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Amino Acid Profile of Goat Milk Kefir with *Lacticasei bacillus casei* AP and Oat Milk during Storage

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ABSTRACT

Background: Accurate and up-to-date information about amino acid profile is crucial in the production and promotion of dairy products and fermented milk because both components contribute to the nutritional and market value of the dairy products.

Methode: This study aims to evaluate the amino acid profile of goat milk kefir supplemented with *Lacticaseibacillus casei* AP, oat milk, or both during 14-day storage using the gas chromatography method.

Result: The results demonstrated that *Lacticaseibacillus casei* AP and oat milk incorporated into kefir production significantly affected amino acids (aspartac acid, glutamic acid, tyrosine, valin, phenylalanine, ileusin and leucine). The dominant amino acids in the treatments were phenylalanine (46.66 ppm), glutamic acid (40.40 ppm) and lysine (23.91 ppm). The storage time only significantly reduced the amount of valine. Conclusively, incorporating *Lacticaseibacillus casei* AP, oat milk, or both into goat milk kefir did not negatively affect the amino acid profile during storage time.

Key words: Amino acid, Lactic acid bacteria, Prebiotic, Probiotic.

INTRODUCTION

Milk contains high nutritional and biological values as a main component of human diet (Yu *et al.*, 2018) but lactose content in milk has been regarded as disease agent, including lactose intolerance. Among other measures, fermentation can reduce lactose content in dairy products, and a previous study (Alm, 1982) reported that yogurt, kefir, buttermilk, *bifidusi* milk can reduce lactose intolerance by 20-52%. In addition to complete proteins which partly improve digestion, kefir contains vitamins, minerals and essential amino acids for convalescence and homeostasis (Otles and Cagindi, 2003). Innovative kefir that can be recommended in the human diet with an eye on their health (Subbalakshmi, 2024). The fermentation process may result in the change of protein profile which depends on the proteolytic activity of lactic acid bacteria (LAB). LAB proteolytic is crucial for milk proteolysis during the fermentation. Proteinase and peptidase are the main enzymes of LAB responsible for the protection of milk protein proteolysis as the source of amino acids (Donkor *et al.*, 2006).

Nutritional value of protein is reflected in the amino acid profile. Kefir fermentation may change the amino acid profile of milk as its main ingredient (Otles and Cagindi, 2003). Meanwhile, fat is the source of energy and the main reservoir for body to function. As a result of the symbiotic co-cultivation of yeasts and lactic acid bacteria within a matrix of polysaccharides and proteins mostly obtained from milk, kefir is a fermented dairy product recognized for its distinctive microbiological composition (Soni *et al.*, 2024). Goat milk is easier to digest and has a higher mineral bioavailability compared to cow's milk, making it a valuable choice for kefir production (Nurliyani *et al.*, 2022).

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Goat milk also has a more balanced protein and fat profile, enhancing the nutritional value of the resulting kefir product. Fresh goat milk and goat milk products, including kefir, offer various potential health benefits such as anti-inflammatory properties, prevention of cardiovascular diseases, anti-diabetic and antihypertensive effects, bone strengthening, immune system boosting and improved metabolism (Subbalakshmi, 2024). However, compared to cow's milk, goat's milk is less stable to heat treatment as it is affected by pH, heating temperature and the addition of calcium chloride, disodium EDTA, disodium hydrogen phosphate and sucrose (Wang *et al.*, 2016). These health benefits make goat milk kefir a valuable functional food option.

Efforts to improve kefir quality include incorporating probiotics and prebiotics into kefir (Buran *et al.*, 2021). The probiotic approach, *i.e.* therapeutically consuming beneficial microorganism cultures of the healthy human microflora, holds great promise for the prevention and

treatment of clinical conditions associated with impaired gut mucosal barrier functions and sustainedinflammatory responses (Sugandhi, 2018). *Lacticaseibacillus casei* AP is a local probiotic bacteria (Widodo *et al.*, 2012a; Widodo *et al.*, 2012b; Widodo and Anindita, 2014), which is able to produce fermented milk products with good quality (Widodo *et al.*, 2017). Consumption of fermented milk containing *Lacticaseibacillus casei* AP bacteria provides anti-hyperglycaemia effects (Widodo *et al.*, 2019) and improves lipid profiles in obese cases (Widodo *et al.*, 2021). By adding probiotic strains to kefir, it is possible to target obesity-related metabolic pathways and promote a healthier gut environment conducive to obesity management. The probiotic function of the product can be enhanced by the presence of prebiotics, which is related to the fact that prebiotics can increase the survival of probiotics in the gut. The content of b-glucan in oats can serve as a substrate for probiotic bacteria and promote the growth and activity of probiotic bacteria in the gut.

Goat milk kefir supplemented with *Lacticaseibacillus casei* AP and oat milk has been reported to improve kefir quality without negative effects and sensorically acceptable by the panelists (Wulansari *et al.*, 2022). Accordingly, product development of goat milk kefir using *Lacticaseibacillus casei* AP, oat milk, or both is considerably feasible. The supplementation of *Lacticaseibacillus casei* AP, oat milk, or both in the production of goat milk kefir allows the production of kefir with different rheology. For this enhanced kefir, two factors mainly contributing to rheology characteristics and duration of coagulation in the fermentation process are different compositions of protein fractions in the combined cow milk and oat milk and the interactions between microorganisms of kefir grain and *Lacticaseibacillus casei* AP as the probiotic bacteria. This study reveals how the addition of *lacticaseibccillus* casei AP and oat milk affects the rheology of kefir products. Amino acids profile of milk can affect the nutritional and market value of dairy products. Accordingly, robust information about amino acid profile is crucial in the production and promotion of dairy products and fermented milk. This study aims to evaluate the amino acids profile of goat milk kefir supplemented with *Lacticaseibacillus casei* AP, oat milk, or both compared to control goat milk kefir during storage time.

MATERIALS AND METHODS

Fresh goat milk was sourced from the dairy goat breeding community 'Susu Poang' (Yogyakarta, Indonesia), grain kefir was from Kefira (Yogyakarta, Indonesia) and oat milk was made by researchers from rolled oats (Quacker Oats, Indonesia). The *Lacticaseibacillus casei* AP strains were obtained from probiotic LABs isolated from the faeces of infants younger than 1 month who were consuming cow milk in Indonesia (Widodo *et al.*, 2012a; Widodo *et al.*, 2012b; Widodo and Anindita, 2014).

Production of kefir starter culture. This study used the Russian method of two-step fermentation for producing kefir (Shah, 2014). In the first step, goat milk was heated at 85°C for 30 minutes and then cooled to room temperature to prepare the culture starter. Then, 3% kefir grains were incorporated into the milk, the incubation was performed at room temperature for 18 hours (Wulansari *et al.*, 2022), then the fermented milk was strained to collect the kefir grains/kefir starter for later use in sample preparation.

Preparation of starter *Lacticaseibacillus casei* AP. *Lacticaseibacillus casei* AP starter was created by creating the mother starter. Exactly 100 ml of 18% (w/v) skim milk was sterilized at 110°C and 13 psi for 10 minutes, let cool to room temperature, then added with *Lacticaseibacillus casei* AP inoculum. The milk was incubated at 37°C for 12-18 hours until it formed curds that would be the mother starter to make bulk starters. After that, 3% (v/v) mother starter was inoculated into 18% (w/v) skim milk and incubated for 12-18 hours to generate bulk starter. The bulk starter was inoculated directly into milk at 4% in the product manufacturing process.

Preparation of oat milk. Prior to kefir production, oat milk (16% w/v) was made according to the method of Demir *et al.* (2021) with minor modifications. Exactly 16 g oats was mixed with 100 ml preheated water and soaked for 15 minutes. The mixture was then homogenized in a mixer (LG brand) for 2 minutes to crush all particles. The characteristics of the oat milk were 1.78%, 12.4% non-fat solids (SNF), 0% lactose, 1.56% protein, 85.8% moisture, 11,399 mPas viscosity and pH 6.6 (Wulansari *et al.*, 2022).

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The production of samples in this study, four kefir samples were developed following the procedures of previous studies (Wulansari *et al.*, 2023). KC (Kefir control) was made of goat milk + kefir starter. KL (Kefir + *Lacticaseibacillus casei* AP) was goat milk + kefir starter + *Lacticaseibacillus casei* AP. KO (Kefir + Oat Milk) was goat milk + oat milk (75:25) inoculated with kefir starter. KLO (Kefir + *Lacticaseibacillus casei* AP + Oat Milk) is goat milk + oat milk + kefir starter + *Lacticaseibacillus casei* AP.

Amino acids profile in every kefir sample was determined individually by Gas Chromatography. Exactly

60mg was added with 4 ml of HCL 6N and heated at 110°C for 24 h. Then, the sample was let cool to reach room temperature, neutralized using NaOH 6N to reach pH 7, added with Aquadest until the volume was 10 ml and then filtered using 0.2 mm Whatman paper. Exactly 50 mm of the filtered solution was added with 30 mm OPA (Orthophalaldehid), stirred for 5 mins, then 10 mm of the solution was poured into an HPLC injector. The amino acid compounds in all samples were observed for filtration and quantification. Amino acid was identified based on the retention time and the comparison between chromatography and the actual standard (Akin and Ozcan, 2017).

All treatments were repeated three times to obtain the mean values and standard deviation. Data interactions were made in a 4×3 structure with two factors, treatment (KC, KL, KO, and KLO) and storage time (1, 7, and 14 days). Data were subjected to ANOVA followed by Tukey's multiple comparison test with $P<0.05$ significance (Norman and Streiner, 1996). All analyses were performed with SPSS statistical program version 16.0.

RESULTS AND DISCUSSION

Fermented dairy products, such as kefir, are beneficial protein-source food due to its unique balance of essential amino acids and high bioavailability. The level of amino acids in goat milk kefir is the effect indicator of supplementing *Lacticaseibacillus casei* AP, oat milk, or both on the kefir samples produced in this study (Table 1). This study found that treatments significantly ($P<0.05$) affected the parameters, including aspartic acid, glutamic acid, tyrosine, valine, phenylalanine, isoleucine and leucine. Among other samples, KL showed the highest number of amino acids. In other words, this study showed that the addition of *Lacticaseibacillus casei* AP, oat milk, or both into kefir production resulted in significant effects on amino acid profile of the kefir yield. Meanwhile, the storage time only significantly ($P<0.05$) affected valine which decreased even after seven days of storage time.

All four kefir samples showed that the order of amino acids from the highest to the lowest concentrations in this study was glutamic acid > lysine > tyrosine > leucine > aspartic acid. This order was different from that of previous studies using kefir grain which reported the sequence of prolin > methionine > leucine > valin > histidin, while using culture starter was leucine > methionine > prolin > valin > threonine (Ozcan *et al.*, 2019). A slight difference was observed from buffalo milk kefir inoculated with either kefir grain or culture starter, namely glutamic acid > alanine > serin > tyrosine > histidine > methionine > lysine (Gul *et al.*, 2015).

Twenty proteinogenic amino acids can be divided into two main subgroups: essential amino acids (EAAs) and non-essential amino acids (NEAAs) (Reeds, 2000). EAAs consist of 9 amino acids: histidine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Meanwhile, 11 amino acids in NEAAs are alanine, aspartic, asparagine, arginine, cysteine, glutamate, glycine, proline, serin and tyrosine (Choi and Coloff, 2019). Unlike NEAAs, EAAs are not synthesized in the body, so human need protein supply from food intake to meet the required amount. Fig 1 shows the comparison between EAAs and NEAAs in each treatment with mean values of 40.65% vs. 59.35%, respectively.

EAAs bound in protein can be found in all food sourced from animal and plant protein, including milk which contains abundant amount of nearly all EAAs except arginine and glycine (Hou and Wu, 2018). Protein serves vital functions that include 1) digest and absorb food nutrients through the small intestines, 2) transport nutrients and 3) oxidize nutrients into water and carbon dioxide. EAAs deficiency is therefore a serious problem to both human and animals because it leads to declining protein synthesis in body cells and tissues, especially skeletal muscles (Wu, 2021). Unfortunately, EAAs deficiency remains the most prominent issue of nutrition in marginalized area in the world. In fact over 50% of senior citizen in the US are lacking ≥1 EAA (Dasgupta *et al.*, 2005). Accordingly, it is crucial to analyze amino acid profile in food product in order to evaluate the prospect of functional food product.

Glutamic acid was identified as the highest amino acid in this study, with an average value of 36.5 ppm or almost 20% of the total amino acids. Similar reports were published on kefir made of cow milk and buffalo milk (Gul *et al.*, 2015) and kefir made of cow milk and soy milk (Gamba *et al.*, 2020). Glutamic acid was detected as the main amino acid in kefir samples, and it declined during shelf life (Gul *et al.*, 2015). Glutamic acid can be formed from proteolytic activities, glutamine deamination, or transamination of other amino acids as the acceptor of selected α-ketoglutarate groups that are converted into glutamic acids (Fernández and Zúñiga, 2006). During storage, the conversion of glutamic acid may be resulted from the decarboxylase into g-aminobutyric acids. During storage, the concentrations of tyrosine, methionine, and serine increased which may be due to the proteolytic activities, but glutamic acids showed a steady decline (Grønnevik *et al.*, 2011). In contrast, this study observed that storage time did not affect glutamic acid content in goat milk kefir.

The KOL treatment (kéfir made of oat milk) produced the highest total amino acids of all other treatments, although not significant, namely 224 ppm. The contributing factors to different concentrations of total amino acids may include proteolytic, assimilation and cell release (Grønnevik *et al.*, 2011). Similarly, microorganism proteolytic activities, peptide assimilation and amino acid releaseduring fermentation result in different amounts of free amino acids (Ozcan *et al.*, 2019). The structure of compounds bond to amino acids may be strong or varied depending on the microorganisms in kefir (Gamba *et al.*, 2020).

Table 1: Concentration of amino acids (ppm) of goat milk kefir containing *Lacticaseibacillus casei* AP and/or oat milk during storage (mean±SD, n= 3).

Parameter	Storage (days)	Treatments[a]			
		KC	KL	KO	KLO
Essential amino acids (EAAs)					
Valine	0	10.46±3.48^{aA}	12.28±2.7^{aA}	8.36±1.14^{bA}	14.02±1.94^{aA}
	7	14.94±3.13^{aB}	14.71±2.77^{aB}	12.49±1.62^{bB}	13.08±2.07^{aB}
	14	15.07±0.18^{aB}	14.71±1.16^{aB}	9.35±2.51^{bB}	13.27±1.88^{aB}
Lysine	0	21.94±10.18	20.13±4.84	16.69±5.05	23.22±2.59
	7	29.04±8.69	26.7±4.47	21.32±4.17	21.87±4.7
	14	29.2±4.28	27.89±9.35	26.57±17.08	23.27±2.32
Threonine	0	9.36±2.2	12.91±4.47	10.26±2.88	15.94±4.27
	7	11.25±1.23	13.21±1.28	10.29±1.69	11.48±3.91
	14	12.55±0.62	13.83±3.88	13.89±4.38	12.83±5.47
Phenylalanine	0	9.9±3.19^{ab}	10.96±2.21^{a}	7.64±0.86^{b}	12.69±3.31^{a}
	7	12.03±2.29^{ab}	13.51±1.24^{a}	10.38±1.59^{b}	10.96±1.59^{a}
	14	13.55±0.98^{ab}	12.27±1.05^{a}	9.25±3.15^{b}	12.51±3.09^{a}
Methionine	0	1.09±1.89	-	0.81±1.39	1.09±1.89
	7	3.09±2.71	4.48±3.89	2.18±1.92	2.03±3.51
	14	2.43±2.21	3.48±1.01	-	2.55±2.51
Leucine	0	15.24±5.32^{a}	17.63±3.57^{a}	11.38±1.36^{b}	19.28±2.94^{ab}
	7	20.65±4.38^{a}	20.43±4.58^{a}	16.93±3.37^{b}	17.89±3.38 ^{ab}
	14	22.18±1.21^{a}	20.76±2.23^{a}	14.43±5.98^{b}	18.65±2.52 ^{ab}
Isoleusin	0	8.5±2.74^{a}	9.81±1.98^{a}	6.79±0.76^{b}	10.95±1.53^{a}
	7	11.22±2.14^{a}	11.44±2.22^{a}	9.59±1.47^{b}	10.16±1.66^{a}
	14	12.04±0.38^{a}	11.26±0.89^{a}	7.69±2.03^{b}	10.45±1.31^{a}
Non-essential amino acids (NEAAs)					
Aspartic acid	0	13.36±4.13^{ab}	16.13±3.08^{a}	10.85±1.13^{b}	18.15±3.22^{a}
	7	17.11±2.31^{ab}	17.19±2.82^{a}	15.74±2.44^{b}	15.92±2.98^{a}
	14	18.8±0.83^{ab}	17.69±1.98^{a}	12.36±2.64^{b}	16.33±3.07^{a}
Glutamic acid	0	32.48±11.97^{ab}	40.99±7.36^{a}	29.76±2.91^{b}	43.98±4.52^{ab}
	7	43.34±8.48^{ab}	44.52±7.93^{a}	36.64±7.24^{b}	40.72±7.28^{ab}
	14	47.08±3.12^{ab}	46.52±4.69^{a}	34.05±9.33^{b}	42.68±6.29^{ab}
Serine	0	11.16±3.04	12.92±2.52	10.08±2.08	14.87±3.05
	7	13.4±1.64	13.59±0.48	12.26±1.53	12.2±3.02
	14	14.69±0.85	14.60±2.22	12.59±4.82	12.87±2.73
Glutamine	0	7.62±2.01	9.32±0.25	2.88±2.94	6.71±1.36
	7	8.97±1.2	7.76±2.75	6.95±0.55	4.99±4.42
	14	9.34±0.93	5.97±5.17	6.86±1.55	7.11±2.93
Glycine	0	7.28±1.02	7.59±0.51	6.9±0.59	8.21±0.74
	7	7.67±0.51	8.24±0.33	7.88±0.48	7.41±1.12
	14	8.13±0.27	8.34±0.77	8.52±2.41	7.92±1.02
Arginine	0	8.73±1.82	6.42±5.62	2.35±4.06	3.48±6.03
	7	10.54±1.72	7.95±6.89	10.37±0.47	6.54±5.89
	14	10.99±0.74	7.12±6.17	-	6.55±5.72
Alanine	0	7.87±1.58	9.1±1.24	7.07±0.77	10.41±1.89
	7	9.24±1.18	9.91±1.24	8.89±0.87	8.77±1.32
	14	9.87±0.57	10.03±0.75	8.67±2.46	9.43±1.5

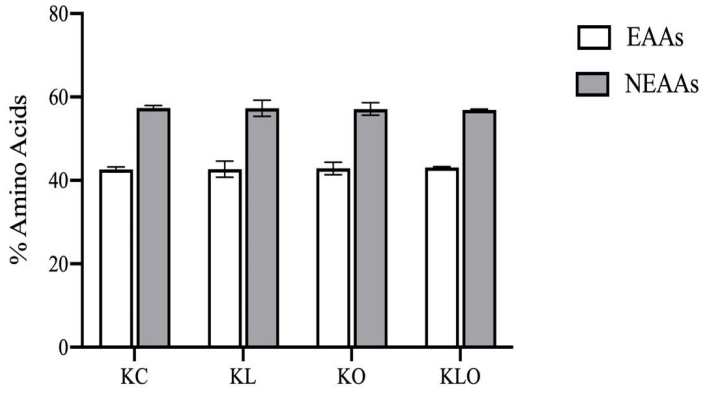
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Tyrosine	0	16.88±5.56^{ab}	20.45±4.18^{a}	13.22±1.79^{b}	21.67±1.68^{ab}
	7	23.73±5.46^{ab}	23.92±5.85^{a}	18.83±5.09^{b}	20.08±4.37^{ab}
	14	25.16±1.69^{ab}	24.13±2.45^{a}	18.31±9.57^{b}	20.61±3.26^{ab}
Total	0	181.87±58.23	206.65±35.09	145. 04±11.81	224.59±25.65
	7	236.25±45.46	237.54±43.81	200.7± 32.79	204.12±39.41
	14	663.22±704.5	238.59±27.05	121.7±115.82	217.03±29.92

Values are expressed as mean±standard deviation ($p<0.05$) for triplicate analysis. Different subscripts within lines for each property analyzed indicate statistically significant differences among the samples ($p<0.05$). Different superscripts within columns for each wall material during storage time for each property analyzed indicate statistically significant differences ($p<0.05$).

[a]Description treatment = KC: Control sample made of goat milk with kefir starter; KL: Sample made of goat milk with starter kefir and *Lacticaseibacillus casei* AP; KO: Sample made of combined goat milk and oat milk with kefir starter; KOL: Sample made of goat milk with *Lacticaseibacillus casei* AP and oat milk with kefir starter.



Description treatment = KC: Control sample made of goat milk with kefir starter; KL: Sample made of goat milk with starter kefir and *Lacticaseibacillus casei* AP; KO: Sample made of combined goat milk and oat milk with kefir starter; KOL: sample made of goat milk with *Lacticaseibacillus casei* AP and/or oat milk.
Description components = EAAs (Essential amino acids): Valine, Lysine, Threonine, Phenylalanine, Methionine, Leucine and Isoleucine. NEAAs (Nonessential amino acids): Aspartic acid, Glutamic acid, Serine, Glutamine, Glycine, Arginine, Alanine and Tyrosine.

Fig 1: Comparison of essential and non-essential amino acids (%) of goat milk kefir produced containing *Lacticaseibacillus casei* AP and/or oat Milk.

CONCLUSION

Goat milk and oat milk have different properties and composition, so when incorporated with kefir grain and *Lacticaseibacillus casei* AP, the microorganisms act as the probiotic bacteria. It could be the main contributing factor to the duration of coagulation and the rheology properties of kefir products during fermentation. Supplementation of oat milk and *Lacticaseibacillus casei* AP significantly affected amino acid parameters: aspartic acid, glutamic acid, tyrosine, valine, phenylalanine, ileucine and leucine. Evaluated from these two parameters, the quality of kefir products was evidently safe for consumption up to 14 days of shelf life. It demonstrated that oat milk and *Lacticaseibacillus casei* AP did not negatively affect amino acid.

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Conflict of interest

The authors declare no conflict of interest.

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