also had lower protein (24.98%), and fat (1.10%) compared to protein (36.56%) and fat (22.02%) of soybean (Gunathilake *et al*., 2016).

Cocoa pod husk extraction aims to obtain pectin. The function of pectin is to form the texture of the meat analogue (Fajri *et al*., 2018). In this study, the cacao pod husk extraction in pH 7 resulted in a high equivalent weight of extract (Table 3), while the study by Susilowati *et al*. (2017) resulted in pH 3.

Methoxyl content of cocoa pod husk extract is low

Table 2. Chemical properties of cowpea flour (%).

Components	Content	Mung Bean Flour (BSN, 1995)	Cowpea flour (Khalid and Elhardallou, 2016)	Cowpea flour (Sreerama *et al.,* 2012)
Water content	9.24±0.37	Max 10.00	-	-
Ash content	3.06±0.05	-	3.87	-
Fat content	2.17±0.11	-	2.30	-
Protein content	25.27±0.31	Min 23.00	26.73	-
Carbohydrate content	60.26±0.71	-	59.78	-
Insoluble fibre content	10.37±0.37	-	-	13.10
Soluble fibre content	3.90±0.20	-	-	1.00
Total dietary fibre content	14.27±0.27	-	-	14.10

Values are presented as mean±standard deviation (*n* = 3).

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Table 3. Chemical properties of cocoa pod husk extract.

Components	Content	IPPA (2003)	Susilowati *et al.* (2017)	Chan and Choo (2013)
Pectin content (%)	4.22±0.10	-	-	3.3 – 3.9%
Equivalent weight	9032±234	600-800	6250	
Methoxyl content (%)	6.72±0.20	High methoxyl (> 7.12) Low methoxyl (2.5-7.12)	-	-
Galacturonic acid content (%)	43.23±0.55	Min. 35%	-	-

Values are presented as mean±standard deviation (*n* = 3).

because the value is below 7.12. Pectin with low methoxyl content does not require a high level of sugar to form gels, but it needs divalent cations, such as calcium (Baker *et al*., 2005). The high galacturonic content in the extract indicates the higher quality of pectin and its ability to form a gel (Antika and Kurniawati, 2017).

3.2 Chemical properties of meat analogue

3.2.1 Moisture content

The water content in a food ingredient significantly affects the quality and shelf life of the food ingredient (Leviana and Paramita, 2017). The moisture content of meat analogue ranges from 38.85-42.84% (Table 4). There is a significant difference in moisture content between the control from treatment meat analogues. As comparison, the meat analogue made from peanuts has moisture content of 40.74% (Rehrah *et al*., 2009). Therefore, the moisture content of the treatment meat analogue is within the reference range. The moisture content in meat analogues tends to increase along with the addition of cocoa pod husk extract because the extract contains high amount of water (Herawati, 2018).

3.2.2 Ash content

The ash content of treatment meat analogue ranges from 4.55-5.04% (Table 4). It is lower value than the results of the ash content of meat analogue made from peanut flour (5.74%) (Rehrah *et al.,* 2009). Increasing the concentration of cowpea flour will increase the ash content. The mineral contents in 100 g cowpea flour are 110 mg calcium, 8.30 mg iron, 184 mg magnesium, 424 mg phosphorus, 1112 mg potassium, 16.20 mg sodium, and 1.53 mg manganese (Dlamini, 2016). Meat analogue control made from commercial pectin has higher ash content than cocoa pod husk extract. The ash content of commercial pectin is 7.00% (Roikah *et al*., 2016) and the ash content of cocoa pod husk extract is 3.60% (Maulidiyah *et al*., 2014). The meat analogue made from formulation A has the highest mineral. The formulation B has the second highest mineral content. The difference between formulation A and B is not significant. The higher the ash content, the higher the mineral content the food has. Minerals are required for metabolic and physiologic functions in the human body (Williams, 2005).

3.2.3 Fat content

The fat content of meat analogue ranges from 3.56 – 4.36% (Table 4). It is lower than the meat analogue made of wheat gluten, soya chunk, and button mushrooms (5.15%) (Ahirwar *et al*., 2015). The result of the fat content analysis shows that there is a significant difference between the control from the one made from cowpea flour and cocoa pod husk extract.

The higher the pectin content added, the higher the fat content. Meat analogue with formulation C has the lowest fat content and the one with formulation B has the second lowest. The difference of the fat content between formulation A from B is not significant. The lower the fat content, the better the product. A low-fat diet can

Table 4. Chemical properties of low fat and high protein meat analogue of cowpea (*Vigna unguiculata*) with stabilizer cocoa pod husk extract (*Theobroma cacao* L.)

Ratio of Cowpea flour: Cocoa husk extract	Control	98.5: 1.5 (A)	99.0: 1.0 (B)	99.5: 0.5 (C)
Moisture Content (%)	38.85±1.29^{a}	42.84±0.62^{c}	41.56±0.98^{bc}	40.35±0.96^{ab}
Ash Content (%)	5.04±0.15^{b}	5.00±0.14^{b}	4.85±0.25^{ab}	4.55±0.28^{a}
Fat Content (%)	4.05±0.46^{ab}	4.36±0.29^{b}	3.89±0.43 ^{ab}	3.56±0.29^{a}
Protein Content (%)	17.88±0.06^{c}	18.37±0.24^{bc}	19.16±0.48^{ab}	19.98±0.76^{a}
Carbohydrate Content (%)	34.18±1.77^{a}	29.89±0.40^{b}	30.54±1.62^{b}	31.12±1.23^{b}
Soluble Fibre (%)	11.35±1.11^{c}	10.821.44^{bc}	8.75±1.32^{b}	6.1±1.23^{a}
Insoluble Fibre (%)	9.82±2.73^{a}	9.83±1.43^{a}	10.21±3.30^{a}	10.79 ±0.36^{a}
Total Dietary Fibre (%)	21.18±1.76^{a}	20.67±2.83^{a}	18.97±2.67^{a}	16.90±1.44^{a}

Values are presented as mean±standard deviation (*n*= 3). Values with different superscript within the same row are statistically significantly different at 95% confidence level.

prevent the complications of diabetes, heart disease, and cancer (Putri, 2007).

3.2.4 Protein content

The protein content of the control and treatment meat analogue ranges from 17.88-19.98% (Table 4). As the comparison, meat analogue made from combination of wheat flour, maize flour, textured soy grit, and mushroom paste has the protein content of 15.79% (Yadav *et al*., 2015). Meat analogue with formulation C has the highest protein content. The second best is the meat analogue with formulation B. However, the difference between formulation C from B is not significant. The higher the protein content is high, the better the quality of the product (Lisa *et al*., 2015). The higher the concentration of cowpea flour, the higher the protein content of the analogue meat. The meat analogue made of cowpea flour has the protein content of 25.27% (Table 4). Cowpea is a potential source of vegetable protein. The higher concentration of cowpea flour addition, the higher the protein content (Lestari *et al*., 2019).

3.2.5 Carbohydrate content

The carbohydrate content of meat analogue ranges from 29.89-34.18% (Table 4). It is higher than the meat analogue made from tempeh and wheat gluten (28.32%) (Stanin,2019). The result of carbohydrate content analysis shows a significant difference between the control meat analogue and the treatment meat analogue.

The carbohydrate content of the control meat analogue is higher than the treatment one. It is likely attributed to the lower water content and protein content of the control meat analogue. The content of other compounds such as water, ash, fat, and protein content can affect the carbohydrate content. Low levels of such compounds will produce high levels of carbohydrates (Sugito and Hayati, 2006).

Meat analogue with formulation A has the lowest carbohydrate content. The one with formulation B is the second lowest. The difference of carbohydrate content between formulation A from B is not significant. High consumption of carbohydrates can increase the risk of heart disease in overweight and obesity (Flight and Clifton, 2006).

3.2.6 Total dietary fibre content

The total dietary fibre was obtained through addition of soluble and insoluble dietary fibre (Agustin *et al*., 2020). The total content of dietary fibre ranges from 16.90-21.18% (Table 4) and it doesn't have significant difference. The result is higher than the dietary fibre content of meat analogue made from red beans and soybeans (14.94%) (Mentari *et al*.,2016).

The higher the concentration of cocoa pod husk extract, the higher the soluble fibre content. The increase in soluble fibre content is due to the pectin content (Fajri *et al*., 2018). Cocoa pod husk soluble fibre content is 16.27% (Edahwati *et al*., 2013) and it is higher than the soluble fibre content of cowpea flour 3.90% (Table 2). Soluble fibre has a potential as a prebiotic (Slavin, 2013).

Insoluble fibre content increased with the addition of cowpea flour concentration. The cowpea has insoluble fibre content (da Silva *et al*., 2018). The insoluble fibre content of cowpea flour is higher than of cocoa pod husk. Cowpea flour contains 10.37% insoluble fibre (Table 2), while cocoa pod husk extract contains 2.60% insoluble fibre (Ramos *et al*., 2008). The insoluble fibre in cocoa pod husk includes 26.10% cellulose, 21.20% lignin, and 12.70% hemicellulose (Alemawor *et al*., 2009).

Meat analogue with formulation A has the highest total dietary fibre content. The one with formulation B is the second highest. The difference of total dietary fibre content between formulation A from B is not significant. The higher the fibre content, the healthier the food (Kusharto, 2006). The increase of dietary fibre consumption can reduce the risk of diseases, such as cardiovascular disease, type 2 diabetes, and cancer (Dahl and Stewart, 2015).

3.2.7 In vitro protein digestibility

In vitro protein digestibility of meat analogue ranges from 62.66-71.86% (Table 5). It is lower than meat analogue made from lupine protein and spirulina (73.70%) (Palanisamy *et al*., 2019). The higher the concentration of cowpea flour, the lower the *in vitro* protein digestibility. It is likely attributed to the phytic acid in cowpea flour. The content of phytic acid ranges from 2.60 to 15.20 g/kg. The interaction between protein and phytic acid may affect protein structure and further it

Table 5. *In vitro* protein digestibility content of low fat and high protein meat analogue of cowpea (*Vigna unguiculata*) with stabilizer cocoa pod husk extract (*Theobroma cacao* L.)

Ratio of Cowpea flour: Cocoa husk extract	*In vitro* Protein Digestibility Content (%)
Control	71.86±1.29^{a}
98.5: 1.5 (A)	70.60±0.62^{c}
99.0: 1.0 (B)	65.94±0.98^{bc}
99.5: 0.5 (C)	62.66±0.96^{ab}

Values are presented as mean±standard deviation (n = 3). Values with different superscript within the same column are statistically significantly different at 95% confidence level.

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reduces protein digestibility (Nissar *et al*., 2017). The higher the concentration of cowpea flour, the higher the phytic acid content and the lower the digestibility.

The more the protein hydrolysed by digestive enzymes, the higher the digestibility (Haris and Nafsiah, 2019). Meat analogue with formulation A as the highest *in vitro* protein digestibility. The one with formulation B is the second highest. The difference of *in vitro* protein digestibility between A from B is significant. The higher the protein digestibility, the better the quality of the food (Gibney *et al*., 2013).

3.2.8 Amino acid quality

Amino acid quality was analysed using chemical score method. It is a method in which the essential amino acid content in food ingredients is compared to reference parameter (FAO, 2013). The chemical score of meat analogue ranges from 8.80-9.68 (Table 6) and the main limiting amino acid is in threonine. The increase of concentration of cowpea flour increases the chemical score because cowpea flour has more threonine.

The lack of threonine in meat analogues can be compensated with the ingredient containing high threonine. Analogue meatballs made from potato, wheat flour, and soy protein concentrate have chemical score of 34.40; the first and second main limiting amino acids are methionine-cysteine and threonine respectively (Youssef, 1988). Meat analogue with formulation C has the highest chemical score. The one with formulation B is the second highest. The difference of chemical score between formulation C from B is not significant. The higher the content of and the more in variety of essential amino acids, the better the protein quality (Gibney *et al*., 2013).

3.3 Texture analysis

The texture is one of the key factors of the assessment of product quality and acceptability (Chen and Opara, 2013). A good meat analogue should have real meat-like texture (Joshi and Kumar, 2015). Meat analogue texture is assessed on the basis of hardness, chewiness, and gumminess using a texture analyzer (Table 7).

The hardness value of meat analogue ranges from 1798 – 1458 g, chewiness value ranges from 425 – 304 gmm, and gumminess value from 193 - 218 g. There is no significant difference between control from meat analogues (Table 7). It is higher than sausage analogue of which hardness was 1339 g, chewiness was 240 gmm, and gumminess 120 g (Kamani, 2019) in analogue sausage. The higher concentration of cocoa pod husk extract, the higher the chewiness and gumminess, but the hardness is lower.

Pectin has hydrocolloid ability to bind water and further it improves the structural integrity of the product which affecting the chewiness value of the product (Maity *et al*., 2013). The addition of pectin also increased the gumminess (Al-Hinai *et al*., 2013). In addition, it can be used as an emulsifying agent due to pectin's ability to bind fat and water (Sharefiabadi and Serdarolu, 2020).

Table 6. Amino acid quality of low fat and high protein meat analogue of cowpea (*Vigna unguiculata*) with stabilizer cocoa pod husk extract (*Theobroma cacao* L.) using chemical score method

Parameter	Ratio of Cowpea flour: Cocoa husk extract			
	Control	98.5:1.5 (A)	99.0: 1.0 (B)	99.5:0.5 (C)
Phenylalanine-Tyrosine	25.52±4.99^{a}	31.08±2.51^{a}	32.27±1.31^{a}	23.32±15.32^{a}
Isoleucine	22.67±1.03^{a}	22.81±0.92^{a}	23.27±0.59^{a}	24.15±0.49^{a}
Valine	20.76±1.02^{a}	20.65±1.19^{a}	20.96±0.40^{a}	21.82±0.60^{a}
Lysine	14.35±1.02^{b}	12.94±0.58^{a}	12.69±0.50^{a}	13.43^{a}±0.39^{b}
Leucine	17.78±0.76^{a}	17.86±0.72^{ab}	18.31±0.48^{ab}	18.98±0.27^{b}
Threonin	8.80±0.95^{a}	9.36±0.86^{a}	9.57±0.27^{a}	9.68±0.31^{a}
Methionine-cysteine	ND	ND	ND	ND

Values are presented as mean±standard deviation (n = 3). Values with different superscript within the same row are statistically significantly different at 95% confidence level. ND: Not detected.

Table 7. Physical properties of low fat and high protein meat analogue of cowpea (*Vigna unguiculata*) with stabilizer cocoa pod husk extract (*Theobroma cacao* L.)

Ratio of Cowpea flour: Cocoa husk extract	Hardness (g)	Chewiness (gmm)	Gumminess (g)
Control	1495±527.83	413±129.40	209±35.47
98.5: 1.5 (A)	1458±229.40	425±146.29	218±47.82
99.0: 1.0 (B)	1462±318.04	383±155.32	211±46.06
99.5: 0.5 (C)	1798±37.04	304±10.69	193±10.44

3.4 Microbiological analysis

3.4.1 Total plate count

The total plate count of meat analogues ranged from 7.30×10^1 – 5.00×10^2 CFU/g (Table 8). The total plate count of the treatment meat analogue is still below the maximum threshold of $1\text{x}10^4$ CFU/g (Kumar *et al.*, 2015). Therefore, the treatment meat analogue is safe for consumption. The result of the total plate number shows a significant difference between the control from treatment meat analogues.

The increase of cocoa pod husk extract increases the total plate count as well. Microbial growth can be affected by environmental factors like nutrients, pH, water, oxygen, and compounds that inhibit bacterial growth (Chrismanuel *et al.*, 2012). Meat analogue with formula C has the lowest total plate count. The one with formula B is the second lowest. The lower the total plate count, the better the quality of the product.

3.4.2 Yeast and mould count

Yeast and mould count on meat analogue ranged from 2.00×10^1 - 2.33×10^2 CFU/g (Table 8), and it shows a significant difference between treatment from control meat analogues. The yeast and mould count of the meat analogue were still below the maximum threshold of $2.95\text{-}3.38\times10^5$ CFU/g (Filho *et al.*, 2005). The higher the concentration of cowpea flour, the higher the counts of yeast and mould. The meat analogue with formula A has the lowest yeast and mould counts. The one with formula B is the second lowest. The lower the yeast and mould counts, the better the quality of the product.

The increase of yeast and mould counts occurred because cowpea flour was susceptible to microbiological contamination during storage and processing. The protein component of the cowpea flour can be nutrients for organism to grow. *Fusarium* spp., *Rhizopus* spp., *Mucor* spp., *Aspergillus* spp., and *A. niger* were types of fungi found in the cowpea beans from Benin, West Africa (Houssou *et al*., 2009).

3.5 Sensory analysis

The colours of treatment meat analogues are brown. The panellist chose the brown colour of meat analogue with formula B as the best (Table 9). The brown colour comes from the cowpea flour and the addition of cocoa pod husk extract. Cocoa pod husk extract can be used as a natural brown colourant (Masiswo *et al*., 2020).

The texture of meat analogue with formula B had the highest score according to the panellists. The aroma score ranged between 2.93 – 3.17 and formula B also have higher aroma and taste acceptance because higher protein content in than formula A.

4. Conclusion

The decrease of cowpea flour and the increase of cocoa pod husk extract increase the water, fat, total dietary fibre, in vitro protein digestibility, total plate count content, chewiness, and gumminess of the meat analogue. However, the ash content, protein, carbohydrates, yeast mould numbers, and hardness decrease. The ratio of cowpea flour 99.0% and cocoa pod extract 1.0% was the preferred formula to make meat analogue due to the physical, amino acid, microbiological, and sensory quality.

Conflict of interest

The authors declare no conflict of interest.

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References

Agustin, A.T., Zaini, M.A. and Handito, D. (2020). The

Table 8. Microbiological properties of low fat and high protein meat analogue of cowpea (*Vigna unguiculata*) with stabilizer cocoa pod husk extract (*Theobroma cacao* L.)

Ratio of Cowpea flour: Cocoa husk extract	Control	98.5: 1.5 (A)	99.0: 1.0 (B)	99.5: 0.5 (C)
Total plate count (CFU/g)	$7.30\pm1.33\times10^{1a}$	$5.00\pm0.78\times10^{2b}$	$4.40\pm1.06\times10^{2b}$	$3.37\pm2.45\times10^{2b}$
Yeast and mould count (CFU/g)	$2.00\pm1.00\times10^{1a}$	$2.17\pm0.72\times10^{2b}$	$2.27\pm0.87\times10^{2b}$	$2.33\pm0.72\times10^{2b}$

Values are presented as mean±standard deviation (*n* = 3). Values with different superscript within the same row are statistically significantly different at 95% confidence level.

Table 9. Hedonic parameter of low fat and high protein meat analogue of cowpea (*Vigna unguiculata*) with stabilizer cocoa pod husk extract (*Theobroma cacao* L.)

Ratio of Cowpea flour: Cocoa pod husk extract	Colour	Texture	Taste	Aroma	Overall
Control	2.90	2.53	2.60	2.97	2.23
98.5: 1.5 (A)	3.00	2.77	2.77	2.93	2.57
99.0: 1.0 (B)	3.40	3.00	2.90	3.17	3.27
99.5: 0.5 (C)	3.10	2.17	2.47	2.93	1.93

https://doi.org/10.26656/fr.2017.7(5).789

effect of methods and temperature of blanching treatment on fiber compounds of banana stem as raw material of ares. *Pro Food*, 6(1), 609-622. https://doi.org/10.29303/profood.v6i1.117 [In Bahasa Indonesia].

Ahirwar, R., Jayathilakan, K., Reddy, K.J., Pandey, M. and Batra, H. (2015). Development of mushroom and wheat gluten base meat analogue by using response surface methodology. *International Journal of Advanced Research,* 3(1), 923-930.

Alayande, L.B., Mustapha, K.B., Dabak, J.D. and Ubom, G.A. (2012). Comparison of nutritional values of brown and white beans in Jos North Local Government markets. *African Journal of Biotechnology*, 11(43), 10135-10140. https://doi.org/10.5897/AJB11.3908

Alemawor, F., Dzogbefia, V.P., Oddoye, E.O. dan Oldham, J.H. (2009). Enzyme cocktail for enhancing poultry utilisation of cocoa pod husk. Scientific Research and Essays, 4(6), 555-559.

Al-Hinai, K.Z., Guizani, N., Singh, V., Rahman, M.S. and Al-Subhi, L. (2013). Instrumental texture profile analysis of date-tamarind fruit leather with different types of hydrocolloids. *Food Science and Technology Research,* 19(4), 531-538. https://doi.org/10.3136/fstr.19.531

Antika, S.R. and Kurniawati, P. (2017). Isolasi dan karakterisasi pektin dari kulit nanas. In Prosiding Seminar Nasional Kimia, p. 218-225. [In Bahasa Indonesia].

AOAC International. (2016). Official Methods of Analysis of AOAC International. 20th ed., p. 3172. Gaithersburg, MD, USA: AOAC International.

Asp, N.G., Johansson, C.G., Hallmer, H. and Siljestroem, M. (1983). Rapid enzymic assay of insoluble and soluble dietary fiber. *Journal of Agricultural and Food Chemistry*, 31(3), 476-482. https://doi.org/10.1021/jf00117a003

Baker, R.A., Berry, N., Hui, Y.H. and Barrett, D.M. (2005). Fruit Preserves and Jams. In Barrett, D.M., Somogyi, L. and Ramaswamy, H. (Eds). Processing fruits: science and technology. 2nd ed, p. 112-125. Boca Raton, USA: CRC Press, LLC

Bhat, Z.F. and Bhat, H. (2011). Tissue engineered meat-future meat. *Journal of Stored Products and Postharvest Research*, 2(1), 1-10.

Chan, S.Y. dan Choo, W.S. (2013). Effect of extraction conditions on the yield and chemical properties of pectin from cocoa husks. Food Chemistry, 141(4), 3752–3758.

Chen, L. and Opara, U.L. (2013). Texture measurement approaches in fresh and processed foods — A review. *Food Research International,* 51(2), 823–835. https://doi.org/10.1016/j.foodres.2013.01.046

Chrismanuel, A., Pramono, Y.B. and Setiani, B.E. (2012). the effect of edible coating's carragenan on pH, total bakteria and h2s meatball 16 hours storage. *Animal Agriculture Journal,* 1(2), 286-292.

da Silva, A.C., da Costa Santos, D., Junior, D.L.T., da Silva, P.B., dos Santos, R.C. and Siviero, A. (2018). Cowpea: a strategic legume species for food security and health. In Jimenez-Lopez, J.C. and Clemente, A. (Eds). Legume Seed Nutraceutical Research. InTech Open E-Book.

Dahl, W.J. and Stewart, M.L. (2015). Position of the academy of nutrition and dietetics: health implications of dietary fiber. *Journal of the Academy of Nutrition and Dietetics*, 115(11), 1861-1870. https://doi.org/10.1016/j.jand.2015.09.003

Dlamini, N.P. (2016). Sensory and nutritional quality of an extruded sorghum and cowpea blend as a complementary food for school age children. South Africa: University of Pretoria, Pretoria, MSc. Thesis.

Edahwati, L., Susilowati. and Harsini, T. (2013). The production pectin from cacao leather. *Jurnal Teknologi Pangan*, 5(2), 121-124.

Fajri, F., Tamrin, T. and Asyik, N. (2018). Effect of the addition of cacao pod husk's pectin on sensory of chocolate bar. *Jurnal Sains dan Teknologi Pangan*, 2 (6), 911-919.

Fasuan, T.O., Gbdamosi, S.O. and Omobuwajo, T.O. (2018). Characterization of protein isolate from *Sesamum indicum* seed: In vitro protein digestibility, amino acid profile, and some functional properties, *Wiley Food Science and Nutrition* 6(6), 1715-1723. https://doi.org/10.1002/fsn3.743

Filho, G.C.S., Penna, T.C.V. and Schaffner, D.W. (2005). Microbiological quality of vegetable proteins during the preparation of a meat analogue. *Italian Journal of Food Science*, 17(3), 269-283.

Flight, I. and Clifton, P. (2006). Cereal grains and legumes in the prevention of coronary heart disease and stroke: a review of the literature. *European Journal of Clinical Nutrition*, 60(10), 1145-1159. https://doi.org/10.1038/sj.ejcn.1602435

Food and Agriculture Organization. (2013). Dietary protein quality evaluation in human nutrition. FAO Food and Nutrition Paper No. 92. Rome, Italy: FAO

Gibney, M.J., Lenham-New, S.A., Cassidy, A. and Vorster, H.H. (*2013).* Introduction to Human Nutrition. New York, USA: Blackwell Publishing

Gonçalves, A., Goufo, P., Barros, A., Domínguez-Perles, R., Trindade, H., Rosa, E.A., Ferreira, L. and

Rodrigues, M. (2016). Cowpea (*Vigna unguiculata* L. Walp), a renewed multipurpose crop for a more sustainable agri-food system: nutritional advantages and constraints. *Journal of the Science of Food and Agriculture,* 96(9), 2941-2951. https://doi.org/10.1002/jsfa.7644

Gunathilake, K.G.T., Herath, T. and Wansapala, J. (2016). Comparison of physicochemical properties of selected locally available legumes varieties (mung bean, cowpea, and soybean). *Potravinarstvo*, 10, 424-430. https://doi.org/10.5219/631

Haris, H. and Nafsiyah, I. (2019). Formulation of mixed fish waste and waste fish on content and digestibility of protein fish meal. *Majalah BIAM*, 15(2), 82-93.

Herawati, H. (2018). The hydrocolloids potential as additive materials to the qualified food and non-food products. *Jurnal Penelitian and Pengembangan Pertanian,* 37(1), 17-25. https://doi.org/10.21082/jp3.v37n1.2018.p17-25

Hesyandi (2018). Optimization of analog meat formulas from Pleurotus flabellatus mycelium, soybean flour, sweet potato flour, and corn flour using Response Surface Methodology (RSM). Bogor, Indonesia: Fakultas Teknologi Pertanian Institut Pertanian Bogor. Thesis.

Hoek, A.C., Luning, P.A., Stafleu, A. and de Graaf, C. (2004). Food-related lifestyle and health attitudes of Dutch vegetarians, non-vegetarian consumers of meat substitutes, and meat consumers. *Appetite*, 42 (3), 265-272. https://doi.org/10.1016/j.appet.2003.12.003

Houssou, P.A., Ahohuendo, B.C., Fandohan, P., Kpodo, K., Hounhouigan, D.J. and Jakobsen, M. (2009). Natural infection of cowpea (*Vigna unguiculata* (L.) Walp.) by toxigenic fungi and mycotoxin contamination in Benin, West Africa. *Journal of Stored Products Research,* 45(1), 40-44. https://doi.org/10.1016/j.jspr.2008.07.002

Indonesia Nasional Standard Board (BSN). (1995). Mung Bean Flour (SNI 01-3728-1995). Jakarta, Indonesia: BSN.

International Pectin Producers Association (IPPA). (2003). What is Pectin*.* Retrieved from website: https://ippa.info/what-is-pectin/.

International Organization for Standardization (ISO). (2003). Microbiology of food and animal feeding stuffs (ISO/DIS Standard No. 4833). Geneva: ISO.

Joshi, V.K. and Kumar S. (2015). Meat Analogues: Plant based alternatives to meat products - A review. *International Journal of Food and Fermentation Technology,* 5(2), 107-119. https://doi.org/10.5958/2277-9396.2016.00001.5

Kale, R., Sawate, A., Kshirsagar, R., Patil, B. and Mane, R. (2018). Studies on evaluation of physical and chemical composition of beetroot (*Beta vulgaris* L.). *International journal of chemical studies*, *6*(2), 2977-2979.

Kamani, M.H., Meera, M.S., Bhaskar, N. and Modi, V.K. (2019). Partial and total replacement of meat by plant-based proteins in chicken sausage: evaluation of mechanical, physico-chemical and sensory characteristics. *Journal of Food Science and Technology,* 56(5), 2660-2669. https://doi.org/10.1007/s13197-019-03754-1

Khalid, I.I. and Elhardallou, S.B. (2016). Factors that compromise the nutritional value of cowpea flour and its protein isolates. *Food and Nutrition Sciences*, 7(2), 112-121.

Kumar, P., Chatli, M.K., Mehta, N., Singh, P., Malav, O.P. and Verma, A.K. (2017). Meat analogues: Health promising sustainable meat substitutes. *Critical Reviews in Food Science and Nutrition*, 57(5), 923-932. https://doi.org/10.1080/10408398.2014.939739

Kusharto, C.M. (2006). Dietary fiber and its role for health. *Jurnal Gizi dan Pangan*, 1(2), 45-54. https://doi.org/10.25182/jgp.2006.1.2.45-54

Larasati, Y.N. (2018). The utilization of cowpea flour on the manufacture of cavigna cake. Yogyakarta, Indonesia: Yogyakarta State University. Thesis.

Lestari, P.A., Yusasrini, N.A. and Wiadnyani, A.A.I.S. (2019). The effect comparative of wheat flour and cowpea flour to characteristics of crackers. *Jurnal Ilmu dan Teknologi Pangan,* 8(4), 457-464. https://doi.org/10.24843/itepa.2019.v08.i04.p12

Leviana, W. and Paramita, V. (2017). Effect of temperature on water content and water activity in material on turmeric (*Curcuma longa*) with tools electrical oven dryer. *Metana*, 13(2), 37-44. https://doi.org/10.14710/metana.v13i2.18012

Lisa, M., Lutfi, M. and Susilo, B. (2015). Effect of temperature variation and long drying of the quality flour white oyster mushroom (*Plaerotus ostreatus*). *Jurnal Keteknikan Pertanian Tropis dan Biosistem*, 3 (3), 270-279.

Maity, T., Raju, P.S. and Bawa, A.S. (2013). Effect of hydrocolloid pre-treatment on instrumental and sensory texture attributes of frozen carrot (*Daucus carota*). Internati*onal Journal of Food Properties,* 16 (2), 461-474. https://doi.org/10.1080/10942912.2011.553756

Masiswo, Haerudin, A., Lestari, D.W., Mandegani, G.B., Satria, Y., Arta, T.K. and Atika, V. (2020). Application of cocoa pod husk (*Theobroma cocoa*

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spp.) for natural dyes powder on silk batik cloth. *IOP Conference Series: Materials Science and Engineering*, 980, 012020. https://doi.org/10.1088/1757-899X/980/1/012020

Maulidiyah, M., Halimatussadiyah, H., Susanti, F., Nurdin, M. and Ansharullah, A. (2014). Pectin isolation of skin fruit of cocoa (*Theobroma cacao* L.) and absorbtion test on metals copper (Cu) dan zinc (Zn). *Jurnal Agroteknos,* 4(2), 113-119.

Mentari, R., Anandito, R.B.K. and Basito, B. (2016). Meat analogue formulations in the form of "meat" balls made from kidney beans (*Phaseolus vulgaris*) and soybeans (*Glycine max*). *Jurnal Teknosains Pangan,* 5(3), 31-41.

Mistry, M., George, A. and Thomas, S. (2020). Alternatives to meat for halting the stable to table continuum–an update. *Arab Journal of Basic and Applied Sciences*, 27(1), 324-334. https://doi.org/10.1080/25765299.2020.1807084

Nanta, P., Skolpap, W. and Kasemwong, K. (2021). Influence of hydrocolloids on the rheological and textural attributes of a gluten-free meat analogue based on soy protein isolate. *Journal of Food Processing and Preservation*, 45(3), e15244. https://doi.org/10.1111/jfpp.15244

Nissar, J., Ahad, T., Naik, H.R. and Hussain, S.Z. (2017). A review phytic acid: As antinutrient or nutraceutical. *Journal of Pharmacognosy and Phytochemistry,* 6(6), 1554-1560.

Olalekan, A.J. and Bosede, B.F. (2010). Comparative study on chemical composition and functional properties of three Nigerian legumes (jack beans, pigeon pea and cowpea). *Journal of Emerging Trends in Engineering and Applied Sciences*, 1(1), 89-95.

Palanisamy, M., Töpfl, S., Berger, R.G. and Hertel, C. (2019). Physico-chemical and nutritional properties of meat analogues based on Spirulina/lupin protein mixtures. *European Food Research and Technology*, 245(9), 1889-1898. https://doi.org/10.1007/s00217-019-03298-w

Perera, C. (2011). Evaluation of meat analogues made with texturized pea protein for their functionality and applications in nutritionally improved meatless formulations. Retrieved on September 24, 2020 from Website: https://www.researchgate.net/publication/280234057.

Putri, A.S. (2007). How to Prevent Common Diseases. New Delhi, India: Sterling Publishers.

Ramos, S., Moulay, L., Granado-Serrano, A.B., Vilanova, O., Muguerza, B., Goya, L. and Bravo, L. (2008). Hypolipidemic effect in cholesterol-fed rats of a soluble fiber-rich product obtained from cocoa husks. *Journal of Agricultural and Food Chemistry,* 56(16), 6985-6993. https://doi.org/10.1021/jf8009816

Ranganna, S. (2005). Handbook of Analysis and Quality Control for Fruit and Vegetable Products. India: Tata McGraw-Hill Publishing Company Limited.

Rehrah, D., Ahmedna, M., Goktepe, I. and Yu, J. (2009). Extrusion parameters and consumer acceptability of a peanut-based meat analogue. *International Journal of Food Science and Technology*, 44(10), 2075-2084. https://doi.org/10.1111/j.1365-2621.2009.02035.x

Roikah, S., Rengga, W.D.P., Latifah, L. and Kusumastuti, E. (2016). Extraction and characterization of pectin from *Averrhoa Bilimbi Linn*. *Jurnal Bahan Alam Terbarukan,* 5(1), 29-36. https://doi.org/10.15294/jbat.v5i1.5432

Sharefiabadi, E. and Serdaroğlu, M. (2020). Pectin: properties and utilization in meat products. *Food and Health,* 7(1), 64-74. https://doi.org/10.3153/FH21008

Slavin, J. (2013). Fiber and prebiotics: mechanisms and health benefits. *Nutrients*, 5(4), 1417-1435. https://doi.org/10.3390/nu5041417

Sreerama Y.N., Sashikala V.B., Pratape V.M. and Singh V. (2012). Nutrients and antinutrients in cowpea and horse gram flours in comparison to chickpea flour: Evaluation of their flour functionality. *Food Chemistry*, 131(2), 462-468. https://doi.org/10.1016/j.foodchem.2011.09.008

Stanin, E., Swamilaksita, P.D. and Mulyani, E.Y. (2019). Tempeh and vital wheat gluten based analogue meat development as vegetarian alternative food, presented at Proceedings of the 1st International Conference on Health, Jakarta, 2019. Jakarta: Science and Technology, Lda. https://doi.org/10.5220/0009591902470256

Sugito, S. and Hayati, A. (2006). The use of gabus (*Ophicepallus strianus* BLKR) fillet fish and application of freezing in making gluten pempek. *Jurnal Ilmu-Ilmu Pertanian Indonesia*, 8 (2), 147-151.

Susilowati, P.E., Fitri, A. and Natsir, M. (2017). The Effect of Edible Coating Made from Pectin of Cacao Fruit Peel to the Shelf Life and Quality of Tomato. *Jurnal Aplikasi Teknologi Pangan*, 6(2), 1-4.

Tjokrokusumo, D., Octaviani, F.C. and Saragih, R. (2019). Fortification of mung bean (*Vigna radiata*) and ear mushroom (*Auricularia auricula-judae*) in dried sago noodles. *Journal of Microbial Systematics and Biotechnology*, 1(2), 34-40. https://doi.org/10.37604/jmsb.v1i2.30

U.S. Food and Drug (FDA). (2017). Yeast, Molds and mycotoxins (BAM Chapter 18). Retrieved from FDA website: https://www.fda.gov/food/laboratory-methods-food/bam-chapter-18-yeasts-molds-and-mycotoxins.

Waters. (2012). H-Class and H-Class Bio Amino Acid Analysis System Guide. Retrieved on November 24, 2021 from FSIS Website: https://www.waters.com/webassets/cms/support/docs/acq_uplc_h-class_aaa_sysgd_rev_b.pdf.

Williams, M.H. (2005). Dietary supplements and sports performance: minerals. *Journal of the International Society of Sports Nutrition*, 2(1), 1-7. https://doi.org/10.1186/1550-2783-2-1-43

Yadav, P., Ahlawat, S.S., Jairath, G., Rani, M. and Bishnoi, S. (2015). Studies on physico-chemical properties and shelf life of developed chicken meat analogue rolls. *Haryana Veterinarian*, 54(1), 25-28.

Youssef, M.M. (1988). Formulation, acceptability, chemical composition and in vitro digestibility of novel snack food and meat ball analogue. *Plant Foods for Human Nutrition,* 38(3), 243-249. https://doi.org/10.1007/BF01092863

