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Quality Yoghurt Set with the Addition *Lactobacillus acidophilus* FNCC 0051 and *Streptococcus thermophilus* FNCC 0040 Encapsulated in Terms of pH, Total Titrated Acid, Syneresis, and Total Lactic Acid Bacteria

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ABSTRACT

Probiotics are generally added in the manufacture of food products because of their function that can provide benefits to human health, especially in the digestive tract. The probiotics used in this study were *Lactobacillus acidophilus* FNCC 0051 and *Streptococcus thermophilus* FNCC 0040 which were encapsulated and added in making yoghurt sets. The purpose of this study was to examine the physicochemical and microbiological quality of the yoghurt set with the addition of encapsulated Lactic Acid Bacteria (LAB). The research method used is a laboratory experiment using a completely randomized design (CRD) pattern with 4 treatments and 3 replications. The treatments given were P0 Addition of LAB without encapsulation (control) (2%); T1 addition of 2% encapsulated LAB (v/v); T2 added 3% encapsulated LAB (v/v) and T3 added 4% (v/v) encapsulated LAB. Data analysis used Analysis of Variance (ANOVA). The results of the analysis showed that the encapsulation using gelatin and Na alginate coatings gave a very significant difference ($P<0.01$) to the syneresis of yoghurt sets with the resulting average T0 (24.51±0.70%), T1 (23.52±0.76%), T2 (20.44±0.44%), and T3 (19.96±0.83), a significant difference ($P<0.05$) on the pH of the yoghurt set with the resulting average T0 (4.56±0.03), T1 (4.55±0.02), T2 (4.45±0.09), and T3 (4.40±0.07), a significant difference ($P<0.05$) to the total acid titrated yoghurt set with the resulting average, namely P0 (0.68±0.14%), T1 (1.05±0.03%), T2 (1.13±0.32%), T3 (1.31±0.21%), the significant difference ($P<0.05$) on the total LAB yoghurt set with the resulting average T0 (8.62±0.30 log CFU/ml), T1 (9.20±0.70 log CFU/ml), T2 (9.36±0.33 log CFU/ml), and T3 (9.51±0.10 log CFU/ml). It was concluded that the addition of the percentage of encapsulated LAB of 4% was able to improve the quality of the yoghurt set optimally.

Keywords: yoghurt set, probiotics, encapsulation, pH, total acid, syneresis, total LAB

INTRODUCTION

Yoghurt is a fermented milk product that is very popular among the public because it contains bacteria that are beneficial to human health. Regular consumption of yoghurt can balance the intestinal microflora, suppressing the number of harmful bacteria so that the intestines will be dominated by beneficial bacteria. Yoghurt contains nutrients, such as protein, calcium, and phosphorus, yoghurt can also be considered a probiotic product.

Probiotic microorganisms generally come from Lactic Acid Bacteria (LAB). LAB such as *Leuconostoc* sp., *Pediococcus* sp., *Lactobacillus* sp and *Streptococcus* sp. *Lactobacillus acidophilus* is a probiotic species belonging to the LAB group, namely bacteria that produce lactic acid as a product of carbohydrate fermentation, such as glucose, galactose, lactose, maltose and sucrose. *Lactobacillus acidophilus* homofermentative, namely in the glycolysis pathway it produces only lactic acid and is known to increase macrophage production and activate phagocytes is (Susanti et al., 2007).

Another species that can be used in the fermentation process, namely *Streptococcus thermophilus*, is a thermophilic, homofermentative LAB which has an optimum pH of 6.5 and will stop its growth at a pH of 4.2-4.4. In addition to producing lactic acid, *Streptococcus thermophilus* also produces the enzyme lactase which functions to digest lactose in milk (Bilang et al., 2018). *Streptococcus thermophiles* has a sensitivity to gastric acid conditions, namely a decrease in cell viability so that it cannot reach the intestine in living conditions at an acceptable level (Pestka et al., 2001). Efforts to increase the viability of bacteria can be through the application of the microencapsulation method.

Microencapsulation is a process of wrapping (coating) a core material using a certain encapsulating material. In this process, probiotics will be trapped in capsule-like particles which can protect probiotics from food processing, storage and extreme environmental conditions as well as facilitate delivery to the large intestine in live conditions and in sufficient quantities (Burgain et al., 2011). The encapsulated core material will be released gradually through the capsule wall, which is known as controlled release or diffusion, or when external conditions trigger the wall capsule to rupture, melt or dissolve (Jyothi et al., 2012). The protective capsule consists of polymers that are easily digested and allow the probiotics to survive in unfavourable environments.

Gelatin is a polymer that is biodegradable, biocompatible, stable over a wide pH range, inexpensive, has good extensibility, and can undergo cross-linking such as Na alginate (Elzoghby, 2013). Due to its amphoteric nature, gelatin can be combined with anionic polysaccharides such as Na alginate (Li et al., 2009). Alginates are the main compounds used for microencapsulation of probiotics, mainly because of their safety, good gelling properties (temperature and pH) and biocompatibility. Alginate degrades at low pH, allowing the release of probiotics under digestive conditions (Moghanjougi et al., 2021) the combination of Na alginate and gelatin as a matrix will reduce porosity, because the matrix formed is more compact, so that the particle size is smaller and the trapping ability is greater (Li et al., 2009).

The purpose of this study was to determine the percentage addition of *Lactobacillus acidophilus* FNCC 0051 and *Streptococcus thermophilus* FNCC 0040 in gelatin and Na alginate coating using emulsion techniques in making yoghurt sets in terms of pH, total titrated acid, syneresis and total LAB.

MATERIAL AND METHOD

Location and Research

This research was conducted on January 04th - February 04th, 2022 at the Laboratory of livestock technology Malang, Faculty of Animal Science, University of Brawijaya.

Research Material

The material used is encapsulated LAB (*Lactobacillus* FNCC 0051 and *Streptococcus thermophilus* FNCC 0040 with gelatin and sodium alginate encapsulated materials). *Lactobacillus acidophilus* FNCC 0051 and *Streptococcus thermophilus* FNCC 0040 were microencapsulated in gelatin and Na alginate-based on Lestari et al., (2019) with several modifications. First, gelatin solution (5% w/v) was dissolved in distilled water heated at 50-55 °C and sodium alginate (1.5% w/v) was dissolved in distilled water and sterilized at 121°C for 15 minutes. Then, 7 ml of a suspension of *Lactobacillus acidophilus* FNCC 0051 and *Streptococcus thermophilus* FNCC 0040 were mixed with a solution of gelatin and sodium alginate mixed evenly in the ratio (1:1); (1:2); (1:3) as treatment T1, T2, and T3 respectively. It was continuously inserted with a sterile syringe into 50 ml of paraffin oil containing 0.5% sterile span 80, then sterile $CaCl_2$ (10 ml 0.1 M) and mixed using a magnetic stirrer at a speed of 450 rpm for 30 minutes. After the bead was established, the microcapsules were centrifuged at 3.500 rpm for 15 min. The beads obtained were washed twice with sterile distilled water.

Research Procedure

The procedure for making yoghurt sets in this study refers to Tan et al. (2016) with several modifications, including the length of the pasteurization process, incubation time, type of starter and encapsulation material. UHT cow low-fat milk at a temperature of 72^{o}C was measured using a thermometer and maintained the temperature for 15 minutes. Put the milk in a small container. Cooled to a temperature of 43^{o}C, then added LAB without T0 encapsulation treatment (2%) and BAL encapsulated with the percentage of T1 (2%), T2 (3%), T3 (4%) and incubated in an incubator at 37^{o}C for 18 hours.

Research Methods

The research used a laboratory experimental method with 4 treatments with 3 replications and a

laboratory experiment using a Completely Randomized Design (CRD). The treatments of this study were as follows: P0: yoghurt using a concentration of 2% starter without encapsulated, T1: yoghurt using a concentration of 2% starter *Lactobacillus acidophilus* and *Streptococcus thermophilus* encapsulated, T2: yoghurt using a concentration of 3% starter *Lactobacillus acidophilus* and *Streptococcus thermophilus* encapsulated, T3: yoghurt using a concentration of 4% starter *Lactobacillus acidophilus*, and *Streptococcus thermophilus* encapsulated.

Research Variables

The variables observed on this research were pH, total acid titrated, syneresis, total Lactic Acid Bacteria (LAB) yoghurt set.

Data Analysis

The data were analyzed with a variety analysis (ANOVA) and continued with the Duncan Multiple Range Test if there were differences.

RESULT AND DISCUSSION

pH Value of the Yoghurt Set

The average pH in yoghurt set with the addition of a different percentage of encapsulated LAB with the use of a combination of gelatin and Na alginate as encapsulation material through the emulsion technique can be seen in Table 1.

Table 1. The average pH value of the yoghurt set

Treatment	pH
T0	4.56 ± 0.03^{b}
T1	4.55 ± 0.02^{b}
T2	4.45 ± 0.09^{ab}
T3	4.40 ± 0.07^{a}

Note: ^{a,b}different superscripts in the same column indicate the treatment using a combination of gelatin and Na alginate through emulsion techniques gave a significant difference ($P<0.05$) to the pH of yoghurt set

The results of the analysis of variance showed that the use of *Lactobacillus acidophilus* FNCC 0051 and *Streptococcus thermophiles* FNCC 040 encapsulated using a combination of gelatin and Na alginate through the emulsion technique gave a significant difference ($P<0.05$) to the average pH of the yoghurt set. The higher the percentage of use of encapsulated LAB, the lower the pH of the resulting yoghurt set. pH of yoghurt set in the control treatment or without the use of encapsulated LAB (T0) was 4.56±0.03, while the average pH of the yoghurt set in the treatment using encapsulated LAB was T1 4.55±0.02, T2 4.45±0.09, and T3 4.40±0.07 (Table 1).

The pH value test is carried out to determine the H+ which indicates the amount of dissociated acid so that the quality and safety of the product can be estimated for consumption. According to Pratama et al. (2020) the quality of fermented milk-based on pH, which is good, is 3.8–4.6. The pH value of the yoghurt set produced in this study ranged from 4.40±0.07 up to 4.56±0.03. The decrease in pH value was caused by the activity of LAB in fermenting lactose into glucose and galactose, then the glucose produced would be converted into lactic acid. The higher the lactic acid formed, the lower the pH of the yoghurt produced (Adamberg et al., 2003). The yoghurt sample set using 2% LAB concentration without encapsulation has the highest pH value of 4.56±0.03. This happens because LAB interacts directly with environmental conditions so that it will inhibit LAB mobility. The absence of an encapsulating material (matrix) to protect LAB from high-temperature environmental conditions resulted in some LAB in yoghurt experiencing a decrease in cell viability, causing the number of H^{+} produced during milk fermentation to be less. Ajlouni et al. (2020) explained that some of the LAB may not be able to survive in high-temperature environmental conditions, causing cell death. As a result, the resulting level of lactic acid production is lower and therefore less acid is detected in the yoghurt.

The lowest pH value was obtained at P3 which was 4.40±0.07. This is thought to occur because LAB is protected by microcapsules so that they can maintain their viability. The decrease in yoghurt pH is one of the consequences of the fermentation process that occurs due to the accumulation of lactic acid as the main product of LAB activity. Ions^{+}, causing the pH to become lower (Rasbawati et al., 2019). According to Jannah et al. (2014) LAB *Streptococcus* is responsible for the decrease in the initial pH of yoghurt to around 5.0. *Lactobacillus* was responsible for a further decrease until the pH reached 4.5. The increase in total acid in fermented milk is balanced with a decrease in pH, in other words, the greater the total acid value, the lower the resulting pH value (Utami et al., 2010). The measured value in determining the pH value is the concentration of H^{+} which indicates the amount of dissociated acid while the total titrated acid is a manifestation of dissociated and undissociated lactic acid levels (Zakaria et al., 2013).

Total Acid Titrated Value of the Yoghurt Set

The average total acid titrated in yoghurt set with the addition of a different percentage of encapsulated LAB with the use of a combination of gelatin and Na alginate as encapsulation material through the emulsion technique can be seen in Table 2.

Table 2. The average total acid titrated value of the yoghurt set

Treatment	Acid Titrated (%)
T0	0.68±0.14[a]
T1	1.05±0.03[ab]
T2	1.13±0.32[b]
T3	1.31±0.21[b]

Note: [a,b]different superscripts in the same column indicate the treatment using a combination of gelatin and Na alginate through emulsion techniques gave a significant difference (P <0.05) to the total titrated acid of yogurt set.

The results of the analysis of variance showed that the addition of *Lactobacillus acidophilus* FNCC 0051 and *Streptococcus thermophilus* FNCC 0040 encapsulated using a combination of gelatin and Na alginate with emulsion technique gave a significant difference (P<0.05) to the average total acid titrated yoghurt set. The higher the percentage of the use of encapsulated LAB, the higher the total acidity of the yoghurt set produced. The average total acid titrated yoghurt set in the control treatment or the addition of LAB without encapsulation (T0) was 0.68±0.14%, while the average total acidity in the yoghurt set in the treatment with the addition of encapsulated LAB T1 1.05±0.03%, T2 1,13±0.32% and T3 1.31±0.21% (Table 2). Lactic acid is the main acid component formed during the yoghurt set fermentation process. The increase in total acid with the addition of encapsulated LAB in the yoghurt set was related to the activity of LAB in breaking down lactose into lactic acid. The level of lactic acid contained in yoghurt is influenced by the ability of LAB to produce lactic acid (Azhar, 2009). The increase in lactic acid in milk fermentation is balanced with a decrease in the pH of the yoghurt set, meaning that the higher the level of lactic acid formed during fermentation, the pH of the yoghurt set will decrease (Utami et al., 2010).

During the fermentation process, lactose is hydrolyzed to glucose and galactose which are then converted to lactic acid. Adrianto et al., (2020) stated that reduced lactose and increased lactic acid in yoghurt resulted from the fermentation process by LAB, lactose was degraded to glucose and galactose which in turn became lactic acid. The process of reshuffling lactose into glucose and galactose occurs due to the help of enzymes in yoghurt. Martharini and Indratiningsih (2017) explained that the -galactosidase enzyme in yoghurt can hydrolyze lactose into glucose and galactose. Furthermore, glucose resulting from this reshuffle is converted into lactic acid by LAB from yoghurt. The acid accumulates in the milk and the increased acidity causes the casein to coagulate and the milk thickens and increases the sour taste. Variations in acidity can change the texture of the yoghurt. At high acidity, yoghurt will be more viscous and sour (Ali et al., 2020). The total acid produced by the yoghurt set with the addition of the percentage of encapsulated LAB is in the range of 0.68%-1.31% this amount is following the quality requirements of yoghurt. According to SNI (2009), the total acidity of yoghurt ranges from 0.5% to 2.0%.

Syneresis Value of the Yoghurt Set

The average syneresis in yoghurt set with the addition of a different percentage of encapsulated LAB with the use of a combination of gelatin and Na alginate as encapsulation material through the emulsion technique can be seen in Table 3.

Table 3. The average syneresis value of the yoghurt set

Treatment	Syneresis value (%)
T0	24.51±0.70[c]
T1	23.52±0.76[c]
T2	20.44±0.44[ab]
T3	19.96±0.83[a]

Note: [a,b,c]different superscripts in the same column showed the treatment using a combination of gelatin and Na alginate through the emulsion technique gave a very significant difference (P<0.01) to the syneresis of the yogurt set.

The results of the analysis of variance showed that the addition of *Lactobacillus acidophilus* FNCC 0051 and *Streptococcus thermophilus* FNCC 0040 encapsulated using a combination of gelatin and Na alginate with emulsion technique gave a very significant difference (P<0.01) to the average syneresis value of yoghurt set. The higher the percentage of the use of encapsulated LAB, the lower the syneresis value of the resulting yoghurt set. The average syneresis value of yoghurt set in the control treatment or the addition of LAB without encapsulation (T0) was 24.51±0.70%, while the

average syneresis of yoghurt set in the treatment of adding encapsulated LAB T1 23.52±0.76%, T2 20.44±0.44% and T3 19.96±0.83% (Table 3).

Syneresis is the event of the release of water from the gel, the higher the syneresis value indicates the instability of the gel bond, so it can be said that the higher the syneresis value, the lower the quality of the yoghurt set (Krisnaningsih et al., 2018). The highest syneresis value was obtained in the control treatment (T0) with the addition of LAB without encapsulation, which was 24.51±0.70%. Syneresis occurs due to the shrinkage of the three-dimensional structure of the protein network which causes a decrease in the water-binding capacity of casein so that *whey* is separated from yoghurt (Djali et al., 2018). The occurrence of syneresis is probably caused by changes in the solubility of casein and shrinkage of casein particles due to increased electrostatic binding capacity accompanied by decreased electrostatic repulsion of casein micelles (Manab, 2008). Yoghurt syneresis is also influenced by the protein content of raw materials and additives (Sawitri et al., 2008).

Yoghurt syneresis at T1, T2 and T3 decreased with the addition of encapsulated LAB. The lowest syneresis was obtained at T3 19.96±0.83% with the highest percentage of the use of encapsulated LAB, which was 4%, although P3 had the highest acidity compared to the acidity of T0, T1 and T2. These results indicate the role of encapsulating materials in the form of Na alginate and gelatin which causes the formation of interactions between casein micelles with Na alginate and gelatin. The formation of interactions between casein micelles with Na alginate and gelatin makes casein micelles protected from the effects of decreasing acidity and increasing H^+. Na alginate used for microencapsulation of probiotic bacteria can form a gel by cations present in yoghurt, such as calcium. The prepared microcapsules have gaps and trap some of the calcium ions in the yoghurt gel (Shafiei, 2018). Na alginate forms protein cross-links, protein cross-links can improve protein structure, which in turn can hold water between casein molecules (Rossa et al., 2011). In addition, gelatin can form a gel that is reversible, easily soluble in hot water and can form a unique bond. The main component of gelatin is a protein whose content ranges from 85%-92% (Eriningsih et al., 2012).

The addition of ingredients that contain protein is considered to increase the amount of protein that can be bound which can improve the yoghurt gel structure because it can hold water between the casein molecules, thereby reducing the free water molecules that come out (Aloglu & Oner, 2013). The higher protein can increase the amount of protein that can be bound so that the protein structure is stronger. A strong protein structure can increase the water holding capacity of yoghurt (Prayitno et al., 2020). In addition, the addition of gelatin in the LAB microencapsulation process in the manufacture of yoghurt can reduce the occurrence of syneresis, this is because gelatin functions as a stabilizer that can increase the total solids of yoghurt to produce a thick texture. According to Sawitri et al. (2008) gelatin is a stabilizer that can reduce syneresis and as a water-binding agent by increasing the hydrophilic properties of proteins. Kolapo (2007) stated that the addition of thickener concentration will significantly reduce the occurrence of syneresis.

Total Lactic Acid Bacteria (LAB) value of the yoghurt set

The average total Lactic Acid Bacteria in yoghurt set with the addition of a different percentage of encapsulated LAB with the use of a combination of gelatin and Na alginate as encapsulation material through the emulsion technique can be seen in Table 4.

Table 4. The average total Lactic Acid Bacteria (LAB) value of the yoghurt set

Treatment	Lactic Acid Bacteria (LAB) (log CFU/ml)
T0	8.62±0.30[a]
T1	9.20±0.70[b]
T2	9.36±0.33[b]
T3	9.51±0.10[b]

Note: [a,b] different superscripts in the same column indicate the treatment using a combination of gelatin and Na alginate through emulsion techniques gave a significant difference ($P < 0.05$) to the total lactic acid bacteria of yoghurt set

The results of the analysis of variance showed that the addition of *Lactobacillus acidophilus* FNCC 0051 and *Streptococcus thermophilus* FNCC 0040 encapsulated using gelatin coating and Na alginate with emulsion technique gave a significant difference ($P<0.05$) to the total LAB yoghurt set. The higher the percentage of the use of encapsulated LAB, the higher the total LAB yoghurt set produced. The average total LAB value of yoghurt set in the control treatment or the addition of LAB without encapsulation (T0) was 8.62±0.30 log CFU/ml while the total amount of LAB in the yoghurt set with the addition of encapsulated LAB was higher

than the control treatment, namely T1 9.20±0.70 log CFU/ml, T2 9.36±0.33 log CFU/ml and T3 9.51±0.10 log CFU/ml (Table 4).

Total LAB contained in the yoghurt set is one of the determining parameters of the feasibility level of products that can be categorized as functional foods. According to SNI (2009), the minimum number of starter bacteria in making yoghurt is 7 log CFU/ml. Hou et al. (2003) added that to lead to health benefits, the recommended amount of LAB is 10^8-10^9 CFU/ml/day. The total amount of LAB produced in this study met the established standards, which ranged from 8.62±0.30 log CFU/ml to 9.51±0.10 log CFU/ml. The number of colonies at T0 was lower than at T1, T2 and T3 because LAB without encapsulation (cells) experienced direct contact with the environment which caused the permeability of the bacterial cell membrane to decrease and even be damaged due to environmental changes. Changes in cell permeability cause intracellular solutions, especially water from cells, to come out which results in osmotic damage to cells and cells become stressed and then loses balance. Bilang et al., (2018) stated that extreme temperatures cause the inactivation of enzymes and cell structure functions. LAB death can be caused by bacteria experiencing stress due to environmental conditions that are much different from the optimum temperature for growth so it affects the viability of life.

Viability was determined based on the cell growth rate of each LAB. The growth rate of *Streptococcus thermopillus* takes two hours to pass the adaptation phase and enter the exponential phase. Cell division in the adaptation phase occurs slowly to adapt to the new LAB environment. After passing through the adaptation phase, the bacteria will grow to divide exponentially and produce a lot of lactic acid as a result of LAB metabolites (Bilang et al., 2018). According to Li et al. (2012) starter *Lactobacillus acidophilus* requires a minimum of 12 hours of incubation to produce a primary metabolite in the form of lactic acid, while the starter *Streptococcus thermophilus* has the nature of liking an atmosphere close to pH 6.5. This starter can stimulate the growth of other starters by synthesizing formic acid (Khoiriyah & Fatchiyah, 2013).

The total growth of LAB is influenced by encapsulation factors that can protect the bacteria in the encapsulation from the environmental conditions of the fermentation which become acidic, while in the control treatment the bacteria interact directly with the acidic environment of the fermentation and are at the bacterial saturation point so that the tendency of the bacteria to become less stable at pH the. The process of lactic acid fermentation is glucose fermentation in LAB cells which produces lactic acid (Yang, 2000). Lactic acid will be secreted out of cells and accumulated in the fermentation liquid, causing a decrease in pH and an increase in product acidity. Encapsulation is an attempt to protect bacteria (nuclear substances) from extreme environmental conditions. In addition, the advantage of encapsulation is that probiotic bacteria that have been trapped in the encapsulation will still be able to carry out metabolic activities and can expel the metabolic products through the pores of their semipermeable membrane (Kailasapathy, 2002).

Various factors can influence the metabolic activity of encapsulated bacteria, including capsule size, permeability, density, and pore size (Vidhyalakshmi et al., 2009). Capsules with the emulsion method have a smaller diameter so that the resulting pore size is also smaller. This small pore size makes the transfer of fluid from outside the capsule into the capsule more limited (Kailasapathy, 2002). The principle is to release cells at a controlled rate of release under specific conditions and still allows the diffusion of small molecules (cells, metabolites and substrates) across the membrane (Vidhyalakshmi et al., 2009). The core substance (bacterial cells) after encapsulation can still carry out their functional activities as free cell activities.

CONCLUSION

The results showed that the addition of encapsulated LAB with different percentages could increase the total LAB and total titrated acid and decrease the pH value and syneresis of the yoghurt set. The best treatment was obtained at P3 with the addition of 4% LAB which was able to increase the quality of the yoghurt set optimally and meet the established SNI.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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