TEST Engineering & Management

May-June 2020
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The Evaluation of Viability of Cuko Pempek Probiotic Encapsulation

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Article Info
Volume 83
Page Number: 9017 - 9023
Publication Issue:
May - June 2020

Article History
Article Received*: 19 November 2019*
Revised*: 27 January 2020*
Accepted*: 24 February 2020*
Publication*: 18 May 2020*

Abstract:
The purpose of this study is to make Cuko Pempek as functional food by supplementing BAL to produce probiotic Cuko Pempek. The existence of anti-microbial and anti-bacterial Cuko Pempek components is an obstacle and resistance, therefore a strategy that is able to answer two main problems is needed, first, allowing the presence of capsaicin and alisin which are the character impacts of Cuko Pempek; and second, protecting the BAL so that it can survive. The strategy is the encapsulation prepared according to Sheu and Marshall, (1993) and the preparation of Cuko Pempek modified from ID, (2012).The result is that encapsulation of probiotic Cuko Pempek with cold storage at 12^{o}C produces viability with an average cell count of 10^9, 10^8, and 10^7 and shelf life up to the 20th day and even several units until the 30th day. Encapsulation of probiotic Cuko Pempek with storage at 27^{o}C produces viability with an average number of cells reaching the range of 10^9, 10^8, and 10^7 and shelf life up to the 10th day and even some units reach the 20th day, but on the 8th day the contamination arises on 5 experimental units, on the 10th day 5 units were contaminated, and on the 12th day 3 units were added and on the 13th day Sacharomyces contaminants were present in all trial units.

Keywords:*Probiotic encapsulation, BAL, Cuko Pempek*

2. INTRODUCTION

Pempek is a traditional culinary from Palembang, South Sumatra, Indonesia, made from flour and tapioca, and fish meat. At this time it has become a culinary industry whose development is so rapid, therefore it must be balanced with the provision of safe, healthy and comfortable distribution and presentation devices. Cuko Pempek is a companion sauce to eat pempek. But Cuko Pempek has specific characteristics, especially the acid content of the vinegar, damaging the teeth (dental caries). This is in line with what was stated by Hoppenbrouwers and Driessens (1988) that acetic acid damages teeth twice as strong as lactic acid. In addition, acetic acid is anti-microbial (Lodovico *et al*., 2002; Snyder, 1997).

However, the anti-microbial properties possessed by the Cuko Pempek component, namely capsaicin and alicin, are categorized as weak (Skrinjar and Nemet, 2009). Although, Zeyrek and Oguz, (2005) stated, capsaicin can act as an effective bactericide. But the study of Farag et al., 1995 concluded that capsaicin from chilies that had been irradiated was still overgrown withbacteria of $4.2x10^3$/g;$14.3x10^3$/g;and$9.2x10^5$/g.

Cuko Pempek is a food product that has the potential to be functional food by making probiotic pempek

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cuko (Dunne *et al*., 2001).A probiotic Cuko Pempek is a Cuko Pempek that contain BAL, and is expected to improve its function (Gardiner et al., 2001; Naito *et al*., 2008). Probiotics are supplementary foods that contain living micro-organisms that provide a host advantage to either humans or animals by balancing microorganisms in the digestive tract (Fuller, 1989). Furthermore Senok *et al*., (2005) probiotics are living microorganisms which if regulated in certain amounts will provide health benefits for their host. Encapsulation is the process of forming a matrix-shaped layer where the inside is spherical shaped like a capsule wall that acts as a casing (Vidhyalakshmi *et al*., 2009). Gbassi and Vandamme (2012) call the term Probiotic Encapsulation Technology (PET), where microbes can be widely immobilized using semipermiable and biocompatible materials that regulate 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.

LAB encapsulation techniques using phase separation techniques from Sheu and Marshall (1993) and using alginate-based ingredients (Anal and Singh, 2007) were selected to study the manufacture of probiotic Cuko Pempek.

II. MATERIALS AND METHODS

Lactic Acid Bacteria and Media

L. bulgaricus and *S. thermopylus* were obtained from the Bogor Vateriner Center. Lactobacilus was transferred to the MRSAgar broth medium while Streptococus was on the Blood Agar Base broth medium. Then it was spread in agar media on petri dishes and incubated at 37^{o}C. BAL is harvested after 18 hours of incubation to obtain BAL culture concentrations in the range of 1011 cells / mL.

Preparation of BAL Encapsulation

Preparation of encapsulation using sodium alginate (Sheu and Marshall, 1993; Sultana *et al*., 2000) respectively 1% (A_1), 2% (A_2), and 3% (A_3) and then mixed with a BAL L. bulgaricus culture solution (L.) B1) and S. thermopylus (B_2) with a ratio of 4: 1. After being evenly mixed, the mixture is dropped using a 5 mL dish in a 0.2% tween 80 solution in vegetable oil in a 1000 mL beaker glass. Subsequently poured 0.05M CaCl solution of 250 mL rapidly through the edge of the glass wall and left for 30 minutes. The capsule granules will drop and the solution of tween 80, vegetable oil and the remaining CaCl solution will be removed by pouring slowly. The capsule granules are centrifuged at 350x for 15 minutes then poured into a filter cup and washed with distilled water. Probiotic encapsulation preparation with three replications.

Preparation of Cuko Pempek

The preparation of Cuko Pempek according to ID (2012) is crushing the brown sugar, garlic, cayenne, red chili and salt. Using yakult as the source of acid. Chili and salt are blended and mixed with yakult and fermented for one week (7 days). Then the water and sugar are heated until it boil, removed and filtered. Then the fermented chili and yakult are put into a mixture of filtered sugar water, added with fine garlic. The mixture is heated to boil and cooled, then the Cuko Pempek is produced.

Preparation of probiotic encapsulation of Cuko Pempek

500 ml of Cuko Pempek was put into 2000 ml plastic cans as many as three replications. Then the encapsulation of BAL probiotics was put into Cuko Pempek. Some are stored at 12^{o}C. and some at 27^{o}C.

Observation of viability of probiotic encapsulation of Cuko Pempek

Observation of the viability of probiotic encapsulation of Cuko Pempek gradually during storage on the 1st day, 10th day, 20th day, and 30th day. Other parameters; shelf life (self life) and pH based on time.

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III. RESULTS AND DISCUSSION

A. Viability of BAL Cuko Pempek at 12°C Cold Temperature

The results of the analysis of the diversity of probiotic encapsulation of Cuko Pempek at a storage temperature of 12°C that the concentration of alginate, type of LAB, interaction, and combination of treatments did not significantly influence the viability of BAL cells. While the group's influence is very real. This means that alginate concentration does not affect the viability of BAL cells and can be relied upon for probiotic encapsulation of Cuko Pempek (Sheu and Marshall, 1993; Sultana *et al*., 2000; Mokarram et al., 2009; Lotfipour *et al*., 2012; Wikstrom, Wikstrom, 1993; 2013), as well as BAL types (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 109 for L. bulgaricus (B_1) and 108 for S. thermopylus (B_2), then the average number of BAL cells both B_1 and B_2 on the 10th day, 20th day, and the 30th day is consecutively 10^8, 10^7 and 10^7 and 10^6. The average number of BAL cells fulfills the requirements to act as probiotics that require an average number of BAL cells before consumption of 10^7. As, Ishibashi and Shimamur, (1993) stated, called probiotic food, must contain probiotic cells before consumption of ≥ 107 cells per gram or per ml of product. Meanwhile, Lee and Salminen, (1995) require that probiotic beverage products contain cells of ≥ 105 per mL of product. Whereas FAO / WHO, (2002) requires that the number of probiotic cells before consumption is 10^6 – 10^7 CFU/g atau CFU/mL

Storing Cuko Pempek at cold temperatures of 12°C, the viability of BAL decreases linearly in ten days of observation down by one log as in A_1B_1, A_2B_1, and A_2B_2 obtained at 30th day observations, the average number of BAL cells was 106. This was caused by two things, first, because BAL cells are stored in calcium alginate capsules; secondly, BAL cells are better protected from adverse environmental influences such as acidity, the presence of a capsaicin component from chili and an alisin component from garlic. As Sheu and Marshall, (1993) asserted that encapsulated BAL cells have viability for up to 2 weeks and their viability is 40-45% higher than BAL cells that are not encapsulated; Sultana et al., (2000), Encapsulation increases viability for up to 8 weeks. Wikstrom, (2013), that encapsulation provides cell viability for a long time. Furthermore, Gbassi and Vandamme, (2012) there are two reasons for encapsulation, *first*, to guarantee the viability of encapsulated probiotic cells; and *second*, to ensure the release of probiotic cells when consumed and in the digestive tract. On the other hand, Lotfipour *et al*., (2012) explained that the encapsulation of BAL made from alginate provides better viability under acidic conditions. This confirms why the viability ofBAL cells in encapsulation of Cuko Pempek has good viability. The pattern of decreased viability of BAL cells encapsulated with calcium alginate at 12°C cold storage, is presented in Figure 1, that A_3B_1 (3% alginate and *L. bulgaricus*) has the highest viability with an average in the four observation points of sequential order according to $4{,}60\text{x}10^9$; $1{,}09\text{x}10^8$; $4{,}96\text{x}10^6$; dan $2{,}43\text{x}10^7$;while the lowest is in A_1B_2 (1% alginate and S. thermopylus) with an average of four points of$6{,}55\text{x}10^8$; $4{,}07\text{x}10^7$; $4{,}18\text{x}10^7$; dan $7{,}16\text{x}10^7$. The average number of cells meets probiotic requirements before consumption.

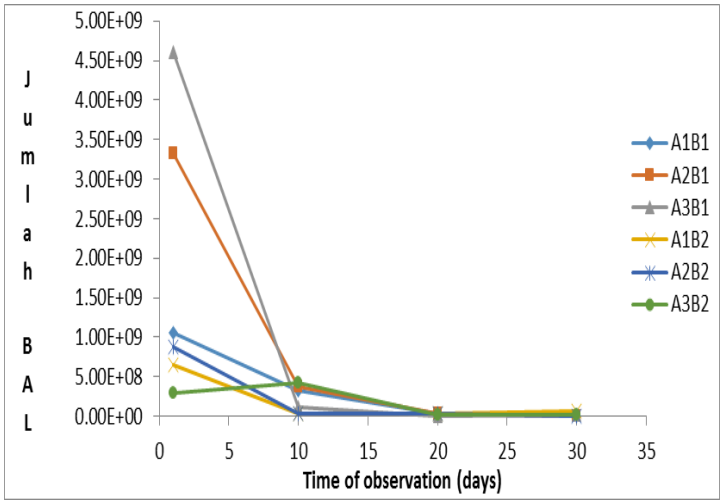

Figure 1. LAB Viability Graph at 12°C Temperature Storage

However, storage at cold temperatures of 12°Csupports the viability of BAL cells. As the

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results of the study of Sheu and Marshall, (1993) that encapsulation of BAL cells stored at cold temperatures has better viability. This pattern of decreased viability is similar to that described by Iyer and Kailasapathy, (2005) that the viability of BAL stored at cold temperatures decreases from $10^8$to 10^7at week 2 and to 10^6 and 10^7 at week 4.

Furthermore, the results of diversity analysis with observations of day 1, day 10, day 20 and day 30, alginate concentration (A) and type of probiotics (B) on the pH of the probiotic encapsulation of Cuko Pempek at a storage temperature of 12°C, that the concentration alginate and its interactions have no significant effect, types of probiotics, combination of treatments and groups have very significant effects. This is due to the existence of probiotic activity over the shelf life which results in changes in pH. *Roberts et al.*, (1994) encapsulation of B. longum BB-79 after 10 days has a pH of 3.9 - 4.2; Iyer and Kailaspathy, (2005) encapsulation of *L. acidophilus* has a pH of 4.6; *L. plantarum* pH 5,6 (Ayama *et al*., 2014).

To see the level of difference of each treatment that has a very significant effect on pH, further tests are carried out, presented in Figure 2, that the pH of probiotic encapsulation of Cuko Pempek is very stable until the 10th day, and relatively stable until the 20th day and partly until the 20th day -30. This shows 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 the activity increased until the 30th day. As stated by Robert *et al*., (1994) that in the encapsulation of BAL probiotics begin to change in pH after the 10th day.

B. Viability of BAL Cuko Pempek at Room Temperature 27°C

The results of the diversity analysis showed that the concentration of alginate, the type of BAL, the combination of treatments and their interactions had no significant effect on the viability of BAL cell of probiotic encapsulation of Cuko Pempek at storage temperatures of 27°C, while the groups had very significant effects. Viability on the first day was 108, then the average number of BAL cells both *L. bulgaricus* (B_1) and *S. thermopylus* (B_2) on the 10th day, 20th day, and 30th day were consecutive 10^7, 10^6 and 10^5. This decrease in BAL cell viability appears to be related to a decrease in pH, because room temperature stimulates microbiological activity which results in a decrease in pH. Noland and Aryana, (2012) observed BAL viability in yogurt, BAL viability decreased if the pH dropped below pH 4.3. However, the average number of BAL cells still meets the requirements as a probiotic that requires a pre-consumption amount of 10^7 (Ishibashi and Shimamur, 1993; Lee and Salminen, 1995; FAO / WHO, 2002). But the number of BAL cells that meet the requirements is only until the 10th day.

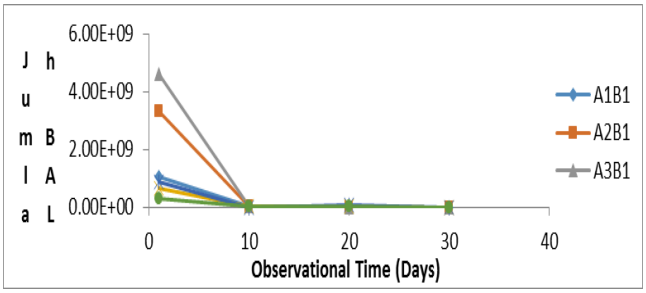

Figure 3. LAB Viability Graph at Storage Temperature of 27°C

Figure 3 shows the decrease pattern of BAL cell viability of probiotic encapsulation of Cuko Pempek at storage temperature of 27°C, that A_3A_1 (3% alginate treatment and L. bulgaricus) has the highest and lowest viability found in A_3A_1 (3% alginate treatment and *S. thermopylus*). The viability of BAL cells at 27°C storage temperature occurs one log lower pattern. It seems that the condition of the room temperature causes the growth rate and probiotic activity to take place.

Furthermore, the results of the analysis of diversity, that the concentration of alginate, the type of LAB, the combination of treatments and their interactions did not significantly affect the pH of the probiotic encapsulation of Cuko pempek at storage temperatures of 27°C. To see the level of difference in the influence of the group followed by further tests shown in Figure 4. The pH pattern of the high

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conditions starting at pH 5 on day 1, then down reaching the lowest point on the 10th day of 3.73 then rising until the day to 20 at 3.87 - 3.9 and rose again until the 30th day at 4. But the increase did not exceed the pH on the first day.

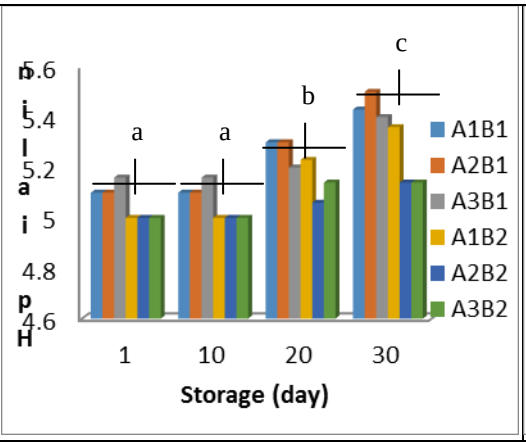

Figure 2. Graphic pattern of pH decreases in the encapsulation of probiotic Cuko pempek at a storage temperature of 12°C

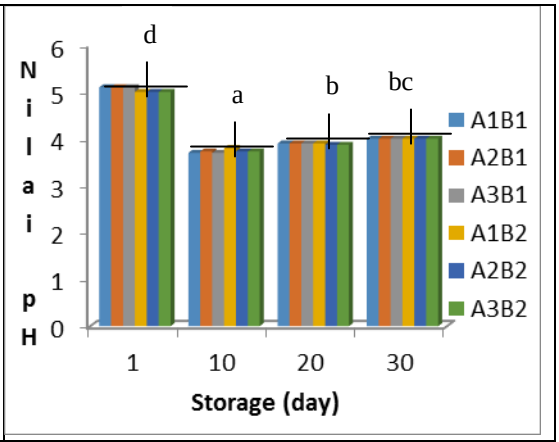

Figure 4. Graphic pattern of pH decreases in the encapsulation of probiotic Cuko pempek at a storage temperature of 27°C

In contrast to storage at cold temperatures of 12°C, storage at 27°C in addition to causing the fermentative rate to produce a decreased pH, also provides a great opportunity for contamination. On the 8th day, A_1B_1 (I), A_1B_2 (II), A_2B_1 ((I), A_2B_1 (III) and A_3B_1 (III) units were contaminated, then on the 10th day the contamination increased in A1B1 (III) units, A_2B_1 (II), A_3B_1 (II), A_3B_1 (III), and A_2B_2 (II), so that on the 10th day all the experimental units using *L. bulgaricus* (B1) were contaminated with *Saccharomyces*.

On the 12th day of the experimental unit using *S. thermopillus* (B_2) which on the 10th day was contaminated by one namely 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 trial units were contaminated with *Saccharomyces.* This phenomenon indicates that Cuko Pempek which is acidic and stored at 27°C prone to contamination and overgrowth of *Sacharomyces*. This confirms the results of the research of Narendranath *et al.*, (2001), that *Saccharomyces* grows in a minimum medium containing acetic acid and lactic acid at 30°C. Furthermore, Thomas *et al.*, (2002) explained that *Saccharomyces* grows on a minimum medium containing lactic acid when the pH is 4.5.

IV. CONCLUSION

1. Probiotic Encapsulation of Cuko Pempek with a storage temperature of 12°C produces viability with an average number of cells reaching 10^9, 10^8, and 10^7 and shelflife until the 20th day and several units until the 30th day, with a relatively constant pH around 5.07 - 5.25.
2. Probiotic Encapsulation of Cuko Pempek with a storage temperature of 27°C produces viability with an average cell count reaching 10^8, 10^7, and 10^6 and shelf life until the 10th day and some units reach the 20th day, with a decrease in pH from the first day, day 10th, 20th day and 30th day pH 5,0 – 5,1; pH 3,70 – 3,80; pH 3,87 – 9,0; dan pH 4.
3. For probiotic encapsulation of Cuko Pempek with a storage temperature of 27°C onthe 8th day contaminants arise in 5 experimental units, on the 10th day 5 units are added and on the 12th day there are three other units and on the

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13th day the contaminants arise in the form of *Sacharomyces* in all trial units.

ACKNOWLEDGMENTS:

This Doctoral Dissertation Research Grant was carried out thanks to the assistance of a research grant fund from the Sim-Litabmas Director General of the Ministry of Education and Culture RI fiscal year 2013-2014.

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