Encapsulation of Yoghurt Starter Using Starch as a Filler Through Emulsification Method

Abubakar1*, S Usmiati1 and M Rahayuningsih2

1Indonesian Center for Agricultural Postharvest Research and Development, Bogor, Indonesia 16114
2Faculty of Agricultural Engineering and Technology, Bogor Agricultural University, Bogor, Indonesia 16002

*Email: abu.028@gmail.com

Abstract. The viability of starter culture (Streptococcus thermophilus and Lactobacillus bulgaricus) in yoghurt declined during storage. Therefore, it needs protection to prevent this condition by encapsulation. The encapsulation has been conducted by the emulsification method. This research aimed to determine the best encapsulant which could protect starter culture and to investigate the characteristic of yoghurt produced from the starter using selected encapsulant. The emulsification method was developed by mixing encapsulant materials (maize resistant starch and maltodextrin) with vegetable oil (canola oil containing 0.5% tween 80%), in a ratio by 1:2:2. Subsequently, 0.1% inoculum (cultivated from initial of log phase) was added. The three composition of encapsulant materials were alginate 2%: maize resistant starch 2%; alginate 3%: maltodextrin 1%; and alginate 4%. The best encapsulant was alginate 3%: maltodextrin 1%. This encapsulation increased the viability of the bacteria by 0.27 log CFU/g (on wet beads) and 5.78 log CFU/g (on dry beads) compared to the free cell. Yoghurt produced using a starter with encapsulant 3% alginate: 1% maltodextrin was more acidic than from the free cells, but it was still lower than commercial starter. Based on hedonic test, yoghurt produced from the best encapsulant was not significantly different from the commercial starter.

1. Introduction

Yoghurt is produced through fermentation of milk with a lactic acid bacteria (BAL) starter consisting of Streptococcus thermophilus (St) and Lactobacillus bulgaricus (Lb). Both types of BAL work synergistically to produce good properties of fermented milk. Generally, yoghurt manufacturers use a liquid starter. However, the viability of bacteria during handling and storage is an issue in the properties of the liquid starter. One attempt to protect the viability of starter bacteria is encapsulation. Common techniques used to encapsulate starter bacteria include extrusion (droplet method) and emulsion (two-phase system). Both methods can increase the viability of bacteria up to 80-95% [1,2,3,4,5,6,7]. In the encapsulation process, the presence of chelating agents such as phosphate, lactate, and citrate must be avoided as they may disturb the stability of beads resulted.

Some attempts to maintain the stability of beads include the application of cryoprotectant compounds (i.e. glycerol), cross-linking with cation polymers (polyethyleneimine, glutaraldehyde), coating with chitosan, blending with starch [8], and coating. Materials that can be used as an encapsulant include fat, wax, glycerol derivatives, sugar, natural and modified starch, dextrin, gum, protein, skim, gelatine, and
cellulose derivatives. Among these materials, starch is quite an abundant encapsulant source, found in tapioca (cassava starch), corn meal (maize starch), and others. According to Asp [9], maize resistant starch is categorized into resistant starch type III (RS III). Both of these starch derivatives are claimed to be suitable as BAL coating agents for their prebiotic properties. Through a biocompatible encapsulation, it was expected that a large number of beads containing \textit{S. thermophilus} and \textit{L. bulgaricus} could be obtained and viability of cells during starter handling and storage before being used for yoghurt production could be maintained. The objectives of this study were to obtain the best encapsulant material and composition that were able to protect and maintain the viability of \textit{Streptococcus thermophilus} and \textit{Lactobacillus bulgaricus} as yoghurt starters, as well as to observe yoghurt characteristics produced from the selected encapsulant material.

2. Materials and methods

2.1. Preparation of bacteria
Preparation of starter bacteria was initiated by growing a culture of pure \textit{S. thermophilus} and \textit{L. bulgaricus} bacteria on deMann Rogosa Sharpe Agar medium. Furthermore, the growing bacteria were propagated into the MRS Broth and used as a stock culture. These bacteria were propagated several times to obtain an adequate population (>10^6 CFU/mL) as the cells would be encapsulated.

2.2. Encapsulation of yoghurt’s starter bacteria

2.2.1. Determination of encapsulation composition.
This stage aimed to obtain the best encapsulation compositions by emulsification method using sodium alginate, maize resistant starch and maltodextrin, and CaCl_2 cross-linking agent. The composition of alginate-maize resistant starch at a ratio of 2%: 2%, experiment was conducted in accordance with Sheu and Marshall, Kailapsapathy, Sultana et al [4,10,11], while the composition of alginate-maltodextrin was examined in six compositions at a total solid of 4% and a ratio of 1: 1. The encapsulation process at this stage applied emulsification method by Sheu and Marshall [4]. Different encapsulation solutions were prepared according to the properties of each material.

The encapsulation of \textit{S. thermophilus} and \textit{L. bulgaricus} bacteria was performed through two mixing methods according to encapsulant compositions, i.e. alginate–maize resistant starch (2%: 2%) and alginates-maltodextrin (six treatments). A 12.5 ml solution of alginate-maize resistant starch was mixed with 0.1% bacteria culture (St; Lb) and 25 mL of canola oil containing 0.5% tween-80. Emulsification process of the three solutions was carried out using a mixer for 20 minutes to form an emulsion. Using a 23g syringe, the emulsion was dripped into 25 ml of 0.1 mL/mole CaCl_2 solution from a distance of 10 cm on 150 rpm magnetic stirrer. The formed beads were stabilized for 12 hours, then were filtered. Beads were observed for parameters of yield, shape, and size. Yield of the beads was calculated by dividing weight of wet beads over total weight of encapsulant solution. Shape of the bead was observed visually and measured using a caliper. The selected encapsulant composition was used as a coating of yoghurt’s BAL. Alginate at a concentration of 4% was used as a control.

2.2.2. Preparation of dried starter;
At this stage, bacterial culture-containing wet beads from the best encapsulant composition were dried in an oven at 40°C for 2 hours. A temperature of 40°C was set in accordance with the optimum temperature range of \textit{S. thermophilus} and \textit{L. bulgaricus}. The population of bacteria was calculated at the beginning of log phase (before encapsulation), in wet beads, in the dried starter, and after a liquid starter was formed. One gram of wet beads (0.1 gram dried starters) was introduced into 9.9 mL buffer 0.1 M at pH 7, and homogenized for 30 minutes. Suspension was then poured upper MRSA, and incubated anaerobically at 37°C for 2 days, and counted.
2.3. Preparation of liquid starter and yoghurt

Before the dried beads were used to prepare yoghurt, a liquid starter was initially made. First, homogenization of bovine milk that has been added with 2% skim milk, followed by heating at 90°C for 20 minutes. After reach to 40-43°C, 0.5% b/v starter was added (each with St-Lb free cell, dried starter, yoghurtmert), and milk was incubated at 40-43°C for 24 hours, allowing production of a liquid starter. The next step, each liquid starter was used to make yoghurt following the same procedure as preparation of a liquid starter, except for the number of starters used was 3% w/v [12]. Yoghurt produced was then tested on TPC, pH, TAT, and viscosity. Organoleptic test of yoghurt was performed to observe consumers’ preference for attributes of colour, aroma, taste, and texture.

2.4. Parameter measurement procedure

Measurement of pH and total acid number / TAN [13], Total Plate Count [14], Viscosity measurement, and Organoleptic Test. Organoleptic test constitutes a hedonic test of attributes of colour, aroma, taste and texture. A total of 20 semi-trained panellists were asked to express their preference level for yoghurt products. The preference level was expressed on a hedonic scale of 1-7: like extremely (7), like moderately (6), like slightly (5), neither like nor dislike (4), dislike slightly (3), dislike moderately (2), dislike extremely (1).

3. Results and discussion

3.1. Modification of encapsulation method using emulsification technique

According to [15], there are two types of the emulsion, namely oil-in-water and water-in-oil emulsion. This research was employed water-in-oil emulsification, e.g. alginate solution (liquid phase) dispersed into canola oil phase. Emulsions were observed under a microscope (1000x magnification) (Figure 1).

![Figure 1](image1.jpg)

**Figure 1.** (a) Stirring emulsion stability 20 minutes; (b) Emulsion stability after dropping into CaCl₂; (c) Emulsion stability after 30 minutes of gelification (Description: Green color was oil and brown color was alginate)

Figure 1 shows that emulsion of alginate and oil (containing 0.5% Tween-80) formed relatively homogenous, small, round particles close each to other. However, after 30 minutes gelation, the particles started to separate and more compact. This might be due to the cross-linking process to form beads. The emulsification technique referred to a study conducted by Sheu and Heymann [5] with a slight modification, including: (i) emulsion was dripped into CaCl₂ solution from 10 cm distance using 23g syringe; and (ii) 24 hour gelation me on a magnetic stirrer to produce more compact and stable beads and to facilitate oil separation so that beads were able to be collected. The ingredient composition consisted of canola oil, alginate solution, CaCl₂ solution, NaCl 0.8% at a ratio of 2:1:2:2. During observation of gelification for 30 minutes (Fig. 2), beads were at 1 mm diameter and fragile, while gelification for 24 hours resulted in the separation of solution into three parts (oil, CaCl₂ solution, beads). Beads appeared to be relatively compact as a result cross-linking of Ca²⁺ ions with two carboxylic groups of alginate. Dripping of sodium alginate into CaCl₂ solution yielded hydrophobic cross-linked lumps [16]. Thus, cross-links between calcium ions and alginites were stronger with the increase gelification time.
3.2. Encapsulation of yoghurt's bacteria starter

3.2.1. Determination of encapsulant composition

The composition of alginate and maize resistant starch in the encapsulant referred by Sheu and Heymann, Kailaspathy, Sultana et al [5,10,11], at 2%:2% ratio. The increment of maize resistant starch composition by more than 2% in yoghurt during storage for eight weeks tend to reduce the yield of beads and increase the viability of encapsulated probiotic as compared to non-encapsulated bacteria [5]. This is possible that during storing product the starter culture metabolizing starch for its growth. Based on the results, an average of beads yield was 21.31% (from 12.31 g sample). The wet beads were round flat shaped with 0.83 mm diameter. Size of beads produced by emulsification method ranged 25 µm-2 mm [17]. The size of wet beads in this research was smaller than the previous study conducted by [11] at the same composition and kind of encapsulant. This may be caused by a number of factors including the difference of distance of drops wise the alginate solution into cross linking agent (calcium solution) in beads making. Beside that there were differences in material specification.

Determination of encapsulant composition using alginate-maltodextrin was performed in six classes. Measurement of parameters in the treatment of alginate-maltodextrin was carried out quantitatively (yield, size of beads) and qualitatively (shape of beads). Results of qualitative observation are presented in Table 1; while, the quantitative measurements are depicted in Fig. 4.

| The composition of alginate: maltodextrin (%:%) | Shape of beads |
|---------------------------------------------|---------------|
| 1 : 3                                        | Globular      |
| 1.33 : 2.67                                 | Convex        |
| 2 : 2                                        | Convex        |
| 2.67 : 1.33                                 | Convex        |
| 3 : 1                                        | Convex        |
| 4 : 0                                        | Convex        |

Table 1. Qualitative measurement of alginate-maltodextrin composition treatment

Figure 2. Yield and beads size at various treatments of alginate and maltodextrin composition.

Remarks: (a) alginate 1%; maltodextrin 3%, (b) alginate 1.33%: maltodextrin 2.67%, (c) alginate 2%; maltodextrin 2%, (d) alginate 2.67%: maltodextrin 1.33%, (e) laginat 3%; maltodextrin 1%, and (f) alginate 4%

Table 1 shows that the shape of beads began to change from globular to convex (the beads shape are not round) when alginate compositions were more than 2% in the alginate-maltodextrin encapsulant. The solution from low maltodextrin and high alginate compositions (to reach 4% TS) showed weak binds between carboxylic groups and Ca$^{2+}$ ions due to the presence of oil in an emulsion system. Based on statistical analysis, treatments did not affect the yield and size of beads. However, there seemed to be important responses of yield that correlated positively with bead size. Generally, bead size was bigger with the increase concentration of alginate. According to Mandal and Singh [18], alginate concentration correlates positively with bead size and cells viability. Overall, bead size produced with emulsification
method conducted by Krasaekoopt and Deeth [17] ranged 25 µm - 2 mm. According to size and shape of beads, the best treatment of maltodextrin incorporated as a filler was the composition of 3% alginate: 1% maltodextrin.

3.2.2. Preparation of dried starter

The amount of encapsulated cultures were 9.85x10⁸ CFU/ml *S. thermophilus* and 1.71x10⁸ CFU/ml *L. bulgaricus* populations. The encapsulated cultures were combined at a ratio of 1:1, and were harvested in the first exponential phase. During this phase, bacteria replicate at a low rate as they have just adapted [19]. In addition, bacteria were expected to ferment milk when released from the beads optimally. Bacteria grow logarithmically when experiencing a lag phase. The population of combined culture of *S. thermophilus* and *L. bulgaricus* after incubation at 37°C for two hours was 4.25x10⁸ CFU/ml, and this culture was ready to be encapsulated. The amount of inoculum mixed into the alginate solution was 0.1% [11]. Encapsulation of a combined culture (*S. thermophilus* and *L. bulgaricus*) was carried out to the selected encapsulants, i.e. 2% alginate: 2% maize resistant starch; 3% alginate: 1% maltodextrin; and 4% alginate. In this steps, the 24 hour gelification was conditioned to be at 10°C (a beaker covered by ice gels) so that both bacteria relatively delayed multiply in the wet beads. The maize resistant starch can be served as a probiotic [20], similar to maltodextrin [21]. According to Rahman et al [22], *S. thermophilus* and *L. bulgaricus* do not grow at 10°C.

Table 2. Bacterial populations in wet beads and dry beads

| Type                        | Bacterial population (log CFU / g) |
|-----------------------------|-----------------------------------|
|                            | Wet beads                         | Dry beads                         |
| Alginate 2%: resistant starch 2% | 6.07 ± 0.32                       | 5.07 ± 0.32                       |
| Alginate 3%: maltodextrin 1% | 5.90 ± 0.22                       | 5.78 ± 0.22                       |
| Alginate 4%                 | 6.08 ± 0.74                       | 5.00 ± 0.74                       |

Table 2 shows that the treatment did not affect the number of bacteria in wet and dry beads. This suggested that the encapsulant worked optimally to maximize the viability of bacteria. The amount of inoculum incorporated in the alginate solution was 5.63 log CFU/ml, giving a slight increase by 0.5 log CFU/ml. Sultana et al [11] reported that the use of 2% alginate: 2% maize resistant starch to encapsulate probiotic (*Lactobacillus acidophilus* and *Bifidobacterium* spp) was able to improve the viability of the bacteria by 0.5 log in yoghurt as compared to free cells during eight weeks of storage.

Dry beads showed a reduction of the number of bacteria by 1 log CFU/ml in the encapsulant prepared from 2% alginate: 2% maize resistant starch and 4% alginate and by 0.12 log CFU/ml in the encapsulant prepared from 3% alginate: 1% maltodextrin. This occurred due to heating at 40°C for two hours which might cause exposure of bacteria to oxygen allowing production of bactericidal hydrogen peroxide. Accordingly, BAL encapsulation using emulsification method provided better protection as compared to encapsulation using spray drying. Homayouni et al [23] reported that the spray drying of *S. thermophilus* and *L. bulgaricus* dropped the number of cells by 1.08 log CFU/ml and 7.17 log CFU/ml, respectively.

3.2.3. Preparation of liquid starter and yoghurt

3.2.3.1. Preparation of liquid starter

Selected dried beads were tested to be applied to yoghurt production by first being prepared into a liquid starter. This preparation aimed to enable the encapsulated bacteria to adapt with pasteurized bovine milk containing 2% skim milk. To prepare a liquid starter, there were three dried starters (i.e 2% alginate: 2% maize resistant starch, 3% alginate: 1% maltodextrin, and 4% alginate). The amount of inoculum used was 0.5% in accordance with application of commercial starters. Parameters studied included formation time, pH, TAT, and bacteria population (Table 3).
Table 3. Result of measurement of culture parameter

| Type                     | Characteristics of work culture |
|--------------------------|---------------------------------|
|                          | Coagulation duration (hr) | pH       | Total acid (%) | Total bacteria (log CFU/mL) |
| Alginate 2% : resistant starch 2% | 13.00 ± 0.71(b) | 5.86 ± 0.02 | 0.33 ± 0.05 | 8.55 ± 0.04 |
| Alginate 3%: maltodextrin 1% | 9.74 ± 0.37(a) | 5.71 ± 0.07 | 0.38 ± 0.04 | 8.04 ± 0.14 |
| Alginate 4%              | 10.75 ± 0.35(a) | 5.95 ± 0.01 | 0.33 ± 0.01 | 8.48 ± 0.30 |

* Different superscript = significant different (P ≤0.05); * same superscript = not significant different (P> 0.05)

Based on statistical analysis, there is no significant difference in all parameters, except for coagulation duration (P<0.05) (Table 3). Coagulation duration was affected by the number of bacteria population in beads, in which the formation was faster with an increasing number of bacteria. Different results were noticed in 2% alginate: 2% maize resistant starch and 4% alginate encapsulants. Dried starter with 4% alginate only had alginate shell and most likely the links were cleaved at acidic pH. The link compounds are the glycosidic chains of alginate. The starter with 2% alginate: 2% maize resistant starch encapsulant had alginate and maize resistant starch layer with most likely tight bonds, thus requiring longer releasing time. A dried starter reached the fastest duration of coagulation with a maltodextrin-containing encapsulant. Blancard and Katz [24] argued that maltodextrin is fast dispersed and has a high solubility in water. Therefore, maltodextrin-encapsulated bacteria released faster into milk and fermented the milk in which allowing fast coagulation to produce and working culture.

Yoghurt flavour and quality are associated with the fermentation process. Streptococcus thermophilus started to ferment lactose into lactic acid, lowering redox potentials by the elimination of oxygen, leading to the disintegration of caseins by proteolytic enzymes. This created an appropriate condition for the growth of L. bulgaricus which started at pH 4.5. Lactic acid bacteria degrades caseins into amino acids through a complex, proteolytic system which involves proteinase, peptidase, amino acid-carriers and peptide-carriers [25]. Enzymes work optimally to break down protein into peptides at pH 5.2-5.8 [22]. Table 3 shows that the starter pH ranging from 5.7 - 5.9, which suggested that the liquid starter was still at protein breakdown stage. Meanwhile, at a pH range of 5.7-5.9, L. bulgaricus did not work optimum as they start to be active when the pH reaches 4.5.

Physically, the beads and liquid culture from 4% alginate in dried starter were not completely soluble. This was because alginate was insoluble in water in which produce insoluble portion floating on the surface of the working culture, while beads in two other cultures were no longer visible as their compositions were made up of water-soluble materials (maize starch, maltodextrin). It is confirmed that the treatment of 3% alginate: 1% maltodextrin was the best in terms of the measurement of parameters. In this treatment, the formation of a working culture occurred the fastest while the treatment also reached the lowest pH and highest TAT. Therefore, a working culture from the dried starter of 3% alginate: 1% maltodextrin encapsulant was used to prepare yoghurt liquid starter.

3.2.3.2. Yoghurt preparation
The Qualitative test of yoghurt prepared with a starter from selected encapsulant material was carried out on coagulation duration, pH, TAT, viscosity and bacterial counts. Testing was performed by comparing yoghurt from three starters (free cell, a starter from selected encapsulant material, and commercial starter). In the quantitative test, organoleptic evaluation of consumer's preference was carried out on yoghurt made from the selected working culture with encapsulation. Results of quantitative test are presented in Table 4.
Table 4. Results of quantitative test of yoghurt

| Treatment | Coagulation time (hr) | pH | Total acid(%) | Viscosity (cp) | Total bacteria (log cfu / ml) |
|-----------|-----------------------|----|---------------|----------------|-----------------------------|
| WCE       | 4.12 ± 0.231(ab)      | 5.73 ± 0.35(ab) | 0.29 ± 0.017(a) | 529 ± 63.789 | 9.14 ± 0.188(a) |
| FC        | 3.41 ± 0.202(b)       | 5.90 ± 0.006(b) | 0.33 ± 0.025(b) | 665 ± 20.429(a) | 8.86 ± 0.191(a) |
| CS        | 2.65 ± 0.173(c)       | 5.36 ± 0.005(a) | 0.56 ± 0.002(a) | 638 ± 8.622(a) | 4.86 ± 0.175(b) |

* Different superscript = significant different (P <0.05); * Same superscript = not significant different (P> 0.05)

Remarks: WCE = Working Culture of Encapsulation (alginate 3%: maltodextrin 1%); FC = Free Cell (not encapsulated, CS = Commercial Starter (yoghurtmert))

Results of the statistical analysis showed that coagulation time (P <0.05) was significantly affected by the treatment. The slowest coagulation duration was shown in yoghurt from 3% alginate: 1% maltodextrin WCE. The slow coagulation duration may be because some bacteria were still trapped in the matrix, and therefore they took a longer time to be released to the substrate. In addition, bacteria were likely still in adaptation phase (pH of 5.71) in which during their activity to break down proteins by their enzyme. The optimum pH for the enzyme to break protein into peptides is 5.2 to 5.8 [21].

The free cell bacteria’s yoghurt has been revealed to adapt with bovine milk. It is therefore the milk coagulation became faster. Similarly, in the freeze-dried commercial starter with a water-soluble encapsulant, bacteria were more readily released from the matrix and immediately exhibited active metabolism. Considering parameters of pH, total acid, and viscosity of yoghurt, the treatments were significantly (P <0.05) affected pH value except for 3% alginate: 1% maltodextrin WCE treatment. A confirmation study conducted by Zain’s [27] the pH of yoghurt prepared from starter through extrusion method ranged from 4.84 to 4.93 with a total acid of 1.20-1.25%. This difference occurred since the study used incubation temperature of 37°C for 10 hours, while incubation performed in the present study was at 43-45°C for 2.65-4.12 hours. Thus, a huge amount of lactose had relatively not been converted to lactic acid, allowing the pH to remain 5.36-5.90 and the total acid of 0.29-0.56%. The acidity and pH value correlate to viscosity. In CS-treated yoghurt, the sample with the lowest pH (5.36) and the highest acidity (0.56%) showed a high viscosity (638 cP), similar to yoghurt from FC treatment (665 cP). The texture was between thick and semi-solid as coagulated milk protein formed a gel structure which was recognized by the formation of pudding-like consistency [26]. Coagulation of milk protein was a response to acidity generated from the activity of starter. Thus, the amount of protein coagulate, and the viscosity was higher as the huge number of bacteria. Overall, yoghurt viscosity from the treatments was lower than that reported by [27], e.g. 1800 cP for yoghurt prepared with FC starter and 1500-1700 cP for yoghurt prepared with WCE.

Statistically, bacterial count in yoghurt was significantly (p <0.05) affected by the starter types (Table 4). The highest number of bacteria was shown by yoghurt with WCE starter (3%alginate:1% maltodextrin). The number of bacteria increased by only 1 log, whereas yoghurt made from other working cultures showed increase numbers of bacteria by up to 3 log. According to [28, 29], yoghurt normally contains 10^7 cells/ml of bacteria. Based on the result, it was known that yoghurt prepared from 3%alginate: 1% maltodextrin WCE contained 9.14 log CFU/ml bacteria and FC yoghurt contained almost equal to 8.86 log CFU/ml. Yoghurt CS contained 4.86 log of CFU/mL bacteria, less than results reported by [13] who evaluated the viability of LAB in the various commercial starter ranged (5.3-23) x10^8 of S. thermophilus and (2.87-3.9) x10^8 CFU/g of L. bulgaricus.

In addition, the organoleptic test was carried out on yoghurt produced from 3% alginate: 1% maltodextrin WCE starter (selected encapsulant). Visually, the average value of preference for the colour attribute was 3.88. Hedonic evaluation of taste and texture showed the value of 3.16 and 3.40, respectively. Preference value of the taste was related to high pH (5.73), which was less acidic. The general acceptance of panellists to the treated yoghurt showed that the product was preferred at hedonic value of 3.08 while commercial yogurt was generally preferred to hedonic value of 3.44 (preferably at medium level).
4. Conclusions
The present study was successful in producing the best encapsulant prepared from 3% alginate:1% maltodextrin. The encapsulation process was able to enhance the viability of yoghurt starter by 0.27 log CFU/g (wet basis) and 5.78 CFU/g (dry basis) as compared to the free starter cells. Yoghurt produced using a starter with encapsulant 3% alginate: 1% maltodextrin was more acidic than from the free cells, but it was still lower than commercial starter. Based on hedonic test, yoghurt produced from the best encapsulant was not significantly different from the commercial starter.

5. References
[1] Audet P, Paquin C and Lacroix C 1988 Applied Microbiology and Biotechnology 29(1) pp 11–18
[2] Rao AV Shiwnarain N and Maharaj I 1989 Canadian Institute of Food Science and Technology Journal 22(4) pp 345–349
[3] Sheu TY and Marshall R T 1991 Journal of Dairy Science. 74 (supplement 1) p 107
[4] Sheu TY and Marshall R T 1993 Journal of Food Science. 54(3) pp 557–561
[5] Sheu TY and Heymann H 1993 Journal of Dairy Science. 76(7) pp 1902–1907
[6] Jankowski T, Zielinska M, and Wysakowska A 1997 Biotechnology Techniques 11(1) pp 31–34
[7] Kebary KMK Hussein S A, and Badawi RM 1998 Egyptian Journal of Dairy Science. 26(2) pp 319–337.
[8] Krasakeoopt W, Bhandari B and Deeth H 2003 Review International Dairy Journal 13 pp 3–13
[9] Asp, NG 1992 Resistant Starch European Journal of Clinical Nutrition 46 (Suppl 2) S1
[10] Kailasapathy K 2000 Food Australia 48 pp 458–461
[11] Sultana K, Godward G, Reynolds, Arumugaswamy R, Peiris P and Kailasapathy K 2000 International Journal of Food Microbiology. 62 pp 47–55
[12] Abubakar and E Purwanti 2008 Mutu Yoghurt Susu Sapi pada Berbagai Presentase Penambahan Starter. Proc.Sem.Nas.Pengembangan Agroindustri Usaha Persusuan Nasional Untuk Perbaikan Gizi Masyarakat dan Kesejahteraan Peternak (Yogyakarta: Fapet UGM)
[13] Dave R I and Shah N P 1997 Int. Dairy Journal 7(1) pp 31–41
[14] Badan Standarisasi Nasional SNI 01-2891-1992 Cara Uji Makanan dan Minuman (Jakarta)
[15] Tamine AY and Robinson 1999. Yoghurt Science and Technology. 2 Ed. (England:Wood Publishing Ltd and CRC Press Ltd)
[16] Winarno FG 1996 Kimia pangan dan gizi (Jakarta: PT Gramedia Pustaka Umum)
[17] Krasaeoak W, Bhandari B and Deeth H 2002 Int. Dairy Journal 13 pp 3–13
[18] Mandal S, Puniya AK and Sigh K 2005 Int. Dairy Journal 16 pp1190-1195
[19] Fardiaz S 1988 Fisiologi Fermentasi (Pusat Antar Universitas Institut Pertanian Bogor: Bogor)
[20] Topping DL, Gooden JM, Brown I L, Biebrick DA, McGrath L, Trimble RP, Choct M and Illman R J 1997 J. Nutr 127 pp 615-622
[21] Gibson GR and Roberfroid MB 2008 Handbook of Prebiotics ( London: CRC Press)
[22] Rahman A, Fardiaz S, Rahaju WP and Nurwitri CC 1991 Teknologi Fermentasi Susu (Bogor: Pusat Antar Universitas IPB)
[23] Homayouni AA, Azizi MR, Elsani MS, Yarman SH and Razavi 2008 Food Chemistry 111 pp 50-55
[24] Blanchard PH and F R Katz 1995 Starch Hydrolysis in Food Polysaccharides and Their Application (New York: Marcell Dekker Inc)
[25] Widodo S and Wahyuni E 2003 J.Tek. Industri Pangan XIV (2) pp 98 – 105
[26] Rukmana HR 2001 Yoghurt dan Karamel Susu (Jogjakarta: Kanisius)
[27] Zain WNH 2010 Karakteristik Mikrobiologis Granul Kultur Starter dengan Sinbiotik Terenkapulasli untuk Menghasilkan Yoghurt dan Dadih Sinbiotik. Tesis. Sekolah Pascasarjana (Bogor: Fakultas Peternakan IPB)
[28] Buckle KA, Edwards RA, Fleet GH and M Wotton 1987 Ilmu Pangan (Jakarta: UI Press)
[29] Garbut, J 1997 Essential of Food Microbiology (London: Arnold)

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