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BIO-ENCAPSULATION OF PROBIOTICS *LACTOBACILLUS ACIDOPHILUS* WITH POLLARD AND WHEAT FLOUR AND ITS VIABILITY DURING YOGHURT FERMENTATION

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Abstract

A research was conducted in order to investigate the viability of bio-encapsulated probiotics *Lactobacillus acidophilus* during yoghurt fermentation. Yoghurt fermentation was prepared on pasteurized 18% of skim milk medium and inoculated with 6% (v/v) of starters consists of *Lactobacillus bulgaricus, Streptococcus thermophillus* and *Lactobacillus acidophilus* with the proportion of 1 : 1 : 1. Fermentation was carried out up to the pH reaching 4.5. Bio-encapsulation was carried out through the formation of calcium-alginate bead gels with the addition of pollard or wheat flour. The result showed that bio-encapsulated probiotics took longer time (11 hours) to get pH 4.5 compared with non-bio-encapsulated one (9 hours). It also showed that bio-encapsulated probiotics resulted in a higher cell viability after fermentation namely at 4.9×10^8 cells / g and at 2.3×10^7 cells / g of probiotics encapsulated with pollard and wheat flour respectively compared with viability of 1.6×10^7 cells/g of non-encapsulated. It is assumed that alginate bio-encapsulation with addition of 2% (w/v) pollard or wheat flour enable to maintain cells viability of probiotics.

Keywords: Bio-encapsulation, probiotics, viability and fermentation

Introduction

Probiotics are defined as viable microorganisms and live-microbial food ingredients that show beneficial effects on human health (Havenaar and Veld, 1992 *cit* Kailasapathy et al., 2000). Lactic Acid Bacteria (LAB) are generally recognized as safe (GRAS) microorganisms which have been applied as probiotics in some dairy products. A species of these bacteria is *Lactobacillus acidophilus*. In the last two decades, *L. acidophilus* has been applied as probiotics in some fermented dairy products (Ouwehand et al., 1999; Fuller, 1989).

Probiotics has gained scientific acknowledgements due to its ability to degrade lactose and reduce lactose intolerance, to degrade protein and provide available amino acids, to inhibit the growth of pathogens, to prevent the growth of cancer and

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tumor, to reduce gastrointestinal diseases, to induce body immunity and to decrease blood cholesterol (Ouwehand et al., 1999, Havenaar and Veld, 1992, Anonim, 1989). However, these roles face hampering factors during fermentation, storage and within the gut system. These factors are highly acidic conditions (pH 1-3), bile acids, anaerobic conditions, gastric juice, and competition with other microflora, antibiotics and toxic metabolites. To overcome these problems, this experiment is designed and aimed at (1) the introduction of prebiotics to support the growth of probiotics and (2) the protection of probiotics by means of bioencapsulation.

A prebiotic is a non-digestible food component, which is selectively fermented by probiotics and resulted in the benefit effects to the hosts by supporting the growth of probiotics (Gibson and Roberfroid, 1995 *cit* Gibson and Fuller, 1998). Bio-encapsulation is a method of forming a protector (capsule) to defend probiotics from the extreme conditions so then enhancing its shelf life and providing a sustained and controlled release (Brazel, 1999). Wheat flour and pollard have a potential capacity as prebiotics. It is because of the presence of potentially prebiotics components such as resistant starch, pentosan (non-starch polysaccharides), oligossacharides and other SDF (soluble dietary fiber) (D'Appolonia, 1973). Meanwhile, the presence of these components has also been known to serve as bioencapulator. This experiment, therefore, would be focussed in studying the role of wheat flour and bran as prebiotics and bioencapsulator.

Materials and Methods

Bacterial strains, growth media and materials used

Bacterial strains used during the experiment are yoghurt starter cultures (*Lactobacillus bulgaricus* FNCC040 and *Streptococcus thermophillus* FNCC041) and probiotics *Lactobacillus acidophilus* D2 were purchased from Food and Nutrition Research Centre UGM. Media used for growing the strains were MRS agar (Oxoid) and MRS broth (Oxoid). Wheat flour and pollard (produced by PT Bogasari Flour Mills) were purchased from the local market. Other materials utilized were Tween 20 solution, skim milk powder, sodium alginate, $CaCl_2.2H_2O$ and other materials used for chemical analysis.

Preliminary evaluation of wheat flour and pollard

The preliminary evaluation of potent probiotics was carried out by analyzing the content of cellulose, fructose, maltose, mannose, starch, dietary fiber and SDF (Soluble Dietary Fibre). Starch analysis was done according to AOAC (1970) as cited Sudarmadji et al. (1984), dietary fiber analysis according to Sudarmadji et al. (1984), Cellulose analysis according to Chesson (1978) as cited Sudarmadji et al. (1984), oligosaccharide analysis was done with High Pressure Liquid Chromatography (HPLC) using Aminex column HPX-87H, 300 mm x 7.8 mm with

mobile phase of H_2SO_4 0.1 N. Soluble Dietary Fiber analysis was performed according to AOAC as cited Furda (1981).

Bio-encapsulation methods

Bio-encapsulation was performed according to Onsoyen (1999) and Sheu and Marshal (1993). It was carried out by means of bead gels formation through the addition of alginate (alginate encapsulation) with pollard or wheat flour. It was done by mixing of overnight-harvested *L. acidophilus* biomass with alginate (1% w/v), 2% of pollard / wheat flour and tween 20 solutions (0.5% v/v). The mixtures were then added into CaCl2.2H2O 5% solution for about 1 minute. The pellets produced were then dried at 45°C for about 4 hours. The proximate content of crude protein, dietary fiber, fat, ash and dry materials of pellets were then analyzed.

Yoghurt fermentation and probiotics viability measurement

Yoghurt fermentation was carried out according to Dave and Shah (1997). Skim milk (18%) was pasteurized at 72°C for about 10 minutes followed by cooling down the temperature up to about 40°C. Yoghurt starters (*L. bulgaricus* and *S. thermophillus*) with the ratio of 1 : 1 was added to the medium correspondingly with the addition of probiotics *L. acidophilus*. The medium was then incubated at 42°C until the pH of 4.5 was reached. The acidification profile was recorded hourly by monitoring the changes of pH. At pH 4.5, fermentation was stopped. The viability of *L. acidophilus* within yoghurt products was counted on Bile-MRS medium by counting total viable cells.

Results and Discussion

Preliminary evaluation

Preliminary evaluation was conducted in order to provide basic information of the potent prebiotics available within pollard and wheat flour. The samples used in the evaluation are pollard, wheat flour *segitiga biru* and *koki*. The results of the analysis can be seen at Table 1.

Table 1 explains that the potent of prebiotics of pollards is much higher compared with that in wheat flour. This is particularly because of the higher content of dietary fiber and SDF. Dietary fiber mainly SDF and cellulose are non digestible but soluble food component which is the main characteristics of prebiotics. Those substances would be only fermented by probiotics so then favoring the optimal growth of probiotics and enhancing its shelf life. Once the growth of probiotics is promoted, it would resulted in short chain fatty acids particularly butyrate which would support the health of human gut systems. The percentage of SDF and cellulose within wheat flour *Segitiga Biru* is higher compared with that in *Koki*. It is,

therefore, the reason to choose *Segitiga Biru* together with pollard to be used for further analysis.

Table 1. Preliminary analysis of pollard, wheat flour *segitiga biru* and *koki*

No	Analysis	Pollard*)	*Segitiga Biru**)	*Koki**)
1.	Starch (%)	42.95	63.52	72.07
2.	Dietary fiber (%)	6.61	0.45	0.65
3.	Cellulose (%)	9.26	2.30	2.05
4.	Fructose (%)	0.66	non detected	non detected
5.	Maltose (%)	0.26	0.69	0.87
6.	Mannose (%)	non detected	non detected	non detected
7.	Soluble Dietary Fiber (% of the total fiber)	3.24	7.62	7.56

*) An average of two repeats

Bio-encapsulation of probiotics

The characteristics of bio-encapsulated cells (pellets) and its viability after the addition of 2% (w/v) of either pollard or wheat flour *Koki* is presented at Table 2. The pellets were taken as samples after being dried at about 45°C for about 4 hours.

Table 2. The characteristics of pellets after the addition of 2% pollard or wheat flour *koki*

Pellet Characteristics	The addition of 2% pollard	The addition of 2% wheat flour koki	Without any filler addition
Appearance	bright brown in colour and small ball in size	bright white in colour and small ball in size	transparent yellow in colour and small ball in size
Weight*)	0.02 g	0.02 g	0.03 g
Solubility at 42°C	Insoluble	Insoluble	Insoluble
iability of probiotics in the pellets*)	2.4 x 10^8 cells / g	3.5 x 10^8 cells / g	3.2 x 10^8 cells / g

*) An average of two repeats

Table 2 reveals that the viability of the probiotics within the pellets is still high, namely at 2.4 x 10^8, 3.5 x 10^8 and 3.2 x 10^8 cells / g samples in the addition of pollard, wheat flour *koki* and without addition of any filler respectively. These results indicate a possible synergistic interaction between alginate and pollard/wheat flour to improve the viability of probiotics. However, pellets produced using this

method showed inability to be soluble at the temperature of fermentation (42°C). This is a problem since the failure to soluble of the pellets in yoghurt would downgrade the physical quality of the products. Therefore, attempts to produce soluble pellets would be of the necessity in other experiments. The pellets produced were then subjected for further analysis of its chemical contents including crude proteins, dry materials, fat, dietary fiber and ash. The results of this analysis are presented at Table 3.

Table 3 explains that the pellets contain a high percentage of crude proteins, that are 43.34% and 28.13% for pellets encapsulated with wheat flour and pollard respectively. It also contains a low level of dietary fiber at 0.92% and 4.76% for pellets encapsulated with wheat flour and pollard respectively. The results also indicate that the content of fat and ash in pellets encapsulated with pollard was higher compared with that with wheat flour. The higher content of dietary fiber in pellets encapsulated with pollard was due to the higher dietary fiber content in pollard compared with that in wheat flour. The pellets produced would be then incorporated into medium for yoghurt fermentation together with yoghurt starter cultures to evaluate the viability of probiotics.

Table 3. Proximate analysis of pellets

Analysis	Pellets encapsulated with wheat flour	Pellets encapsulated with pollard
Crude proteins (%)	16.54	20.19
Dry materials (%)	43.34	28.13
Fat (%)	3.09	4.30
Dietary fiber (%)	0.92	4.76
Ash (%)	12.83	15.78

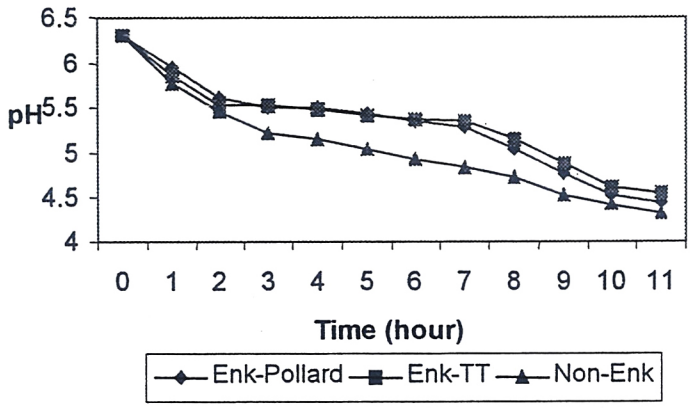
Yoghurt Fermentation and Viability of Probiotics

Yoghurt fermentation was performed at 16% of skim milk medium and harvested after the pH reaching 4.5. The main parameter monitored during fermentation was the time required for reaching pH 4.5 and the viability of probiotics before and after fermentation. The rate of acidification is presented at Figure 1.

Figure 1 describes time taken for the bio-encapsulated cells to get the pH 4.5 is longer than that reached by the free cells. Time which is taken to get pH 4.5 was 10, 11 and 9 hours for cells bio-encapsulated with pollard, wheat flour and non-encapsulated cells respectively. This may due to a slow uptake of nutrients and the slow release of metabolites across the bio-encapsulant alginate shell of the beads. A similar result was reported by Kailasapathy et al. (2000). They found that bio-encapsulated *L. acidophilus* took 20 hours longer than free cells to reach pH 4.5.

Another result was reported by Larisch et al. (1994). They explained that alginate bio-encapsulated cells took 17% longer than free lactococci to reduce the pH of milk to 5.5.

Graphic 1. The rate of acidification during yoghurt fermentation with the addition of Bio-encapsulated (pellets) and non-encapsulated L. *acidophillus*



Yoghurt fermentation was stopped after the pH reaching 4.5. For measuring viability of bio-encapsulated and non-encapsulated probiotics during fermentation, total viable probiotics was counted before and after fermentation. The results can be seen at Table 4.

Table 4. Total viable cells of bio-encapsulated and non-encapsulated probiotics

L. acidophillus	Total viable cells (cells/g) before fermentation	Total viable cells (cells/g) after fermentation
Bio-encapsulated with pollard	1.06×10^8	4.9×10^8
Bio-encapsulated with wheat flour	1.2×10^8	2.3×10^7
Non-encapsulated	2.4×10^8	1.6×10^7

*) An average of two repeats

Table 4 exposes total viable cells of probiotics after fermentation stopped. This result indicate that bio-encapsulation with pollard was able to maintain the highest viability of probiotics during fermentation. The viability of bio-encapsulated with was 4.9×10^8 cells/g compared with that encapsulated with wheat flour at 2.3×10^7 cells/g. Meanwhile, the viability of non-encapsulated probiotics was is the lowest at 1.6×10^7 cells/g. The low viability of unprotected cells is possibly due to the antagonistic effects of bacteriosin-like inhibitory substances (BLIS) produced by yoghurt bacteria during fermentation.

Conclusions

Bio-encapsulation by means of bead gels formation of complex alginate / pollard and alginate / wheat flour has been developed. Pollard and alginate as well as wheat flour and alginate are synergistically interacting during calcium-alginate gel formation and as a result able to provide additional protection to the entrapped bacterial cells.

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