

Application of *Lactobacillus paracasei* spp. Paracasei 1 SKG 44 to Improve Cheese Quality with Extract of Rampelas (*Ficus ampelas* Burm F)

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Abstract: This research was conducted to find out the application of *Lactobacillus paracasei* spp. paracasei 1 SKG 44 to improve the quality of cheese by additional of Rampelas Bark (*Ficus ampelas* Burm F.) extract as a coagulant agent. Completely Randomized Design with 5 levels concentration of *Lactobacillus paracasei* spp. paracasei 1 SKG 44 ie, 0%, 2%, 4%, 6%, and 8% were used in this study. Each treatment was repeated three times to get 15 experimental units. The data obtained were analyzed by ANOVA, and the significant differences effect were followed by Duncan Multiple Range Test (DMRT). The best effect resulted by 2% concentration of *Lactobacillus paracasei* spp. paracasei 1SKG44, that contain of: 12.26% cheese result, pH 4.31; 54.62% water, 26.57% fat; 24.62% protein; LAB population of 3.9x10⁸ cfu / g, and accompanied by panelists acceptancy to. : flavor, taste, color, and overall favored.

1 INTRODUCTION

1.1 Background

Cheese is a dairy product made through milk clotting process using acid or rennet which made from the abomasum extract (Purnomo in Permainy et al, 2013). Rennet preparation take a long time and expensive process, as well as raw material (abomasum) from cow, therefor needs coagulant alternatif as good as rennet. Alternative coagulants that have been studied include pineapple, lime and starfruit (Sumarmono and Suhartati, 2012), fruit of the *Solanum dubium*, sap of *Sideroxylon obtusifolium* (Silva et al, 2013) and bark of rampelas (Silva, 2010; Suardika, 2015).

The bark of *Rampelas* (BR) has long been used by the East Timor leste community as a clotting milk to produce a traditional food known as *Ami Maka-Ana* (AMA) (Silva, 2010). The tannin compound in BR is thought to be able for clotting cacein, so that it can be used as a natural coagulant instead of rennet (Santini, 2014). The use of extract BR as a cheese coagulant has several disadvantages, such as a small yield (9.8%) and a distinctive cheese flavor (Suardika, 2015). Therefore it is necessary the

addition of lactic acid bacteria to correct these deficiencies.

Lactobacillus paracasei spp. paracasei 1 SKG44 is an isolated lactic acid bacteria from wild horse milk, was chosen because it strain of indogenous LAB, thought potentially to be used as a probiotic because it has the ability to inhibit pathogenic bacteria and has been tested as a curd starter, both in the form of dry (powder) and wet (Sugitha, 2008). Therefore, in this study, the application of *Lactobacillus paracasei* spp paracasei 1 SKG44 to quality improvement of cheese with extract coagulant was made lightly. This study aims to determine the additional effect of *Lactobacillus paracasei* spp. paracasei 1 SKG44 to improve the quality of cheese.

2 MATERIALS AND METHODS

2.1 Place and Time of Research

This research was conducted at Integrated Laboratory of Bioscience and Biotechnology (ILBB), Udayana University; Food Analysis Laboratory; Biochemistry and Nutrition laboratory of Food Science and Technology Department,

Faculty of Agricultural Technology, Udayana University

2.2 Tools and Materials

The tools used in this study were test tubes (Iwaki pyrex), petri dishes (Duran), measuring cups (Iwaki pyrex), beaker cups (Iwaki pyrex), erlenmeyer (Iwaki pyrex), centrifuge tube (Falcon), microcentrifuge tube (Nesco), micropipette (Gilson), incubator (Memmert BE 400), autoclave (Tomy ES 315), Laminar Flow Cabinet (ESL JSCB-900SB), Centrifuge (Clement GS 150), Waterbath (Eyela SB 35), blue tips (QSP), yellow tips (QSP),

The material to be used in this study is milk (Greenfield Fresh Milk) which was purchased at a local supermarket, isolate *Lactobacillus paracasei* spp. *paracasei* 1SKG44, which has been stored in 30% glycerol at ILBB. The chemicals used The Mann Rogosa Sharpe Agar (MRSA) (Pronadisa), de Mann Rogosa Sharpe Broth (MRSB) (Pronadisa), 0.85% NaCl (Merck), 50% NaOH (Brataco), H₂SO₄ (JT Baker), 1N HCl (JT Baker), Aquades (Chemical Sabha), Petroleum Ether (Bratachem), 3% Boric Acid (Merck), Kjeldahl Tablets, Phenolphthalein Indicators, Kitchen Salt (Rafina), and CaCl₂ (Merck).

2.3 Research Design

The completely randomized design (CRD) was used in the research with a concentration of 25% bark extract (Suardika, 2015) and the concentration of *Lactobacillus paracasei* spp. *paracasei* 1SKG44 which consists of 5 levels (v/v), such as :1=0%;2= 2%;3= 4%;4= 6% and 5= 8%.

Each treatment was repeated 3 times to obtain 15 experimental units. The data obtained were analyzed by ANOVA and if there were significant effects should continue by the Duncan Multiple Range Test (DMRT) test. The variables observed in this study were recovery, pH, water content, total lactic acid bacteria, protein content, fat content, and sensory properties

2.4 Research Implementation

Stock isolates *Lactobacillus paracasei* spp. *paracasei* 1SKG44 was inoculated as much as 50 µl into 10 ml MRSB media and incubated at 37 ° C for 24 hours. Then confirmation to gas and catalase test, Gram staining (Dewi et al, 2014) and morphological observation. The mass production of cells by transferring 10 ml of MRSB which was positively

overgrown with isolates into 100 ml of sterile MRSB, and incubated at 37 ° C for 24 hours, then inserted into several centrifuge tubes, at 3500 rpm for 10 minutes separating pellets and supernatants. Once separated, the supernatant was removed and the pellets washed using saline solution 0.85% 1 times, and re-centrifuged. Pre-washed pellets are inoculated into milk.

The making of crude bark extract was done by extraction method (Santini, 2014). The 60 mesh sifted sample was weighed 160 g and included in to 1000 ml erlenmeyer. The result of sandpaper (powder) was dissolved with a 960 ml water solvent which was heated at 90 ° C. Comparative comparison with solvent is 1: 6. A sanding powder solution is heated to a temperature of 90 ° C, and left for 15 minutes. The solution is filtered by using Whatman filter paper No.1. Extract obtained is placed in a dark bottle.

Cheese making is done according to the modified method of Law and Tamime (2010). 400 ml of milk for each treatment was heated to 80 ° C, then the bark of 100% (25%) was taken into account and the temperature was maintained for 30 minutes and then cooled to temperature (\pm 30 ° C) and inoculated *Lactobacillus paracasei* spp. *paracasei* 1SKG44 according to treatment (population 10¹⁰ CFU / ml). Milk was then incubated for 18 hours at 41 ° C. After producing curd the whey was separated, curd is cut into small pieces, then scalding or cooking at 40 ° C for \pm 1 hour while stirring. Then separation of curd and whey was done using cheese cloth, and whey was left to drip for several minutes. Strain strips containing curd are then inserted into the mold and pressed using a load of \pm 300g for 24 hours. Printed cheese, then soaked (Brining) with a solution consisting of sterile water, 3% kitchen salt and CaCl₂0.3% for 24 hours, then drained and analyzed

3 RESULTS AND DISCUSSION

3.1 Cheese Quality

Nutritional composition and total LAB of Cheese produced of coagulant extract and the treatment of *Lactobacillus paracasei* spp. *paracasei* 1SKG 44 can be seen in Table 1.

Table 1: Average nutritional composition and total LAB cheese of the sample.

Treatment	Yield(%)	pH	Water(%)	Fat(%)	Protein(%)	Total LAB (cfu/g)
KB1	12,87 a	5,35 d	55,45 a	26,67 b	25,27 a	$5,7 \times 10^7 \pm 0,27$
KB2	12,26 a	4,31 c	54,62 a	26,57 b	24,65 a	$3,9 \times 10^8 \pm 0,89$
KB3	13,12 a	4,03 b	52,72 a	22,06 ab	23,6 a	$5,2 \times 10^8 \pm 0,82$
KB4	14,44 a	3,81 a	54,19 a	23,68 ab	24,32 a	$1,07 \times 10^9 \pm 0,59$
KB5	16,9 b	3,78 a	62,27 b	19,7 a	26,27 a	$2,8 \times 10^9 \pm 0,62$

Description: KB1 = 0%, KB2 = 2%, KB3 = 4%, KB4 = 6%, KB5 = 8%. The same letter behind the average value in the same column shows a non-significant effect ($P > 0.05$)

3.1.1 Recovery

The results of the analysis showed that the concentration of *Lactobacillus paracasei* spp. *paracasei* 1 SKG44 had a significant effect ($P < 0.01$) on the yield of cheese produced. Table 1 shows the highest yield average of cheese obtained in KB5 treatment with a concentration of 8%. The cheese yield has a tendency to increase with increasing isolate concentration, although statistically the KB1 to KB 4 treatment has no significant effect ($P > 0.05$). This shows that even though tannins in extracts and *Lactobacillus paracasei* spp. *paracasei* 1 SKG44 has a different mechanism of milk clotting, but the use of both can increase the yield of cheese at a concentration of 25% extract and 4-8% *Lactobacillus paracasei* spp. *paracasei* 1 SKG44. Addition of lactic acid bacteria such as *Lactobacillus paracasei* spp. *paracasei* 1 SKG44 causes a decrease in pH, increases the degree of syneresis, and the release of calcium phosphate colloid from casein (Law and Tamime, 2010). Polished babakan extract contains tannin compounds that can agglomerate milk protein to produce cheese (Suardika, 2015). According to Makkar et al (2007) in Firdausi et al (2013) tannin compounds contained in plants naturally have the ability to interact with proteins and form complex proteins

3.1.2 pH

Based on Table 1, the pH of cheese tends to decrease, along with the increasing concentration of LAB used. The decrease in pH was statistically high significant in each treatment ($P < 0.01$), only in the KB4 and KB5 treatments the difference was not significant ($P > 0.05$). This shows that the higher the concentration of bacteria, the lower the pH produced due to the increasing activity of LAB in the process of lactose fermentation to lactic acid. According to Walstra et al (2006), the percentage of inoculums which are higher will increase acid production and a decrease in pH value can increase the degree of

syneresis. Lactic acid is responsible for acid flavors in raw cheese and plays an important role in the formation and texture of curd, synthesis of proteolytic and lipolytic enzymes involved in ripening cheese, and suppressing pathogenic microbes and spoilage microorganisms (Jamilatun, 2009)

3.1.3 Water Content

The results of the analysis showed that the concentration of *Lactobacillus paracasei* spp. *paracasei* 1 SKG44 had a significant effect ($P < 0.05$) on the average water content of cheese produced. The results showed that the increase in water content in each treatment tended to change. In the KB1, KB2, KB3 treatment the water content tends to decrease. In the KB4 and KB5 treatment an increase in water content. Statistically, KB1 to KB4 does not have a real effect. The KB5 treatment has the highest effect and the highest water content, which is 62.27%. Cheese made by using acid as a clot, such as lactic acid as a result of lactic acid bacteria fermentation, is classified into acid-coagulated cheese. Lactic acid bacteria such as *Lactobacillus paracasei* spp. *paracasei* 1 SKG44 ferments lactose into lactic acid, acidifies milk and collects casein micelles into a net matrix, and converts Micellar Calcium Phosphate (MCP) to a soluble form (Kinstedt, 2014). The more concentration of *Lactobacillus paracasei* spp. added *paracasei* 1 SKG44, water content tends to increase. This is consistent with the statement of Lucey (2004) in Kinstedt (2014) that casein matrices which lose MCP tend to be more difficult to release whey, therefore, acid-coagulated cheese has a higher water content.

3.1.4 Fat Level

The analysis showed that the concentration of *Lactobacillus paracasei* spp. *paracasei* 1 SKG44 significantly affected ($P < 0.05$) on the average fat content of the cheese produced. The average fat

content tends to decrease as more bacterial concentrations are inoculated. Giving 0% and 2% *Lactobacillus paracasei* spp. *paracasei* 1 SKG44 has no significant effect on fat content. Decrease in fat levels began to be seen at the 4% and 6% concentration of *Lactobacillus paracasei* spp. *paracasei* 1 SKG44 and significantly decreased at the concentration of 8%. Bottazi (1983) in Sunarlim et al (2007) states that lactic acid bacteria have lipolytic activity, which can break down milk fat into simple chemical compounds. The higher the concentration of bacteria, the lower the fat content of cheese because the increases activity of lactic acid bacteria.

3.1.5 Protein Levels

The results of the analysis showed that the concentration of *Lactobacillus paracasei* spp. *paracasei* 1 SKG44 had no significant effect ($P > 0.05$) on the average levels of cheese protein produced. Addition of *Lactobacillus paracasei* spp. *paracasei* 1 SKG44 at a concentration of 0%, 2%, 4%, 6%, and 8% gave a non-significant effect on the levels of cheese protein produced. Based on research by Mardiani et al. (2013), increasing the concentration of LAB which will increase the level of protein in cheese, because LAB has a protease enzyme that breaks down protein into amino acids. In cheese making with the coagulant extract, the tannin is extracted, the tannin has the ability to bind to proteins forming protein-tannin compounds. Tannin naturally has the ability to inhibit the action of enzymes (enzyme inhibitors) (Smith et al., 2005; This was thought to cause *Lactobacillus paracasei* spp. *paracasei* 1 SKG44 cannot break down cheese protein so that the levels of protein produced are not significantly affected

3.1.6 Total Lactic Acid Bacteria

The total calculation of lactic acid bacteria shows that the total LAB per 1 gram of cheese increases with the concentration of *Lactobacillus paracasei* spp. *paracasei* 1 SKG 44 added. The KB1 treatment

which did not get the addition of *Lactobacillus paracasei* spp. *paracasei* 1 SKG44 produced an total average LAB of 5.7×10^7 . This number is greater than the total average LAB of Ami-Maka Ana (Sugitha et al 2014), which ranges from 0.88×10^4 to 5.36×10^4 . This is thought to be caused by natural lactic acid bacteria in milk which still survive during the pasteurization and develop during the fermentation process. The concentration of bacteria added to KB2, KB3, KB4, and KB5 treatments sequentially are 2%, 4%, 6%, 8%, with the population of *Lactobacillus paracasei* spp. *paracasei* 1 SKG44 $\pm 1 \times 10^{10}$ cfu / ml. The total lactic acid bacteria shown in Table 1 represents the viabilities of *Lactobacillus paracasei* 1 spp. *paracasei* SKG44 during the cheese making process.

3.2 Sensory Analysis

The results of the analysis datas showed in Table 2.

3.2.1 Color

The analysis showed that the concentration of *Lactobacillus paracasei* spp. *paracasei* 1 SKG44 had a very significant effect on the color of the cheese produced ($P < 0.01$). In the KB1 treatment showed a significant difference ($P < 0.05$) compared to other treatments with the color of yellow cheese, while the KB2, KB3, KB4, and KB5 treatments were not significantly different (score 4 = yellowish white). The color of cheese is influenced by the color of milk do to carotene pigments that dissolve in fat (Jamilatun, 2009)

3.2.2 Flavour

The results of the analysis showed that the concentration of *Lactobacillus paracasei* spp. *paracasei* 1 SKG44 has a very significant effect on the flavour of cheese produced ($P < 0.01$). The KB1 treatment showed significant differences ($P < 0.05$) with a scent test scale of 2.46 (not typical of cheese Flavour). The KB2 treatment produces the highest

Table 2: The score average of sensory cheese analysis of the sample.

Treatment	Color	Flavor	Texture	Taste	Acceptace
KB1	3.87 a	2.46 a	4.2a	2.06 a	2.33 a
KB2	4.2 b	4.06 b	4.13a	3.33 b	3.53 b
KB3	4.33 b	3.46 b	4.00a	3.20 b	3.46 b
KB4	4.33 b	3.53 b	4.20a	3.40.b	3.20 b
KB5	4.47 b	3.46 b	4.40a	3.13 b	3.46 b

Note: KB1=0%, KB2=2%, KB3=4%, KB4=6%, KB5=8%. Superscript in the same colom was not significant different ($P > 0.05$)

Flavour). The KB2 treatment produces the highest Flavour sensory value, which is 4.06 or somewhat typical of the Flavour of cheese. The treatment of KB3, KB4, and KB 5 was not significantly different ($P > 0.05$) with a scent test scale 3 (usual). In generally, the taste and Flavour of cheese appeared due to the volatile component formed after starter microbial inoculation which could result in biochemical changes include proteolysis, lipolysis, and lactose fermentation.

3.2.3 Texture

The results of the analysis showed that the concentration of *Lactobacillus paracasei* spp. *paracasei* 1 SKG 44 did not significantly affect the texture of the cheese produced ($P > 0.05$). In this study, panelists were asked to assess the texture of cheese by pressing cheese with the fingertips. The results showed that KB1, KB2, KB3, KB4, and KB5 were not significantly different from the 4 (rather soft) texture test scale. The formation of texture is influenced by lactic acid produced by lactic acid bacteria. Lactic acid is a metabolite of lactic acid bacteria. Lactic acid can agglomerate milk protein through a mechanism for decreasing pH. Texture can be interpreted as an attribute of cheese resulting from a combination of physical attributes, including the size, shape, number and adjustment of structural elements perceived by a combination of the sense of taste, sense of sight and sense of hearing.

3.2.4 Taste

The results of the analysis showed that the concentration of *Lactobacillus paracasei* spp. *paracasei* 1 SKG 44 had a very significant effect on the taste of cheese produced ($P < 0.01$). In the taste test hedonic test (preference) was carried out. The KB1 treatment was significantly different ($P > 0.05$) with a taste test scale 2 (disliked). Whereas KB2, KB3, KB4, and KB5 treatments were not significantly different ($P < 0.05$) with taste test scale 3 (usual). Flavor is built by starter culture, and enzymatic modification of various components of milk. Lipolysis is very important in the formation of the flavor of the cheese. Bacterial starters have a direct effect on fresh cheese taste, for example cottage cheese. In the process of making cheese there is also a process of adding salt consisting of 3% salt and CaCl_2 0.3%. Salt was added in the form of crystals which have been dissolved in sterile water.

3.2.5 Overall Acceptance

The ANOVA test results showed that the concentration of *Lactobacillus paracasei* spp. *paracasei* 1 SKG44 had a very significant effect on the overall acceptance of cheese produced ($P < 0.01$). The KB1 treatment received the smallest evaluation from the panelists, namely 2.33 (somewhat dislike). The treatment of KB2, KB3, KB4, and KB5 is assessed on a scale of 3 (normal). This shows that cheese with the addition of *Lactobacillus paracasei* spp. *paracasei* 1 SKG44 can be accepted by panelists.

4 CONCLUSION

Based on the research that has been done, it can be concluded as follows:

- Addition of *Lactobacillus paracasei* spp. *paracasei* 1SKG44 in making cheese with bark extract of rampelas can improve cheese quality and highly significant effect on yield, pH, sensory color, Flavour, taste and overall acceptance, and significantly affect the water content and fat of cheese, but not significantly affect to protein.
- The Treatment of the addition of *Lactobacillus paracasei* spp. *paracasei* 1 SKG44 is KB2 with a yield of 12.26%; moisture 54.62%; fat 26.57%; pH 4.03; protein 24.65% and total LAB 3.9×10^8 cfu / g with the highest sensory value among other treatments.
- Suggestion for further research is needed regarding the shelf life, probiotic of cheese with coagulants of rampelas bark extract and *Lactobacillus paracasei* spp. *paracasei* 1SKG44.

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