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EVALUATION OF VIABILITY ENCAPSULATION OF PROBIOTIC CUKO PEMPEK

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Abstract

The purpose of this research made Cuko Pempek as functional food by supplementing BAL to produce Cuko pempek probiotic. The existence of anti-microbial and anti-bacterial Cuko pempek components became obstacles, therefore it needed strategy to answer two main issues that was *first*, still allowe the existence of capsaicin and alisin which was caracter impact of Cuko pempek; and *second*, to protect BAL in order to survive. The strategy was the encapsulation prepared according to Sheu and Marshall, (1993) and the preparation of Cuko pempek modified from ID, (2012). The result showed that the encapsulation of Cuko pempek probiotic with cold storage at temperature of 12^{o}C produced viability with the average number of cells reaching the range of 10^{9}, 10^{8}, and 10^{7} and the shelf life until the 20th day even some units until the 30th day. The encapsulation of Cuko pempek probiotic with storage at temperature of 27^{o}C produced viability with the average number of cells reaching the range of 10^{9}, 10^{8}, and 10^{7} and the shelf life until the 10th day even some units reaching the 20th day, but in the 8th day there was contamination in 5 experimental units, on the 10th day increased 5 contaminated units, and on the 12th day increased 3 units and on the 13th day occurred *Sacharomyces* contaminant on all experimental units.

Key words: Encapsulation Probiotic, BAL, Cuko pempek

I. INTRODUCTION

Pempek is a typical culinary Palembang, South Sumatra, Indonesia, made from wheat flour and tapioca, and fish. At this time it has become a culinary industry that development so rapidly therefore must be balanced with the provision of equipment distribution and presentation of a safety, healthy, and comfortable. Cuko pempek is a companion sauce to eat pempek. But Cuko pempek has specific characteristics, especially its cuka acid content, tooth decay (dental caries). This is in line with those proposed by Hoppenbrouwers and Driessens (1988) that acetic acid damages teeth twice as strongly of lactic acid. In addition acetic acid is anti-microbial (Lodovico et al., 2002; Snyder, 1997).

However, the anti-microbial characteristic possessed by Cuko pempek components that is capsaicin and alisin include weak category (Skrinjar and Nemet, 2009). Although, Zeyrek and Oguz, (2005) states, capsaicin can act as an effective bactericide. But the study of Farag *et al*., 1995 concluded that capsaicin from irradiated chilies was still overgrown with 4,2 x 10^{3}/g; 14.3 x 10^{3}/g; and 9,2 x 10^{5}/g.

Cuko pempek is a food product that has potential to be functional food by making Cuko pempek probiotik (Dunne et al., 2001). Cuko pempek probiotic is cuko pempek containing BAL, and is expected to improve its functionality (Gardiner et al., 2001; Naito *et al*., 2008). Probiotics are supplementary foods that contain living micro-organisms that provide either human or animal host benefits by balancing the microorganisms in the digestive tract (Fuller, 1989). Further Senok *et al*., (2005) probiotics are living microorganisms when arranged in certain amounts will provide benefits for the health of its host.

Encapsulation is the process of forming a matrix-shaped layer in which the inner-shaped interior resembles a capsule wall acting as a cloaking (Vidhyalakshmi *et al*., 2009). Gbassi and Vandamme (2012) call the term Probiotic Encapsulation Technology (PET), in which microbes can be widely immobilized using semipermiabel and biocompatible materials that govern the delivery of microbial cells. (Vidhyalakshmi et al., 2009) encapsulation tends to stabilize cells, potentially increasing cell viability and stability during production, storage, and handling.

BAL encapsulation techniques use phase separation techniques from Sheu and Marshall (1993) and use alginate ingredients (Anal and Singh, 2007) were selected to conduct a study of Cuko pempek probiotic.

II. MATERIALS AND METHODS

Lactic Acid Bacteria and Media

L. bulgaricus and *S. thermopylus* were obtained from Balai Besar Vateriner Bogor. Lactobacilus was transferred to media of broth MRSAgar while Streptococus to media of broth Blood Agar Base. Then spread in the media agar of petri dish and incubated at 37°C. BAL was harvested after 18 hours incubation to obtain a BAL culture concentration with a range of 10^{11}sel/mL.

Preparation of BAL Encapsulation

The preparation of encapsulation used alginate natrum (Sheu and Marshall, 1993; Sultana *et al*., 2000) was 1% (A_1), 2% (A_2), and 3% (A_3) then mixed with BAL *L. bulgaricus* (B_1) and *S. thermopylus* (B_2) culture solution with 4: 1 ratio. After a flat stirring, the mixture was dropped using a 5 mL syringe into a 0.2% tween 80 solution in vegetable oil in a 1000 mL beaker glass. It was then poured 0.05M CaCl solution as much as 250 mL rapidly through the edge of the glass wall and left for 30 minutes. The capsule granules would descend and the tween 80 solution, the vegetable oil and the remaining CaCl solution were removed by pouring slowly. The capsule granules were centrifuged at 350x for 15 minutes and then poured into a filter dish and washed with aquades. The preparation of Probiotic encapsulation was with three replications.

The Preparation of Cuko pempek

The preparation of Cuko pempek was according to ID (2012), brown sugar, garlic, cayenne and red chili mashed, and salt. Sour source was used yakult. Chili and salt blend and then mixed yakult and fermented for one week (7 days). Then water and sugar were heated to boil, removed and filtered. Then the fermented chili and yakult were fed into the filtered sugar water mixture, plus the fine garlic. The mixture was heated to boiling and cooled, then Cuko pempek was produced.

The Preparation of Encapsulation of probiotic Cuko pempek

500mL cuko pempek put in a container of plastic cans size 2000mL as much as the amount of treatment with three replications. Then encapsulation probiotic BAL inserted into Cuko pempek. Some were stored at a temperature of 12^{o}C and some were at temperature of 27^{o}C.

Observation of viability Encapsulation of probiotic Cuko pempek

Proactive observation of probiotic Cuko pempek encapsulation during storage on 1st day, 10th day, 20th day, and 30th day. Other parameters; shelf life and pH based on time.

III. RESULTS AND DISCUSSION

A. BAL Viability of Cuko Pempek in Cold Temperature of 12^{o}C

The results of diversity analysis of encapsulation of probiotic Cuko pempek at storage temperature of 12^{o}C, that alginate concentration, BAL type, interaction, and treatment combination had no significant effect on BAL cell viability. While the group was very significant effect. This meant that alginate concentration did not affect BAL cell viability and was reliable for the encapsulation of probiotic Cuko pempek (Sheu and Marshall, 1993; Lotus et al., 2000; Mokarram et al., 2009; Lotfipour et al., 2012; Wikstrom, 2013), as well as the types of BAL (Speck and Myers, 1946; Drakes et al., 2004; Denou et al., 2008; Jimenez et al., 2010). The average number of BAL cells on the first day was 10^9 for L. bulgaricus (B_1) and 10^8 for S. thermopylus (B_2), then the average number of BAL cells both B_1 and B_2 on the 10th day, the 20th day, and the 30th day is sequential 10^8, 10^7 serta 10^7 and 10^6. The average number of BAL cells was eligible to act as probiotic requiring an average number of BAL cells before consumption of 10^7. As, Ishibashi and Shimamur, 1993) suggests, called probiotic food, should contain probiotic cells before consumption of $\geq 10^7$ cells per gram or per-mL of the product. Meanwhile, Lee and Salminen, (1995) require for probiotic drink products to contain as big a cell ≥ 105 per mL of product. While FAO/WHO, (2002) requires the number of probiotic cells before consumption by 106 - 107 CFU/g or CFU/mL.

For storage of Cuko Pempek at cold temperature of 12^{o}C, BAL viability decreased linearly in ten days observation down by one log as in A_1B_1, A_2B_1, and A_2B_2 was obtained on observation the 30th day of the average number of BAL cells by 10^6. This was caused by two things, first, because the BAL cells were stored in calcium alginate capsules; second, BAL cells were better protected from less favorable environmental influences such as acidity, the presence of capsaicin components of chili and the alisin component of garlic. As Sheu and Marshall, (1993) assert that encapsulated BAL cells have viability for up to 2 weeks and their viability is 40-45% higher than un-encapsulated BAL cells; Sultana et al., (2000), Encapsulation improves viability for up to 8 weeks. Wikstrom, (2013), that encapsulation provides cell viability capability for long periods of time. Furthermore, Gbassi and Vandamme, (2012) there are two reasons for encapsulation, first, to ensure the viability of encapsulated probiotic cells; and second, to ensure the release of probiotic cells when consumed and within the digestive tract. On the other hand, Lotfipour et al., (2012) explains that BAL encapsulation made from alginate provides better viability in acidic conditions. This confirms why the viability of BAL cells in Cuko pempek encapsulation had good viability.

Pattern of BAL cell viability reduction that was encapsulated with calcium alginate ingredients in cold storage temperature of 12°C, was shown in Figure 1, that A^3B^1 (alginate 3% and L. bulgaricus) had the highest viability on average in the four consecutive observation points in sequence $4{,}60x10^9$; $1{,}09x10^8$; $4{,}96x10^6$; and $2{,}43x10^7$; while the lowest was in A^1B^2 (alginate 1% and *S. thermopylus*) with average at the four in sequence $6{,}55x10^8$; $4{,}07x10^7$; $4{,}18x10^7$; and $7{,}16x10^7$. The average number of cells met the probiotic requirements before consumption.

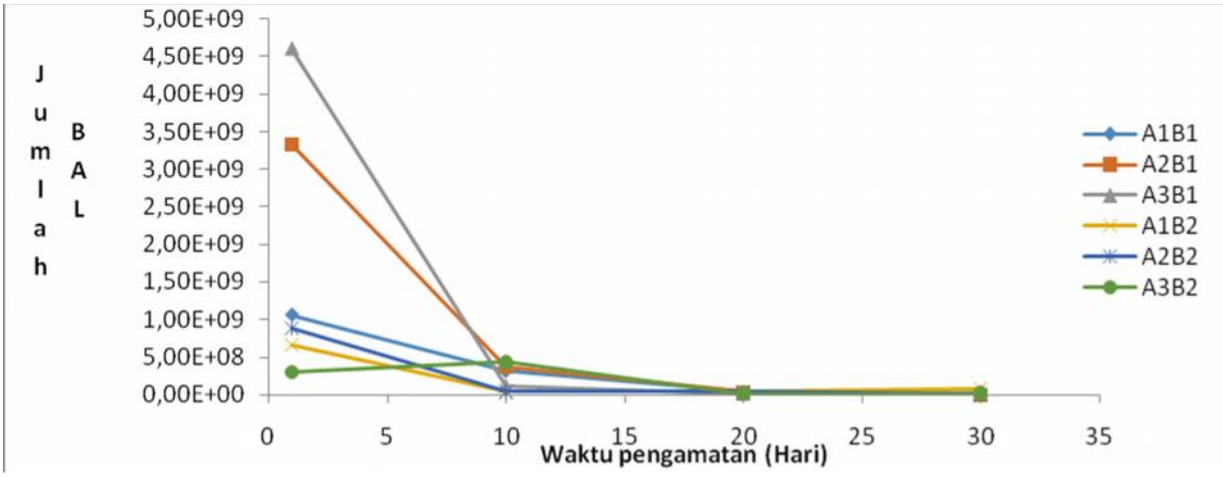


Figure 1. Graph of BAL Viability at Temperature Storage 12°C

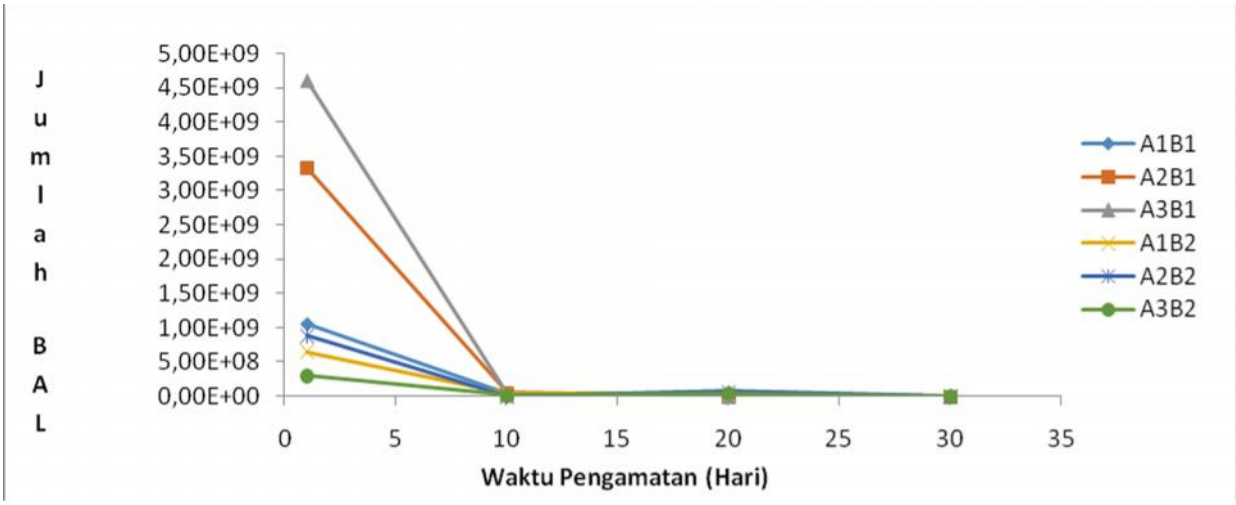
However, storage at cold temperatures of 12°C supported the viability of BAL cells. As the result of the research of Sheu and Marshall, (1993) that the encapsulation of BAL cells stored at cold temperatures had better viability. This pattern of decreased viability was similar to that described by Iyer and Kailasapathy, (2005) that BAL viability stored at cold temperatures decreases from 10^8 to 10^7 at the 2nd week and to 10^6 and 10^7 at the 4th week.

Furthermore, the results of the analysis of diversity with observations on the 1st day, 10th day, 20th day and 30th day, alginate concentration (A) and probiotic type (B) to pH encapsulation probiotik Cuko pempek at storage temperature of 12°C, that the concentration alginate and its interactions had no significant effect, the type of probiotics, combinations of treatments and groups had a very significant effect. This was due to the presence of probiotic activity over time which results in changes in pH.Roberts et al., (1994) B. longum BB-79 encapsulation after 10 days had a pH of 3,9 - 4,2; Iyer and Kailaspathy, (2005) encapsulation of L. acidophilus had a pH of 4,6; L. *plantarum* pH 5,6 (Ayama *et al*., 2014).

To look at the degree of difference in each treatment that had a very significant effect on the pH of further tests, it was shown in Figure 2 that the pH encapsulation of probiotic Cuko pempek was very stable until the 10th day, and relatively stable until the 20th day and partly until the 30th day. This showed that during the period of time until the 10th day there was no significant microbiological activity, then until the 20th day there was little microbiological activity and increased activity until the 30th day. As expressed Robert et al., (1994) that in the encapsulation BAL probiotics begin to change pH after day 10.

B. BAL Viability of Cuko Pempek at Room Temperature of 27^{o}C

The result of diversity analysis of alginate concentration, BAL type, combination of treatment and its interaction had no significant effect on cell viability of BAL encapsulation of probiotic Cuko pempek at storage temperature of 27^{o}C, while the group was very significant. The viability on the first day was 10^8, then the average number of BAL cells both L. bulgaricus (B1) and S. thermopylus (B_2) on the 10th, 20th, and 30th days was sequential 10^7, 10^6 and 10^5. This decrease in BAL cell viability appears to be associated with a decrease in pH, since room temperature induced microbiological activity that affects pH. Noland and Aryana (2012) observes BAL viability in yogurt, BAL viability decreases when the pH falls below pH 4.3. However, the average number of BAL cells still qualifies as probiotics requiring a pre-consumption amount of 10^7 (Ishibashi and Shimamur, 1993; Lee and Salminen, 1995; FAO / WHO, 2002). However, the number of eligible BAL cells was only until the 10th day .



Picture 3. Graph of VAL Viability at storage temperature of 27^{o}C

Figure 3 about the decreased pattern of BAL cell viability of encapsulation of probiotic Cuko pempek at storage temperature of 27^{o}C, that A_3B_1 (3% alginate treatment and *L. bulgaricus*) had the highest viability and lowest available in A_3B_1 (3% alginate treatment and *S. thermopylus).* The viability of BAL cells at storage temperature of 27^{o}C occured in a lower log pattern decrease. This appears to be the condition of the room temperature caused the growth rate and activity of probiotic take place.

Furthermore, the results of the analysis of diversity, that the concentration of alginate, BAL type, combination of treatment and its interaction had no significant effect on pH encapsulation of probiotic Cuko pempek at storage temperature of 27^{o}C. To see the level of difference of group effect continued test shown in Figure 4. The pH pattern from high condition started pH 5 on the 1st day, then fell to its lowest point on the 10th day of 3,73 and then up to the the 20th day of 3,87 – 3,9 and up again until the 30th day of 4. But the increase did not go beyond pH on the first day.

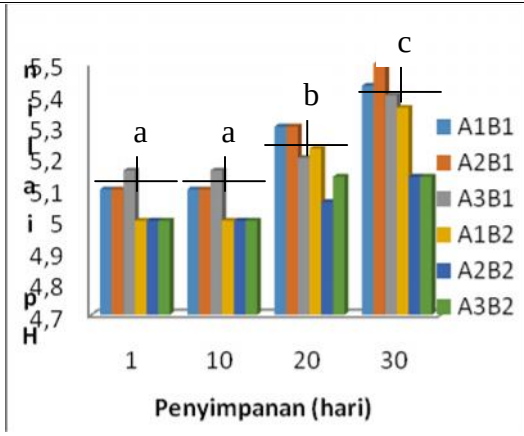
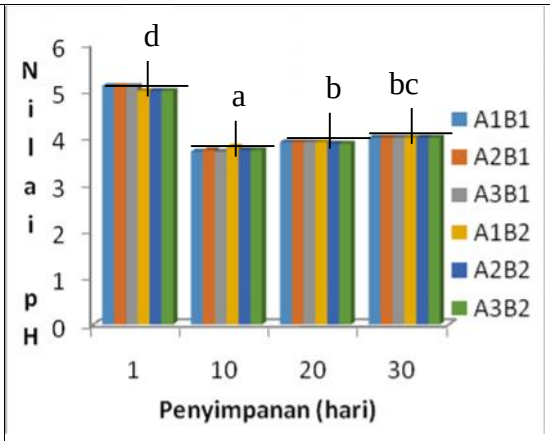


Figure 2. Graph of decreasing pattern of pH encapsulation of probiotic Cuko pempek at storage temperature of 12°C



Gambar 4. Graph of decreasing pattern of pH encapsulation of probiotic Cuko pempek at storage temperature of 27°C

Unlike storage at cold temperatures of 12°C, storage at temperature of 27°C in addition to caused the fermentative rates to produce decreasing pH, also provided a great chance of contamination. On the 8th day, the units A_1B_1(I), A_1B_2(II), A_2B_1((I), A_2B_1(III), and A_3B_1(III) were contaminated. Then on the 10th day the contamination increased in unit A_1B_1(III), A_2B_1(II), A_3B_1(II), A_3B_1(III), and A_2B_2(II), so that on the 10th day all experimental units using *L. bulgaricus* (B_1) had been contaminated *Saccharomyces*.

On the 12th day of the experimental unit using *S. thermopillus* (B_2) which on the 10th day was contaminated one of A_2B_2(II), three units were added, namely A_1B_2(I), A_1B_2(II), and A_1B_2(III). Then, on the 13th day all the experimental units were contaminated with *Saccharomyces*. This phenomenon indicated that acidic of Cuko pempek and stored at 27°C prone to contaminated and overgrown with *Sacharomyces*. This confirms the results of the study of Narendranath *et al*. (2001),that *Saccharomyces* grows in a minimum medium containing acetic acid and lactic acid at temperature of 30° C. Furthermore, Thomas *et al*. (2002) explains that Saccharomyces grows on a minimum medium containing lactic acid when its pH is 4.5.

IV. CONCLUSIONS

1. The encapsulation of probiotics Cuko pempek with storage temperature of 12°C produced viability with average number of cells reaching 10^9, 10^8, and $10^7$and shelf life until the 20th day and some units until the 30th day, with a relatively constant pH ranging from 5,07 – 5,25.
2. Encapsulation of Cuko pempek probiotic with storage temperature 27°C produced viability with average number of cells reaching 10^8, 10^7, dan $10^6$and shelf life up to the 10th day and some units reaching the 20th day, with decreasing pH from the 1st day, the 10^{th} day, the 20th day and the 30th consecutive day pH 5,0 – 5,1; pH 3,70 – 3,80; pH 3,87 – 9,0; and pH 4.
3. For encapsulation of probiotic Cuko pempek with storage temperature of 27°C on the 8th day arose contaminant at 5 unit experiment, on the 10th day added 5 unit and on the 12th

day arose in three other unit and on the 13th day arose contaminant in the form *Sacharomyces* on all experimental units.

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Evaluation of Viability Encapsulation of Probiotic Cuko Pempek

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Abstract**—** *The purpose of this research made Cuko Pempek as functional food by supplementing BAL to produce Cuko pempek probiotic. The existence of anti-microbial and anti-bacterial Cuko pempek components became obstacles, therefore it needed strategy to answer two main issues that was first, still allowe the existence of capsaicin and alisin which was caracter impact of Cuko pempek; and second, to protect BAL in order to survive. The strategy was the encapsulation prepared according to Sheu and Marshall, (1993) and the preparation of Cuko pempek modified from ID, (2012). The result showed that the encapsulation of Cuko pempek probiotic with cold storage at temperature of 12ºC produced viability with the average number of cells reaching the range of 10^9, 10^8, and 10^7 and the shelf life until the 20th day even some units until the 30th day. The encapsulation of Cuko pempek probiotic with storage at temperature of 27ºC produced viability with the average number of cells reaching the range of 10^9, 10^8, and 10^7 and the shelf life until the 10th day even some units reaching the 20th day, but in the 8th day there was contamination in 5 experimental units, on the 10th day increased 5 contaminated units, and on the 12th day increased 3 units and on the 13th day occurred Sacharomyces contaminant on all experimental units.*

Keywords**— Encapsulation Probiotic, BAL, Cuko pempek.**

I. INTRODUCTION

Pempek is a typical culinary Palembang, South Sumatra, Indonesia, made from wheat flour and tapioca, and fish. At this time it has become a culinary industry that development so rapidly therefore must be balanced with the provision of equipment distribution and presentation of a safety, healthy, and comfortable. Cuko pempek is a companion sauce to eat pempek. But Cuko pempek has specific characteristics, especially its cuka acid content, tooth decay (dental caries). This is in line with those proposed by Hoppenbrouwers and Driessens (1988) that acetic acid damages teeth twice as strongly of lactic acid. In addition acetic acid is anti-microbial (Lodovico et al., 2002; Snyder, 1997).

However, the anti-microbial characteristic possessed by Cuko pempek components that is capsaicin and alisin include weak category (Skrinjar and Nemet, 2009). Although, Zeyrek and Oguz, (2005) states, capsaicin can act as an effective bactericide. But the study of Farag *et al*., 1995 concluded that capsaicin from irradiated chilies was still overgrown with 4,2 x 10^3/g; 14.3 x 10^3/g; and 9,2 x 10^5/g.

Cuko pempek is a food product that has potential to be functional food by making Cuko pempek probiotik (Dunne et al., 2001). Cuko pempek probiotic is cuko pempek containing BAL, and is expected to improve its functionality (Gardiner et al., 2001; Naito *et al*., 2008). Probiotics are supplementary foods that contain living micro-organisms that provide either human or animal host benefits by balancing the microorganisms in the digestive tract (Fuller, 1989). Further Senok *et al*., (2005) probiotics are living microorganisms when arranged in certain amounts will provide benefits for the health of its host.

Encapsulation is the process of forming a matrix-shaped layer in which the inner-shaped interior resembles a capsule wall acting as a cloaking (Vidhyalakshmi *et al*., 2009). Gbassi and Vandamme (2012) call the term Probiotic Encapsulation Technology (PET), in which microbes can be widely immobilized using semipermiabel and biocompatible materials that govern the delivery of microbial cells. (Vidhyalakshmi et al., 2009) encapsulation tends to stabilize cells, potentially increasing cell viability and stability during production, storage, and handling.

BAL encapsulation techniques use phase separation techniques from Sheu and Marshall (1993) and use alginate ingredients (Anal and Singh, 2007) were selected to conduct a study of Cuko pempek probiotic.

II. MATERIALS AND METHODS

Lactic Acid Bacteria and Media

L. bulgaricus and *S. thermopylus* were obtained from Balai Besar Vateriner Bogor. Lactobacilus was transferred to media of broth MRSAgar while Streptococus to media of broth Blood Agar Base. Then spread in the media agar of petri dish and incubated at 37°C. BAL was harvested after 18 hours incubation to obtain a BAL culture concentration with a range of 10^{11}sel/mL.

Preparation of BAL Encapsulation

The preparation of encapsulation used alginate natrum (Sheu and Marshall, 1993; Sultana *et al*., 2000) was 1% (A_1), 2% (A_2), and 3% (A_3) then mixed with BAL *L.*

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bulgaricus (B_1) and *S. thermopylus* (B_2) culture solution with 4: 1 ratio. After a flat stirring, the mixture was dropped using a 5 mL syringe into a 0.2% tween 80 solution in vegetable oil in a 1000 mL beaker glass. It was then poured 0.05M CaCl solution as much as 250 mL rapidly through the edge of the glass wall and left for 30 minutes. The capsule granules would descend and the tween 80 solution, the vegetable oil and the remaining CaCl solution were removed by pouring slowly. The capsule granules were centrifuged at 350x for 15 minutes and then poured into a filter dish and washed with aquades. The preparation of Probiotic encapsulation was with three replications.

The Preparation of Cuko pempek

The preparation of Cuko pempek was according to ID (2012), brown sugar, garlic, cayenne and red chili mashed, and salt. Sour source was used yakult. Chili and salt blend and then mixed yakult and fermented for one week (7 days). Then water and sugar were heated to boil, removed and filtered. Then the fermented chili and yakult were fed into the filtered sugar water mixture, plus the fine garlic. The mixture was heated to boiling and cooled, then Cuko pempek was produced.

The Preparation of Encapsulation of probiotic Cuko pempek

500mL cuko pempek put in a container of plastic cans size 2000mL as much as the amount of treatment with three replications. Then encapsulation probiotic BAL inserted into Cuko pempek. Some were stored at a temperature of 12^{o}C and some were at temperature of 27^{o}C.

Observation of viability Encapsulation of probiotic Cuko pempek

Proactive observation of probiotic Cuko pempek encapsulation during storage on 1st day, 10th day, 20th day, and 30th day. Other parameters; shelf life and pH based on time.

III. RESULTS AND DISCUSSION

A. BAL Viability of Cuko Pempek in Cold Temperature of 12^{o}C

The results of diversity analysis of encapsulation of probiotic Cuko pempek at storage temperature of 12^{o}C, that alginate concentration, BAL type, interaction, and treatment combination had no significant effect on BAL cell viability. While the group was very significant effect. This meant that alginate concentration did not affect BAL cell viability and was reliable for the encapsulation of probiotic Cuko pempek (Sheu and Marshall, 1993; Lotus et al., 2000; Mokarram et al., 2009; Lotfipour et al., 2012; Wikstrom, 2013), as well as the types of BAL (Speck and Myers, 1946; Drakes et al., 2004; Denou et al., 2008; Jimenez et al., 2010). The average number of BAL cells on the first day was 10^9 for L. bulgaricus (B_1) and 10^8 for S. thermopylus (B_2), then the average number of BAL cells both B_1 and B_2 on the 10th day, the 20th day, and the 30th day is sequential 10^8, 10^7 serta 10^7 and 10^6. The average number of BAL cells was eligible to act as probiotic requiring an average number of BAL cells before consumption of 10^7. As, Ishibashi and Shimamur, 1993) suggests, called probiotic food, should contain probiotic cells before consumption of $\geq 10^7$ cells per gram or per-mL of the product. Meanwhile, Lee and Salminen, (1995) require for probiotic drink products to contain as big a cell $\geq$ 105 per mL of product. While FAO/WHO, (2002) requires the number of probiotic cells before consumption by 106 - 107 CFU/g or CFU/mL.

For storage of Cuko Pempek at cold temperature of 12^{o}C, BAL viability decreased linearly in ten days observation down by one log as in A_1B_1, A_2B_1, and A_2B_2 was obtained on observation the 30th day of the average number of BAL cells by 10^6. This was caused by two things, first, because the BAL cells were stored in calcium alginate capsules; second, BAL cells were better protected from less favorable environmental influences such as acidity, the presence of capsaicin components of chili and the alisin component of garlic. As Sheu and Marshall, (1993) assert that encapsulated BAL cells have viability for up to 2 weeks and their viability is 40-45% higher than un-encapsulated BAL cells; Sultana et al., (2000), Encapsulation improves viability for up to 8 weeks. Wikstrom, (2013), that encapsulation provides cell viability capability for long periods of time. Furthermore, Gbassi and Vandamme, (2012) there are two reasons for encapsulation, first, to ensure the viability of encapsulated probiotic cells; and second, to ensure the release of probiotic cells when consumed and within the digestive tract. On the other hand, Lotfipour et al., (2012) explains that BAL encapsulation made from alginate provides better viability in acidic conditions. This confirms why the viability of BAL cells in Cuko pempek encapsulation had good viability.

Pattern of BAL cell viability reduction that was encapsulated with calcium alginate ingredients in cold storage temperature of 12^{o}C, was shown in Figure 1, that A^3B^1 (alginate 3% and L. bulgaricus) had the highest viability on average in the four consecutive observation points in sequence $4{,}60x10^9$; $1{,}09x10^8$; $4{,}96x10^6$; and $2{,}43x10^7$; while the lowest was in A^1B^2 (alginate 1% and *S. thermopylus*) with average at the four in sequence $6{,}55x10^8$; $4{,}07x10^7$; $4{,}18x10^7$; and $7{,}16x10^7$. The average number of cells met the probiotic requirements before consumption.

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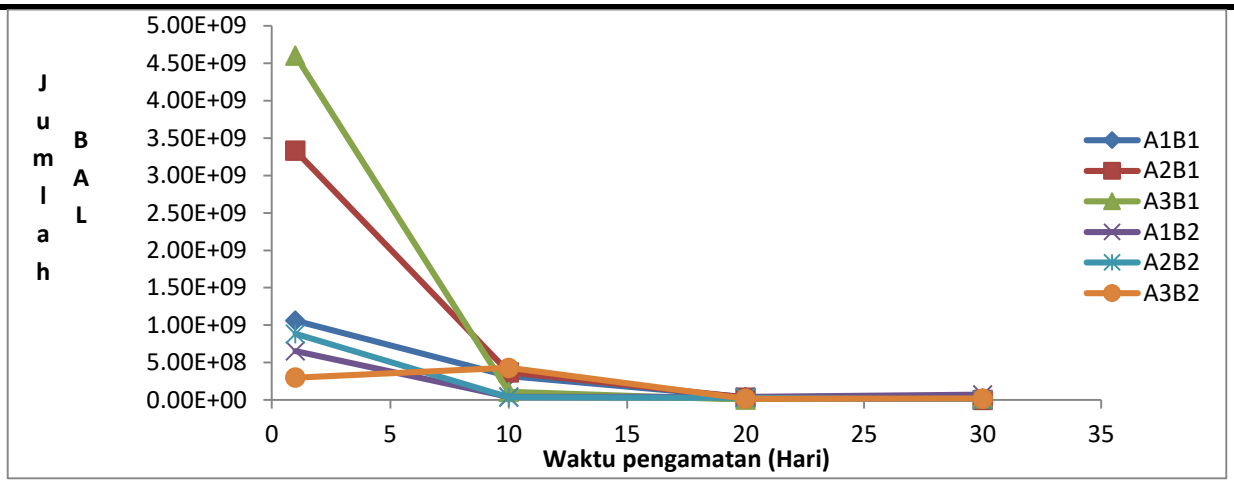

Fig.1: Graph of BAL Viability at Temperature Storage 12ºC

However, storage at cold temperatures of 12ºC supported the viability of BAL cells. As the result of the research of Sheu and Marshall, (1993) that the encapsulation of BAL cells stored at cold temperatures had better viability. This pattern of decreased viability was similar to that described by Iyer and Kailasapathy, (2005) that BAL viability stored at cold temperatures decreases from 10^8 to 10^7 at the 2nd week and to 10^6 and 10^7 at the 4th week.

Furthermore, the results of the analysis of diversity with observations on the 1st day, 10th day, 20th day and 30th day, alginate concentration (A) and probiotic type (B) to pH encapsulation probiotik Cuko pempek at storage temperature of 12ºC, that the concentration alginate and its interactions had no significant effect, the type of probiotics, combinations of treatments and groups had a very significant effect. This was due to the presence of probiotic activity over time which results in changes in pH.Roberts et al., (1994) B. longum BB-79 encapsulation after 10 days had a pH of 3,9 - 4,2; Iyer and Kailaspathy, (2005) encapsulation of L. acidophilus had a pH of 4,6; L. *plantarum* pH 5,6 (Ayama *et al*., 2014).

To look at the degree of difference in each treatment that had a very significant effect on the pH of further tests, it was shown in Figure 2 that the pH encapsulation of probiotic Cuko pempek was very stable until the 10th day, and relatively stable until the 20th day and partly until the 30th day. This showed that during the period of time until the 10th day there was no significant microbiological activity, then until the 20th day there was little microbiological activity and increased activity until the 30th day. As expressed Robert et al., (1994) that in the encapsulation BAL probiotics begin to change pH after day 10.

B. BAL Viability of Cuko Pempek at Room Temperature of 27ºC

The result of diversity analysis of alginate concentration, BAL type, combination of treatment and its interaction had no significant effect on cell viability of BAL encapsulation of probiotic Cuko pempek at storage temperature of 27ºC, while the group was very significant. The viability on the first day was 10^8, then the average number of BAL cells both L. bulgaricus (B1) and S. thermopylus (B_2) on the 10th, 20th, and 30th days was sequential 10^7, 10^6 and 10^5. This decrease in BAL cell viability appears to be associated with a decrease in pH, since room temperature induced microbiological activity that affects pH. Noland and Aryana (2012) observes BAL viability in yogurt, BAL viability decreases when the pH falls below pH 4.3. However, the average number of BAL cells still qualifies as probiotics requiring a pre-consumption amount of 10^7 (Ishibashi and Shimamur, 1993; Lee and Salminen, 1995; FAO / WHO, 2002). However, the number of eligible BAL cells was only until the 10th day.

Figure 3 about the decreased pattern of BAL cell viability of encapsulation of probiotic Cuko pempek at storage temperature of 27ºC, that A_3B_1 (3% alginate treatment and *L. bulgaricus*) had the highest viability and lowest available in A_3B_1 (3% alginate treatment and *S. thermopylus).* The viability of BAL cells at storage temperature of 27ºC occured in a lower log pattern decrease. This appears to be the condition of the room temperature caused the growth rate and activity of probiotic take place.

Furthermore, the results of the analysis of diversity, that the concentration of alginate, BAL type, combination of treatment and its interaction had no significant effect on pH encapsulation of probiotic Cuko pempek at storage temperature of 27ºC. To see the level of difference of group effect continued test shown in Figure 4. The pH pattern from high condition started pH 5 on the 1st day, then fell to its lowest point on the 10th day of 3,73 and then up to the the 20th day of 3,87 – 3,9 and up again until the 30th day of 4. But the increase did not go beyond pH on the first day.

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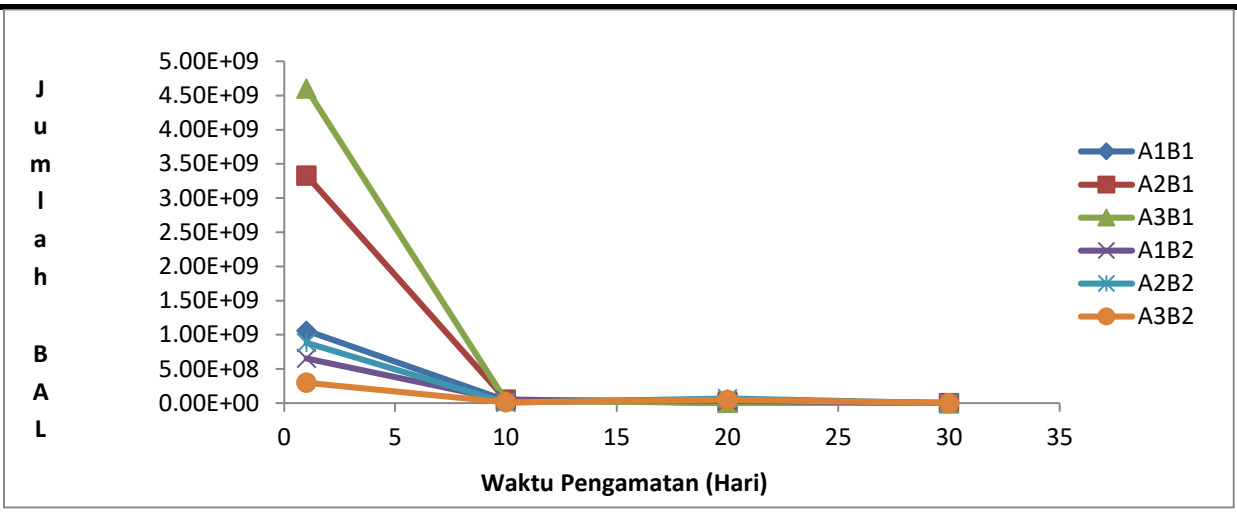


Picture 3. Graph of VAL Viability at storage temperature of 27°C

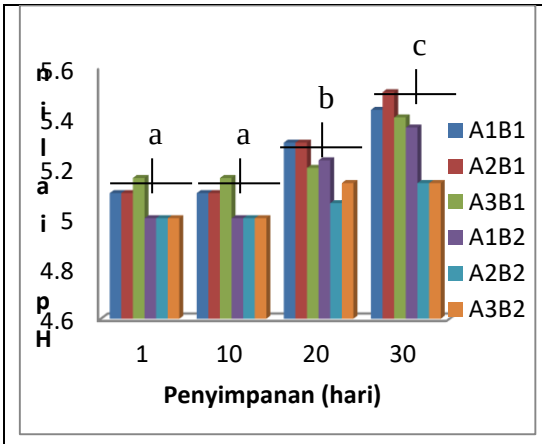
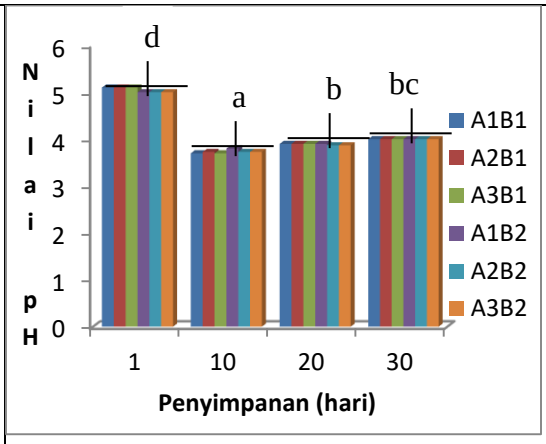


Figure 2. Graph of decreasing pattern of pH encapsulation of probiotic Cuko pempek at storage temperature of 12°C



Gambar 4. Graph of decreasing pattern of pH encapsulation of probiotic Cuko pempek at storage temperature of 27°C

Unlike storage at cold temperatures of 12°C, storage at temperature of 27°C in addition to caused the fermentative rates to produce decreasing pH, also provided a great chance of contamination. On the 8th day, the units A_1B_1(I), A_1B_2(II), A_2B_1((I), A_2B_1(III), and A_3B_1(III) were contaminated. Then on the 10th day the contamination increased in unit A_1B_1(III), A_2B_1(II), A_3B_1(II), A_3B_1(III), and A_2B_2(II), so that on the 10th day all experimental units using *L. bulgaricus* (B_1) had been contaminated *Saccharomyces*.

On the 12th day of the experimental unit using *S. thermopillus* (B_2) which on the 10th day was contaminated one of A_2B_2(II), three units were added, namely A_1B_2(I), A_1B_2(II), and A_1B_2(III). Then, on the 13th day all the experimental units were contaminated with *Saccharomyces*. This phenomenon indicated that acidic of Cuko pempek and stored at 27°C prone to contaminated and overgrown with *Sacharomyces*. This confirms the results of the study of Narendranath *et al*. (2001),that *Saccharomyces* grows in a minimum medium containing acetic acid and lactic acid at temperature of 30° C. Furthermore, Thomas *et al*. (2002) explains that Saccharomyces grows on a minimum medium containing lactic acid when its pH is 4.5.

IV. CONCLUSIONS

1. The encapsulation of probiotics Cuko pempek with storage temperature of 12°C produced viability with average number of cells reaching 10^9, 10^8, and $10^7$and shelf life until the 20th day and some units until the 30th day, with a relatively constant pH ranging from 5,07 – 5,25.
2. Encapsulation of Cuko pempek probiotic with storage temperature 27°C produced viability with average

number of cells reaching 10^8, 10^7, dan $10^6$and shelf life up to the 10th day and some units reaching the 20th day, with decreasing pH from the 1st day, the 10^{th} day, the 20th day and the 30th consecutive day pH 5,0 – 5,1; pH 3,70 – 3,80; pH 3,87 – 9,0; and pH 4.

3. For encapsulation of probiotic Cuko pempek with storage temperature of 27ºC on the 8th day arose contaminant at 5 unit experiment, on the 10th day added 5 unit and on the 12th day arose in three other unit and on the 13th day arose contaminant in the form *Sacharomyces* on all experimental units.

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