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Utilisation of chicken eggshell nanopowder on physicochemical quality, microstructure, and amino acid profile of beef patties

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Abstract: Chicken eggshells are a source of calcium and protein that can be added to restructured meat products, including patties. This study aimed to improve the chemical quality of beef patties with the addition of chicken eggshell nanopowder, including pH, protein content, ash content, fat content, antioxidant activity, calcium, microstructural quality, and identification of amino acid profiles. The method used in this study was a laboratory experiment arranged in a completely randomised design with five treatments: without the addition of chicken eggshell nanopowder as a control, the addition of chicken eggshell nanopowder 0.1, 0.3, 0.5, and 0.7% (*w*/*w*) with five replications. Data were analysed using the analysis of variance. It was followed by Duncan's multi-range test when the differences were significant or very significant. The research results show that the addition of chicken eggshell nanopowder to beef patties had a very significant effect ($P < 0.01\%$) on pH, protein content, ash content, fat content, antioxidant activity, calcium, microstructural quality through scanning electron microscopy, and several amino acids. The addition of 0.7% chicken eggshell nanopowder could improve the chemical quality of the beef patties. The patty matrix was uniform and compact, and the air voids were getting tighter with the addition of chicken eggshell nanopowder, which was increasing based on scanning electron microscopy observations. Moreover, beef patties had various types of amino acids to achieve the best treatment.

Keywords: waste; calcium; enrichment

Livestock development will increase the consumption of livestock products, accompanied by an increase in by-products and livestock waste. In addition, this has an impact on environmental pollution and environmental safety issues. High egg consumption from the food industry and households will result in more shell waste. This waste product will have a bad impact on the environment and increase pollution (Ngayakamo et al. 2020). Eggshell waste comes from the food industry, including the bakery, egg powder processing, culinary, and other food industries that use eggs as raw material. Increasing added and economic value is sought to address environmental problems and solutions. Eggshell applications are widely used as plant fertiliser, animal feed, and in the food industry. Several food applications use eggshell products for enrichment and improvement of the quality of food processing products. Though it is categorised as waste, eggshell

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still contains useful contents, including calcium, protein, and other components.

Calcium is one of the constituent components of tooth and bone formation (Raskh 2020). Calcium supplementation is necessary for postmenopausal women and osteoporosis patients due to calcium deficiency (Capozzi et al. 2020). Additionally, many lactose-intolerant sufferers require new calcium supplements in micronutrient-enriched foods to meet their dietary calcium requirements (Kobus-Cisowska et al. 2020). 95% of the eggshell is made up of calcium carbonate ($CaCO_3$), along with other important elements like boron and strontium that are crucial in preventing osteoporosis (Arnold et al. 2021). It is possible to separate about 37–39% of calcium from chicken eggshells. Specific minerals are present in the eggshell, i.e. ash content 89.9 g per 100 g, calcium 35.10 mg per 100 g, magnesium 370 mg per 100 g, sodium 150 mg per 100 g, and phosphorus 120 mg per 100 g eggshell (Al-awwal et al. 2015). Eggshells processed into flour or powder and into nanoparticle size can be applied to restructured products. Utilising eggshells can have a major positive impact on nutritional value and, most importantly, on calcium content (Arnold et al. 2021).

Nanoparticles are the result of technological modification, i.e. nanotechnology. The nanotechnology has developed in all fields, one of which is the food sector. The principle of nanotechnology is the process of product improvement which includes physicochemical and biological properties of the product. An important role of nanoparticles is increasing the bioavailability of calcium components (Lou et al. 2020; Kobus-Cisowska et al. 2020). The inner eggshell contains bioactive components utilised and applied in cosmetics, nutrition, and nanotechnology (Leite et al. 2017; Lou et al. 2020; Baláž et al. 2021). In previous research shell flour was added to muffins, sausages, biscuits, chocolate cake, white bread, candy, and yoghurt.

One of the popular meat-based restructured products is patties. Patties are produced as a ready-to-eat food product, as a convenience for consumers. Patties are generally used as a burger filling with mayonnaise, sauce, and vegetables added in fast food restaurants. Patties commonly use chicken, beef, rabbit, and goat as basic ingredients (Ren et al. 2022; Shen et al. 2022; Evanuarini et al. 2023). Beef processed into various products aims to increase the shelf life of the meat. Patties have a good nutritional content but they still have a drawback, i.e. low calcium. The quality of beef patties can be improved by applying chicken eggshell nanopowder. This research aimed to identify the use of chicken eggshells to improve physicochemical quality, microstructure, and amino acid profile of beef patties.

MATERIAL AND METHODS

Research material. The research materials used in this study were beef patties made from beef thigh (70%) which was bought at a traditional market in Malang city, ice cubes (12%), tapioca flour (10%), egg white (0.75%), pepper (0.75%), onions (2.25%), garlic (1.125%), salt (0.75%), mushroom stock (0.75%), and carboxy methyl cellulose (CMC) (0.75%). Optional ingredients added were purchased at the supermarket in Malang city. The treatment carried out included adding chicken eggshell nanopowder to beef patties. Chicken eggshell nanopowder was produced in-house in the livestock products technology laboratory of the Faculty of Animal Science, Universitas Brawijaya. Beef patty samples with the addition of chicken eggshell nanopowder at different percentages were used for the analysis of the material.

Research methods. A completely randomised design with five treatments and replications was used in this study. The treatments carried out in this research were the addition of chicken eggshell nanopowder, without the addition of chicken eggshell nanopowder as a control (C) and with the addition of chicken eggshell nanopowder 0.1% (CE1), 0.3% (CE2), 0.5% (CE3), and 0.7% (CE4) (*w*/*w*).

Procedure for making chicken eggshell nanopowder. The eggshells were obtained from one of the bread producers in Malang. The eggshell was cleaned with running water, and the sticky white layer inside the eggshell was removed. Then, the eggshells were put into boiling water for ± 30 min. Once the eggshells were boiled, they were drained and dried for 3 h. Dried eggshells were prepared on a baking sheet lined with parchment and turned upside down. After that, the eggshells were put in a preheated oven at 80 ± 2 °C for 2 h. Then, the eggshells were drained and left for 1 × 24 h. The eggshells were ground in two stages. Stage 1 was grinding them with a pestle and mortar to reduce the size, while Stage 2 was in a dry mill to get a finer flour texture. Fine eggshells were sieved with a 300-mesh sieve. Chicken eggshell nanopowder was ready to be applied in food production.

Procedure for making beef patties with the addition of chicken eggshell nanopowder. The basic ingredient for making patties is a fresh boneless and skinless beef thigh fillet. Beef was cut in the size of 2 × 3 × 2 cm. Then, the beef was ground with a meat grinder (HR 2939 N; PT Philips, Indonesia) for 20 s and

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added ice cubes. Then other ingredients were added: tapioca, egg white, pepper, shallots, garlic, salt, sugar, and mushroom broth. The final ingredient added was chicken eggshell nanopowder following certain treatments, namely 0, 0.1, 0.3, 0.5, and 0.7%. An amount of 15 g of dough was then weighed and pressed into moulds to form loaves of the same size with a thickness of 2 cm (PT Tupperware Indonesia, Indonesia). Beef patties with chicken eggshell nanopowder were put on a baking sheet for 5 min at 120 ± 2 °C. Then, the beef patties were removed, cooled down at 25 ± 2 °C for 30 min, and baked over low heat for 8 min.

Physicochemical analysis. The chemical analysis of beef patties with chicken eggshell nanopowder followed the Association of Official Analytical Chemists (AOAC) methods for protein content (AOAC 2001.11) by Kjeldahl method, ash content (AOAC 942.05) using a gravimetric method using L 5/11/B furnace (Nabertherm, Germany), and fat content (AOAC 920.39) by Soxhlet extraction with Soxhlet extractor ISO K-6 set SOXH-SET-100 (Iwaki, Indonesia). pH measurements were carried out at room temperature using a digital pH meter (HI-8424N Waterproof pH Meter with Automatic Calibration; Hanna, UK). Antioxidant activity was determined using the method of Bondet et al. (1997). It was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The results were expressed as scavenging percentage, and measurements were performed in triplicate.

Determination of mineral content. The atomic absorption spectrophotometer sample was conditioned first. Then the absorbance of each sample solution was measured using an atomic absorption spectrophotometer instrument with a wavelength of 422.67 nm with an air-acetylene flame.

Sensory properties. The samples weighed roughly 10 g each after they were cooked and cut into smaller pieces. A five-person sensory panel evaluated the cooked samples for flavour, taste, and colour using a 5-point hedonic scale (1 being extremely disliked; 5 being highly liked) (Tolentino et al. 2021).

Scanning electron microscopy observation. The beef patties were prepared by coating with gold or platinum and observed using scanning electron microscope (SEM) (JSM-6510; Jeol, Japan) with the magnification of 100, 400, and 600 times, the resolution of 100 µm (Rahmawati et al. 2020).

Proximate amino acid profile. The amino acid profile analysis was carried out using a modified configuration in accordance with the Geldenhuys et al. (2015) method. After adding 30 g·kg^{-1} of beef patty sample, a selection of raw and cooked samples was defatted, dried, and flushed with N_2 gas for a duration of 30 s. After that, the samples were hydrolysed in a vacuum oven at 110 °C for 24 h using 6 mol·L^{-1} HCl and 150 g·L^{-1} phenol. The hydrolysed samples were then kept in microtubes at –20 °C until they were further examined, which could take up to two months. The use of Nexera (X2 HPLC; Shimadzu, Japan) allowed for the determination of the amino acid profile of the samples. Phthaldialdehyde was used to derivatise the sample for two min before injection. The temperature was 40 °C, 338 nm detection, and the Zorbax Eclipse AAA column (150 × 4.6 mm, 3.5 µm particle size; Agilent Technologies, USA) was used. The separation was carried out at a flow rate of 1.5 mL·min^{-1}. Mobile phase A consisted of 40 mmol·L^{-1} NaH_2PO_4 at pH 7.8, while mobile phase B consisted of 45% (*v*/*v*) acetonitrile (high-performance liquid chromatography (HPLC) quality, ≥ 99.8%, Merck, Germany), 45% (*v*/*v*) methanol (HPLC grade, ≥ 99.8%, Merck), and 10% (*v*/*v*) Milli-Q water (Merck). The gradient program was as follows: 2% B from 0 to 0.5 min; 57% B for 20 min; 100% B from 20.1 to 23.5 min; and 2% and 0% B from 23.6 to 25 min. The proportion (in percent) against the total identified was calculated by the amino acid.

Data analysis. Statistical analysis was performed using analysis of variance (ANOVA) to determine the effect of the chicken eggshell nanopowder treatment. Statistical analysis was performed using IBM SPSS (version 25). Duncan's test is at a 1% level of significance.

RESULTS AND DISCUSSION

pH value. The results of the analysis of various beef patties with the addition of chicken eggshell nanopowder show a very significant effect ($P < 0.01$) on pH. Table 1 shows the average pH of beef patties with the addition of chicken eggshell nanopowder. The addition of chicken eggshell nanopowder could increase the pH value. The pH of eggshells can change the concentration of OH^- ions during the reaction, and calcium (Ca^{2+}) helps stabilise the pH value (Goh et al. 2021). In addition, an increase in pH can be caused by the presence of carbonate groups in the eggshell, which can potentially increase the number of negative ions, so the pH balance reaches 6 (Abatan et al. 2020). According to the research of Suryanto et al. (2014), the pH value increases in the processing of meat products, which is caused by the enrichment with calcium in the eggshell.

Protein content. The results of the analysis of various beef patties with the addition of chicken eggshell nanopowder show a very significant effect ($P < 0.01$) on pro-

https://doi.org/10.17221/156/2023-CJFS

Table 1. Physicochemical quality of beef patties with chicken eggshell nanopowder

Parameters	C	CE1	CE2	CE3	CE4
pH value	$6.03^{a} \pm 0.01$	$6.14^{a} \pm 0.04$	$6.30^{b} \pm 0.05$	$6.42^{b} \pm 0.03$	$6.62^{c} \pm 0.16$
Protein content (%)	$15.95^{a} \pm 0.04$	$16.15^{ab} \pm 0.10$	$17.14^{bc} \pm 0.10$	$17.49^{cd} \pm 0.17$	$18.15^{d} \pm 0.02$
Ash content (%)	$2.24^{a} \pm 0.09$	$2.66^{b} \pm 0.10$	$2.94^{c} \pm 0.09$	$3.35^{d} \pm 0.11$	$3.71^{e} \pm 0.04$
Fat content (%)	$17.88^{a} \pm 0.08$	$18.14^{b} \pm 0.03$	$18.27^{b} \pm 0.04$	$18.38^{c} \pm 0.04$	$18.39^{c} \pm 0.08$
Antioxidant activity (%)	$2.42^{a} \pm 0.29$	$4.25^{b} \pm 0.38$	$8.16^{c} \pm 0.67$	$10.16^{d} \pm 0.49$	$11.44^{d} \pm 0.57$
Calcium content (g)	$0.19^{a} \pm 0.01$	$0.24^{b} \pm 0.01$	$0.29^{c} \pm 0.01$	$0.35^{d} \pm 0.01$	$0.41^{e} \pm 0.02$

$^{a–e}$ in the same column show a highly significant difference ($P < 0.01$); C – without the addition of chicken eggshell nanopowder as control; CE1 – the addition of chicken eggshell nanopowder 0.1%; CE2 – the addition of chicken eggshell nanopowder 0.3%; CE3 – the addition of chicken eggshell nanopowder 0.5%; CE4 – the addition of chicken eggshell nanopowder 0.7%

tein levels. Table 1 shows the average protein content in beef patties with the addition of chicken eggshell nanopowder. The main source of protein levels in beef patties is beef. The largest component found in meat protein is myofibrillar protein consisting of myosin, actin, tropomyosin, and troponin (Liu et al. 2021). In addition, adding chicken eggshell nanopowder also contributed to an increase in the amount of protein. Eggshells contain hundreds of proteins that can interact with other minerals to improve the structure (Gautron et al. 2021). The protein in high-quality eggshells has acid and alkali resistance, so it has the potential to be converted into bioactive components (Zhao et al. 2019).

Ash content. The results of the analysis of various beef patties with the addition of chicken eggshell nanopowder show a very significant effect ($P < 0.01$) on the ash content. Table 1 shows the average ash content of beef patties with the addition of chicken eggshell nanopowder. The eggshell has chemical mineral components, including calcium, calcium carbonate, phosphorus, and ash content. Eggshell flour extracted using an acid has an ash content of 86.48% (Rosnah et al. 2021). The increased ash content of beef patties occurred in each treatment. The ash content shows the amount of minerals contained in the eggshell (Ooi et al. 2012). Chicken eggshell flour is indicated to be rich in calcium and phosphate content, so it can increase the amount of ash content (Omelka et al. 2021).

Fat content. The results of the analysis of various beef patties with the addition of chicken eggshell nanopowder show a very significant effect ($P < 0.01$) on fat content. Table 1 shows the average fat content in beef patties with the addition of chicken eggshell nanopowder. The source of fat content in beef patties is beef used as a basic ingredient. The increase in fat content was caused by the difference in the amount of addition of chicken eggshell nanopowder and possibly also due to different fat content in the same amount of meat. Previous study showed that eggshell powder added to biscuits increased fat content (Arif et al. 2022). Other similar studies also found that the fat content increased with the use of eggshell powder by as much as 8% (Chilek et al. 2018).

Antioxidant activity. The results of the analysis of various beef patties with the addition of chicken eggshell nanopowder show a very significant effect ($P < 0.01$) on antioxidant activity. Table 1 shows the average antioxidant activity in beef patties with the addition of chicken eggshell nanopowder. The protein in eggshells can increase antioxidant activity. Small peptides contain aromatic amino acids and hydrophobic amino acids. Two types of amino acids reduce oxidative stress. Using eggshells can increase and maximise antioxidant activity (Neimi et al. 2022). Eggshell proteins can be converted into bioactive peptides as antioxidants, producing various functional properties (Zhao et al. 2019). Previous study mentioned the existence of peptide proteins that have the potential as bioactive antioxidants (Zhao et al. 2020).

Calcium content. The results of the analysis of various beef patties with the addition of chicken eggshell nanopowder show a very significant effect ($P < 0.01$) on calcium. Table 1 shows the average of calcium content in beef patties with the addition of chicken eggshell nanopowder. Beef patties without the addition of chicken eggshell nanopowder have little calcium while beef patties with chicken eggshell nanopowder treatment showed an increased content with increasing amounts added. This is due to the amount of calcium in the chicken eggshell nanopowder. The calcium content in chicken eggshell nanopowder is 11.81 g. The chicken eggshell has high calcium content and other forms of inorganic calcium carbonate, making it effective for improving the bone structure with respect to osteoporosis (Omelka et al. 2021). Another study showed that the amount of calcium in eggshells extracted with two different ingredients produced 35% and 27% calcium (Rosnah et al. 2021).

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Sensory properties. The sensory scores for beef patties with the addition of 0.5% and 0.7% chicken eggshell nanopowder were almost the same as in the control treatment. Significant changes occur in taste, flavour, and colour. The higher the addition of chicken eggshell nanopowder, the more the panellists liked the taste, flavour and colour produced. The beef patties tend to have a sandy taste. This is because chicken eggshell nanopowder was ground into nanoparticles, thereby minimising the sandy taste of beef patties. The research results of Chilek et al. (2018) indicated that they had a sandy taste after adding more and more eggshell. The flavour produced by beef patties is smoky. Apart from that, the colour produced in beef patties with chicken eggshell nanopowder tends to be brighter than in the control treatment. The colour of the beef patties with the addition of chicken eggshell nanopowder was liked by the panellists.

Scanning electron microscopy. The results of scanning electron microscopy can be seen in Figure 1.

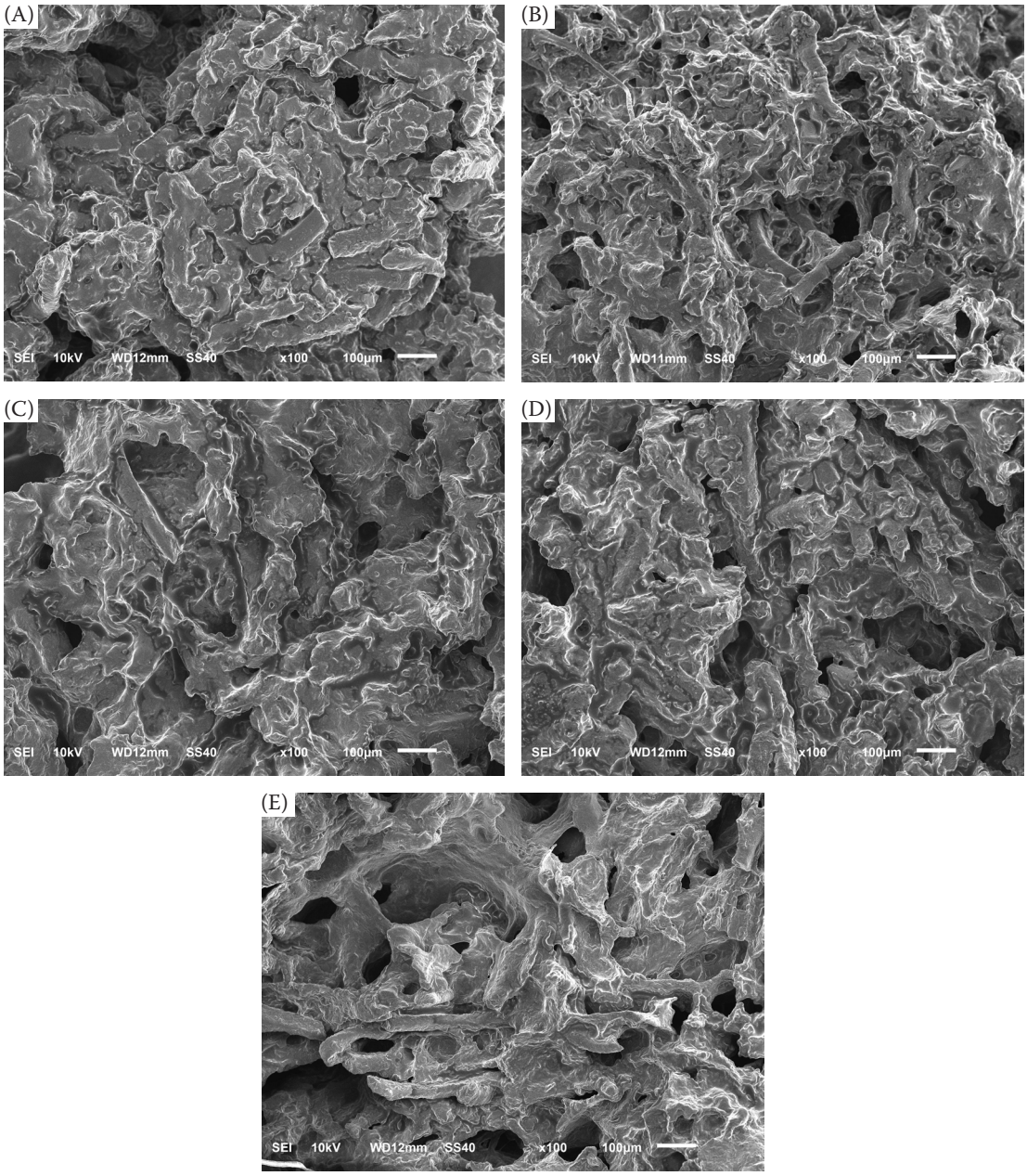

Figure 1. Results of observations of the microstructure of beef patties using scanning electron microscopy at 100 × magnification: (A) without additions, (B) with 1% chicken eggshells nanopowder, (C) with 3% chicken eggshells nanopowder, (D) with 5% chicken eggshells nanopowder, and (E) with 7% chicken eggshells nanopowder

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Microstructural observations using SEM clearly show that the addition of chicken eggshell nanopowder at different percentages can change the microstructure of beef patties. The meat protein network is more compact and denser with 0.7% chicken eggshell nanopowder than with 0.1% and no addition of chicken eggshell nanopowder. The increase in gel network compactness was followed by increased tissue integrity, indicating that the beef patties experienced cohesiveness. The air voids produced in beef patties with the addition of 0.7% chicken eggshell nanopowder were fewer than with other treatments. The fewer air voids indicate increased compactness of the tissue structure. Improvements in the microstructure of patties can be influenced by the presence of protein and calcium in chicken eggshell nanopowder. Calcium incorporation can improve the microstructure of the gel network in the presence of polysaccharide components (Zhao et al. 2020). The addition of $CaCl_2$ to meat can facilitate the formation of a meat gel structure with smaller voids and an increased level of uniformity (Gong et al. 2023).

Amino acid profile. The amino acid profile of beef patties with the addition of chicken eggshell nanopowder shows the content of various types of amino acids (Table 2). The number of amino acids did not change significantly in the samples before and after cooking. Amino acids in beef patties can be converted into bioactive components, i.e. antioxidant compounds, to prevent lipid oxidation to extend the product shelf life. Calcium in chicken eggshell nanopowder can act as an antioxidant agent in a product (Zhao et al. 2020). The total amount of amino acids in beef patties before cooking was 14.77% and after cooking, it was 14.83%. The detected amino acids were essential and non-essential amino acids. The essential amino acids in beef patties included lysine, threonine, valine, methionine, isoleucine, leucine, phenylalanine, and arginine. In this case, the highest essential amino acid was lysine.

Furthermore, there were essential amino acids, namely glutamic acid, aspartic acid, and alanine. The highest conditionally essential amino acid found was glutamic acid. Finally, there were non-essential amino acids, namely serine, glycine, tyrosine, and histidine. The highest non-essential amino acid found in beef patties was glycine.

Table 2. Amino acid profile of beef patties with chicken eggshell nanopowder (%)

Type of amino acid	Raw (*w*/*w*)	Cooked (*w*/*w*)
Aspartic acid	1.59	1.57
Threonine	0.73	0.75
Serine	0.64	0.65
Glutamate	3.04	3.05
Glycine	0.70	0.68
Alanine	0.95	0.97
Valine	0.81	0.84
Methionine	0.41	0.42
Ileucine	0.77	0.79
Leucine	1.32	1.36
Tyrosine	1.32	0.51
Phenylalanine	0.53	0.41
Histidine	0.63	0.61
Lysine	1.41	1.46
Arginine	0.75	0.76
Amino acid total	14.77	14.83

CONCLUSION

Beef patties with 0.7% chicken eggshell nanoparticles could enrich nutrition with chemical properties. Apart from that, microstructural observations were also carried out to produce beef patties with a more compact, dense, and uniform structure characterised by fewer air es and various types of amino acids in beef patties. Product shelf life needs to be developed to maximise the use of chicken eggshell nanopowder in food products.

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