








## Amino Acids Profile of Dangke Cheese Ripening by *Lactobacillus delbrueskii* subsp. *bulgaricus* Ropy Strain

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### ABSTRACT

Dangke is a traditional cheese typical of Enrekang Regency, South Sulawesi Province, a processed food made from milk. Functional cheese is currently being developed to support its benefits for human health, including probiotic cheese from lactic acid bacteria. This study aimed to determine and compare the amino acid profile of probiotic cheese from developing dangke using starter culture *Lactobacillus delbrueckii* spp. *bulgaricus* and coating by various types of biodegradable film. The results of this study indicate that the highest amino acid profile in all cheese groups is glutamic acid, with an average of 43.60mg/kg. Coating use has a significant influence on the amino acid profile of ripened cheese. In addition, the cheese group with the highest amino acid component is in the 2% Konjac cheese group, which is coated with Konjac.

**Keywords:** Amino acids, Biodegradable film, Dangke, *Lactobacillus delbrueskii*

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### INTRODUCTION

Dangke is a traditional cheese typical of Enrekang Regency, South Sulawesi Province, a processed food made from milk. Traditional cheese is processed from fresh milk from buffalo, cow, goat, or sheep, which is produced enzymatically by adding papaya latex (Malaka et al., 2022). Cheese is a processed dairy food rich in nutritional components and contains protein, short-chain fatty acids, vitamins, and minerals (Santiago-López et al., 2018). Cheese is widely developed into functional products with health benefits and probiotic cultures. Probiotics are living microorganisms that, when given in sufficient quantities, will provide health benefits to their hosts (Hill et al., 2014; Hammam et al., 2019; Das et al., 2022)

Probiotic is developed by adding lactic acid bacteria microorganisms in sufficient quantities to provide health benefits to the community (Araujo et al., 2024; Usman et al., 2024). The benefits contained in the development of probiotic cheese include protection against various types of

pathogens, lowering cholesterol levels, improving the immune system, and even improving lactose intolerance. Currently, probiotic products, especially dairy products, are widely consumed worldwide; cheese is one of the most famous functional dairy products.

Cheese quality depends on its chemical properties, and understanding their influence on the quality of the product is essential in the dairy industry (Hanlon et al., 2022). Understanding the chemical composition of cheese is necessary because specific chemical components in the product contribute to beneficial sensory aspects, while other microorganisms may contribute the opposite, causing off-flavors or reducing quality through spoilage; this is especially relevant in fermented and aged foods (Mayo et al., 2014).

During the cheese ripening process, very complex biochemical events occur. One of the biochemical processes is lipolysis, which is the breakdown of free fatty acids, which play a role in the taste and aroma of cheese. Proteolysis affects the development of the cheese structure during

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cheese ripening. This involves improving the cheese flavor through amino acids and peptides that directly affect the cheese flavor. Free amino acids are revealed through protein proteolysis (Kavas et al., 2022).

The use of biodegradable packaging materials in the food industry has become essential due to concerns about human health and environmental issues. Among these materials, edible films and coatings are widely studied biopolymers. Edible films and coatings help maintain the quality of food products by controlling the transfer of moisture, oxygen, carbon dioxide, lipids, flavors, and aromas. Therefore, this article aims to determine and compare the amino acid profile of probiotic cheese developed from Dangke using different biodegradable packaging.

## MATERIALS & METHODS

### Culture Propagation of *Lactobacillus delbrueckii* subsp. *bulgaricus*

Starter culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* was obtained from the Dairy Processing Biotechnology Laboratory's collection. Before use, the culture was thawed, transferred to sterile MRS broth, and incubated at 37 °C for 24 hours. Up to three times a week, the culture was transferred to a new sterile MRS broth. After stable growth, it was propagated into 10% Reconstituted Skim Milk before being used as an inoculum for healthy cheese.

### Dangke Cheese Making

Dangke cheese was made using methods from Sabil et al. (2017) Fresh cow's milk from an Enrekang Regency, South Sulawesi, smallholder dairy farm. Homogenized and pasteurized cow's milk with low-fat content (<1%), after separating the cream with a cream separator, was cooled to 40°C. *Lactobacillus delbrueckii* subsp. *bulgaricus* culture of 1% (vol/vol) was stirred for approximately 2 minutes. The milk was then ripened for 45 minutes and coagulated with 0.3% dried papain. The curd is then cut into 1-cm<sup>3</sup> cubes and stirred for 20 minutes at 40°C. The whey was removed,

and the curd pieces are put into a cheese mold weighing 250g for 90 minutes. The cheese was soaked overnight in 10% (wt/vol) brine at 4°C. The cheese was stored in a vacuum after coating and acidification treatment at 4°C.

### Amino Acid Profile Analysis

Amino acid testing using the Ultra Performance Liquid Chromatography (UPLC) method involves several stages. The sample is weighed at 0.1g, crushed, and placed into a closed test tube. Five to ten milliliters of 6N HCl are added to the sample solution, which is then hydrolyzed in an oven at 110°C for 22 hours. After cooling to room temperature, it is transferred to a 500mL measuring flask. Aquabides are added up to the boundary mark, filtered through a 0.45µL filter, and 10µL and 70µL of AccQ Fluor Borate are pipetted and vortexed together. Next, 20µL of Flour A dan reagent is added, vortexed for 1 minute, and incubated for 10 minutes at 55°C. Finally, one µL is injected into the UPLC under the following chromatography conditions: an ACCQ-Tag Ultra C18 column, a temperature of 49°C, a mobile phase composition system gradient detector PDA, a flow rate of 0.7 µL/min, and a wavelength of 260 nm.

### Data Analysis

The collected data were then processed and analyzed using the analysis variant with Excel and SPSS. If there is a significant difference in the results analysis variance, a difference test, such as Duncan's test, is conducted. The best results were interpreted based on the related conclusion statistics.

## RESULTS & DISCUSSION

Every type of cheese has a profile of unique free amino acids (Kabelová et al., 2009), which are affected by enzymatic activity, degradation, and inter-conversion amino acids. Profile results of amino acids from analyzed cheese are presented in Table 1 and Fig. 1. No significant difference was observed in all content amino acids in every cheese group, except in groups KA and KJ cheeses, which have a 1.5% U2 difference significance (P<0.05).

**Table 1:** Profile of prebiotic cheese amino acids from development dangke cheese (mg/kg)

Amino acids	KA	K. BW	KR 1%	KR 2%	KR 3%	KJ 1% U1	KJ 2% U1	KJ 1.5% U2
Ala	5.77 ±0.01 <sup>a</sup>	6.23 ±0.01 <sup>ab</sup>	9.08 ±0.03 <sup>ab</sup>	8.95±0.03 <sup>ab</sup>	8.69 ±0.01 <sup>ab</sup>	8.00±0.00 <sup>ab</sup>	9.23±0.02 <sup>ab</sup>	8.34±0.01 <sup>b</sup>
Arg	7.03±0.01 <sup>a</sup>	7.90 ±0.02 <sup>ab</sup>	12.48 ±0.04 <sup>ab</sup>	12.77 ±0.04 <sup>ab</sup>	10.76 ±0.02 <sup>ab</sup>	10.96±0.00 <sup>ab</sup>	13.06 ±0.04 <sup>ab</sup>	11.30 ±0.01 <sup>b</sup>
Asp	11.36±0.01 <sup>a</sup>	11.56 ±0.02 <sup>ab</sup>	16.04 ±0.05 <sup>ab</sup>	15.84 ±0.04 <sup>ab</sup>	15.39 ±0.02 <sup>ab</sup>	14.96 ±0.03 <sup>ab</sup>	16.89±0.04 <sup>ab</sup>	14.61 ±0.02 <sup>b</sup>
Gly	3.50 ±0.00 <sup>a</sup>	3.76 ±0.01 <sup>ab</sup>	5.65±0.02 <sup>ab</sup>	5.44 ±0.01 <sup>ab</sup>	5.02 ±0.01 <sup>ab</sup>	4.91 ±0.01 <sup>ab</sup>	5.86±0.02 <sup>ab</sup>	5.16 ±0.01 <sup>b</sup>
Glu	34.19±0.00 <sup>a</sup>	35.42 ±0.08 <sup>ab</sup>	47.53 ±0.18 <sup>ab</sup>	46.41 ±0.14 <sup>ab</sup>	44.65 ±0.09 <sup>ab</sup>	45.68±0.10 <sup>ab</sup>	52.08±0.15 <sup>ab</sup>	42.89 ±0.07 <sup>b</sup>
His	6.38 ±0.00 <sup>a</sup>	7.36 ±0.01 <sup>a</sup>	12.25 ±0.02 <sup>b</sup>	12.68 ±0.2 <sup>ab</sup>	9.97 ±0.00 <sup>ab</sup>	9.58 ±0.00 <sup>ab</sup>	12.69 ±0.01 <sup>b</sup>	10.85±0.01 <sup>b</sup>
Iso	9.75 ±0.02 <sup>a</sup>	10.65 ±0.02 <sup>ab</sup>	15.84 ±0.01 <sup>ab</sup>	15.22±0.02 <sup>ab</sup>	14.57 ±0.03 <sup>ab</sup>	14.31±0.04 <sup>ab</sup>	17.05±0.05 <sup>ab</sup>	14.52±0.03 <sup>b</sup>
Leu	18.83±0.03 <sup>a</sup>	20.94 ±0.05 <sup>ab</sup>	31.40 ±0.06 <sup>ab</sup>	30.32 ±0.07 <sup>ab</sup>	28.99 ±0.06 <sup>ab</sup>	28.82±0.07 <sup>ab</sup>	34.25 ±0.10 <sup>ab</sup>	28.75±0.05 <sup>b</sup>
Lys	13.39±0.02 <sup>a</sup>	14.58 ±0.03 <sup>ab</sup>	18.73 ±0.05 <sup>ab</sup>	18.13 ±0.04 <sup>ab</sup>	18.51 ±0.03 <sup>ab</sup>	18.37±0.02 <sup>ab</sup>	20.61 ±0.04 <sup>ab</sup>	17.21±0.02 <sup>b</sup>
Val	11.69 ±0.01 <sup>a</sup>	13.07 ±0.02 <sup>ab</sup>	19.38 ±0.06 <sup>ab</sup>	18.57±0.04 <sup>ab</sup>	17.79 ±0.03 <sup>ab</sup>	17.49±0.02 <sup>ab</sup>	21.07±0.05 <sup>ab</sup>	17.56±0.01 <sup>b</sup>
Phe	10.84 ±0.01 <sup>a</sup>	12.15 ±0.02 <sup>ab</sup>	20.55 ±0.02 <sup>ab</sup>	21.86 ±0.02 <sup>ab</sup>	17.10 ±0.02 <sup>ab</sup>	17.33±0.02 <sup>ab</sup>	21.52 ±0.05 <sup>ab</sup>	18.50±0.00 <sup>b</sup>
Pro	15.76±0.03 <sup>a</sup>	18.08 ±0.04 <sup>ab</sup>	26.11 ±0.09 <sup>ab</sup>	24.70 ±0.07 <sup>ab</sup>	23.62 ±0.04 <sup>ab</sup>	21.64 ±0.05 <sup>ab</sup>	28.13 ±0.08 <sup>ab</sup>	23.25±0.04 <sup>b</sup>
Ser	11.53 ±0.02 <sup>a</sup>	12.85 ±0.03 <sup>ab</sup>	20.10 ±0.07 <sup>ab</sup>	19.63 ±0.06 <sup>ab</sup>	17.45 ±0.03 <sup>ab</sup>	18.13 ±0.04 <sup>ab</sup>	21.36 ±0.06 <sup>ab</sup>	18.15±0.03 <sup>b</sup>
Thr	7.59 ±0.01 <sup>a</sup>	8.84 ±0.02 <sup>ab</sup>	14.57±0.04 <sup>ab</sup>	14.24 ±0.03 <sup>ab</sup>	12.50 ±0.02 <sup>ab</sup>	11.17 ±0.02 <sup>ab</sup>	14.86±0.04 <sup>ab</sup>	13.06±0.02 <sup>b</sup>
Thy	9.75 ±0.00 <sup>a</sup>	11.18 ±0.02 <sup>ab</sup>	17.34 ±0.04 <sup>ab</sup>	19.07 ±0.03 <sup>ab</sup>	15.13 ±0.02 <sup>ab</sup>	15.89±0.01 <sup>ab</sup>	17.80 ±0.04 <sup>ab</sup>	16.02±0.02 <sup>b</sup>

x ± sd; mean values ± standard deviation ab: statistically significant differences between the mean values (P<0.05) on signs with different letters in a row (KA: negative control Agar; K.BW: Positive control (beewax coating); KR 1%: coating with 1% carrageenan; KR 2%: coating with 2% carrageenan; KR 3%: coating with 3% carrageenan; KJ 1%: coating with konjac 1%; KJ 1.5%: Ala= L-alanine; Arg=L-arginine; Asp=L-aspartic Acid; Gly=glycine; Glu=L-glutamic acid; His=L-histidine; Iso=L-isoleucine; Leu=L-leucine; Lys=L-lysine; Val=L-valine; Phe=L-Phenylalanine; Pro=L-proline; Ser=L-serine; Thr=L-threonine; Thy=thyrasin

Prebiotic Dangke cheese contained 15 types of amino acids, with glutamate having the highest concentration. In all cheese groups, glutamate was the dominant amino acid component, followed by leucine, proline, valine, serine, aspartate, phenylalanine, and thymine. Glutamate is one of the most abundant amino acids in cheese products. Glutamate plays a role in protein structure, is also important as a nutrient, in body metabolism, and nerve cell signaling. (Brosnan and Brosnan et al., 2013).

Analysis results in the study show that the content of individual amino acids was the highest found in the group cheese coated with 2% konjac (KJ 2% U1), and the lowest are in the group coated cheese with Agar (KA). Table 1 shows the 15 amino acids contained in each type of cheese with different coatings. Profile of the amino acid consists of essential amino acids (histidine, leucine, lysine, phenylalanine, valine, threonine) and non-essential amino acids (alanine, arginine, aspartic acid, glycine, glutamic acid, isoleucine, proline, serine, tyrosine). This matters to the study of Sadievich (2021), which states that the amino acids found in cheese vary in content, the most caused by glutamate acid, leucine, and proline.

L-glutamate has an important role in microbial metabolism in the intestine which can improve human health (Le Drian & Blottiere, 2024). Glutamate is essential for amino acid metabolism, but most dietary glutamate is catabolized in the intestine (Burrin and Stoll, 2009; Le Drian & Blottiere, 2024). Glutamate is the main excitatory neurotransmitter in the central nervous system.

Glutamate serves as the principal excitatory neurotransmitter in the central nervous system, while its metabolite, gamma-aminobutyric acid (GABA), functions as the main inhibitory neurotransmitter (de Leon and Tadi, 2023). The interaction between glutamate and specific taste receptors on the tongue plays a critical role in eliciting the umami taste sensation (Yamaguchi and Ninomiya, 2000). Furthermore, the identification of glutamate receptors throughout the gastrointestinal tract has provided new perspectives on its physiological roles (Diepeveen et al., 2022). As a functional amino acid, glutamate contributes to the bioactivity of Dangke cheese, thereby enhancing its functional food properties (Brosnan and Brosnan, 2013).

The essential amino acids—Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, and Valine—are indispensable nutrients that cannot be synthesized endogenously and must therefore be obtained through dietary intake (Agrez et al., 2012; Wunderle et al., 2024). Due to this dietary dependence, nine of the 20 amino acids required for proper physiological function are categorized as essential. Essential amino acids have many benefits for the body, including helping to form proteins and repair tissues, supporting metabolism and producing energy, producing hormones and neurotransmitters, increasing endurance, and maintaining healthy skin, hair, and nails (La Pelusa and Kaushik, 2022).

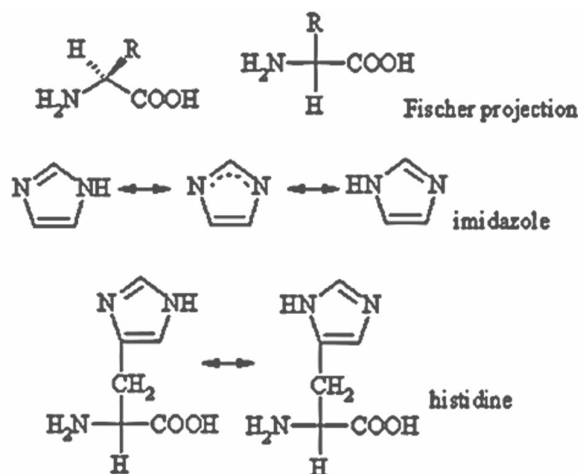
In Dangke cheese, only methionine and tryptophan are not found in the cheese content. Methionine plays a more significant role in metabolism and detoxification in the body (Agrez et al., 2012). Methionine can also help the body absorb zinc and selenium minerals from food

(Limwachirakhom et al., 2022). Methionine can be found in egg whites. In addition, fish and meat also contain quite a lot of methionine.

This amino acid is commonly found in a variety of foods, including chocolate, oats, durian, mango, dried dates, milk, yogurt, cheese, red meat, eggs, poultry, sesame seeds, chickpeas, sunflower seeds, pumpkin seeds, spirulina, and various nuts. Tryptophan is a precursor of vitamin B3, which induces relaxation and drowsiness. Therefore, consuming foods containing tryptophan can improve sleep quality (Kapalka, 2010).

Histidine is an essential amino acid detected in this Dangke cheese. The amount ranges from 6.38 in the control of uncoated cheese to the highest in dangke cheese coated with konjac; the concentration of this amino acid is 12.69mg/kg. Histidine is an essential amino acid that plays a vital role in various body processes (Moro et al., 2020), including protein formation, purine synthesis, conversion into other substances, such as histamine, glutamate, or hemoglobin; as a precursor to carnosine, which functions as a buffer and antioxidant in muscles and the brain; growth and repair of damaged tissue; blood cell production; protection of nerve cells. Histidine is considered a crucial amino acid, particularly for children. Due to its imidazole side chain and a relatively neutral pKa of approximately 6.0, even minor fluctuations in cellular pH can significantly influence its ionization state (Kim et al., 2013; Manville et al., 2024). This property makes histidine a frequent part of the enzyme catalytic group and a coordinating ligand in metalloproteins (Chen and Zhou, 2015).

Histidine is a precursor to histamine, an amine in the nervous system, and carnosine, an amino acid (Bae and Majid, 2013). There are two enantiomers of histidine: D-histidine and L-histidine, with L-histidine (or S-histidine) being the more dominant form (Murphy et al., 2011). The molecular formula of histidine can be seen in Fig. 1.



**Fig. 1:** Histidine. The systematic name of Histidine is S-2-amino-3-(3H-imidazol-4-yl) propanoic acid with the molecular formula C<sub>6</sub>H<sub>9</sub>N<sub>3</sub>O<sub>2</sub>. (<https://id.wikipedia.org/wiki/Histidina>)

Isoleucine is one of the nine essential amino acids needed by the body. It is important in producing hemoglobin, the oxygen-carrying pigment in red blood

cells, and helps control blood sugar (Amaya-Farfan and Pacheco, 2003). Like other branched-chain amino acids (BCAA), it is associated with insulin resistance (Lu et al., 2013). The isoleucine content is relatively high in dangke, ranging from 9.75mg/kg in the control group, which is not inoculated with *Lactobacillus delbrueckii* subsp. *bulgaricus* and is not coated. In contrast, samples ripened with the inoculation of *Lactobacillus delbrueckii* subsp. *bulgaricus* and coated with konjac showed an increase in this essential amino acid, reaching the highest level of 17.05mg/kg. This increase is likely due to adding konjac components to the cheese.

Following glutamate, Leucine is the second most abundant amino acid in Dangke cheese. As an essential branched-chain amino acid (BCAA), leucine plays a vital role in protein synthesis and muscle growth (Wolfe, 2017). It naturally occurs in various foods, including chicken, turkey, and cheese (Rehman et al., 2023). Multiple studies have demonstrated that leucine may contribute to increased muscle mass, improved athletic recovery, reduced inflammation, and regulation of blood glucose levels. The concentration of this amino acid ranged from 18.83% in the uncoated control Dangke cheese to a maximum of 34.25% in Dangke cheese coated with 2% konjac. Another name for leucine is S-2-amino-4-methylpentanoic acid, which has the chemical formula  $C_6H_{13}NO_2$ .

Lysine (Fig. 2) is a water-soluble, basic essential amino acid. The average daily requirement is 1 - 1.5 g. It functions as a framework for thiamine (vitamin B1), is antiviral, and helps calcium absorption and the formation of hormones, collagen, and antibodies (Munteanu et al., 2024). In addition, it allows the liver to detoxify and produce digestive enzymes to increase and stimulate appetite. Lysine also plays a role in the production of carnitine (Shekhawat et al., 2013), which converts fatty acids into energy, thereby helping to lower cholesterol.

The lysine content in dangke ripening coated with Agar was 13.39%, and that coated with beeswax was 14.58%, while that coated with 18.73% carrageenan was not significantly different from 2% carrageenan, which was 18.13%. Almost the same as cheese coated with 3% carrageenan, which was 18.51%. This was also not significantly different from the lysine level in dangke ripening cheese coated with 1, 1.5, and 2% konjac, with values of 18.37, 17.21, and 20.61%, respectively.

Phenylalanine is an essential amino acid, along with tyrosine and tryptophan, a group of aromatic amino acids with benzene rings. Phenylalanine, taurine, and tryptophan function as conductors or messengers in the brain's nervous system (Fig. 3). The control ripening dangke (CRD), coated with Agar, contained 10.84% phenylalanine, significantly different from the Dangke Ripening coated with beeswax with a value of 12.15%. CRD KR 1%, CRD KR 2%, and CRD 3% also had phenylalanine content of 20.55, 21.86, and 17.10%, respectively. Likewise, CRD KJ 1, 1.5, and 2% contained phenylalanine of 17.33, 18.5, and 21.52%, respectively.

The human body cannot form threonine, but it needs this amino acid, which must be obtained from outside the body (Radonic, 2018). Plants and microorganisms can synthesize threonine from the amino acid aspartic acid

(Ferreira et al., 2004). Another name for threonine is 2S,3R-2-amino-3-hydroxybutanoic acid, with the chemical formula  $C_4H_9NO_3$  (Fig. 4). Threonine helps treat uncontrolled muscle disorders, with symptoms of involuntary and tight movements in the muscles (spasticity), multiple sclerosis, congenital disorders characterized by weakness and stiffness in the legs (familial spastic paraparesis or FSP) and Lou Gehrig's disease or ALS. However, further research is still needed to prove these benefits.

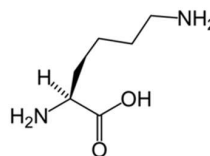


Fig. 2: Lysine has the systematic name S-2,6-diamino hexanoic acid.  
(<https://id.wikipedia.org/wiki/Lisin#/media/Berkas:L-lysine-skeletal.png>)

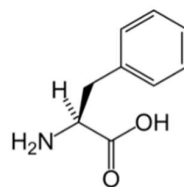


Fig. 3: Another name for phenylalanine is 2-amino-3-phenylpropanoic acid with the molecular formula  $C_9H_{11}NO_2$ .  
(<https://id.wikipedia.org/wiki/Fenilalanina>)

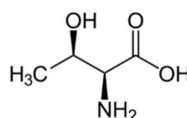


Fig. 4: Another name for threonine is 2S,3R-2-amino-3-hydroxybutanoic acid, with the chemical formula  $C_4H_9NO_3$ .  
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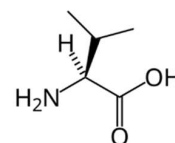


Fig. 5: Valine has another name: (S)-2-amino-3-methyl-butanoic acid with the chemical formula  $C_5H_{11}NO_2$ .  
([https://en.wikipedia.org/wiki/Valine\\_\(data\\_page\)#/media/File:L-valine-skeletal.svg](https://en.wikipedia.org/wiki/Valine_(data_page)#/media/File:L-valine-skeletal.svg))

Threonine is abundant in dairy products, meat, fish, and sesame seeds. Dangke cheese coated with Agar contains 7.59% threonine, and that coated with beeswax is 8.84%. While in Dangke Cheese coated with 1, 2, and 3% Carrageenan, the threonine content is 14.57, 14, 24, and 12.5%, respectively. This is also significantly different from the threonine level in dangke cheese coated with 1, 1.5, and 2% Konjac, which are 11.17, 13.06, and 14.86%, respectively.

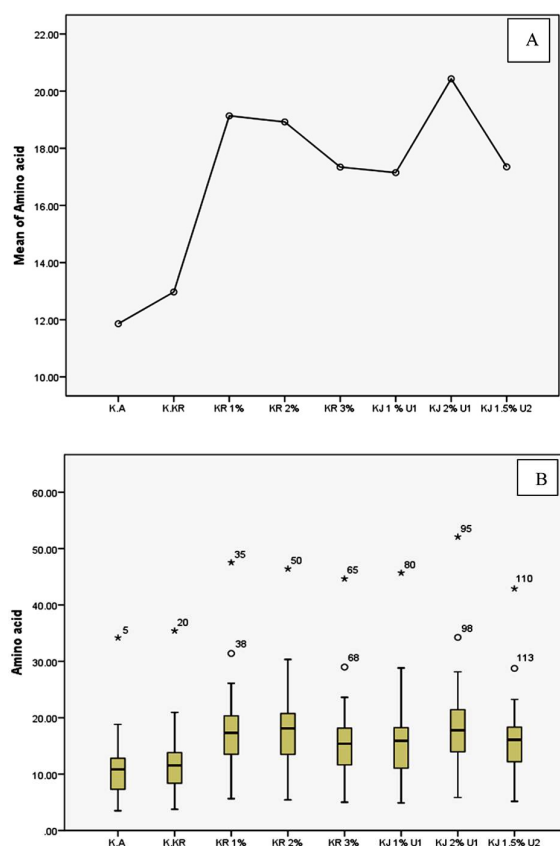
Valine is an essential amino acid that plays a role in muscle growth, tissue repair, and energy production (Sharma et al., 2024). It is one of three amino acids that the body cannot produce and must be supplied with food (Akal, 2017). Food sources rich in valine are livestock products such as meat, eggs, milk, and cheese (Hertzler et al., 2020), and grain products containing oil, such as peanuts and sesame. The molecular formula of valine can be seen in Fig. 5.

In this study, the control Dangke cheese coated with agar contained 11.69% valine, while the cheese coated with beeswax had a valine concentration of 13.07%. Coating with carrageenan resulted in a significantly higher concentration than the control, specifically carrageenan at 1, 2, and 3%, yielding 19.38, 18.57, and 17.39%, respectively. Additionally, using konjac coating at 1, 1.5 and 2% also increased the levels of this amino acid, measuring 17.49, 17.56 and 2%, respectively.

Glutamic acid is the predominant amino acid present in all types of cheese, with an average concentration of 43.60mg/kg. It is a non-essential amino acid that the body can

synthesize and is typically produced during the fermentation of milk. This amino acid plays a crucial role in the body's metabolism and acts as a neurotransmitter in the brain (Karjadidjaja, 2009). The glutamic acid content in cheese made from cow's milk is particularly high, reaching 9847mg/100g of cheese (Jain et al., 2012).

Fig. 6 shows the composition plot of Dangke ripening cheese fermented with *Lb. delbrueckii* subsp. *bulgaricus* ropy strain with various coating treatments. The highest average percentage of amino acids in the coating, 2% Konjac, indicates the best coating treatment.



**Fig. 6:** (a) Mean plot; and (b) component boxplot graph profile Dangke cheese amino acids by *Lb. delbrueckii* subsp. *bulgaricus* fermentation. X-axis legend, KA = negative control (agar); KKR = positive control (beeswax coating); KR 1%, 2%, 3% = coating with 1%, 2%, and 3% carrageenan; KJ 1.5% U1/U2 = coating with 1.5% konjac (unit 1 and 2); KJ 2% U1 = coating with 2% konjac (unit 1).

Cheese contains a high level of glutamic acid, primarily due to its fermentation process. During cheese production, bacteria or mold ferment milk proteins, breaking them into various components, including amino acids such as glutamic acid. Glutamic acid is a natural amino acid found in many foods; however, its concentration can increase in fermented foods like cheese due to this process (Zheng et al., 2021). Glutamic acid is an amino acid that plays a role in forming chemicals in the brain. This amino acid has several health benefits, one of which includes regulating blood sugar levels. Glutamic acid is one of the eleven nonessential amino acids that the body can produce naturally. Glutamate helps form chemicals that transmit signals between nerve cells in the brain (neurotransmitters).

The concentration of glutamic acid is typically regarded as an indicator of the degree of deep protein hydrolysis in cheese. Enzymes that cleave amino acids from the amino end produce flavors and aromatic compounds, including 3-methyl-butanol, methionyl-propyl aldehydes, sulfides, and aromatic esters (Suzuki-Iwashima et al., 2020).

Glycine is the least abundant amino acid in each cheese group, with an average concentration of 4.90mg/kg. It is found in cow's milk but at a relatively low concentration, averaging 4.54mg per 100mg (Landi et al., 2021). This is noteworthy because glycine is not a primary amino acid in milk proteins such as casein and whey. Instead, it tends to be found in higher concentrations in connective tissue and gelatinous cuts than in muscle tissue, which is the source of milk protein (Razak et al., 2017).

Cheese coated with agar has one of the lowest amino acid content profiles. This happens because the agar layers interact with the cheese curd Dangke, resulting in a noticeable appearance of wetness and a reduction in protein content. The absorption and interplay of the agar layer (Ceylan & Atasoy, 2023) with the curd can influence the amino acids present in cheese and diminish its quality composition (Malaka et al., 2017).

Cheese coated with carrageenan can affect the amino acid content and the levels of proteolysis, which breaks down proteins into amino acids during ripening. The proteolysis process can be influenced by the water content and the quantity of non-fat substances in cheese that contains carrageenan (Blaszak et al., 2018), thereby enhancing the cheese's quality compared to an agar coating. Coating, besides increasing and maintaining the nutritional value of the product, also aims to avoid microorganism contamination. In their research, At-Tayyar et al. (2020) used bionanocomposite films for food packaging, which showed superior antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*, S33R) and Gram-negative bacteria *Escherichia coli* (IRAQ3).

Group cheese coated with konjac is rich in amino acids. This is significant due to the behavior of gelation cheese, which features a related konjac coating with a limited presence of acetyl groups in the chain (Silva et al., 2015). Incorporating konjac into edible films triggers complex protein bonds that enhance the film's molecules. The konjac coating can affect the amino acid composition in food, potentially boosting the protein content alongside the konjac. This can result in improved nutritional value for cheese (Fahrullah et al., 2020).

## Conclusion

The highest amino acid profile in all cheese groups is glutamic acid, with an average of 43.60mg/kg. The cheese group with the highest amino acid component is the 2% Konjac cheese group, which is coated with Konjac.

## DECLARATIONS

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**Data Availability:** The data supporting the findings of this study are openly available online in the research report.

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**Author's Contribution:** RM: Conceived and designed the experiments; RWK, SSMY, FAA: conducted field experiments, analyzed data, and wrote the paper; RM, WSP: supervised, analyzed data, and wrote the paper. All authors read and approved the final manuscript.

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