

Original Article

Antioxidant Activity and Amino Acid Profile of "Dadih" from Various Regions in West Sumatra, Indonesia

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ABSTRACT

Dadih or curd is a traditional product typical of West Sumatra which is made through natural fermentation using the lactobacillus plantarum bacteria in bamboo. Each region in West Sumatra makes curd in various ways that affect its quality. This study aims to determine the amino acid profile and antioxidant activity of curd from various regions in West Sumatra. The study was conducted by testing curd from 4 main curd center regions, namely Bukittinggi, Payakumbuh, Batusangkar and Alahan Panjang. Certain tests were applied to assess the protein content using the Kjeldahl method, amino acids content using the HPLC method and antioxidant activity using the DPPH method. The test results showed that curd from Payakumbuh had a higher protein and amino acid content compared to other regions. Furthermore, such curd had the highest level of antioxidant activity towards free radicals compared to other regions, with an IC50 value of 95.11 ± 0.40 mg/kg, which was included in the strong category. There was a significant relationship between amino acid content and antioxidant activity of curd. It can be concluded that curd from Payakumbuh had the best amino acid content and antioxidant activity compared to other regions in West Sumatra.

Keywords: Curd, Antioxidant, Amino Acids

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INTRODUCTION

Dadih or curd is a traditional fermented milk product originating from West Sumatra, Indonesia. This product is made by processing fresh buffalo milk using local lactic acid bacteria of *Lactobacillus plantarum* to ferment naturally for approximately one day in bamboo. Curd is considered to have good health benefits due to its amino acid composition and antioxidant activity ¹.

The fermentation process in curd is considered to produce free amino acids which can be beneficial for the body. These amino acids can be essential or non-essential. The amino acid content determines its ability to

supply protein needs in various body metabolisms². Amino acids are known to have a strong relationship to antioxidant activity³.

The antioxidant activity of curd refers to its ability to fight harmful free radicals and reduce oxidative stress in the body⁴. Antioxidants are essential for maintaining cellular health and reducing the risk of chronic diseases such as cardiovascular disorders, neurodegenerative conditions, and some types of cancer⁵. Evaluation of antioxidant activity in curd is considered important to determine its potential as a functional food.

West Sumatra has several regions as the centers of curd production, including Bukittinggi, Alahan Panjang, Payakumbuh and

Batusangkar. The curd production process in each region is very diverse regarding preparation, processing to distribution. In addition, the type of buffalo and the feed used are also different. Such differences mean that the quality of the curd produced will be different. Therefore, this study aims to evaluate the antioxidant activity and amino acid profile of curds originating from various regions in West Sumatra, Indonesia.

METHOD

The study was conducted by observing several curd samples from several regions in West Sumatra, including the Payakumbuh, Batusangkar, Bukittinggi and Alahan Panjang areas. The study observed the antioxidant capacity and amino acid content of curd originating from various regions.

Materials and Tools. The materials used in this study included curd from Payakumbuh, Batusangkar, Bukittinggi and Alahan Panjang which was taken on the second day of fermentation, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ DPPH (1,1-diphenyl-1-picrylhydrazyl) and other chemicals. The tools used included an ice box, UV-VIS spectrophotometer, burette and stand, Erlenmeyer, rubber suction cup, watch glass, dropper pipette, measuring flask and volume pipette.

Protein Content Test. Protein content was tested using the Kjeldahl method and AOAC method⁶. Through the following stages:

Destruction stage. The sample was taken, then ground thoroughly, then weighed 1 gram and put it in a Kjeldahl flask. To make sample destruction easier, 2 grams of mixed catalyst and 25 ml of concentrated H_2SO_4 were added while stirring gently until the solution was homogeneous. Then the solution was heated until it boiled and the color changed to clear green.

Distillation stage. The cooled destructed solution was diluted with 100 ml of distilled water in a 100 ml volumetric flask and pipetted 5 ml into the distillation flask. To make it easier to separate ammonia from the sample solution, 30% NaOH was added to make the solution alkaline. Some boiling stones were also added. The solution was distilled and the distillate was collected in an Erlenmeyer flask

containing 10 ml of 2% boric acid solution and a few drops of mixed indicator (methylene red + bromothymol blue). Distillation was performed for approximately 5-10 minutes.

Titration stage. The resulting distillate was titrated with a standard solution of 0.01 N hydrochloric acid. The titration point was reached when the color changed from blue to orange. Blanks were made according to the sample treatment. Calculation of % Protein was performed using the following formula:

$$\begin{aligned} \%N &= (\text{ml NaOH blanko} - \text{ml NaOH sample}) \\ &\times \\ &\quad \frac{\text{Normality of NaOH} \times 14.008}{\text{gr sample} \times 1000} \times 100\% \\ \% \text{ Protein} &= \% N \times 6.25 \end{aligned}$$

Antioxidant Activity Test. The curd samples were weighed as much as 10 grams, dissolved in 10 mL of ethanol p.a (1000 ppm). Furthermore, a dilution was made from this solution with a concentration of 100-500 ppm, 2.5 mL was pipetted into a 5 mL volumetric flask and 1 mL of DPPH was added. Samples were incubated for 30 minutes at room temperature. Absorbance was determined using the UV-Vis spectrophotometric method at a wavelength of 517 nm. Samples were made in three replications. Further, an equation was made between the concentration of the solution and the absorbance⁷. The equation was made into the following formula:

$$\text{Antioxidant Capacity} = (\text{V sample} \times [\text{sample}] \times \text{Dilution factor}) / \text{Sample weight (g)}$$

Amino Acid Profile Test. The amino acid profile was analyzed using the High-Performance Liquid Chromatography (HPLC) method to determine the content of essential amino acids such as L-arginine, L-histidine, L-isoleucine, L-leucine, L-phenylalanine, L-valine, L-threonine, as well as non-essential amino acid profiles such as L-alanine, L-aspartic acid, glycine, L-glutamic acid, L-lysine, L-proline, L-serine, L-threonine, the mobile phase used consists of water and sulfuric acid. Meanwhile, the stationary phase used is column C18. Detection was carried out at a wavelength of 254 nm².

RESULTS

Table 1. Protein content of curd from various regions in West Sumatera

Parameter	Unit	Payakumbuh	Batu sangkar	Alahan panjang	Bukittinggi
Protein Level	%	8.91±0.22	7.70±0.17	7.94±0.18	7.66±0.01

Table 2. Amino acid profile of curd from various regions in West Sumatera

Parameter	Unit	Payakumbuh	Batu sangkar	Alahan panjang	Bukit tinggi
Essential amino acids					
L-Arginine	mg / kg	2546.94±1.70	1865.83±0.30	2063.39±0.42	2347.63±1.1
L-Histidine	mg / kg	2891.65±3.90	2329.49±2.26	2102.38±6.02	2532.96±5.6
L-Isoleucine	mg / kg	4555.92±8.75	4121.03±3.46	4199.02±0.76	3905.79±5.7
L-Leucine	mg / kg	8588.33±3.08	7483.63±5.18	7556.36±1.095	7148.97±7.1
L-Phenilalanin	mg / kg	4993.27±3.57	4100.96±3.51	3729.85±5.41	4692.96±7.3
L-Valine	mg / kg	5403.23±2.56	4797.27±4.72	4746.56±3.82	4405.91±2.9
L-Threonine	mg / kg	4881.31±2.76	4224.61±3.04	3924.22±0.84	4206.66±5.8
Non-essential amino acids					
L-Alanine	mg / kg	2763.06±0.22	2380.14±1.06	2651.94±4.00	2356.93±0.2
L-Aspartic Acid	mg / kg	5602.30±3.38	4698.05±3.79	5604.85±1.62	4932.51±3.2
Glisine	mg / kg	2002.84±0.16	1698.22±1.02	1631.21±0.36	1675.01±1.1
L-Glutamic Acid	mg / kg	17378.74±11.64	14611.48±8.74	16631.87±19.45	14674.93±18.1
L-Lysine	mg / kg	5980.03±3.25	5451.36±2.60	6500.24±4.66	5101.19±1.9
L-Proline	mg / kg	8884.55±4.56	7756.81±2.06	7886.49±7.29	7227.07±10.3
L-Serine	mg / kg	5816.78±0.63	5034.03±3.40	4718.41±3.71	4882.03±3.4
L-Thyrosine	mg / kg	4385.40±1.60	3518.98±1.07	3422.52±0.80	4168.46±1.7

Curd is a traditional functional drink that is naturally fermented using buffalo milk as its raw material. Buffalo milk is known to contain quite good animal protein compared to other food ingredients. Table 1 shows the protein content values in curd from several regions in West Sumatra. The highest protein content in curd comes from the Payakumbuh region. This protein content is greatly influenced by the quality of milk produced by each type of

buffalo⁸.

As a protein source functional drink, curd contains very diverse amino acids. Table 2 shows the amino acid profile of curd. Curd has several essential amino acids including argine, histidine, isoleucine, leucine, phenylalanine, valine and threonine. Such amino acid content shows that curd is a functional drink that has quite complete ingredients.

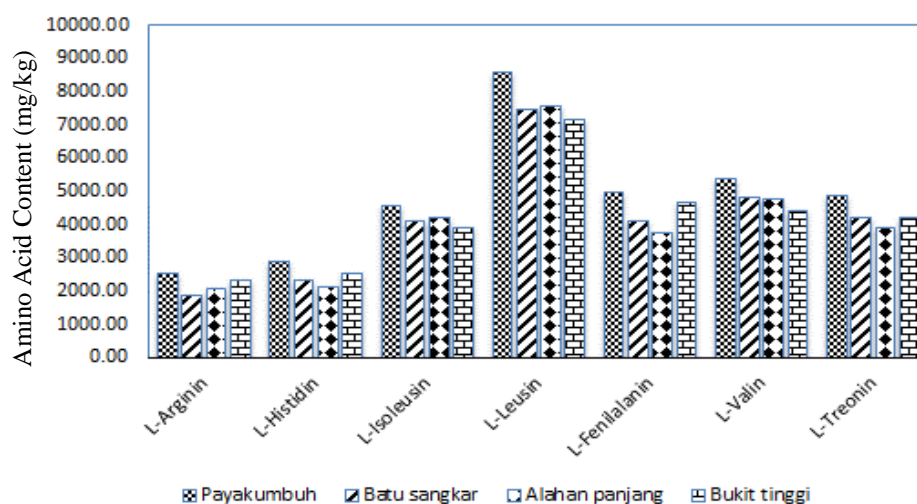


Figure 1. Graph on Amino Acid Profile of Curd from various regions in West Sumatera

Table 3. Absorbance value of curd towards free radicals of DPPH

Area	Concentration (ppm)	Absorbance DPPH	Absorbance Sample + DPPH	% Inhibition
Payakumbuh	100	0.714	0.380	48
	200	0.714	0.266	63
	300	0.714	0.172	76
	400	0.714	0.103	86
	500	0.714	0.076	89
Batusangkar	100	0.714	0.465	35
	200	0.714	0.245	66
	300	0.714	0.178	75
	400	0.714	0.123	83
	500	0.714	0.087	88
Alahan Panjang	100	0.714	0.402	44
	200	0.714	0.256	64
	300	0.714	0.177	75
	400	0.714	0.105	85
	500	0.714	0.08	89
Bukittinggi	100	0.714	0.455	36
	200	0.714	0.32	55
	300	0.714	0.205	71
	400	0.714	0.145	80
	500	0.714	0.112	84

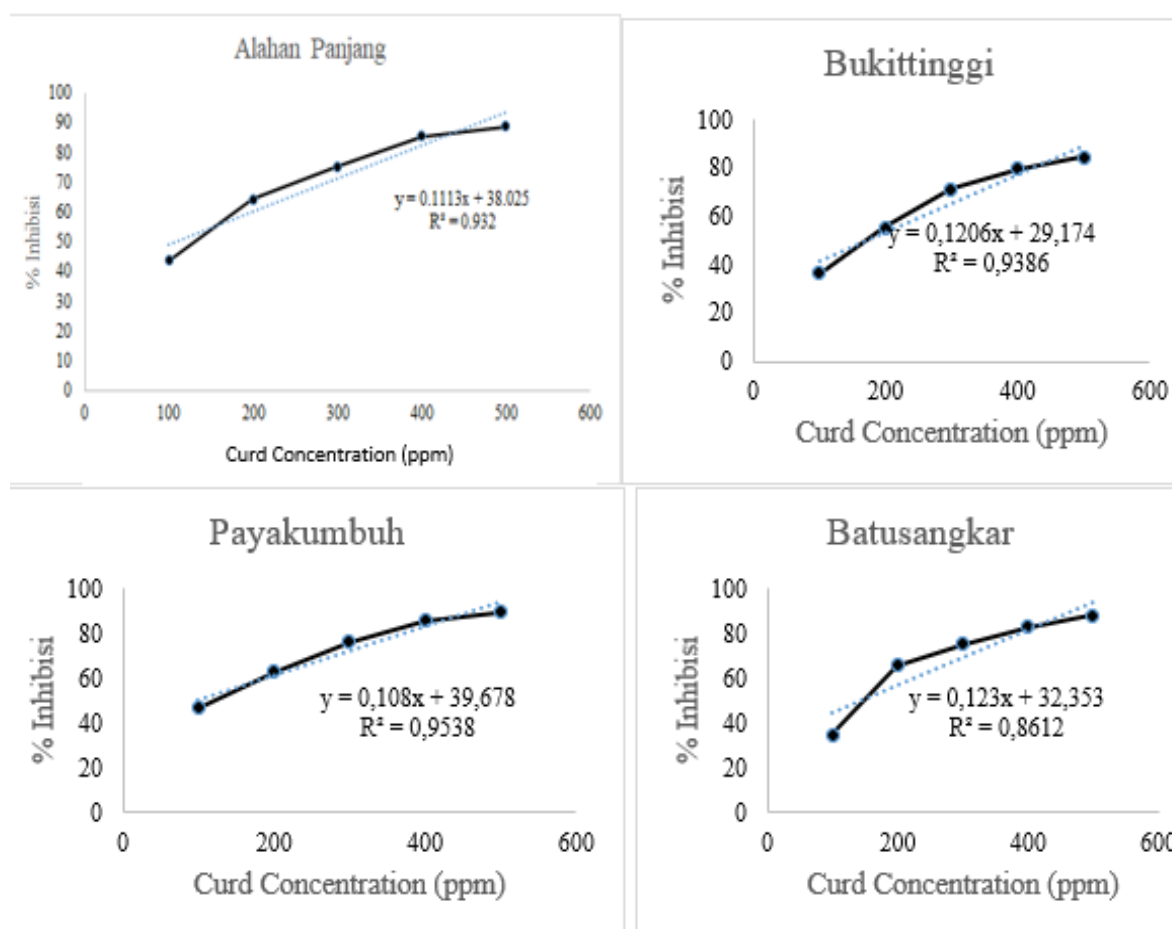


Figure 2. Similarity curve between curd concentration and % inhibition

Table 4. Calculation of antioxidant activity (IC 50)

Area	Equation	Antioxidant Activity (IC50) (mg / kg)	Category
Payakumbuh	$Y=0.1080x + 39.678$	95.11± 0.40	Strong
Batusangkar	$Y=0.1230x + 32.353$	143.76 ±0.19	Moderate
Alahan Panjang	$Y=0.1113x + 38.025$	107.59 ±1.09	Moderate
Bukittinggi	$Y=0.1206x + 29.174$	172.49 ±0.26	Weak

The presence of amino acids in curd is known to provide antioxidant activity. Table 3 shows the absorbance value of curd against DPPH which can be used as an indicator of antioxidant activity in the product. Curd from Payakumbuh had the highest level of antioxidant activity with an IC50 value of 95.11 ± 0.40 mg/kg, which was included in the strong category.

DISCUSSION

Protein Level

Buffalo milk generally has a protein content of 4.68 -5.14%⁹. The fermentation process can increase the protein content of milk after it becomes curd. It is known that there is an increase in protein from 4.68 -5.14% buffalo milk to 7.66-8.91% or around 15-30% after it becomes curd. This increase is due to the fermentation process of the *Lactobacillus plantarum* microorganism which utilizes carbohydrates as an energy source and produces carbon dioxide as a by-product. The fermentation process results in a decrease in carbohydrate content, with further impact on an increase in the protein concentration in the curd³.

Fermentation of curd is considered not only to increase protein quantitatively, but also to improve the protein quality of the curd. The protein in curd is generally in the form of small peptides, making it easier for the body to digest it after consumption. Under such condition, curd has higher bioavailability than buffalo milk¹¹.

Amino Acid Profile

Amino acids are chemical compounds that make up protein and contain an amine group (NH₂) and a carboxyl group (COOH)¹². There are at least around 20 amino acids that make up protein¹². Every protein that enters the body must first be digested into amino acids before it can be used in various functions such as immunity, muscle building and various other body metabolisms¹³.

Amino acids are divided into two groups, namely essential and non-essential amino acids. Essential amino acids are amino acids that cannot be made in the body and must be obtained from food sources of protein, which are also called exogenous amino acids. On the other hand, non-essential amino acids are amino acids that can be made in the body, or so called endogenous amino acids¹⁴.

Table 2 shows the amino acid content of curd from various regions. Curd contains both essential amino acids and non-essential amino acids. The essential amino acids contained in curd include L-arginine, L-histidine, L-iso leucine, L-leucine, L-Phenylalanine, L-valine and L threonine. On the other hand, curd in West Sumatra also contains non-essential amino acids including L-alanine, L-aspartic acid, glycine, L-glutamic acid, L-lysine, L-proline, L-serine and L-tyrosine. The complete amino acid content in curd makes this fermented drink considered very beneficial for individuals who consume it¹⁵.

Figure 1 revealed that curd from Payakumbuh had the highest amount of essential amino acids compared to curd from other regions. The amino acid content in curd increases due to the fermentation process carried out by microorganisms, generally the *Lactobacillus plantarum* bacteria¹⁶. The fermentation process in curd causes the breakdown of casein protein in milk resulting in smaller peptide molecules and free amino acids¹⁷. Amino acid content in curd reaches its highest amount after being fermented for 8 hours at an incubation temperature of 42°C or the equivalent of 16-20 hours at room temperature³. Curd that is fermented for too long will cause the amino acids in the curd to be reused by the *Lactobacillus plantarum* bacteria for their nitrogen. This condition will reduce the amino acid content in curd³.

The essential amino acid content in

curd is very useful for maintaining individual health, for example L-arginine and L valine which have benefits for reducing fatigue due to exercise¹⁸. Furthermore, L-arginine also has a good effect in lowering blood pressure because it can influence the synthesis of Nitric Oxide. (NO) which can relax blood vessels¹⁹. Another amino acid, namely isoleucine has a good influence on improving digestive tract health, digestive ability, and improving intestinal microbiota²⁰. There is also the amino acid called phenylalanine which plays a role in nervous system regulation. Consuming this amino acid can increase concentration, motivation and mood for those who consume it²¹. With the various benefits presented by the amino acids in curd, this fermented drink has the potential to be a functional drink that is good for health.

Antioxidant Activity

The antioxidant activity of curd was tested using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The curd sample was mixed with DPPH free radicals which had been dissolved in several concentrations. Furthermore, the ability of the curd to fight free radicals was examined using a UV-Vis spectrophotometer.

Table 3 shows the absorbance values of free radicals in various concentrations. Figure 2 shows the equation obtained between free radical concentration and absorbance. After making an equation between the concentration of free radicals and the absorbance value, an equation was obtained: $y = a(x) + b$ per each sample. This equation was further used as a basis for calculating antioxidant activity (IC₅₀). Table 4 shows the results of antioxidant activity calculation of curd from various regions in West Sumatra.

The antioxidant activity of curd from the Payakumbuh region was included in the strong category. Meanwhile, curd from Batusangkar and Alahan Panjang was in the moderate category, and curd from Bukittinggi was in the weak category. There was a very strong relationship between antioxidant activity and the number of amino acids in curd³. Each amino acid has antioxidant activity, of which there are seven amino acids with the best antioxidant activity including tryptophan, methionine, histidine, lysine, cysteine, arginine, and tyrosine²².

The antioxidant activity of amino acids varies based on constituent structures. For example, the amino acids of histidine and arginine contain a nitrogen atom with one free electron. This condition will make it easier for such amino acids to fight free radicals and then neutralize them. Other amino acids such as tyrosine have a benzene ring which is very active in eliminating free radical ions²². The good antioxidant activity in curd makes this drink an alternative functional food in treating various degenerative diseases, such as dyslipidemia, atherosclerosis, obesity and others²³⁻²⁵.

Differences in antioxidant activity in each region were also caused by differences in the amino acids content, as well as antioxidants presented in milk before processing. The feed consumed by buffalo greatly influences the quality of the milk produced. Cows or buffalo that are fed a diet high in antioxidant sources such as vitamin E, carotene and retinol produce milk that contains higher antioxidant activity²⁶. The antioxidants in raw milk include β -lactoglobulin, vitamins A, E, C, and β -carotene²⁶. Efforts to increase the stability of antioxidants in curd can be done in various ways, including adding antioxidants²⁷, or by making the curd into powder using the freeze drying method²⁸.

CONCLUSION

Curd from various regions in West Sumatra had various antioxidant activities and amino acid profiles. Curd from Payakumbuh had the highest level of antioxidant activity towards free radicals compared to other regions, with an IC₅₀ value of 95.11 ± 0.40 mg/kg (strong category). Furthermore, such curd also had the highest essential amino acid content compared to other regions, including the amino acids of histidine, arginine, isoleucine, leucine, valine, phenylalanine and tyrosine.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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