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RESEARCH ARTICLE

Beetroot as a Source of Antioxidants and Natural Colouring in Fresh Cheese Making from Cow's Milk

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Abstract

Beetroot has potential as an additional ingredient in cheese making. Beetroot contains betalain which can be used as a natural colourant and has antioxidant properties. The objective of this research was to investigate the characteristics of fresh cheese with the addition of beetroot extract. The treatment in this study was cheese making with the addition of beetroot extract (1%, 2%, 3%, and 4%). The results showed no difference in yield, pH, moisture content, total solids, gumminess and chewiness of cheese. Beetroot extract can increase antioxidant activity, and can change the colour of cheese. L, b*, chroma, and whiteness index values decreased, while a* and hue values increased as the level of beetroot extract added increased. This indicates that the higher the level of beetroot extract added, the more intense the cheese colour will be. It can be concluded that the addition of beetroot extract can increase the antioxidant activity and change the colour of cheese starting from 1% level.

Keywords: Cheese, Beetroot, Antioxidant, Instrumental Colour, Texture Profile.

1. Introduction

Cheese is a dairy product that has high economic and nutritional value. Cheese consumption has beneficial health effects. Cow's milk cheese is an important source of protein for muscle repair and growth (Pratelli et al., 2024)"lactase-deficient" individuals cannot digest milk's main carbohydrate, lactose, depriving themselves of highly beneficial milk proteins like casein, lactoalbumin, and lactoglobulin due to lactose intolerance (LI . Cheese contains 3% to 40% protein, including essential amino acids that are important for the body (O'Callaghan et al., 2017) . Cheese is beneficial for health as an antioxidant, antimicrobial, and immunological (Santiago-López et al., 2018) cheese is manufactured by converting fluid milk to a semisolid mass through the use of a coagulating agent, such as rennet, acid, heat plus acid, or a combination thereof. Cheese can vary widely in its characteristics, including color, aroma, texture, flavor, and firmness, which can generally be attributed to the production technology, source of the milk, moisture content, and length of aging, in addition to the presence of specific molds, yeast, and bacteria. Among the most important bacteria, lactic acid bacteria (LAB. Cheese can also promote gut health and improve digestion as it contains probiotics (Chon et al., 2016)milk-derived bioactive peptides have been identified as potential ingredients found in health promoting functional foods. These bioactive peptides target diet-related chronic diseases, particularly non-communicable ones such as cardiovascular disease, diabetes and obesity. Additionally probiotics such as lactic acid bacteria (LAB . Cheese is a versatile ingredient used in various food products. Cheese can enhance the flavour, texture and nutritional value of various dishes (Guinee, 2011). Many coloured cheeses use synthetic

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colouring, making them less appealing to consumers who prefer natural ingredients. Cheese with beetroot added is one way to provide a healthier natural colour.

Beetroot offers many health benefits. Beetroot is high in bioactive compounds such as betalains and nitrates. The bioactive compounds in beetroot contribute to its anti-inflammatory, anticancer properties, support cardiovascular health and aid liver function (Stoica et al., 2025). Beetroot exhibits antioxidant properties that can fight free radicals in the body. Beetroot is rich in vitamins, minerals and amino acids, thus enhancing its status as a functional food (Rehman et al., 2024). Beetroot can reduce clinical systolic blood pressure in hypertensive patients (Grönroos et al., 2024). Beetroot has been shown to lower LDL cholesterol levels which contribute to cardiovascular health (Chen et al., 2021). Beetroot consumption has also been linked to improved athletic performance due to its nitrate content.

Cheese with beetroot added provides several benefits due to its nutritional and functional properties. The incorporation of beetroot can enhance the sensory attributes of cheese, improve nutrition, and provide health benefits. Beetroot is high in fibre, vitamins and minerals, thus contributing to a more nutritious cheese product (Dhawan & Sharma, 2019). Beetroot acts as a natural colourant, thus enhancing the visual appeal of cheese. This is especially beneficial in cheeses marketed as health foods.

Researching cheese with added beetroot is of great importance due to its potential health benefits, nutritional enhancement and natural colouring properties. Beetroot, which is rich in biologically active compounds, can enhance the functional value of cheese while providing a unique flavour and attractive colour. This research explores the novel application of beetroot extract to enhance fresh cheese by providing natural colouration and increased antioxidant activity, aiming to create an innovative, healthier functional food product as an alternative to cheeses using synthetic additives. Therefore, the objective of this study was to investigate the characteristics of fresh cheese with the addition of beetroot extract.

2. Materials and Methods

2.1 Research Material

This study used fresh cow's milk from the Experimental Farm of Jenderal Soedirman University, Purwokerto, Indonesia. Milk from the morning milking was mixed before pasteurisation for each replicate. Mesophilic bacteria (*Lactococcus lactis subsp lactis and Lactococcus lactis subsp cremoris*), rennet enzyme and CaCl2 were purchased from online market. Beetroot was purchased from local supermarket.

2.2 Preparation of Beetroot Extract

Beetroot was cut into small pieces, added 10% water, then juiced using a blender. Beetroot juice was filtered using 300 mesh filter cloth to obtain beetroot extract.

2.3 Cheese Making

Cow's milk was pasteurised at 72^0C for 15 seconds. Mesophilic bacteria of 0.01 gram were added to the milk after the temperature dropped between 37-45^0C and incubated for 45 minutes. Beetroot extract was added according to the treatment (1%, 2%, 3%, and 4%) after the incubation was completed. Milk curdling was carried out for 60 minutes at room temperature by adding 0.2 grams of CaC12 and 1 gram of rennet. The curd was cut and allowed to stand again for 60 minutes. Curd was filtered using cheesecloth and hung overnight at $15^{(0)C}$. Curd was moulded with a press. The cheese was conditioned overnight at 15^0C and then analysed.

2.4 Yield Calculation

The yield was calculated by weighing the fresh cheese and then calculated using the following formula:

$$Yield\ (\%) = \frac{weight\ of\ fresh\ cheese\ (g)}{weight\ of\ milk\ (g)} x\ 100\%$$

2.5 Acidity (pH) Measurement

The pH of the cheese was measured using a digital pH meter (Spear Tip pH Electrode PE-06HDA). The pH meter was calibrated using pH-7 and pH-4 buffer solutions before use. The pH measurement was performed twice for each sample.

2.6 Antioxidant Activity Measurement

Antioxidant activity was measured using a spectrophotometer (Mapada V-1100D) (Samadi & Fard, 2020). Antioxidant activity was calculated using the following formula:

$$Antioxidant\ activity\ (\%) = \frac{fDPPH\ absorb. - sample\ absorb}{DPPH\ absorb} x\ 100\%$$

2.7 Moisture Content and Total Solids

Moisture content and total solids were measured following the guidelines from Association of Official Analytical Chemists (AOAC, 2012) . Moisture content and total solids were calculated using the following formula:

$$Moisture\ Content\ (\%) = \frac{bInitial\ weight\ (g) - Final\ weight\ (g)}{Initial\ weight\ (g)} x100\%$$

$$Total\ solid\ (\%) = 100\ moisture\ content\ (\%)$$

2.8 Instrumental Colour

Colour was measured using a portable Colourimeter (DS-200 series) to determine the colour coordinates of the cheese sample (L, a*, b*). L indicates brightness from dark to light, a* indicates reddish or greenish, and b* indicates yellowish or bluish. Measurements were taken twice for each sample on different surfaces. Hue, chroma, and whiteness indices were calculated using the following formula (Kamal-Eldin et al., 2020) .

$$Hue = tan^{-1}\left(\frac{a^*}{b^*}\right) \quad 1$$

$$Chroma = \sqrt{(a^{*2} + b^{*2})} \quad 2$$

$$Whiteness\ indeks = 100 - \sqrt{((100 - L^*)^2) + a^{*2} + b^{*2}} \quad 3$$

2.9 Texture Profile

Texture was measured using a Food Texture Analyser (Stable Micro System TA.XT Plus, Surrey, UK) with a cylindrical probe (5 mm) (Setyawardani et al., 2023). Cheese samples were prepared at 1x1x1 cm and measured at 5 mm/sec.

2.10 Experimental Design and Data Analysis

The research was conducted at the Animal Product Technology Laboratory, Faculty of Animal Husbandry, Universitas Jenderal Soedirman, Purwokerto. Each treatment was repeated as many as four replicates. The treatments consisted of control (without beetroot extract) and the addition of beetroot extract as much as 1%, 2%, 3% and 4%. The data obtained were analysed by unidirectional analysis of variance (ANOVA). Tukey test was used when necessary. Data analysis was performed using Graphpad Prism version 9.5.1.733 (GraphPad Software, San Diego, California USA).

3. Results and Discussion

The graph below shows the effect of beetroot extract addition on cheese making at various levels (Figure 1). Parameters measured included yield, pH, moisture content, total solids, and antioxidants.

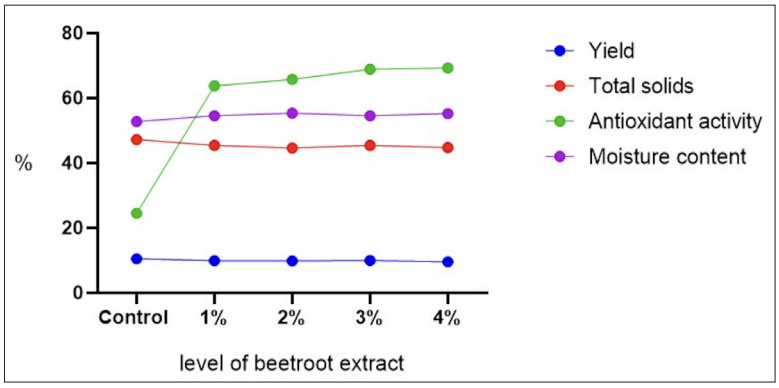

Figure 1. *Graph of characteristic values of cheese with beetroot extract added.*

3.1 Yield

The yield value was relatively stable in all variations of beetroot extract level, including the control. There was no significant increase in the yield value as the level of beetroot extract increased. Cheese yield depends on the amount of protein and fat in milk, which play a role in curd formation during coagulation. Beetroot does not contain significant protein or fat, so it does not affect the curd formation process. The average cheese yield in this study was 9.95%, while the cheese yield from cow's milk with beta-casein A1A2 genotype was 13.1% (Ladyka et al., 2023) . This means that the cow's milk used in this study has low quality, especially in the amount of protein and fat.

Cheese yield is the percentage of final cheese product obtained from raw milk at the end of the production process. Yield is calculated to determine the production efficiency and quality of cheese produced. Cheese yield is highly influenced by the quality of milk as raw material. Milk with high fat and protein content tends to produce higher cheese yields. The yield of soft cheese is higher than hard cheese due to the higher water content. Calculating cheese yield is an important step to ensure the production process is efficient, economical and produces a consistently high quality product.

3.2 pH

The pH value of cheese was constant despite the addition of beetroot extract (Figure 1). This indicates that beetroot extract does not affect the acidity of the cheese product. The pH of beetroot extract is 6.1-6.6 (Shakir & Simone, 2024) . The pH of beetroot extract is similar to the pH of control cheese, so the addition of beetroot extract has no effect on the pH of cheese. The addition of beetroot to mozzarella cheese also showed no change in the pH value (Setiaji *et al* . . 2019)

The average pH value of the cheese produced ranged from 6.52-6.66. These pH values are higher than the pH for beetroot cream cheese which is 4.54-4.9 (Sandhya & Priya, 2017) . Cheese with pH 6 showed better sensory acceptance (Talbot-Walsh et al., 2019) . Factors that can affect cheese pH include the type of milk used, starter culture, and processing conditions. Cheese pH can be influenced by the fermentation of lactic acid bacteria (*Lactococcus lactis subsp lactis and Lactococcus lactis subsp cremoris*), which produce lactic acid from lactose. Understanding these factors is critical to optimising cheese quality and characteristics.

3.3 Antioxidants

The antioxidant activity of cheese increased significantly with increasing levels of beetroot extract. The graph shows that the higher the level of beetroot extract, the higher the antioxidant content in the cheese product. This is because beetroot contains powerful antioxidants, such as betalains, vitamin C, flavonoids, and polyphenols. These antioxidants are soluble in the cheese matrix and remain stable during the production process. Therefore, beetroot can be a natural additive to make functional cheese, which is not only delicious but also healthier for consumers. The lowest antioxidant activity of cheese was obtained from the control treatment at 24.5%. The antioxidant activity of cheese with the addition of beetroot extract at 1%, 2%, 3%, and 4% levels were 63.77%, 65.77%, 68.93%, and 69.29%, respectively. Antioxidant activity in mozzarella cheese also increased after adding beetroot (Setiaji *et al* . ., 2019)

Beetroot contains vitamins, minerals, carotenoids, phenolics, nitrates, ascorbic acid and betalains (Chhikara et al., 2019) . This content is an antioxidant that can cause beetroot to have the potential to increase antioxidants in cheese. Cheese with added beetroot not only improves its nutritional profile, but also utilises its antioxidant properties to improve health outcomes. Beetroot antioxidants are associated with a range of health benefits, including improved blood pressure, enhanced athletic performance, and anti-inflammatory effects (Masih et al., 2019) .

3.4 Moisture Content and Total Solids

The moisture content value experienced a small change. The total solids value also tended to be stable, showing no drastic increase or decrease. This may indicate that beetroot extract does not significantly affect the moisture content and total solids in cheese products. The moisture content of cheese depends on the pressing process of the curd after coagulation. Cheese is moulded and pressed with the same process and time to obtain cheese with a stable moisture content. The average moisture content of cheese in this study reached 54.5%, which was obtained from cheese with 1-4% beetroot extract added. This moisture content is similar to that of beetroot cheese in previous studies which was 55% (Sandhya & Priya, 2017) .

Moisture content is an important factor in determining the textural properties of cheese, affecting chewiness, melting ability and overall sensory attributes. Increased moisture content in cheese generally results in a softer texture. Moisture content can also affect the flavour of cheese, as it affects flavour release and perception. Moisture content is closely related to the functional properties of cheese, such as spreadability and melting ability. An increase in moisture content can enhance certain texture and flavour attributes, so it is important to balance them. This is done to avoid issues such as excessive softness or syneresis, which can negatively impact product quality. The addition of beetroot should be controlled to maintain the desired characteristics of the cheese.

3.5 Cheese Colour

Beetroot is a root vegetable known for its bright natural colour and nutritional profile. Beetroot is used in the food industry as a natural colourant and additive due to its stable and non-toxic properties. It can replace synthetic additives and increase the nutritional value of foods such as cheese (Thiruvengadam et al., 2022). The colour change graph of cheese with beetroot extract added can be seen in Figure 2.

Colour parameters including L, a*, b*, hue, whiteness index, and chroma in cheese supplemented with beetroot extract had significant differences. This is because beetroot has pigments that can be utilised as natural food colourants that are harmless and non-toxic (Masih et al., 2019) . The L value of cheese decreased after the addition of beetroot extract. The higher the L value, the lighter the colour and vice versa. Beetroot cheese becomes darker due to the red pigment from beetroot. Hue indicates the direction of colour change in an object, based on the colour wheel. If the hue value increases in cheese after adding beetroot extract, it means that there is a change in colour hue towards more red or purple. The increase in hue value means that the cheese changes from a yellowish white colour to a red or purple colour due to beetroot pigments. The more beetroot added, the higher the hue change towards red-purple. Whiteness index measures how white a product is. If the whiteness index value

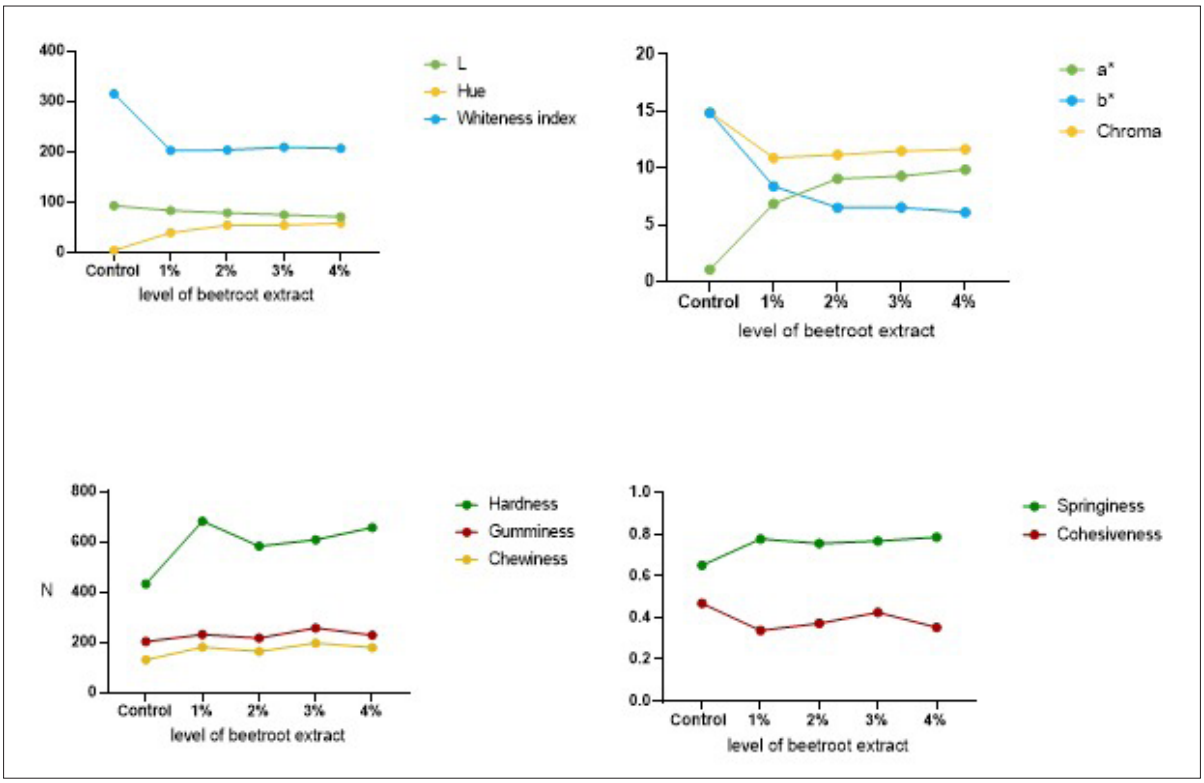

Figure 2. *Instrumental colour and texture of cheese with beetroot extract added*

decreases, the cheese becomes less white and more colourful. This is influenced by betalain pigments from beetroot. Beetroot contains betalains (mainly betasianin) which give the cheese its red-purple colour. The addition of beetroot extract reduces the whiteness of the cheese, as red pigments predominate. Cheese with beetroot has a more attractive appearance and can be used as a naturally coloured cheese variant. This can be an added value in cheese products that want to appear unique and healthier. Potential as an innovative product for functional cheese with a distinctive colour. The a* value increases and the b* value decreases as the cheese colour shifts towards red after the addition of beetroot. Chroma is the overall colour intensity. Decreased chroma value means the colour of the cheese becomes less bright and more muted.

3.6 Texture Profile

The results showed that the addition of beetroot extract influenced several cheese texture parameters. One of the parameters that experienced a significant change was hardness, where there was an increase in hardness value after the addition of beetroot extract. This increase indicates that the cheese becomes denser and harder, which is likely due to the interaction of components in the beetroot extract with the cheese protein matrix. Beetroot is rich in polyphenols and betalains, which can interact with proteins in cheese, potentially resulting in a harder texture (Ubeira-Iglesias et al., 2019) . Beetroot also contains magnesium and sodium, which contribute to its functional properties in food products (Paciulli et al., 2016) . Despite the increase in hardness, gumminess and chewiness values remained relatively stable. This indicates that the addition of beetroot extract did not have a significant impact on the chewiness of the cheese. Thus, modification of the formulation through the addition of beetroot extract does not reduce the chewiness of the cheese when consumed.

The springiness parameter increased after the addition of beetroot extract, indicating that the elasticity of the cheese increased. Springiness is an indicator of the ability of cheese to return to its original shape after being subjected to pressure. An increase in springiness value indicates that the cheese becomes more elastic and does not crumble easily when chewed, which is a desirable texture characteristic in cheese products. Meanwhile, the cohesiveness value did not show a significant change up to the 4% level of beetroot extract addition. Cohesiveness describes the cohesiveness of the internal structure of cheese, as well as its ability to maintain integrity during chewing. The stable cohesiveness value indicates that the addition of beetroot does not disrupt the structural integrity of the cheese.

In general, the texture of cheese is highly influenced by the type of cheese and its production method. In cow's milk-based fresh cheese, high moisture content generally results in a soft and elastic texture. The results of this study indicate that the addition of

beetroot extract can modify some textural properties of cheese without drastically reducing its sensory quality. Thus, beetroot could be a potential additive to improve certain characteristics of cheese, without compromising the integrity or comfort of the texture when consumed.

4. Conclusion

The addition of beetroot extract can increase antioxidant activity and change the colour of cheese starting from 1% level. The addition of beetroot extract up to 4% is a safe and effective way to enhance both the antioxidant content and visual appeal of cheese.

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