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Antioxidant Activities and Profile of Amino Acid of Yoghurt from Beef Milk Fermentation with Dadih Starter

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Abstract

Dadih is naturally fermented buffalo milk in bamboo tubes and known to have antioxidant, antibacterial, and antihypertensive activity. Lactic acid bacteria in dadih can use as a starter to produce yogurt. The study aimed to produce yogurt of cow's milk with dadih as a starter, to determine the antioxidant activity and amino acid composition. Dadih is added with concentration variation of 2,5; 5; 7,5 and 10% (v/v) and it fermented for 48 hours at room temperature. Lactic acid bacteria cell counts of dadih were calculated by the total plate count method. Yogurt was tested by organoleptics with 33 panelists and proximate analysis water, ash, fat and protein contens based on the Association of Official Analytical Chemist (AOAC) in 2005. Antioxidant activities of yogurt were tested by DPPH method (1,1-diphenyl-2-picrylhydrazyl). Amino acid composition of yogurt was analyzed using ultra performance liquid chromatography (UPLC). The research result of amount of LAB on dadih are 1,01 x 1011 CFU/mL, has fulfilled the requirements of SNI 2981: 2009. Yogurt produced with the addition of dadih by 10% (v/v) is most accepted by panelists. The highest antioxidant activity was obtained by yogurt with 2.5% addition of dadih with IC_{50} value of 78.28 ppm. Yogurt almost contain all essential and non-essensial amino acids including tyrosine and phenylalanine as the antioxidants. Measurement of water, ash, protein and fat content in the sample meets the requirements of SNI 2981: 2009.

Keywords: Antioxidant, lactic acid bacteria, dadih, DPPH, yogurt

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1. INTRODUCTION

Antioxidants are compounds that can prevent and protect the body from free radicals (Wildman, 2001). The state when the number of free radicals is higher than the antioxidants in the body can accelerate the aging process and cause degenerative diseases such as cardiovascular disease, diabetes mellitus, tumors, and cancer (Cooper and Hausman, 2003). Natural antioxidants in the human body have not been effective at warding off free radicals (Sen *et al.*, 2010), so it is necessary to consume foods that contain antioxidants such as dairy products.

Yogurt as fermented milk is one way to increase the benefits of milk. One of the studies regarding the benefits of fermented milk is an antioxidant (Chalid and Hartiningsih, 2013; Sah *et al.*, 2014). A starter that is commonly used in the manufacture of yogurt is a pure isolate of lactic acid bacteria (BAL): *Streptococcus thermophilus* dan *Lactobacillus bulgaricus* (Winarno *et al.*, 2003). The duration of milk fermentation is 48 hours to produce yogurt with the highest activity (Mulyani *et al*., 2019)

Chalid and Hartiningsih (2013) stated that fermented buffalo milk has antioxidant activity with an IC_{50} value of 241.80 ppm. Dadih as an indogenic food is used as a starter in making yogurt for the development of previous research ssince yogurt products are widely liked by various groups. The bacteria involved in the fermentation of buffalo milk is BAL indigenous from the *Lactobacillus plantarum* IS-10506 strain which comes from bamboo tubes and banana leaves as a curd

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cover. A total of 36 strains of BAL were found in dadih from the genus *Lactobacillus*, *Streptococcus*, *Leuconostoc*, and *Lactococcus* (Pato, 2008). The amount of BAL starter for making yogurt must be in accordance with the minimum standard of SNI (2009), namely 107 CFU/mL, therefore the dadih must be analyzed for the amount of LAB contained in it.

The quality of yogurt is determined by an organoleptic test and proximate analysis to determine whether the yogurt meets the SNI requirements. According to SNI (2009), good yogurt has a thick to semi-solid texture, a distinctive aroma of yogurt, a sour taste, and homogeneous consistency. BAL in dadih produces protease enzymes which break down milk proteins to produce bioactive peptides that have antioxidant activity hence yogurt is expected to have antioxidant activity.

Antioxidant activity is related to the amino acid composition of peptides (Korhonen and Pihlanto, 2006). Amino acids that have the potential to act as antioxidants are hydrophobic amino acids such as tyrosine, tryptophan, and phenylalanine. The results of the research by Kusumaningtyas *et al*. (2015) showed that high antioxidant activity in goat milk protein hydrolyzate was caused by the total amount of hydrophobic and aromatic amino acids. In this study, the production of cow's milk yogurt using a dadih starter and measuring the antioxidant activity of yogurt by the DPPH method and the amino acid content was analyzed by UPLC.

2. MATERIALS AND METHODS

Materials

The tools used in this research are glass tools, closed glass bottles, oven (Memmert UN55), magnetic stirrer, centrifuge (Hettich), analytical balance (OHAUS AX124), vortex, hot plate, micro pipette, micro tube, tip, distillation apparatus, socklet device, UV-Vis spectrophotometer (ThermoFisher Scientific G10S), Ultra Performance Liquid Chromatography / UPLC (Water type Breeze with ACCQ-Tag Ultra C18 column), autoclave (Hirayama HVE50), incubator (Memmert BM500), desiccator (Normax 250mm), coarse filter paper.

The main ingredient used in this research is fermented buffalo milk from Koto Malintang village, Agam district, West Sumatera. Other ingredients are skim cow's milk (IndoPrima), distilled water, DPPH (1,1-diphenyl-2-picrylhydrazyl) 0.1 mM (Sigma-Aldrich Co), deMan's Rogosa Sharpe Agar (MRSA) medium, Buffer Peptone Water (BPW) 0, 1% (Merck KGaA), Bradford reagent (Merck KGaA), bovine serum albumin (BSA) (Merck KGaA), 30% sodium hydroxide solution (Merck KGaA), 10% trichloroacetic acid (TCA) (Sigma-Aldrich Co), ethanol 75% (Merck KGaA), indicator Brom Cresol Green-Methyl Red (Merck KGaA), 6 N and 0.05 N hydrochloric acid solution (Merck KGaA), borax solution (Merck KGaA), aquabides, α-Aminobutyric Acid (AABA), AccQ Fluor borate, fluorine reagent A.

Autoclave Tool Sterilization

Glass utensils used are washed clean. Tools such as tweezers, scalpels, Petri dishes, bottles, and Erlenmeyers are covered with paper. Tool sterilization was carried out using an autoclave with a pressure of 15 psi at a temperature of 121°C for 15 minutes.

Total BAL Dadih Test with the Total Plate Count (TPC) Method (Pelczar and Chan, 1988)

Calculation of the number of lactic acid bacteria is carried out on curds using 10^{-1}, 10^{-2}, 10^{-3}, to 10^{-9} dilutions, namely by moving 1 mL of the sample into a test tube containing 9 mL of 0.1% BPW solution (v/v), then homogenized using a tube shaker. 1 mL of a 10^{-7} to 10^{-9} dilution was planted in a petri dish containing MRS Agar, then incubated at 37°C in an inverted position. The count of the growing colonies is carried out after 48 hours.

Making Yogurt Based on Dadih Starter (SNI, 2009) and Measuring the pH Value

A total of 157.5 g of powdered skim milk were dissolved in 900 mL of warm mineral water, then 5 sterile glass bottles with lids were prepared as fermentation containers. The first bottle was used as a control, which contained 100 mL of skim milk without adding a dadih starter. The second to fifth bottles are added with a starter of dadih as much as 2.5; 5.0; 7.5 and 10% (v/v) and the liquid skim milk is added to a total volume of 100 mL. The five bottles were shaken until homogeneous and fermented at room temperature for 48 hours. Yogurt pH measurement was carried out at the 48 hour fermentation time. Yogurt pH is measured using a pH meter that has been calibrated

Organoleptic Test for Dadih Starter Based Yogurt (SNI, 2009)

Organoleptic tests were carried out by 33 poorly trained panelists. Organoleptic test parameters include texture, aroma, taste, consistency, and general preferences. The range of values used is 1-3 where each number indicates the level of preference for the panelist to the product. The value of the panelists' preference level is added and averaged for each parameter then a statistical analysis (ANOVA) is carried out on the data from the organoleptic test results.

Extraction of Dadih Starter Based Yogurt Protein (Chalid et al., 2018)

A total of 100 mL of yogurt sample was dissolved with 100 mL of 75% (v/v) ethanol, then stirred until it was homogeneous, then filtered with coarse filter paper. The precipitate is removed and the filtrate is evaporated using a rotary vaccum evaporator to remove ethanol solvent. The filtrate was centrifuged and the yogurt protein extract was obtained.

Measurement of Yoghurt Dissolved Protein Levels (Bradford, 1976)

The protein extract of yogurt and skim milk as much as 50 µL was put into each test tube, 2.5 mL of Bradford reagent was added, vortexed and allowed to stand for 5 minutes, then the absorbance of the solution was measured by a UV-Vis spectrophotometer at λ = 595 nm. The results were obtained by making a standard curve of bovine serum albumin (BSA) to determine the protein content.

Hydrolysis Degree Test (Hoyle and Merritt, 1994)

The 5 mL protein extract was added with 5 mL 10% (v/v) TCA. The mixture is then left to stand for 30 minutes to allow for precipitation, then centrifuged at a speed of 6000 rpm for 15 minutes. The supernatant was analyzed for protein content using the Bradford method, resulting in data on the concentration of TCA dissolved protein.

Yogurt Antioxidant Activity Test (Molyneux, 2004)

A total of 2 mL of protein extract was added with 2 mL of 0.1 mM DPPH. The mixture was homogenized and left for 30 minutes in a dark place, the absorption was measured by a UV-Vis spectrophotometer at a wavelength of 517 nm. Methanol is used as blank. The IC_{50} value is determined using the linear regression equation y=ax+b, where y is the% inhibition value 50 and x is the sample concentration to which the IC_{50} value will be determined from the sample concentration series. Vitamin C was used as a positive control.

Analysis of the Amino Acid Composition of Skim Milk and Yogurt with Ultra Performance Liquid Chromatography (UPLC) (Gandjar and Rohman, 2007)

The optimum amino acid composition of yoghurt antioxidant was analyzed using Ultra Performance Liquid Chromatography. A sample of 0.1-1.0 g was added to 5 mL of 6 N HCl solution, then oven for 22 hours at a temperature of 110 ℃. The solution was cooled and transferred to a 50 mL volumetric flask, then aquabides were added to the limit. The solution was filtered off with a 0.45 µm filter. A total of 500 µL of solution was added with 40 µL α Aminobutyric Acid (AABA) and 460 µL of aquabides. The solution that had been homogenized was taken as much as 10 µL and added with AccQ Fluor borate and 20 µL of Fluor A reagent and homogenized again. The solution was allowed to stand for 1 minute and incubated at 55 ℃ for 10 minutes. A total of 10 µL of solution was injected into the UPLC.

Yogurt Proximate Test Based on Dadih Starter Moisture Content (AOAC, 2005)

Measurement of moisture content was carried out using an oven. Empty porcelain plates were dried in an oven at 105℃ for 15 minutes, cooled in a desiccator, then weighed (A). A total of 2 g of sample (B) was weighed and placed in a dish. The plate containing the sample was dried in an oven with a temperature of 105 ℃ for 6 hours. The sample was cooled in a desiccator and weighed (C).

Ash Content (AOAC, 2005)

The sample was weighed as much as 2 g (B) and put into a porcelain dish whose weight was known (A). Samples were oven at 105 ℃ for 6 hours, then put in a furnace at 500-600 ℃ until they turned ash white for 3 hours. The plate containing the ash is cooled in

a desiccator and weighed until a fixed weight is obtained (C).

Protein analysis of the Kjeldahl Method (AOAC, 2005)

The protein content contained in the yogurt product was analyzed through the total nitrogen analysis stage using the Kjeldahl method which consisted of three stages, namely digestion, distillation and titration. The digestion stage was carried out by inserting 0.25 g of the sample into a Kjeldahl flask and adding 0.25 g of selen catalyst mixture (SeO_2 + K_2SO_4 + $CuSO_4$) and 3 mL concentrated H_2SO_4, then digested for 1 hour with a gradual increase in temperature until liquid become clear, then cooled down.

The digestion sample was diluted with distilled water to 50 mL. The distillation stage was carried out by adding 20 mL of 40% NaOH solution. The results of the distillation are collected in an Erlenmeyer flask containing 10 mL of boric acid solution and 2 drops of Brom Cresol Green-Methyl Red indicator. The distillation is carried out up to 20 minutes after the first drop until the distillate turns green tosca. The distillation solution was titrated with 0.1 N HCl solution which had previously been standardized using 0.1 N borax solution. The titration was carried out until the color changed from Tosca green to red. The volume of HCl used for titration is measured.

Fat Analysis (AOAC, 2005)

Fat content was analyzed using the sokhletation method. 2 g of sample is weighed (B) and wrapped in filter paper. The wrapped sample is put into a sokhlet extraction flask which has been previously dried in an oven, then cooled in a desiccator and weighed (A). The condenser is placed at the top and the round flask is placed at the bottom. A total of 150 mL of n-hexane solvent was put into a round flask, then refluxed for 6 hours. The solvent in the flask is distilled off and the solvent is reclaimed. The flask containing the extracted fat is heated in an oven at 105 ℃ until it reaches a constant weight, then cooled in a desiccator. The pumpkin with the fat in it is weighed (C) and the weight of fat can be found using the equation.

LCMS/MS Analysis

The yogurt protein extract with the highest antioxidant activity was analyzed using Liquid Chromatography-Mass Spectrometry (LCMS). The molecular weight (BM) of the yoghurt protein was analyzed from the mass spectrum results through deconvolution of protein BM.

3. RESULTS AND DISCUSSION

Dadih is known to contain various bacteria *Lactobacillus plantarum*, *Lactobacillus brevis*, *Streptococcus agalactiae*, *Bacillus cereus*, and *Streptococcus uberis* (Pato, 2008), therefore that dadih can be used as a starter in making yogurt in this study. The total BAL test was carried out using the Total Plate Count (TPC) method.

The purpose of testing the total BAL on dadih is to determine the amount of BAL for dadih used meets the SNI requirements. According to SNI (2009), the amount of BAL as a starter in making yogurt must contain a total BAL of at least 10^7 colony-forming units per milliliter (CFU/mL).

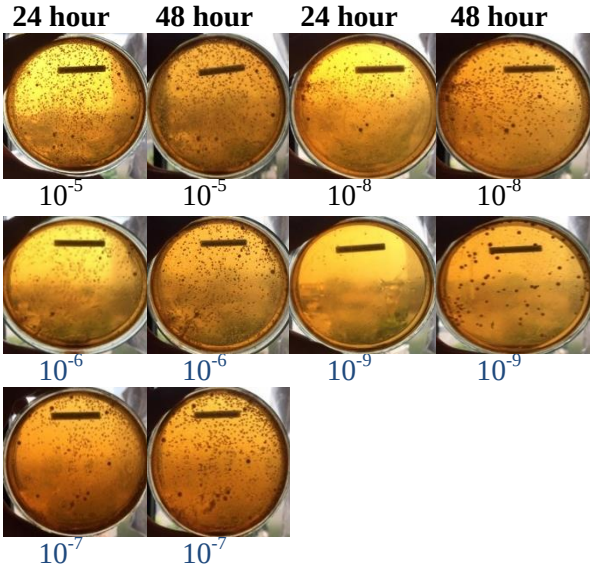

Figure 1. Colony of BAL dadih on MRS medium in order to dilute 10^{-9}, incubation time of 24 and 48 hours

The results of the total BAL dadih test according to SNI standards were obtained at 10^{-9} dilution, namely 1.01 x 10^{11} incubation for 24 hours with a dilution factor of 10^{-5}-10^{-9} while the 48 hour incubation time, the number of colonies exceeded 300 colonies, was not used as a starter. The growth of BAL colonies on MRS Agar media continued to increase with the length of the incubation time. The smaller the dilution, the less BAL colonies will

grow. BAL growth on MRS medium in order to dilute 10^{-9} (**Figure 1**).

Yogurt is a food or beverage product obtained from milk fermentation using *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria and/or other suitable lactic acid bacteria (SNI, 2009). Yogurt produced in this study uses skim milk with dadih starter. Dadih was obtained from the village of Koto Malintang, Agam Regency, West Sumatra. Characteristics of yogurt with the addition of 2.5 curds; 5; 7.5 and 10% are visually almost the same, namely cloudy white and thick. Yogurt with a 10% dadih starter concentration has the thickest texture than the others. It is estimated that the viscosity of the BAL dadih is higher than that of collecting almost all the chemical components of milk.

Table 1. The pH value of yogurt based of dadih starter

Concentration on adding dadih % (v/v)	Yoghurt pH value
2.5	4.27
5.0	4.28
7.5	4.18
10.0	4.20

The pH value of yogurt is in the range 4.27 to 4.20 (Table 1). Umar and Novita (2014) state that during the milk fermentation process, the lactose breaks down into lactic acid which causes acidity to increase and a decrease in the pH value.

The yoghurt organoleptic test aims to determine the level of preference and acceptance of the panelists towards yogurt products with several concentrations of adding curd. The organoleptic test in this study included texture, consistency, aroma, taste and general preference (hedonic) tests for yogurt (Figure 2). According to SNI (2009), yogurt has the characteristics of thick, homogeneous, has a distinctive aroma of yogurt, sour taste with a cloudy white color. The organoleptic test used was the hedonic test of 33 untrained panelists.

Rukmana (2001) stated that when the pH of milk is low, the casein becomes unstable and coagulates (clots) hence the texture of the yogurt is thicker. The results of the analysis of variance on the texture of yogurt showed that the panelists preferred the highest level of yogurt with the addition of starter dadih with a concentration of 10% (v / v), namely a score of 2.90. The average yogurt texture among the four formulations showed significant differences (P <0.05). The thicker the yogurt texture, the more preferred the panelists. According to Pangestu et al. (2017), the viscosity of the texture of yogurt is caused by the material which has a very good binding power in forming a matrix to trap water, causing the viscosity to become thicker.

Yogurt consistency is related to the level of yogurt homogeneity. The results of statistical analysis of variance on the consistency of yogurt showed that the panelists preferred the highest level of preference to yogurt with the addition of dadih with a concentration of 10% (v / v) with an average value of 2.6. Djali et al. (2018) stated that the increased amount of protein causes the formation of coagulants hence yogurt has a higher consistency. The results of the analysis of variance showed that there was a significant difference (P <0.05) in all variations in the concentration of yoghurt.

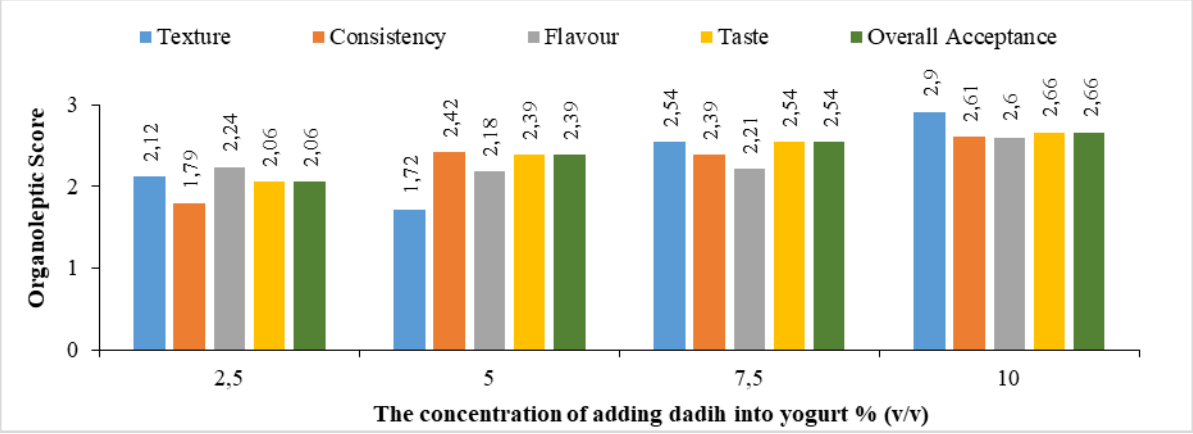

Figure 1. The graph of the panelists' preference for yogurt variations

Yogurt has a distinctive aroma because of the acidity produced from lactic acid bacteria in the milk fermentation process (Yunus et al., 2017). The results of the analysis of variance statistically on aroma showed the highest preference for the panelists in yogurt with the addition of curd with a 10% (v/v) concentration of 2.60. The analysis of the variance in the aroma of yogurt showed significant differences (P <0.05).

The taste of yogurt is sour (Yunus et al., 2017). Djaafar and Rahayu (2006) state that the formation of lactic acid in the fermentation process causes an increase in the acidity of yogurt. Based on the panelists' assessment of the taste of yogurt, it shows that the more the proportion of adding curd as a starter, the more sour the yogurt taste. This is in line with the pH value, the greater the addition of curd, the lower or acidic pH value of yogurt is. The results of statistical data processing are the highest preference level for the taste of yogurt with the addition of curd with a concentration of 10% (v/v) of 2.66. Based on the analysis of the taste variance in all variations in the concentration of yoghurt, it showed a significant difference (P <0.005).

General preference is the level of preference for the panelists to a product as a whole, which is influenced by the parameters of appearance, texture, consistency, aroma, and taste (Figura and Teixeira, 2007). The highest score was obtained for yogurt produced from fermentation with the addition of 10% (v/v) dadih, which is 2.06. The overall parameters showed that the most preferred result for the panelists was yogurt with a concentration of 10% (v / v) dadih addition. The results of the analysis of variance on the general preference of yogurt among the four formulations did not show a significant difference (P> 0.005).

The dissolved protein content of the control (skimmed milk without curds) was 2265 ppm (Fiigure 3). This shows that the protein content is still very high because the protein hydrolysis process has not yet occurred. The highest protein content was yogurt with the addition of 2.5% (v/v) dadih concentration, which was 210.875 ppm. Protein levels decrease when the cleavage activity of the number of bacteria increases, therefore the bacteria need amino acids as a source of energy in greater amounts. This is in accordance with the research of Kartika (2011), which states that the optimal formulation and fermentation time for BAL produces the highest dissolved protein content. The breakdown of bacterial cells affects the increase in protein levels because these bacterial cells will mix with the substrate, causing an increase in protein levels in the substrate (Yusmarini and Efendi, 2004).

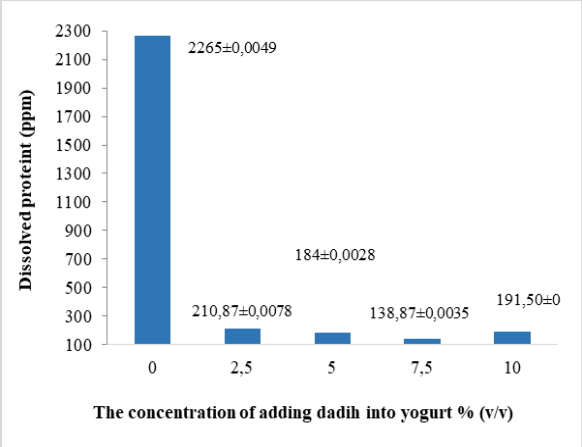

Figure 2. Graph of yogurt dissolved protein content

The value of the degree of hydrolysis is related to the amount of hydrolyzed product produced, or in other words, the value of the degree of hydrolysis has the same tendency to the amount of dissolved protein or free amino groups (Adler-Nissen, 1979).

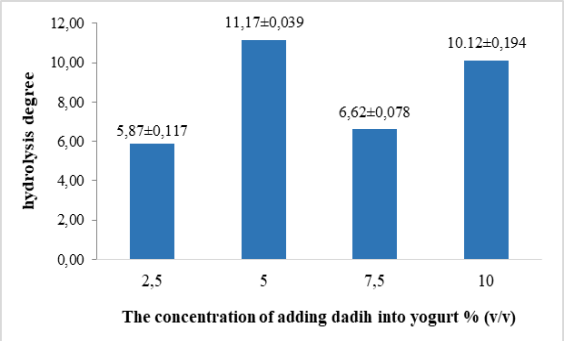

Figure 3. The value of the hydrolysis degree of yogurt

Yogurt with dadih concentration of 5% (v/v) had the highest degree of hydrolysis value, namely 11.165%. According to Hernandez *et al.*, (2011), the degree of hydrolysis is directly proportional to the dissolved protein concentration, but in this study the value of the degree of hydrolysis is not directly proportional to the dissolved protein content. This is presumably because there are still many proteins that have not been

degraded into peptides, so that the hydrolysis is incomplete.

The highest inhibition percentage in this study was yogurt with an added concentration of curd 2.5% (v/v) of 88.86%. The IC_{50} of the concentration that produces the highest percent inhibition is determined by constructing the relationship curve between the concentration and the percent inhibition shown in Figure 5.

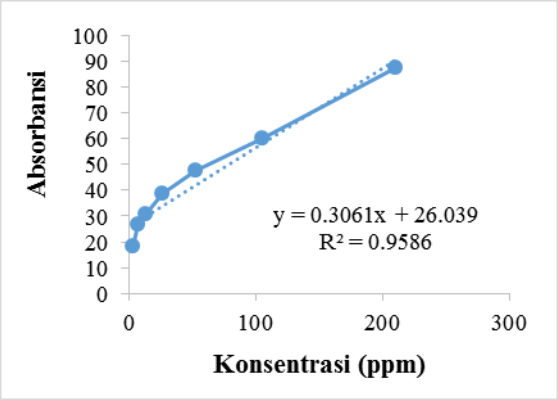

Figure 4. % Inhibition dadih of yogurt with the addition of 2.5% dadih (v/v)

From the% inhibition curve, the IC_{50} value of yogurt was obtained with a concentration of addition of curd 2.5% (v/v), which was 78.27 ppm. Yogurt based on curd starter shows strong antioxidant activity because it has an IC_{50} value in the range of 50-100 ppm (Jun *et al.*, 2003). The positive control used in testing antioxidant activity was ascorbic acid. The IC_{50} value of ascorbic acid is 4.20 ppm.

Amino acids are the building blocks of protein, their composition is unique for each type of protein. Proteins can be broken down into simpler units (amino acids) through the hydrolysis process (Nelson and Cox, 2004). The following is the result of amino acid analysis in skim milk and yogurt with the addition of 2.5% (v/v) curd which is presented in table 1.

The amino acid with the highest levels in skim milk and yogurt is glutamic acid, which is 4343 mg / kg and 338.315 mg / kg. The amino acids in skim milk and yogurt showed a decrease, due to the protein hydrolysis process during the fermentation process due to the activity of BAL. BAL in the fermentation process requires protein as an energy source so that the amino acid levels in yogurt are smaller. The amino acid composition of yogurt that is thought to have potential as an antioxidant is tyrosine and phenylalanine with levels of 67.87 mg/kg and 97.91 mg/kg. This is consistent with Pownall *et al.*, (2010) which states that antioxidant activity is related to the total content of hydrophobic amino acids. Tyrosine, phenylalanine and tryptophan are hydrophobic amino acids that have an aromatic ring.

Tabel 2. The amino acid composition of yogurt with the addition of dadih 2,5% (v/v)

Amino acid classification	Amino acid	Amino acid levels (mg/kg)	
		Skim milk	Yoghurt
Esential	L-Threonin	1213.97	131.05
	L-Lisin	1523.86	131.58
	L-Fenilalanin	1520.11	97.91
	L-Isoleusin	1265.98	106.93
	L-Leusin	2499.98	187.12
	L-Metionin	-	-
	L-Arginin	967.21	61.58
	L-Valin	1477.47	119.53
	L-Triptofan	339.27	-
Non esential	L-Asam aspartat	1391.35	138.33
	L-Prolin	2338.12	277.65
	L-Tirosin	1284.46	67.79
	L-Alanin	737.92	230.15
	L-Serin	1552.77	107.17
	L-Asam glutamat	4343.73	338.32
	Glisin	553.56	66.24
	L-Sistin	63.72	<16.12
	L-Histidin	757.69	62.54

Table 3. Yogurt proximate test results

Parameter (%)	Yoghurt		Milk solution skimmed	Dadih**	Yoghurt (SNI, 2009)
	2.5%	10%			
Water content	84.72±0.26	82.72±0.07	85.26±0.43	76.05	-
Ash content	0.85±0.01	0.93±0.004	0.90±0.01	0.83	Maks 1.0
Protein content	3.24±0.13	3.74±0.12	21.00*	6.04	Min 2.7
Fat content	0.84±0.14	2.54±0.49	0.00*	13.62	0.6-2.9

* Citation from the information on the nutritional value of IndoPrima skim milk on the packaging
**Chalid *et al.* (2018)

Proximate analysis was carried out on yogurt which had the highest antioxidant activity and the result of the general preference most favored by panelists in the organoleptic test, namely yogurt with the addition of dadih with concentrations of 2.5% and 10% (v / v). Following are the proximate results of yogurt and compared with the literature.

Food products have different content or moisture content. Water content greatly affects the properties of the product, chemical changes, and damage by microbes because water can be used by microorganisms for their growth. The water content of the yogurt with the addition of dadih concentrations of 2.5 and 10% (v/v) were 84.72 and 82.72%. The fermentation process is one of the factors that affect water content, because the breakdown of carbohydrates by enzymes and the increasing length of fermentation time will further increase the water content (Buckle, 2007).

Ash content is analyzed to determine the amount of mineral content contained in a foodstuff (Sudarmadji *et al.*, 2007). The high value of ash content is related to the fermentation process, in accordance with the statement of Tamime and Robinson (2007) that the fermentation process, apart from converting glucose into lactic acid, also produces minerals as a by product. The results of the ash content test showed that 2.5% concentration of yogurt had an ash content of 0.85%, while 10% concentration of yogurt had an ash content of 0.93%. The increase in ash content is thought to occur due to the concentration of the addition of a larger starter. The ash content of the two yoghurt samples still conforms to the SNI quality standard, where according to SNI (2009) the ash content of yogurt is a maximum of 1%.

According to Winarno and Fernandez (2007), the more lactic acid bacteria in yogurt, the higher the protein content since most of the components of lactic acid bacteria are protein. The results of testing the concentrations of yoghurt protein content of 2.5% and 10% were 3.24% and 3.74%. The increase in protein content occurs due to the use of more starters so that the protein produced from the fermentation process increases. The protein content of the yogurt sample is in accordance with the SNI quality standard, which is a minimum of 2.7%.

The fat content of yogurt depends on the raw materials used in making yogurt. High fat content in food is one thing that must be considered so as not to cause negative effects on health when consumed (Ekafitri and Isworo, 2014). During fermentation, fat is hydrolyzed into simpler compounds by the enzyme lipase and produces fatty acids and glycerol. Yogurt with added curd concentration of 2.5 and 10% (v/v) had fat content of 0.84 and 2.54%, respectively. These results meet the requirements for yogurt fat content in SNI, namely 0.6-2.9%. with the addition of 2.5% (v/v) curd which has the highest antioxidant activity in the form of chromatogram and spectrum. Antioxidant protein chromatogram of yogurt protein extract (Figure 6).

The extract of the yogurt protein sample was then characterized using LCMS / MS to determine the molecular weight of the protein contained in the sample. Results of protein analysis from yogurt.

Figure 7 shows that the analyzed yoghurt sample produces the highest chromatogram peak. The chromatogram peaks appeared at the retention time of 16.79 minutes. The peaks of the chromatogram are further analyzed based on their mass spectrum. The mass spectrum at the retention time of 16.79 can be seen in Figure 7. Based on the m/z data from each mass spectrum produced, then a molecular weight analysis is carried out. The weight of the peptide molecule from the peak that appears on the chromatogram is 5324 Da.

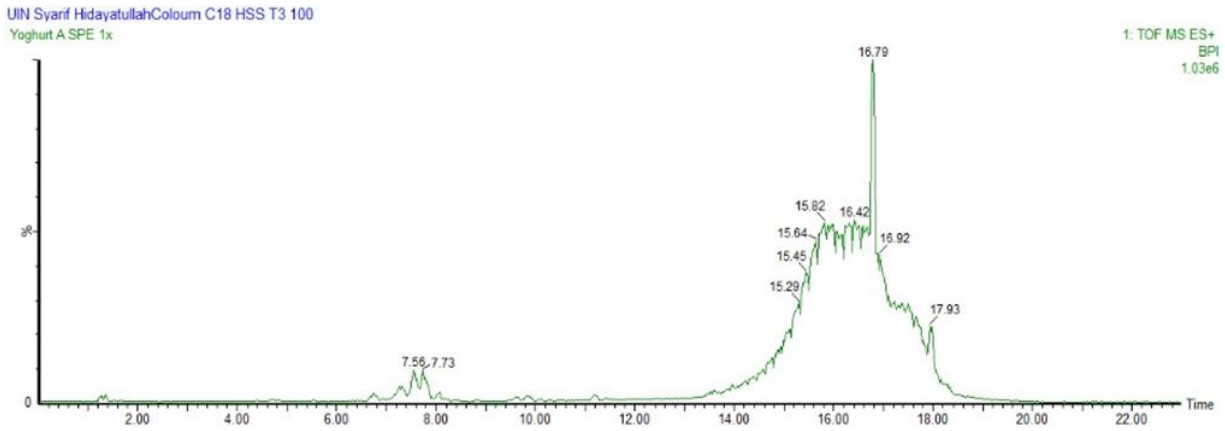

Figure 5. Antioxidant peptide chromatogram of yogurt protein extract

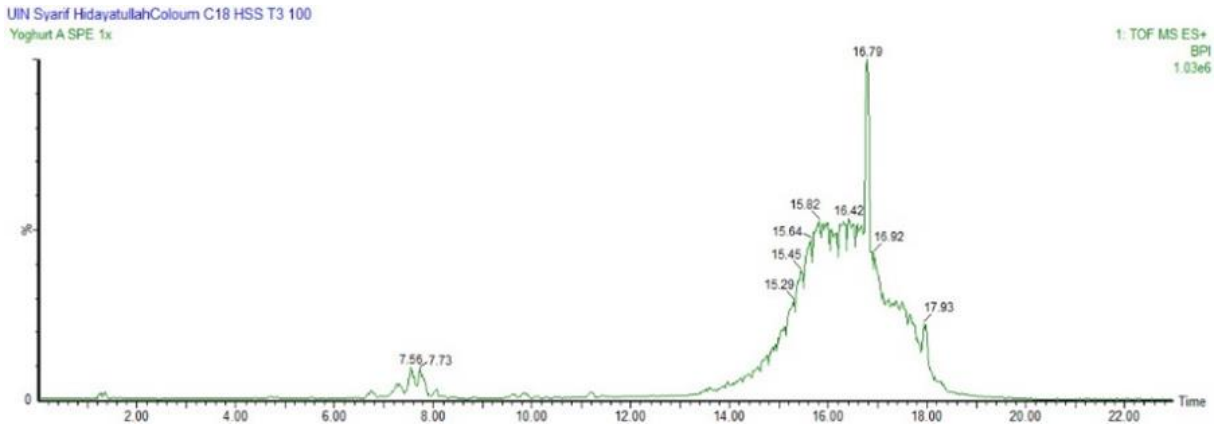

Figure 6. Mass spectrum of yoghurt antioxidant peptide chromatogram

4. CONCLUSION

Yogurt with curd starter meets the SNI quality requirements for yogurt including water content, ash content, fat content and protein content. The highest antioxidant activity was yogurt with the addition of 2.5% (v/v) curd with an IC_{50} value of 78.28 ppm. Amino acids that have the potential to act as antioxidants from yogurt are hydrophobic amino acids such as tyrosine and phenylalanine.

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