Physico-chemical properties, amino acid and fatty acid profile of chicken patties added with beetroot peel flour as natural colourant

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Summary

Ground meat is prone to oxidation and thus shall be added with spices and antioxidants whether natural or the synthetic ones. Beetroot (*Beta vulgaris*) peel is an agro-industrial waste material, which contains a natural pigment that can be used as a natural dye and a source of antioxidants for processed meat products. The objective of this study was to determine the proper addition level of the beetroot peel flour for enhancing the physico-chemical quality of chicken patties. A completely randomized design was used with four groups, i. e. control without addition of beetroot peel flour and with 10, 20 and 30 $g\cdot kg^{-1}$ addition per meat weight with five replications. The treatment had very significant ($P < 0.01$) effect on colour (L^*, a^* and b^*), water-holding capacity, cooking loss, proximate composition and antioxidant activity of chicken patties. The addition of beetroot peel flour at 30 $g\cdot kg^{-1}$ improved redness, antioxidant activity and water-holding capacity, while reduced cooking loss, moisture and fat content as well as prevented the loss of polyunsaturated fatty acids and changes in amino acids content at cooking. The results suggest that the addition of beetroot peel flour at 30 $g\cdot kg^{-1}$ improves the physico-chemical quality of chicken patties.

Keywords

Beta vulgaris; agro-industrial waste; amino acid; antioxidant; fatty acid; natural colouring; restructured meat product

Chicken meat is the most consumed livestock product. The fact is that the consumption of chicken meat has no restrictions in any religion or culture. Consumption of chicken meat has increased rapidly due to its availability, affordable price, acceptable taste, that it can be consumed by all groups of population and provides good quality of nutrition. Chicken meat is rich in essential amino acids, vitamins, lipids and low in cholesterol [1]. The main drawback of chicken meat is its high content of moisture, which makes it susceptible to spoilage by bacteria and other microorganisms. Another drawback is that auto-oxidation of lipids causes a short shelf life and low quality of chicken meat products [2]. Further weakness of chicken meat is the unattractive pale colour when it is processed into derivative products [2].

Several types of processed chicken meat products have become available. These include nuggets, sausages, meatballs, corned beef and patties. Patties contain processed meat that was ground or crushed and added with seasonings and additives. A restructuring method is used to produce meat patties and, therefore, meat patties can be classified as processed meat products of the restructured type [3]. Patties are usually made from red meat such as beef or pork. Patties made from red meat do not need the addition of red dyes. In contrast, chicken meat patties can be more attractive when added with red dye.

From the nutritional point of view, meat patties are good sources of proteins and lipids. However, processed meat products such as meat patties have a low fibre content [4]. This can be overcome by adding fibre from cereals, vegetables or fruits, which improve the texture of the patties, add the nutritional value, while reducing energy and lipid content [5]. Consumers are starting to realize the importance of foods that are high in dietary fibre and composed of natural ingredients [6].

The quality of ground chicken patties can be improved by the addition of red beetroot peel flour. Beetroot peel flour is an agro-industrial waste or a by-product of beetroot processing. The

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use of beetroot peel can increase the added value and selling value of a product, due to the utilization of plant waste [7]. Beetroot peel is rich in nutrients including carbohydrates, proteins, lipids, dietary fibre and minerals, which can be beneficial for human health. In addition, beetroot peel contains natural pigments that can be utilized and the phenolic compounds present in beetroot peel possess antioxidant activity [8].

Beetroot peel can be applied to food products in the form of extracts or flour. Recently, El-Beltagi et al. [9] found that the aqueous extract from dried red beetroot peel contains 8.32 g·kg^{-1} of total phenolic compounds, 2.43 g·kg^{-1} of flavonoids and 5.35 g·kg^{-1} of betalains that can be used for preserving Nile *Tilapia* fish fillet. The addition of antioxidant dietary fibre materials to meat products can prevent lipid and protein oxidation together with maintaining their texture [6]. The use of beetroot peel is expected to improve the texture of chicken patties in the presence of fibre and carbohydrates as well as improve its appearance when applied in the form of flour.

The potential of beetroot peel as a natural dye and antioxidant source for chicken patties, however, has not been observed. Therefore, it is necessary to determine the right addition level that improves the physico-chemical quality and prevents the loss of amino acids and polyunsaturated fatty acids at thermal processing.

MATERIALS AND METHODS

Beetroot peel flour preparation

Beetroot was purchased from the local market in Malang City, Indonesia. It was selected visually based on the requirement of clean peel, without stains or fungi. Beetroot was peeled, and the peel was soaked in water and washed. The peel was then sliced to 4 cm × 2 cm pieces and dried in an oven with a heating temperature of 60 ± 2 °C for 24 h. The dried material was ground using a dry mill HR 2115 (PT Philips Indonesia, Jakarta, Indonesia) at medium speed using stainless blades and then sieved with a 0.15 mm sieve to produce fine granular powder. This beetroot peel flour contained 119 g·kg^{-1} moisture, 32 g·kg^{-1} ash, 49 g·kg^{-1} proteins, 4 g·kg^{-1} lipids and 796 g·kg^{-1} carbohydrates.

Materials

Fresh boneless and skinless chicken breast fillet was purchased at the local butcher shop in Malang City, Indonesia. Wheat flour, ice cubes, garlic powder, onion powder, whipped egg (albumen and yolk), salt, ground white pepper, carboxymethyl cellulose and mushroom stock powder were purchased from the local supermarket in the city.

Chicken patties preparation

The chicken meat was cleaned of fat and visible connective tissue, then cut into 4 cm × 4 cm × 2 cm pieces. The meat cubes were ground with half of the ice cubes using a meat chopper HR 2939 N (PT Philips Indonesia). Tapioca flour, palm oil, garlic, onions, eggs, salt, ground white pepper, mushroom stock powder, beetroot peel flour and the remaining ice cubes were then added according to the formula shown in Tab. 1. Beetroot peel flour was added at levels of 10, 20 and 30 g·kg^{-1}. Patties were formed using a non-stick plastic burger press with a thickness of 2.5 cm (PT Tupperware Indonesia, Jakarta, Indonesia). Patties were made five times for treatment replication purposes. Then, they were cooked in an oven with a heating temperature of 120 ± 2 °C for 25 min (final core temperature was 75 °C) and cooled down at 25 ± 1 °C for 30 min.

Surface colour measurement

The surface colour of raw chicken patties was recorded in five repetitions by measuring lightness (L^*), redness (a^*) and yellowness (b^*) using a chromameter CS-10 (Wenzhou Sanqi Technology Machinery Equipment, Zhejiang, China). The light source of illuminant C (2° observer) with 4 mm aperture and attached-closed cone was calibrated using a white plate ($Y = 93.6$, $X = 0.3134$, $y = 0.3194$).

Tab. 1. Chicken patties formulae.

Item [%]	Beetroot peel flour addition level [g·kg^{-1}]			
	0	10	20	30
Chicken fillet	70.0	70.0	70.0	70.0
Wheat flour	10.0	10.0	10.0	10.0
Ice cubes	12.0	12.0	12.0	12.0
Beetroot peel flour	0.0	1.0	2.0	3.0
Garlic powder	1.1	1.1	1.1	1.1
Onion powder	2.3	2.3	2.3	2.3
Salt	0.7	0.7	0.7	0.7
Whipped egg	0.7	0.7	0.7	0.7
Carboxymethyl cellulose	0.7	0.7	0.7	0.7
Ground white pepper	0.7	0.7	0.7	0.7
Mushroom stock powder	0.7	0.7	0.7	0.7

Water-holding capacity and cooking loss

The water-holding capacity of the raw chicken patties were measured according to the press method [10]. Cooking loss was determined according to weight differences of samples before and after cooking in an oven with a heating temperature of 120 ± 2 °C for 25 min (final core temperature was 75 °C) and cooled down at 25 ± 1 °C for 30 min.

Proximate amino acid composition

Moisture, lipid, protein and ash content of cooked samples were determined using AOAC methods for moisture in feed AOAC 930.15, crude fat in feed AOAC 920.39, crude protein in feed AOAC 2001.11, and ash in feed AOAC 942.05 [11]. The amino acid profile analysis was performed according to the method of GELDENHUYS et al. [12] with modification in configuration. Selected raw and cooked samples from the group of 30 g·kg^{-1} addition of beetroot flour were dried, defatted and flushed with N_2 gas for 30 s. Then, they were hydrolysed using 6 mol·l^{-1} HCl and 150 g·l^{-1} phenol under vacuum in an oven set at 110 °C for 24 h. Hydrolysed samples were then stored at –20 °C in microtubes until further analysis for a maximum of 2 months. The amino acid profile of samples was determined by the use of Nexera X2 HPLC (Shimadzu, Kyoto, Japan). Sample derivatization was performed using opthaldialdehyde for 2 min prior to injection. The Zorbax Eclipse AAA column (150 mm × 4.6 mm, 3.5 μm particle size; Agilent Technologies, Santa Clara, California, USA), detection at 338 nm and a temperature of 40 °C were used. Mobile phase A was 40 mmol·l^{-1} NaH_2PO_4 at pH 7.8, and mobile phase B was 45 % (v/v) acetonitrile (HPLC grade, ≥ 99.8%, Merck, Darmstadt, Germany), 45 % (v/v) methanol (HPLC grade, ≥ 99.8%, Merck), and 10 % (v/v) Milli-Q water (Merck), with the separation performed at a 1.5 ml·min^{-1} flow rate. The gradient program was: 2 % B from 0 min to 0.5 min; 57 % B for 20 min; 100 % B from 20.1 min to 23.5 min; 2 % and 0 % B from 23.6 min to 25 min. The results were read as the amount of moles per millilitre sample and converted to milligrams per kilogram sample.

Proximate fatty acid composition

Fatty acid composition of raw and cooked samples of the selected group (30 g·kg^{-1} addition of beetroot flour) was determined using a gas chromatography system 6890 N (Agilent Technologies). Meat lipids were extracted according to FOLCH et al. [13] with a chloroform-methanol mixture (2 : 1, v/v, HPLC grade for both, ≥ 99.8%, Merck). Fatty acids were then converted to methyl esters with 250 g·l^{-1} boron trifluoride in methanol at 80 °C for 1 h. Fatty acid methyl esters were then dissolved in 1.5 ml of *n*-hexane (suitable for HPLC, ≥ 97.0% for gas chromatography, Merck). Sample of 1 μl was injected into the gas chromatograph port by the automatic sampler. The injector temperature was set at 250 °C with a split ratio of 1 : 100. Fatty acid methyl esters were separated using a wall coated open tubular (WCOT) fused silica capillary column (100 m long, 0.25 mm internal diameter, 0.20 μm film thickness; Agilent Technologies), the carrier gas was helium at a flow rate of 1.0 ml·min^{-1}. The oven was programmed at 150 °C for 1 min, 150 °C to 200 °C at elevating rate of 7 °C·min^{-1} for 10 min, 200 °C for 5 min, 200 °C to 250 °C at elevating rate of 5 °C·min^{-1} for 10 min, and hold at 250 °C for 10 min. The temperature of the detector was set at 275 °C. The fatty acid peaks were identified by comparison with the retention time of fatty acid standards (47015-U, Sigma-Aldrich, St. Louis, Missouri, USA). The peak area of each identified fatty acid was used to calculate the proportion (in percent) against total identified peak area.

Antioxidant activity

The antioxidant activity of cooked chicken patties was determined using 2 2-diphenyl-1-picrylhydrazyl (DPPH) assay. The reference method used was similar to that described by BONDET et al. [14]. The results were expressed as scavenging percentage, and measurements were performed in triplicate.

Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA) to determine the effect of the treatment. Statistical analyses was conducted using SPSS IBM v. 20 (IBM SPSS, Chicago, Illinois, USA). Normality distributions were checked using the Shapiro-Wilk test. Duncan's multiple range test was conducted at a significance level of 1 %. Bartlett's test was used to test the homogenity of variance between sample groups. Significance of the differences among results were analysed using Duncan's post-hoc test at $P < 0.05$.

RESULTS AND DISCUSSION

Colour of chicken patties with red beet peel flour

The beetroot flour had a very significant effect ($P < 0.01$) on the surface colour of the raw chicken patties (Tab. 2). With the increase in

Tab. 2. Surface colour of raw chicken patties added with beetroot peel flour.

	Beetroot peel flour addition level			
	0 g·kg^{-1}	10 g·kg^{-1}	20 g·kg^{-1}	30 g·kg^{-1}
Lightness *L**	69.81 ± 0.48 ^{d}	61.54 ± 0.65 ^{c}	57.59 ± 0.16 ^{b}	54.48 ± 0.22 ^{a}
Redness *a**	–1.71 ± 0.36 ^{a}	–0.11 ± 0.40 ^{b}	0.26 ± 0.66 ^{c}	0.74 ± 0.88 ^{c}
Yellowness *b**	9.95 ± 0.45 ^{a}	10.61 ± 0.38 ^{b}	11.45 ± 0.42 ^{c}	12.42 ± 0.38 ^{d}

Mean values ± standard deviation within the same column with the different letters in superscript are significantly different among treatments ($P < 0.01$).

the addition of beetroot flour, lightness (L^*) decreased, while redness (a^*) and yellowness (b^*) increased. Beets contain purple pigments, namely betacyanin and betalain, and yellow pigments, such as betaxanthin, so they tend to be dark red and purplish in colour. El-Beltagi et al. [9] reported that beetroot peel aqueous extract contains 5.35 g·kg^{-1} of betalains. These cause the lightness of the chicken patties to decrease. Chicken patties with the addition of 30 g·kg^{-1} beetroot peel flour turned dark red almost like ground beef patties. Previous studies using other natural purple red colouring agents from grapes and tomatoes flour to be added to patties caused a decrease in lightness, an increase in redness and yellowness [15]. Beef patties with the addition of acetate maize starch had increased lightness caused by the bright colour of the maize starch [16]. These results indicated that the beetroot peel powder effectively turned the colour of pale chicken patties into red meat-like patties.

Water-holding capacity

Water-holding capacity is used to describe the quality of protein-rich food products and is related to juiciness. Chicken patties with the addition of red beetroot flour showed an increase in water-holding capacity along with the addition level significantly ($P < 0.01$) as shown in Tab. 3. Previously, pomegranate peel and bagasse powder added to chicken patties increased water-holding capacity of chicken patties [17]. Samard et al. [18] reported that addition of vegetable proteins to meatless patties increases their water-holding capacity and improved the texture and reduced the amount of fat needed to be used at production.

Cooking loss

Thermal process can affect the quality of meat products. Cooking loss measurement can also determine the economical efficiency in meat products manufacture. Minor loss is preferable from efficiency aspect. The addition of beetroot peel flour to chicken patties had a very significant effect ($P < 0.01$) and reduced the amount of loss during the thermal process (Tab. 3). Salt used among the ingredient helps to reduce cooking loss as it is able to retain moisture along with increasing solubility of proteins [19]. Cooking loss is also decreased by the inhibition of degradation of both sarcoplasm and myofibrillar proteins [20]. Protein oxidation causes a decrease in the ability of proteins to retain moisture in meat products [20].

Tab. 3. Physico-chemical properties of chicken patties added with beetroot peel flour.

Variable	Beetroot peel flour addition level [g·kg^{-1}]			
	0	10	20	30
Raw sample				
Water-holding capacity [%]	50.6 ± 0.4 ^{a}	51.9 ± 0.7 ^{b}	54.0 ± 0.5 ^{c}	55.5 ± 0.2 ^{d}
Cooking loss [%]	8.5 ± 0.2 ^{d}	7.2 ± 0.1 ^{c}	6.5 ± 0.1 ^{b}	5.7 ± 0.1 ^{a}
Cooked sample				
Moisture content [g·kg^{-1}]	68.3 ± 0.2 ^{d}	67.7 ± 0.3 ^{c}	66.5 ± 0.4 ^{b}	65.4 ± 0.3 ^{a}
Ash content [g·kg^{-1}]	2.3 ± 0.0 ^{a}	2.4 ± 0.1 ^{b}	2.5 ± 0.1 ^{bc}	2.7 ± 0.1 ^{c}
Crude protein content [g·kg^{-1}]	15.6 ± 0.3 ^{a}	16.4 ± 0.3 ^{b}	17.4 ± 0.2 ^{c}	18.5 ± 0.3 ^{d}
Crude fat content [g·kg^{-1}]	9.6 ± 0.3 ^{d}	8.4 ± 0.3 ^{c}	7.4 ± 0.1 ^{b}	6.3 ± 0.1 ^{a}
Antioxidant activity [%]	2.2 ± 0.6 ^{a}	6.3 ± 0.6 ^{b}	7.5 ± 0.2 ^{c}	8.3 ± 0.2 ^{d}

Mean values ± standard deviation within the same row with the different letters in superscripts are significantly different among treatments ($P < 0.01$).

Moisture content

The moisture content is influenced by the ingredients and the treatment of the chicken patties. The addition of beetroot peel flour to chicken patties had a very significant effect ($P < 0.01$) on moisture content of cooked samples (Tab. 3). The higher the addition level of beetroot peel flour added, the lower the moisture content of the chicken patties was. Beetroot peel flour used in this study had a moisture content of 119 g·kg^{-1} with the remaining 891 g·kg^{-1} dry matter. The addition of dry matter from beetroot peel powder may prolong the shelf life of chicken patties by decreasing their moisture content. The decrease in the moisture content of the chicken patties was also caused by carbohydrates in the beetroot peel flour, which were present at 804 g·kg^{-1}. Starch is a glucose polymer, which is composed of amylose and amylopectin [21]. The moisture content plays an important role in the functional structural capacity of starch, namely water absorption and dispersion in an aqueous medium by starch granules [22].

Ash content

The addition of beetroot peel flour to the chicken patties had a very significant ($P < 0.01$) effect on the ash content (Tab. 3). Ash content increased along with the amount of flour added. The ash content of beetroot peel flour was 32 g·kg^{-1}. Beetroot peel flour contained phosphorus, manganese, calcium, iron and sodium as major minerals [23]. These affect the amount of ash contained in the chicken patties.

Proteins content

Chicken meat patties contain proteins from the chicken meat and other ingredients such as beetroot peel flour. The protein content of beetroot peel flour was 49 g·kg^{-1} and it increased the proteins content of the patties ($P < 0.01$). The highest proteins content had samples added with 30 g·kg^{-1} of beetroot flour (Tab. 3). Proteins in the ingredients help to hold water in the meat and are assisted by hydrocolloids to trap water [24].

Lipids content

The results showed that beetroot peel flour had a very significant effect ($P < 0.01$) on the lipids content of chicken patties (Tab. 3). The lipids content of the chicken patties decreased with the addition of flour. Beetroot peel flour used in this study had a lipids content of 4 g·kg^{-1}. In contrast, it was shown that the addition of 10 g·kg^{-1} lemon albedo powder to chicken patties reduced the fat content and did not lead to a significant difference in proteins and ash contents [25].

Antioxidant activity

The results showed that chicken patties with the addition of beetroot peel flour had a very significant effect ($P < 0.01$) on antioxidant activity, which increased compared to control (Tab. 3). The antioxidant activity of beetroot peel flour itself was 61.2 %. The antioxidant compound present in beetroot peel is betacyanin. Betacyanin is a phenolic compound that is used as a dye and antioxidant for protection from oxidation [9].

Proximate amino acid profile

The amino acid profile of chicken meat patties with the addition of beetroot peel flour with an addition of 30 g·kg^{-1} showed that the chicken meat patties contained several types of amino acids (Tab. 4). Their content did not change significantly after the samples were cooked, indicating that the addition of beetroot peel powder at 30 g·kg^{-1} could prevent a significant loss of any amino acid. The antioxidant compounds in beetroot peel flour could prevent the oxidative deterioration of amino acids in chicken patties. Fruit waste contains various antioxidant compounds that can be used as antioxidant and antibacterial agents for meat products [26]. The amino acids contained included essential, conditionally essen-

Tab. 4. Amino acid profile of raw and cooked chicken patties added with 30 g·kg^{-1} beetroot peel flour.

Amino acid [mg·kg^{-1}]	Chicken patties	
	Raw	Cooked
Valine	7891.71	7943.02
Leucine	12668.72	12734.77
Isoleucine	7252.15	7274.36
Lysine	12036.59	12107.03
Arginine	12668.10	12800.53
Threonine	9001.96	9049.21
Methionine	1680.99	1682.12
Phenylalanine	8755.72	8813.42
Tryptophan	1796.89	1798.30
Glycine	7838.37	7876.28
Serine	7878.86	7896.96
Cysteine	7452.55	7456.28
Tyrosine	5993.27	6024.71
Histidine	6694.96	6675.75
Alanine	8919.95	8999.99
Glutamatic acid	25144.66	25312.43
Aspartic acid	13002.31	13085.34
Proline	5450.64	5488.75

Tab. 5. Fatty acid composition of raw and cooked chicken patties added with 30 g·kg^{-1} beetroot peel flour.

Fatty acid [%]	Chicken patties	
	Raw	Cooked
Lauric acid (C12:0)	1.3	1.2
Myristic acid (C14:0)	1.2	1.2
Palmitic acid (C16:0)	30.4	30.0
Stearic acid (C18:0)	9.6	9.5
Palmitoleic acid (C16:1*n*7)	2.2	2.2
Oleic acid (C18:1*n*9)	53.9	54.0
Linoleic acid (C18:2*n*6)	6.2	6.7
α-Linolenic acid (C18:3*n*3)	6.3	6.4
Arachidic acid (C20:0)	0.5	0.5
Eicosenoic acid (C20:1)	0.4	0.5
Eicosadienoic acid (C20:2)	0.5	0.5
Eicosatrienoic acid (C20:3*n*6)	0.5	0.5
Arachidonic acid (C20:4*n*6)	1.7	1.7
Eicosapentaenoic acid (C20:5*n*3)	0.3	0.3

Proportion of fatty acid is expressed as area peak percentage.

tial and non-essential amino acids. Chicken patties with the addition of 30 g·kg^{-1} of beetroot peel powder contained essential amino acids valine, leucine, isoleucine, lysine, arginine, threonine, methionine, phenylalanine and tryptophan. Essential amino acids are types of amino acids that cannot be synthesized by the human body. The essential amino acid with the highest content was arginine.

Conditionally essential amino acids contained in chicken meat patties with the addition of beetroot peel flour with an addition of 30 g·kg^{-1} were glycine, serine, cysteine, tyrosine and histidine. Conditionally essential amino acids are amino acids that can be synthesized by the body from other amino acids. The one with the highest content of them was serine.

Non-essential amino acids contained in chicken meat patties with the addition of beetroot peel flour at 30 g·kg^{-1} included alanine, glutamic acid, aspartic acid and proline. Non-essential amino acids are amino acids that can be synthesized by the body. The highest non-essential amino acid was glutamic acid.

Proximate fatty acid composition

Selected treatment group (30 g·kg^{-1} addition level) was used to analyse whether fatty acid composition of chicken patties was altered during cooking. Data on fatty acid composition of chicken patties before and after thermal process (oven heating) are shown in Tab. 5. The addition of 30 g·kg^{-1} beetroot peel flour to chicken patties apparently prevented changes in fatty acid composition during cooking.

CONCLUSION

Utilization of beetroot peel as natural colouring agent and source of antioxidants for ground chicken patties was proven. It improved the physico-chemical properties and prevented changes in fatty acid composition during oven heating. Beetroot peel in the flour form can be added at 30 g·kg^{-1} per meat weight, thus obtaining a high-quality product with increased attractiveness and market value within the trend of low-energy functional foods containing exclusively natural ingredients.

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