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Identification And Characterisation Of Bioactivpeptides Of Fermented Goat Milk As A Sources Of Natural Product For Medicine

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Abstract: The increasing of functional food is risingin line with public awareness for healthy food consumption. Provision of functional food source is developed through enhanced bioactive that has a regulatory function for body. Bioactivepeptides in milk is known have variety of beneficial function of the body such as immunomodulator, immunostimulatory, anti-hypertension, anti-hypercholesterol, as well as a variety of other beneficial function. The aim of this study is to obtain a fermentation method to product functional dairy product contain bioactive peptides and beneficial of fermented goat milk. The result of this study showed that goat milk fermented using 3 % commercial starter able to produce the best yoghurt than using local yoghurt starter. Analysis of protein content showed that the fermentation processing increased the amount of protein in goat milk sample. Using SDS- PAGE showed that the breakdown of protein into fraction of fermented milk greater than unfermented goat milk. The result of fractional protein was analyzed by LC Ms/MS and showed that there were three kind bioactive sequences of bioactive peptides. Each of which consist of 16 amino acids that are safely protected from gastrointestinal animal model feddietary treatment of hypercholesterolemia.
Keywords: Food functional; bioactive peptides; fermented goat milk; anti-hypercholesterol.

1. Introduction

Food protein is one of food component of today be attention by researcher and industries because as well known protein is a source of amino acids and protein known have biology activities that to influence functional and body system of both on whole protein or hydrolysis product. Hydrolysis product of food protein that have biologist function have an important function on regulation body function and body healthy are known as bioactive peptides[1]. Bioactive peptides bonded on protein precursor of food and will be released through hydrolysis process by proteolysis enzyme in gastrointestinal tract, or maybe by in-vitro using proteolysis enzyme that isolated from plat or microorganism or by fermentation processing[2]. Several biological function of bioactive peptides that have known are as an antioxidant, antimicrobial, anti-hypertension, anti-cholesterol, cyto-modulator and immunomodulator[3].

Bioactive peptides have be isolated and identified from several of food sources, for instance milk, egg, meat of cattle, chicken, product of fermented milk, fish, soybean [4]. Milk is the main sources of bioactive peptides and have several biological function if be compared with throw foodstuff[2]. But in protein bonding condition, bioactive peptides are inactive form, So are needed to hydrolysis to release bioactive peptides, so milk can be used as food sources with high content of bioactive and used as maximal as sourceof bioactive [4]. That fore it was important to study the effective methods milk product with high bioactive content and available.

Fermentation using lactic acid bacteria as afamous method that is used to produce fermented milkis believed to give healthy benefits and cure several diseases ([2];[5] Several results of research showed that bioactive peptides from fermented milk have many benefits for body regulation system and enhance body healthy [6].That fore developing and optimization fermentation method to product bioactive peptidestube focus of researcher in many states.

Goat milk as one of natural functionalfood has chemical characteristic that different from cattle milk, but equally the same as human milk. Lactose and protein contained in goat milk is almost equal

to cattle milk, but there are some differences on structure and immunological protein. Beside that goat milk contains several middle chain fatty acid and lipid globularthat are relatively smallcompared to cattle milk. Therefore It so very important to do research with local spirit basic that use goat mil as source of bioactive peptides.

Now days development of goat milk industry in Indonesia is very fast, It was important to develop processing technology to receive milk product processing that has economical value than fresh milk..Foods products with basic source of goat milk have been develop as a food medicinal product or biomedicine that is useful for body healthy. Thus it is very important and urgent to explore bioactive peptides from goat milk through fermentation processing method.

The purpose of this study is to know the level concentrationof lactic acid bacteria that is needed and how long fermentation processing needed to produce bioactiveof goat milk. We hope with to find the optimal of fermentation through this study, it will be used to develop milk fermentation product with have better value if be compared with fresh milk.

2. Research Methodology

2.1. Starter Preparation

Starter preparation was done according method instruction of starter product industry (www.yogourmet.com) : 100 mL of fresh goat milk was pasteurized at 72 0C for 5 minute, then cooled to 400C – 450C. 0,5 gram frozen starter was weighted then be solved to 5 mL of pasteurized milk (from 100 mL of Pasteurized milk). Then the starter was added into 100 mL pasteurized milk, then homogenized and incubated at 400C to 450C for 4 to 8 hoursrespectively until milk starter reached 4.0 – 4.5 pH.

2.2. Yogurt Preparation

The yogurt preparation method was done according Position et al. (2005). 500 mL of goat fresh mil was pasteurized at 72 0C for 5 minutethen cooled at 40 0C- 45 0C, Then it was incubated with milk starter solution 3% concentration and 5 % concentration (According study of treatments), then homogenized and incubated at 40 0C to 45 0C for 4 to 8 hours respectively until pH value of yogurt reach 4.5 to 5.0 respectively. The product of yogurt then was saved at 4 0C to 5 0C for the next analysis purpose.

2.3. Protein Yogurt ≤ 3kD preparation

Hydrolised product of yogurt protein was prepared by solve 100 mg of sample of dry frozen yogurt to 1 mL buffer ammonium bicarbonate solution (50 mM,; pH 8.5), then it was sonicated (10 second, 40 Hz), and replicated 4 times it was centrifugated of cold centrifugation (12,000 Xg ; for 10 minutes at 4 0C). The supernatant (500 µL) was entered through in ultra filtration membrane 3 kDa molecular weight cut of (MWCO) and then was centrifugated in cold centrifugation for 15 minutes (12.,000 x g ; 4 0C), and 100 µL buffer ammonium carbonat was added to avoid waste protein, it was then be centrifugated with cold centrifugation for 7 menits (12,000 x g ; 4 0 C).The bottom of solution phase was protein fraction ≤ 3 kDa, was collected for profile peptides identification immediately. The solution of protein fraction was frizzed at -20 0c for next laboratory analysis purpose.

2.4. Kjehdahl Method Protein Determination

Weighed 0,50 g of sample was put into Kjehdahl flask, a tablet ofKjehdahl and added 10 mL concentrated.H2SO4was added Installed digest tube of digestion unit. To control the temperature of instrument, it should be turn off when reached 400 0C..Destruction processing will be running as a programming. After raised color changer from black to green color. The processing of digestion was stopped by turn off. Please wait the tube for moment (until cold room temperature reached), and continued to distillation and titration processing.

The dry sample of peptides sample was solved in 5 % of acetonitril and 0,1 % of formic acid in deionization water for LC-MS/MS analysis. LC- MS/MS analysis method was done by used LCQ Deka XP System MAX Thermo with ionization electrospray (ESI) Thermo Scientific InC. USA) used C 18 Bio-basic column, with 150 x 2,1 mm diameter, 5 µm particle size. LC- MS condition was gas flow rate 50 absorb prayer potential 4 kV, Capillary potential 20 v, Capiller temperature 3000C.

Interval of MS Scan was between m/z 100 – m/z 1600 with flow rate 200 µL/min. Peptides separation was used with gradient linier gradually from 5 % B solution to 70 B solution (Formic acid 0,1 % in acetonitril) for 90 menit more. Massa spectra data reading was done by thermo– Xialibor TM program (Thermo Scientific USA). Data of MS/MS were calculated by using Format File MGF with Mascot Distiler V2.3.2.0. (Matrix Saints, London United Kingdom), and continued with Blast by MGF file to Mascot search engine V 2. 3 (Matrix Science, UK.Sequence peptides were identified by Based of peptides sequence in Base data.

3. Result and Discussion

Table 1. Result of Qualitative analyzed of yoghurt

Treatment	pH	Viscosity consistency	Taste	Aroma
A 3 %	5.2	Less	Acid less	Milk
A 5 %	5,0	Less	Acid less	Milk
B 3 %	4.3	Viscous	Yoghurt	Yoghurt
B 5 %	3.8	Viscous	Acid	Acid

Note: A : Local yoghurt starter B : Commercial yoghurt starter

Good quality yoghurt starter was able to produce good yoghurt with good viscosity as needed. Table1 shows that there are different of yoghurt quality were produced by two kind of yoghurt starter in both of concentrationdifferent of 3 % and 5 %. Based d on data of Table 1, showed that product with high quality were produced by yoghurt starter commercial with 3 % concentration. Yoghurt starter with 3 % concentration was used for protein analysis.

Table 2. Result of proteinanalysis of yoghurt of 3% concentration commercial starter

Kind of sample	Protein content (% dry matter)
Milk	20.81
Yoghurt	22.80

Result of content protein analysis of both on goat milk and yoghurt were produced from 3% of starter yoghurt commercial showed to produce yoghurt with high quality, if be compared to yoghurt produced by local yoghurt starter. The purpose of analysis protein content was to know protein content is both yoghurt and fresh milk of goat. Protein content of yoghurt was 22.80 % it was higher than protein in the milk content in fresh goat milk (unfermented goat milk) was 20.81 % (Table 2). Many milk proteins possesspecific biological properties that make components potential ingredients of health- promoting food. Increasing attention is being focused to physiologically active peptides derived from milk protein. These peptides are inactive within the sequence of the parent protein molecule and can be liberated by fermentation of milk with proteolytic starter culture[2]. For this reason it can be concluded that yoghurt of goat milk is potential to be developed as drinking product as a functional food.

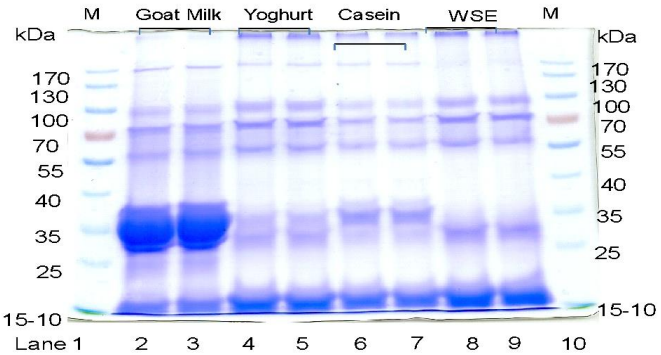

Figure 1. Figure of protein bands of yoghurt and fresh milk of goat

As what can be seen inFigure 1 fermentation processing treatment peptides of protein milk became hydrolyzed and were breakdown ,, it was known from total band of peptides protein formed at SDS- PAGE electrophoresis processing the total of peptides protein bands more than peptides protein band of fresh milk. The hydrolyzed or breakdown of peptides protein fermented milk was reached optimally, because there were presenting of lactic acid bacteria. It was according to Korhonenand Pihlanto (2006)[2], that peptides are inactive within the sequence of the parent protein molecule and can be liberated by (1) gastrointestinal digestion of milk, (2) fermentation of milk with proteolytic starter cultures or (3) hydrolysis byproteolytic enzymes.

So since thereis variation difference of total amount of peptides bands in fermented goat milk or yoghurt, so the nextresearch is suggested to determine peptides character that is useful to decrease cholesterol in blood. The peptides characterization is done by protein hydrolyzed fractionation method, that have molecule with weight less than 3 kDa, that is known has potential of both as an antioxidants and anti hyper-cholesterol.

The functional properties of bioactive peptides are largely determined by the amino acid composition of the bioactive peptide, eg peptides with compositions and the amino acid composition Val-Lys-Glu-Ala-Meta-Pro-Lys have antioxidant functions (1). In general the functional properties of bioactive peptides derived from milk derivatives can be divided into four groups, namely the functional properties associated with the circulatory system (cardiovascular system), the nervous system (nervous system), the gastrointestinal system (immune system) and immune system (2).

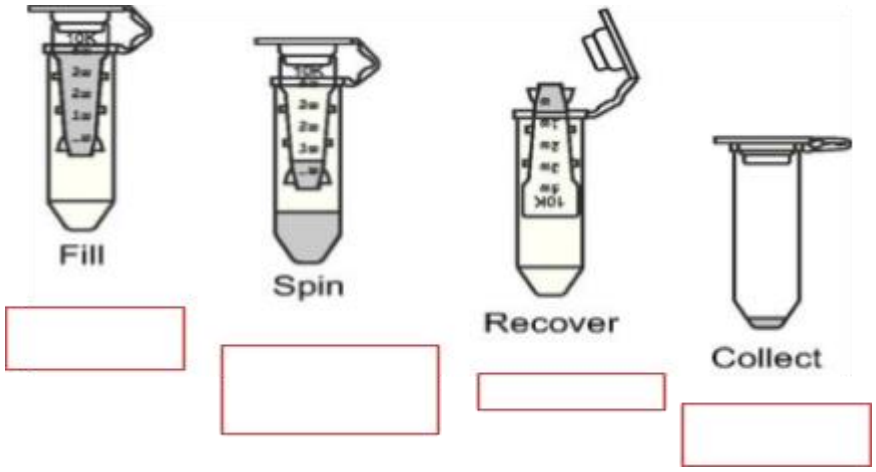

Figure 2. Hydrolisat protein fractionation processing (<3 kDa) used ultra filtration method

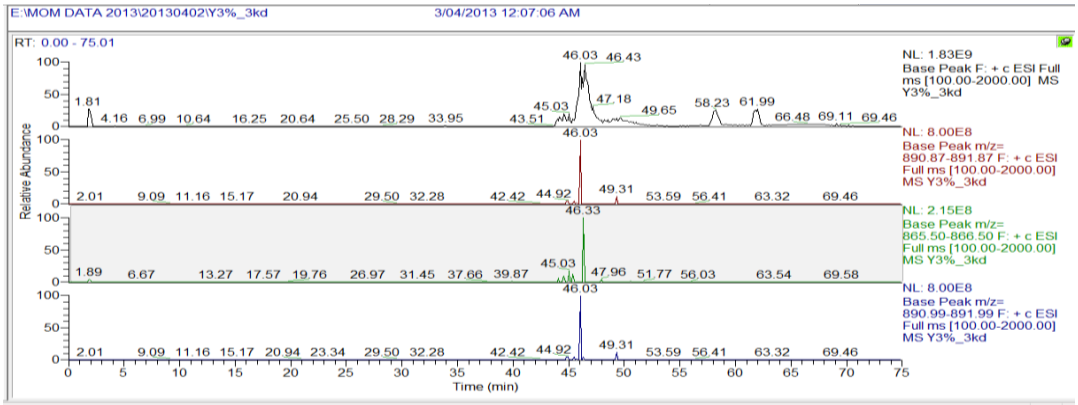

Figure 3. LC-MS/MS Chromatogram of yoghurt sample <3 kDa

Figure 3 showed that a dominant peak at 4603 (A) is selected ion chromatogram at retention time 46.03; 46.32 ; 46.03 (B,C, D) showed dominant peak have m/z 891.37 ; 891.49 and 866.48. Result of blast using mascot distillerTM showed peak identity come from Beta casein Capra hercus with sequentLYQEPVLGPVRGPFPI, YQEPVLGPVRGFPIL, and VQSWMHQPPQPLSPT.

Table 3. Result of identification of yoghurt sample peptides <3kDa using Mascot Distiller and NCBI basedata.

Sequent peptide yang identified	m/z	Molecule weigh (Dalton)	Score	Retention time
LYQEPVLGPVRGPFPI	891. 37	1780.90	50 (homology)	46.03
YQEPVLGPVRGPFPIL	891. 49	1780.90	52 (homology)	46.03
VQSWMHQPPQPLSPT	866.88	1731.84	51 (homology)	45.03; 46.33

Based on data in Table 3. Showedthat result of isolation characterization of bioactive peptides of each were 16 amino acids were saved from protease activities of gastrointestinal. Its Have potential antiloxidant activivities.

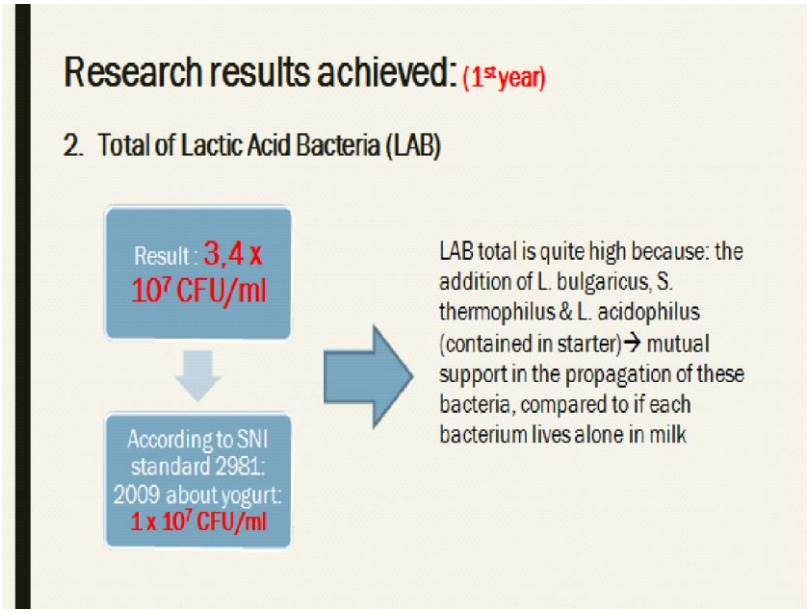

Figure 4. Total of Lactic acid bacteria (LAB)

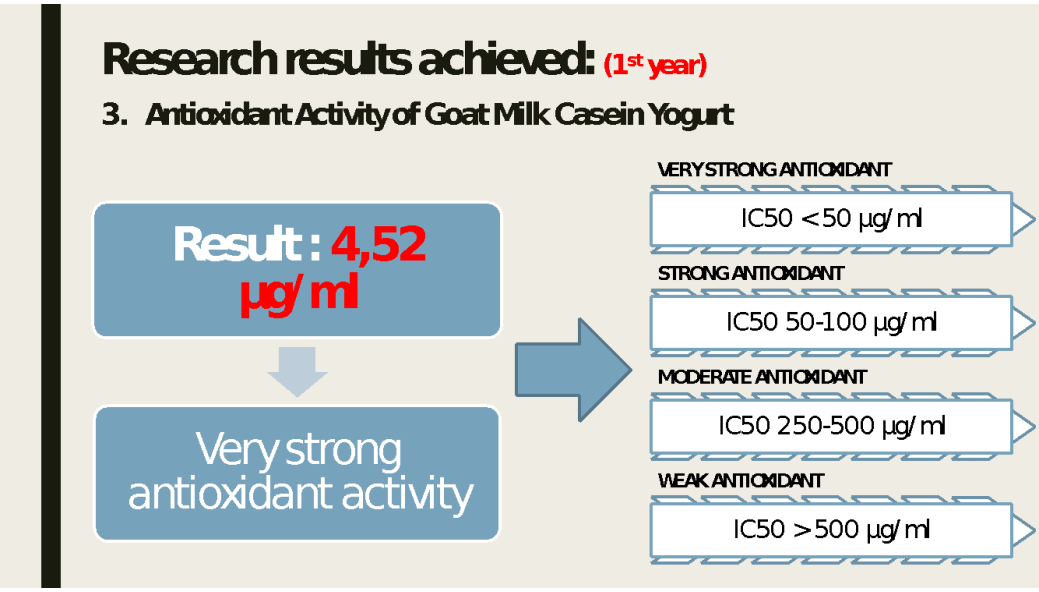

Figure 5. Antioxidant activity of Goat milk casein yogurt

From figure 4 and 5 showed that fermented of goat milk especially casein and Water soluble extract (WSE) have sources of lactic acid bacteria and natural antioxidant. Its according to (2) and (7) That According to their functional properties, bioactive peptides may be classified as antimicrobial, antithrombotic, antihypertensive, opioid, immunomodulatory, mineral binding andantioxidative. These peptides play an important role human health. In this review, we describe above stated properties of bioactive peptides especially derived from milk.

4. Conclusion

The best quality of yoghurt was produced by Canadian commercial yoghurt starter by 3 % concentration. The content of dry matter protein fermented goat milk was 22,80 percent, it was higher than Protein content of fresh goat milk. The Result of isolation and characterization of peptides bioactive were found 3 kinds of bioactive peptides sequences each switch 16 amino acids . These peptides play important role on human health.

5. Thank You Note

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6. References

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