

Nutrient Profile and Antioxidant Activity of Functional Processed Cheese Cube Fortified with Nano Moringa Extract

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Abstract. Cheese is a dairy product with high nutritional value and continues to experience increasing consumption in Indonesia. Innovation in the development of functional processed cheese cubes (PCC) made from natural ingredients is needed to increase nutritional value and health benefits. *Moringa oleifera* (MO) is a source of bioactive compounds such as flavonoids, phenolics, beta carotene, and macro and micro nutrients that have the potential to improve the functional quality of food products. This study aims to encourage the effect of the addition of nano moringa extract (NME) on the content of carbohydrates, protein, fat, crude fiber, and antioxidant activity in processed cheese cubes. MO was extracted using the maceration method, then converted into nano form through spray drying techniques to increase the bioavailability and stability of active compounds. PCC formulations were made with the addition of NME at concentrations of 0%, 0.2%, 0.4%, and 0.6%. The results showed that the addition of NME significantly affected all tested parameters. Carbohydrate content increased in the 0.2% and 0.4% treatments, with the highest value of 18.71% at 0.4% NME, but decreased at 0.6% due to nanoparticle aggregation that hindered dispersion homogeneity. The highest protein content was found at 0.2% NME at 7.46%, while higher concentrations showed a decrease due to the dilution effect and potential protein denaturation during the nanoencapsulation process. Fat content decreased with increasing NME concentration, with the lowest value of 27.54% at 0.4% NME, influenced by the activity of bioactive compounds that play a role in binding lipids. Crude fiber content tended to decrease at high concentrations due to degradation of the fiber fraction and protein-polysaccharide interactions. Antioxidant activity increased significantly, marked by the lowest IC₅₀ value of 7.98 µg/mL at 0.6% NME which is included in the very strong category.

Keywords: antioxidant, cheese cubes, *Moringa oleifera*, nano, nutritional profile

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1 Introduction

Cheese is a dairy product with high nutritional value and has become a staple in many food products, both as a primary ingredient and as an additive in various food products. In recent years, cheese consumption in Indonesia has increased significantly, along with the increasing variety of cheese products on the market, from processed cheeses to traditional cheeses, which meet the needs of various consumer segments. Cheese, particularly processed cheeses such as cheddar and mozzarella, is popular for its nutritional content, rich in protein, fat, minerals, and vitamins, all of which are beneficial for health. Processed cheeses have the advantage of better shelf life and a more stable texture than natural cheeses. Innovation in the development of functional processed cheese products is increasingly needed to increase the utility and health benefits of cheese products.

MO is known as a functional plant because it contains a variety of nutrients and bioactive compounds such as protein, carbohydrates, crude fiber, minerals, beta-carotene, and flavonoids, which act as natural antioxidants. This plant is also known as The Miracle Tree due to its comprehensive nutritional content and extensive health benefits, including its ability to ward off free radicals and protect cells from oxidative damage [1]. Several studies have shown that adding MO to food products can increase the protein, fat, fiber, and mineral content, potentially enhancing the functional value of these products. Based on proximate analysis, 5 g of MO contains high levels of crude protein (17.01%), carbohydrate (63.11%), crude fiber (7.09%), ash (7.93%), and fat (2.11%). Mineral content per million (ppm) includes Ca 1.91%, K 0.97%, Na 192.95%, Fe 107.48%, Mn 81.65%, and P 30.15% [2]. The presence of antioxidant compounds such as beta-carotene and flavonoids also make MO a potential ingredient for improving the oxidative stability of dairy products.

The developing MO nano extracts is an innovative strategy to increase the effectiveness and bioavailability of its active compounds. The extraction process using the maceration method is considered capable of maintaining the stability of thermolabile compounds in MO, while converting the extract into nano form through a spray-drying technique allows for increased particle surface area so that the active compounds are more easily absorbed by the body. The extract was then made into nano-form using a spray-drying technique with maltodextrin and whey protein as coating materials to protect the active compounds. The addition of NME to PCC is expected to increase the carbohydrate, protein, fat, crude fiber, and antioxidant content. Thus, this processed cheese innovation has the potential to provide more optimal nutritional benefits and added health value for consumers.

2 Materials and methods

2.1 Material

The main ingredient in making NME is dried Moringa leaves obtained from the Pusat Kelor Madura Marongghi, Bluto, Sumenep, East Java, then Moringa leaf powder production is carried out at the UPT Herbal Materia Medica Laboratory, Batu, East Java. Additional ingredients used include 96% food grade ethanol, maltodextrin, tween 80, and emulplex. Ingredients for making processed cheese consist of fresh cow's milk from Toromiri Farm, Bumiaji, Batu, East Java, liquid starter of lactic acid bacteria from CV Brawijaya Dairy, Dau, Malang, East Java. Other ingredients used include rennet, calcium chloride (CaCl_2), curd, coconut oil, emulsifier SP (Koepoe Koepoe), skim milk (NZMP), maltodextrin, carrageenan, distilled water (Hydrobat), lactic acid, and salt.

2.2 Equipment

The equipment used in the research consisted of, digital thermometer (Taffware), gloves, micropipette (Dragonlab), blue tip, 250 mL measuring cup (Herma), 500 mL Erlenmeyer flask (Iwaki), glass funnel (Pyrex), 50 mL beaker glass (Pyrex), aluminum foil, chopper (Signora), cheese cloth, spray dryer (Buchi), ultra turrax homogenizer (IKA T25 Digital), digital scale (Taffware Digipounds 1-2000), analytical scale (BC Series OHAUS Centrogram Balance), refrigerator (Midea), autoclave (Hiramaya HL-36Ae), cheese mold, and baking paper.

2.3 Research Methods

This study used an experimental method in a laboratory with a completely randomized design (CRD) consisting of four treatments, namely P0 (control), P1 (0.2% NME), P2 (0.4% NME), and P3 (0.6% NME) each carried out in three replications. The parameters measured in this study were carbohydrate, protein content, fat content, fiber content and antioxidant activity.

2.4 Nano Moringa Extract

The process of making NME begins with the extraction stage using the maceration method. A total of 100 g of Moringa leaf powder was soaked in 96% ethanol solvent with a ratio of 1:5, then stored at room temperature for 24 h. The maceration filtrate was then evaporated using a rotary vacuum evaporator at 60°C until a thick extract was obtained. The Moringa leaf extract was then prepared as an oil phase by adding 5% tween 80 and 0.5% emulplex. The mixture was then homogenized using an ultraturax homogenizer at a speed of 36.000 rpm at room temperature (27-30°C) for 7 min until a stable emulsion was formed. The next step was the production of NME. The resulting extract was mixed with 5% maltodextrin previously dissolved in 250 mL of distilled water, then homogenized for 2 min. The mixture was then spray-dried at an inlet temperature of 180°C and an outlet temperature of 70°C to produce a powdered form of NME [3]. The final product was then analyzed to determine the yield and size of the nanoparticles.

2.5 Processed cheese cubes

The process of making PCC involves two main stages, curd production and cheese processing. The curd production process uses 1 L of fresh cow's milk, 0.05% rennet, 3% LAB starter culture, and 0.04% calcium chloride (CaCl₂). The resulting curd is then used as the base ingredient in the PCC formulation. The ingredients include 15% curd, 35% cheddar, 0.414% vegetable oil, 0.525% salt, 0.30% maltodextrin, 0.75% skim milk, 0.30% SP as an emulsifier, 0.018% lactic acid, 0.60% carrageenan, 0.75% distilled water, and NME at 0% (P0), 0.2% (P1), 0.4% (P2), and 0.6% (P3) of the total weight of the ingredients.

The curd production process begins with pasteurizing the milk using a double boil method to 72°C for 15 s. Afterward, the milk temperature is lowered to 42°C and 30 mL of LAB starter is added. The mixture was covered with aluminum foil and incubated for 1 h for initial fermentation. Then, 0.5 mL of rennet and 0.4 mL of CaCl₂ were added, covered again with aluminum foil, and left for 2 h until a coagulum formed. The curd was then cut into small cubes, filtered to separate the whey, and the resulting curd was weighed to determine its final weight.

The process of making PCC began by placing the cheddar cheese into a chopper, followed by the addition of curd, SP, and vegetable oil. The mixture was chopped for 5 min, then, while the machine was running, skim milk, maltodextrin, and carrageenan, previously dissolved in distilled water, were added. Once the carrageenan was mixed, the chopping

process was continued for another 5 min, followed by salt and lactic acid. The resulting homogeneous mixture was transferred to a stainless steel container, covered with aluminum foil, and allowed to stand for 1 h. Next, it was heated using the double boil method for 2.5-3 min. NME is then added and stirred quickly until evenly mixed, then the mixture is molded into cheese cubes.

2.6 Carbohydrate

Carbohydrate determination was carried out using the by difference method [4]. The dried and homogenized samples were analyzed for moisture, ash, protein, and fat content. The moisture content was determined by drying at a temperature of $105 \pm 2^\circ\text{C}$ to a constant weight, the ash content was obtained by combustion in a furnace at a temperature of $550 \pm 25^\circ\text{C}$, the protein content was analyzed using the Kjeldahl method with a conversion factor of 6.25, and the fat content was determined by Soxhlet extraction. The total carbohydrate content was calculated indirectly by subtracting the total percentage of moisture, ash, protein, and fat content from 100%.

2.7 Protein content

Protein content testing using the Kjeldahl method, which includes three main stages, namely the destruction, distillation and titration processes. First, the sample is accurately weighed and placed into a digestion tube, then concentrated sulfuric acid (H_2SO_4) and a catalyst such as selenium are added to facilitate decomposition. The mixture is heated until all organic components break down, resulting in a clear solution containing nitrogen. In the distillation stage, the digested solution is diluted with distilled water and neutralized using sodium hydroxide (NaOH) to release ammonia (NH_3), which is then collected in a boric acid (H_3BO_3) solution containing an indicator, producing a bluish-green distillate. The final stage is titration, which involves standardizing 0.02 N HCl, titrating the distillate with the standardized acid until a color change occurs, and performing a blank determination as a control. Nitrogen content was obtained from the HCl titration volume and converted to protein content using a conversion factor of 6.25 [4].

2.8 Fat content

Fat content was determined using the modified Soxhlet method [4]. Cotton and filter paper were first dried in an oven at 105°C for 30 min, then cooled in a desiccator for 30 min and weighed. Cheese samples with known moisture content were placed in filter paper and cotton, then weighed to determine the sample weight. The samples were wrapped in a sleeve and tied with wool string, then placed in the Soxhlet apparatus. 500 mL of petroleum ether was added to the extraction flask, and the extraction process was carried out for 4 h. After completion, the sample sleeve was removed, dried in an oven at 105°C for 24 h, then cooled in a desiccator for 30 min and reweighed. Fat content (%) was calculated based on the difference in weight before and after extraction using the formula:

$$\text{Fat content (\%)} = \frac{\text{Final flask weight} - \text{Initial flask weight}}{\text{Dry sample weight}} \times 100\% \quad (1)$$

2.9 Crude fiber

Determination of crude fiber content was carried out using the Gravimetric method [4]. A total of 2 g of sample was weighed and placed into a 500 mL Erlenmeyer flask, then added a

boiling 0.25 N H₂SO₄ solution (1.25 g concentrated H₂SO₄/100 mL) and refluxed using a reverse condenser for 30 min while gently shaking. The suspension was filtered, then the residue was washed with boiling water until neutral. The residue was transferred quantitatively back into the Erlenmeyer flask and added 200 mL of boiling 0.31 N NaOH solution (1.25 g NaOH/100 mL), then refluxed again for 30 min with a reverse condenser. After that, the mixture was filtered using filter paper of known weight, then the residue was washed successively with a 10% K₂SO₄ solution, boiling water, and 15% alcohol of ±15 mL. The filter paper and residue were dried in an oven at 100°C for 1-2 h until constant weight, then cooled in a desiccator and weighed. The crude fiber content (%) was calculated based on the difference in the weight of the dry residue to the initial weight of the sample using the formula:

$$\text{Crude fiber (\%)} = \frac{\text{Residual weight (g)} \times 100\%}{\text{Sample weight (g)}} \quad (2)$$

2.10 Antioxidant activity

Antioxidant activity was determined using the DPPH method [5]. A 5 g sample was added with 10 mL of 95% ethanol, then vortexed until homogeneous and centrifuged at 4000 rpm for 10 min to separate the antioxidant extract from the sediment. A 1 mL 0.2 mM DPPH solution in 95% ethanol was mixed with 4 mL of the antioxidant extract, then the mixture was left for 10 min at room temperature until a color change from purple to yellowish indicating free radical scavenging activity. The absorbance of the solution was measured at a wavelength of 517 nm using a spectrophotometer. Free radical scavenging activity (% RSA) was calculated using the formula:

$$\% \text{ RSA} = \frac{\text{Blank absorbance} - (\text{Absorbance of sample without DPPH} - \text{Sample absorbance with DPPH})}{\text{Blank absorbance}} \times 100 \quad (3)$$

The calculation results are then entered into a regression equation with the extract concentration (ppm) as the abis (X-axis) and the % value (antioxidant) as the coordinate (Y-axis). The IC₅₀ value from the calculation when the % inhibition is 50% is y = ax-b.

2.11 Statistical Analysis

The data obtained from this study were tabulated using Microsoft Excel (2020) and subsequently analyzed statistically using Analysis of Variance (ANOVA). If significant or highly significant differences were found among treatments, the analysis was followed by Duncan’s Multiple Range Test (DMRT).

3 Results and discussion

Table 1. Nutritional profile of PCC with various percentages of natural fortification.

PCC	Carbohydrate (%)	Protein (%)	Fat (%)	Crude Fiber (%)	IC ₅₀ (µg/mL)
NME 0%	6.07±6.90 ^b	5.83±0.25 ^b	31.85±0.48 ^a	0.58±0.14 ^a	161.19±1.54 ^a
NME 0.2%	13.16±4.24 ^a	7.46±0.26 ^a	31.43±1.04 ^a	0.34±0.16 ^b	44.43±8.01 ^b
NME 0.4%	18.71±2.34 ^a	5.40±0.64 ^b	27.54±1.93 ^b	0.28±0.07 ^b	29.76±4.09 ^c
NME 0.6%	13.71±1.72 ^a	5.58±0.46 ^b	31.18±0.78 ^a	0.47±0.12 ^{ab}	7.98±0.87 ^d

Description: a,b different superscripts in the same row indicate that the provision of NME had a significant difference (P<0.05) on PCC.

3.1 Carbohydrate

Analysis of variance showed that the addition of NME to PCC had a significant effect on the carbohydrate content. This indicates that fortification with different NME concentrations altered the carbohydrate composition of processed cheese. The increase in carbohydrate content at 0.2%, 0.4%, and 0.6% NME compared to the control (0% NME) was mainly due to the high content of phytonutrients and carbohydrates naturally present in MO leaves [6]. PCC with 0.4% NME showed the highest carbohydrate content (18.71%), indicating that this concentration represents the optimal level at which carbohydrates from NME can be evenly dispersed within the cheese matrix and effectively contribute to the total carbohydrate fraction. NME improves stability and dispersibility in food systems, facilitating better integration of carbohydrate components into the matrix. At 0.6% NME concentration, the carbohydrate content decreased to 13.71%, which may be related to the excessive concentration of nanoparticles causing aggregation phenomena. Nanoparticle aggregation can reduce homogeneity and limit the availability of carbohydrate components, thereby reducing their functional contribution to the food matrix [7]. NME fortification significantly increased the carbohydrate content compared to the control, the difference between 0.2%, 0.4%, and 0.6% NME treatments was not completely significant, indicating that the increase in carbohydrate content was concentration-dependent but not completely linear.

3.2 Protein content

The addition of NME significantly affected the protein content of PCC. The highest protein content was observed at 0.2% NME (7.46%), while the lowest protein content occurred at 0.4% NME (5.40%). The increase in protein content at low NME concentrations can be attributed to the presence of essential amino acids in MO, such as leucine, valine, and lysine, which contribute to the vegetable protein fraction in the product [8]. In nano, the increased surface area of MO particles facilitates better dispersion and interaction of amino acids and proteins with the casein matrix during cheese processing. Conversely, at higher NME concentrations (0.4% and 0.6%), a decrease in protein content was observed, which can be explained by the dilution effect caused by the increased proportion of non-protein components of NME, including fiber, phenolic compounds, and complex carbohydrates. High-energy mechanical processing during nanoencapsulation can cause partial protein denaturation, which affects the structure and solubility of the protein and consequently reduces the protein values measured by proximate analysis [9]. The increase in protein levels at 0.2% NME and the decrease at 0.4% NME and 0.6% indicate that the addition of NME effectively increases protein only at low concentrations, but is less than optimal or even reduces protein levels at higher concentrations.

3.3 Fat content

The fat content of PCC was significantly affected by the addition of NME, with the lowest fat content observed at 0.4% NME (27.54%) and the highest in the control treatment (31.85%). The decrease in fat content may be related to the presence of bioactive compounds in MO, such as flavonoids, saponins, tannins, and phytosterols, which are known to have lipid-lowering properties [10]. Nanoencapsulation of MO extract increased the surface area and reactivity of these bioactive compounds, promoting stronger interactions with the protein and aqueous phases of the cheese matrix. This interaction may accelerate lipid binding and partially inhibit fat retention, thus contributing to the measured decrease in fat content [11]. The higher the concentration of NME added the lower the resulting PCC fat content,

suggesting that NME play a role in modifying the distribution and stability of fat in the cheese matrix through lipid binding and partial substitution by non-fat components.

3.4 Crude Fiber

The results showed that the addition of NME significantly affected the crude fiber content of PCC. Treatment with 0.4% NME resulted in the lowest crude fiber value (0.28%), while the control without NME (0%) showed the highest crude fiber content (0.58%). This indicates that the addition of NME does not directly increase the crude fiber content. Although MO contains high amounts of insoluble fiber, its contribution to the processed product depends on the extraction rate, particle size, and interaction with the food matrix [12]. During nanoencapsulation, the fiber component of MO can be dispersed more homogeneously and can undergo mechanical degradation, thereby reducing the measured crude fiber content. The fiber fraction of MO, consisting of cellulose, hemicellulose, and lignin, can be partially degraded during processing, including nanoencapsulation. This explains the low crude fiber values observed with 0.4% and 0.6% NME. In addition, the interaction between finely dispersed NME and milk proteins, such as casein, can form complexes that are not detected as crude fiber in gravimetric analysis [13]. The addition of NME significantly changes the crude fiber content, but this change is not linear. The interaction mechanism between nanofibers and cheese components, the nanoencapsulation process, protein-polysaccharide bonds, and mechanical degradation of the fiber are factors that determine the final crude fiber content in PCC products.

3.5 Antioxidant activity

The addition of NME significantly increased the antioxidant activity of PCC, as indicated by a decrease in the IC_{50} value with increasing NME concentration. The control treatment (0% NME) showed the highest IC_{50} value (161.19 $\mu\text{g/mL}$), indicating weak antioxidant activity, while the lowest IC_{50} value was observed at 0.6% NME (7.98 $\mu\text{g/mL}$), which corresponds to very strong antioxidant activity. The lowest IC_{50} value (7.98 $\mu\text{g/mL}$) was obtained at 0.6% NME, indicating very strong antioxidant activity. This increase was due to the high content of bioactive compounds in nano MO, such as flavonoids, alkaloids, phenolics, β -carotene, and quercetin, which act as free radical scavengers by donating hydrogen atoms, thereby increasing the antioxidant capacity [14]. Nanoencapsulation increases the dispersion and availability of these antioxidant compounds, thereby enhancing their ability to donate hydrogen atoms and neutralize free radicals. Based on the IC_{50} classification, values below 50 $\mu\text{g/mL}$ indicate very strong antioxidant activity, while values between 150–200 $\mu\text{g/mL}$ are considered weak [15]. Thus, PCC enriched with 0.6% NME showed superior antioxidant potential compared to the control, which highlights the effectiveness of NME in enhancing the functional properties of processed cheese.

4 Conclusion

The addition of NME significantly affected the nutritional and functional characteristics of PCC. Carbohydrate levels increased at 0.2-0.6% NME with the highest value at 0.4%, while protein content increased only at 0.2% NME and decreased at higher concentrations due to dilution and possible denaturation effects. Fat content consistently decreased with increasing NME levels as a result of lipid-binding activity of Moringa bioactives, whereas crude fiber tended to decrease at higher concentrations due to fiber degradation and interaction with the

cheese matrix. Antioxidant activity improved substantially, with 0.6% NME showing very strong activity.

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