

BAB IV BAHAN DAN METODE PENELITIAN

4.1. Bahan

Bahan-bahan yang digunakan dalam penelitian ini adalah susu UHT *Full Cream Ultra Milk* Ultra Jaya, kultur bakteri *Lactobacillus acidophilus* FNCC 0051, akuades, larutan NaCl merk Riedel-de Haen 31434 0,85%, larutan Na-sitrat 0,1 M teknis, Na Alginat murni merk Zigma A2033-100G, larutan CaCl₂ 1% teknis, air pepton 0,1% (*Peptone from meat* merk “MERCK 1.07224.1000”), alkohol 96%, larutan *Crystal Violet* modifikasi Hucker, larutan iodin, larutan alkohol aseton, larutan Safranin Gram Stain, minyak immerse, kertas lensa, sumbat kapas, aluminium foil, kertas coklat dan korek api.

Media yang digunakan untuk analisa mikrobiologi adalah deMan, Rogosa, Sharpe bouillon (yang selanjutnya disebut MRS) Broth (Pronadisa Cat. 1215.00), Bacto Agar (MERCK 214010), dan MRS Agar (Pronadisa Cat. 1043.00). Komposisi dan cara pembuatan media dan larutan yang digunakan dalam penelitian ini dapat dilihat pada Lampiran 1.

4.2. Alat

Alat-alat yang digunakan dalam penelitian ini adalah erlenmeyer, beker glass, tabung reaksi, rak tabung reaksi, cawan petri, pipet ukur, kawat ose, batang pengaduk, sendok porselen, sendok plastik, thermometer 0-100°C, bunsen, kaki tiga, kassa asbes, penangas air, sumbat kapas, spiritus, *syringe* “Termuno”, spuit injeksi “Terumo Needle” *single use* (1,20x38mm), cup plastik PP Lionstar kapasitas 145 mL, plastik PP Lionstar kapasitas 100 mL, cup plastik PP Lionstar kapasitas 45 mL, enkast,

timbangan digital “Mettler Toledo GB 1302”, vortex “ThermolyneSybron Type 37.600 mixer”, inkubator “WTB Binder” dan “Memmert”, autoklaf “All American Model No.25X”, oven “WTB Binder”, *laminar flow* “Telstar AV-100”, lemari es “Rotary Compressor Mitsubishi”, Mikroskop “Nikon”, Mikrometer “Link’s Brand”, *Texture Profile Analyzer* “Stable Micro Systems Texturometer model TA-XT2i”. Spesifikasi cup dan cara sterilisasi cup dapat dilihat pada Lampiran 2.

4.3. Waktu dan Tempat Penelitian

4.3.1. Waktu Penelitian

Penelitian pendahuluan dilaksanakan pada bulan Juni 2013 sampai dengan bulan Oktober 2013. Penelitian utama dilaksanakan pada bulan November 2013 sampai dengan bulan Desember 2013.

4.3.2. Tempat Penelitian

Penelitian akan dilakukan di Laboratorium Mikrobiologi Industri Pangan, Laboratorium Kimia, Laboratorium Teknologi Pengolahan Pangan, Laboratorium Analisa Pangan, Laboratorium Biokimia Pangan dan Gizi Pangan, dan Laboratorium Penelitian Program Studi Teknologi Pangan, Fakultas Teknologi Pertanian, Universitas Katolik Widya Mandala Surabaya.

4.4. Rancangan Penelitian

Rancangan penelitian yang digunakan adalah Rancangan Acak Kelompok (RAK) faktorial yang terdiri dari dua faktor yaitu konsentrasi Na alginat yang terdiri dari 3 (tiga) level dan lama penyimpanan yang terdiri dari 3 (tiga level), sehingga diperoleh 9 kombinasi perlakuan. Masing-masing kombinasi perlakuan akan dilakukan pengulangan sebanyak tiga

kali sehingga akan diperoleh total 27 unit eksperimen. Rancangan penelitian yang dilakukan dapat dilihat pada Tabel 4.1.

Parameter yang akan diuji adalah viabilitas sel imobil, diameter, dan tekstur *beads*. Data yang diperoleh dari masing-masing pengujian akan dianalisa secara statistik menggunakan uji ANOVA (*Analysis of Varians*) pada $\alpha=5\%$, untuk mengetahui apakah perlakuan memberikan pengaruh nyata pada setiap parameter pengujian. Apabila hasil uji ANOVA menunjukkan perbedaan nyata, maka dilanjutkan dengan uji beda jarak nyata Duncan (*Duncan's Multiple Range Test/ DMRT*) pada $\alpha = 5\%$ untuk menentukan taraf perlakuan mana yang memberikan perbedaan nyata.

Tabel 4.1. Rancangan Penelitian Pembuatan Sel Imobil

Perlakuan		Konsentrasi Na alginat (A)		
		A ₁	A ₂	A ₃
Lama penyimpanan (L)	L ₁	L ₁ A ₁ (1)	L ₁ A ₂ (1)	L ₁ A ₃ (1)
		L ₁ A ₁ (2)	L ₁ A ₂ (2)	L ₁ A ₃ (2)
		L ₁ A ₁ (3)	L ₁ A ₂ (3)	L ₁ A ₃ (3)
	L ₂	L ₂ A ₁ (1)	L ₂ A ₂ (1)	L ₂ A ₃ (1)
		L ₂ A ₁ (2)	L ₂ A ₂ (2)	L ₂ A ₃ (2)
		L ₂ A ₁ (3)	L ₂ A ₂ (3)	L ₂ A ₃ (3)
	L ₃	L ₃ A ₁ (1)	L ₃ A ₂ (1)	L ₃ A ₃ (1)
		L ₃ A ₁ (2)	L ₃ A ₂ (2)	L ₃ A ₃ (2)
		L ₃ A ₁ (3)	L ₃ A ₂ (3)	L ₃ A ₃ (3)

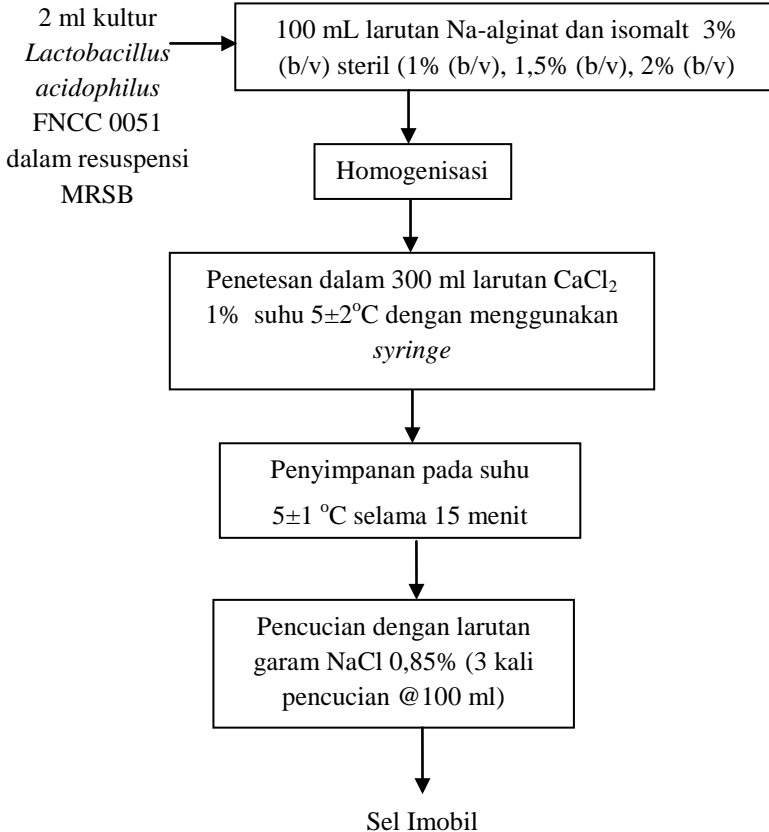
Keterangan:

- A₁ : Konsentrasi Na alginat 1%
- A₂ : Konsentrasi Na alginat 1,5%
- A₃ : Konsentrasi Na alginat 2%
- L₁ : Lama penyimpanan 0 hari
- L₂ : Lama penyimpanan 10 hari
- L₃ : Lama penyimpanan 20 hari
- (1) : Ulangan 1
- (2) : Ulangan 2
- (3) : Ulangan 3

4.5. Pelaksanaan Penelitian

4.5.1. Pembuatan Sel Imobil

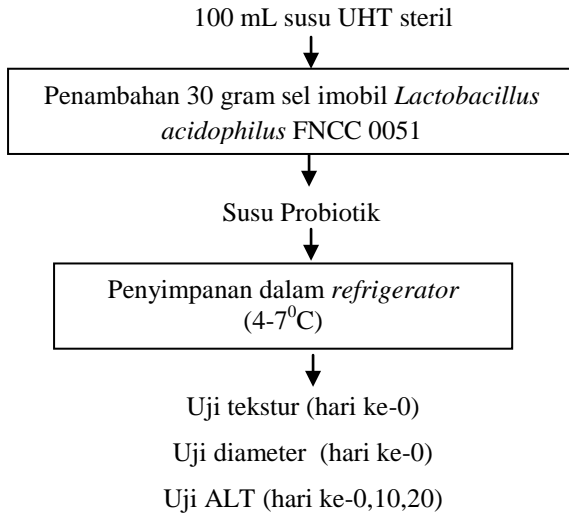
Tahapan pembuatan sel imobil dapat dilihat pada Gambar 4.3.



Keterangan : jumlah *beads* dihasilkan dalam satu perlakuan ± 200 *beads*

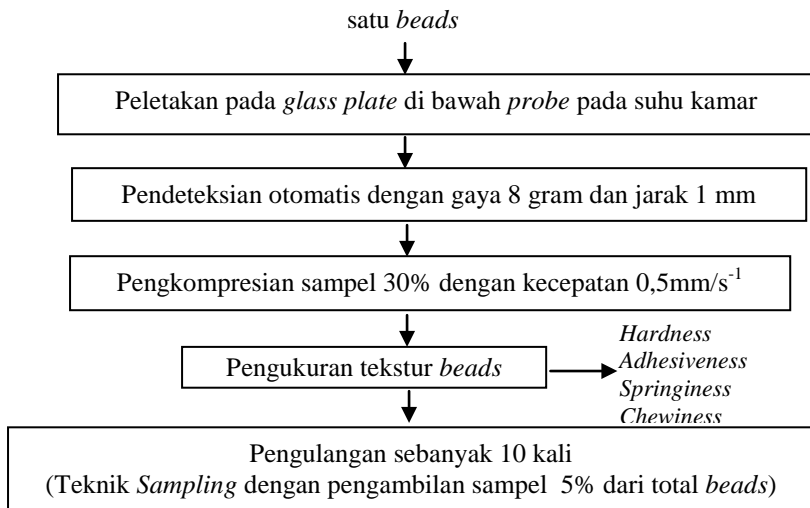
Gambar 4.1. Skema Pembuatan Sel Imobil dalam Ca-alginat
 Sumber: Sheu and Marshall (1993); Leo and Heo (2000); Klinkenberg, *et al.* (2001)

4.5.2. Pembuatan Minuman Susu Sinbiotik



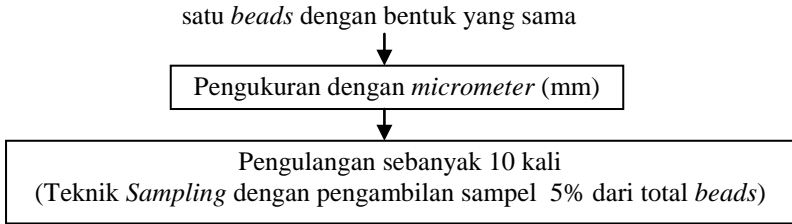
Gambar 4.2. Skema Pembuatan Minuman Susu Sinbiotik
Sumber : Hartati, dkk. (2001) dengan modifikasi

4.5.3. Pengujian Tekstur



Gambar 4.3. Diagram Alir Pengujian Tekstur Sel Imobil
Sumber : Rodriguez-Huezo *et al.*(2011) dengan modifikasi

4.5.3. Pengujian Diameter *Beads*



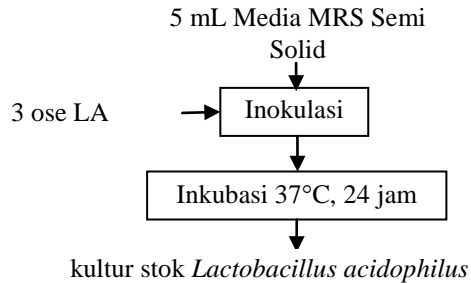
Gambar 4.4. Diagram Alir Pengujian Diameter *Beads*
 Sumber : Huevo *et al.* (2011)

4.5.4. Pengamatan dan Pengujian

1. Uji Angka Lempeng Total (ALT) Sel Imobil (Lampiran 3)
 - a. Penimbangan sebanyak 3 gram sel imobil dan dilarutkan dalam 27 ml larutan Na-sitrat 0,1 M sambil dikocok hingga sel imobil terlarut semua (\pm 10 menit). Setelah sel imobil terlarut semua, dilakukan uji ALT.
 - b. ALT sel imobil menyatakan jumlah sel terimobil yang mampu bertahan pada produk minuman probiotik selama penyimpanan. Data ini menunjukkan viabilitas sel *Lactobacillus acidophilus* yang terimobil selama penyimpanan. Sel yang bertahan dihitung dari jumlah koloni BAL yang tumbuh pada media MRS Agar dan dinyatakan sebagai log cfu/gram.

4.5.5. Peremajaan Kultur *Lactobacillus acidophilus*

Kultur yang digunakan dalam enkapsulasi sel imobil adalah kultur stok *Lactobacillus acidophilus*. Tahapan peremajaan kultur stok *Lactobacillus acidophilus* adalah sebagai berikut.



Gambar 4.5. Diagram Alir Peremajaan Kultur Stok *Lactobacillus acidophilus*
 Sumber: Fardiaz (1989)

Penjelasan proses:

1. Inokulasi

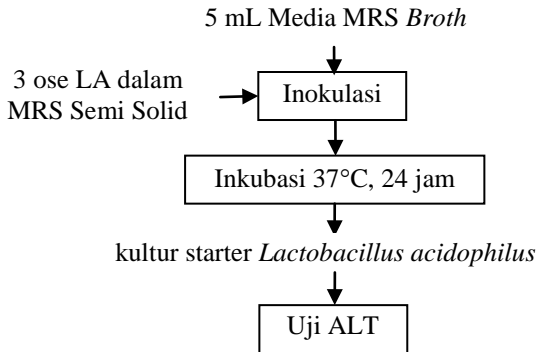
Tahapan ini bertujuan untuk menginokulasikan starter *Lactobacillus acidophilus* ke dalam masing-masing media de Man, Rogosa and Sharpe (MRS) Broth agar dengan menggunakan ose berkolong sebanyak 3 ose. Proses inokulasi dilakukan secara aseptis yaitu dengan dilakukan di dekat nyala api.

2. Inkubasi

Tujuan dari tahapan ini adalah untuk memberi kesempatan bagi *Lactobacillus Acidophilus* untuk tumbuh dengan memanfaatkan nutrisi yang ada pada media MRS agar. Proses ini dilakukan pada suhu 37°C selama 24 jam karena pada suhu dan waktu ini merupakan suhu dan waktu yang optimal bagi pertumbuhan BAL dan BAL masih berada pada fase log (Hui, 1992).

4.5.6. Pembuatan Kultur Starter *Lactobacillus acidophilus*

Tahapan pembuatan kultur *starter* dapat dilihat pada gambar 4.2. Penghitungan jumlah bakteri pada kultur *starter* terdapat pada Lampiran 2. Tahapan peremajaan kultur BAL adalah sebagai berikut.



Gambar 4.6. Diagram Alir Pembuatan Kultur Starter *Lactobacillus acidophilus*
 Sumber: Fardiaz (1989)

Penjelasan proses:

1. Inokulasi Starter

Tahapan ini bertujuan untuk menginokulasikan starter *Lactobacillus acidophilus* ke dalam masing-masing media de Man, Rogosa and Sharpe (MRS) broth dengan menggunakan ose berkolong sebanyak 3 ose. Proses inokulasi dilakukan secara aseptis yaitu dengan dilakukan di dekat nyala api.

2. Inkubasi

Tujuan dari tahapan ini adalah untuk memberi kesempatan bagi *Lactobacillus acidophilus* untuk tumbuh dengan memanfaatkan nutrisi yang ada pada media MRS broth. Proses ini dilakukan pada suhu 37°C selama 24 jam karena pada suhu dan waktu ini merupakan suhu dan waktu yang optimal bagi pertumbuhan BAL dan BAL masih berada pada fase log (Hui, 1992).

DAFTAR PUSTAKA

- Adams, C. 2009. *The Promising Potential of Prebiotics and Probiotics : Research Reveals Interesting Applications for Probiotic Bacteria and Their Frequent Partners in Health, Prebiotics*. Retrieved Available at : http://www.nutraceuticalsworld.com/issues/2009-05/view_features/the-promising-potential-of-prebiotics-amp-probioti/. [2 September 2013].
- Aditya, A.M. 2012. *Pengaruh Pemberian Lactobacillus acidophilus terhadap Angka Kuman Usus Halus yang Diinfeksi Shigella dysentriae*. Retrieved Available at : <http://publikasi.umy.ac.id/index.php/pendokter/article/view/3931/3264>. [5 September 5 2013].
- Akhiar, N.S.A.M. 2010. Enhancement of Probiotics Survival by Microencapsulation with Alginate and Prebiotics. Michigan. *MMG 445 Basis Biotechnology* 6:13-18.
- Amir, M., S.H. Razavi, M.R. Ehsani, and S. Sohrabvandi. 2007. Principle and Methods of Microencapsulation of Probiotic Microorganisms. *Review Article*. Iran : Department of Food Science and Engineering, University of Tehran.
- Alexandra, Drakoularakou, O. Hasselwander, M. Edinburgh, and A.C. Ouwehand. 2007. Lactitol, an Emerging Prebiotic: Functional Properties with a Focus on Digestive Health. USA. *Food Science and Technology Bulletin : Functional Foods* 3 (7) 73-82.
- Anal, A.K. and H. Singh (2007) Recent Advances in Microencapsulation of Probiotics for Industrial Applications And Targeted Delivery. *Trends in Food Science and Technology* 18 240-251.
- Ann, E.Y. Y.Kim, S.Oh, J.Y. Imm, D.J. Park, K.S. Han, and S.H. Kim. 2007. Microencapsulation of *Lactobacillus acidophilus* ATCC 43121 with Prebiotic Substrates using a Hybridisation System. *Inter. Journ. Of Food Sci. and Tech.* 42:411-419.

- Ari, A, R. Muyorini, dan Y. Sri. 2010. Encapsulation of *Lactobacillus casei* Using Extrusion Technique As Starter Culture For Production of Dadih from Cow Milk. *Skripsi*. Bogor : Institut Pertanian Bogor.
- Audet, P., C. Paquin, and C. Lacroix. 1988. Immobilized Growing Lactic Acid Bacteria with K-Carrageenan-Locust Bean Gum Gel. *Appl Microbiol Biotechnol* 29:11-18.
- Badan Standar Nasional. 1998. *SNI Susu UHT* (SNI 01-3950-1998). Dewan Standardisasi Nasional : Jakarta.
- Bar, A. 1990. *Factorial Calculation Model for the Estimation of the Physiological Caloric Value of Polyols in Caloric Evaluation of Carbohydrates*. [N Hosoya, editor]. Tokyo: Research Foundation for Sugar Metabolism. pp. 209-257
- Bernardeau, M., J. P. Vernoux, S. H. Dubernet, and M. Gueguen. 2008. Safety Assessment of Dairy Microorganisms: The *Lactobacillus* Genus. *International Journal of Food Microbiology* 126:278-285.
- Bolhuis, G.K, E.G. Rexwinkel, and K. Zuurman. 2009. Polyols as Filler-Binders for Disintegrating Tablets Prepared by Direct Compaction. Netherlands. *Drug Dev Ind Pharm*. 35(6):671-7.
- Brachkova, M.I., M.A. Duarte, and J.F. Pinto. 2010. Preservation of Viability and Antibacterial Activity of *Lactobacillus spp.* in Calcium Alginate Beads. Portugal. *European Journal of Pharmaceutical Sciences* 41 : 589-596.
- Buttris, J. 1997. Nutritional Properties of Fermented Milk Products. *International Journal of Dairy Technology* 50(1):21-27.
- Cahoney. 2013. *Lactobacillus achidophilus*. Retrieved available at : <http://cahoney-l-acidophilus.pbworks.com/w/page/6327467/Structure>. [1 September 2013]

- Capela, P., T.K.C. Hay, and N.P. Shah. 2006. Effect of Cryoprotectants, Prebiotics and Microencapsulation on Survival of Probiotic Organisms in Yoghurt and Freeze Dried Yoghurt. *Food Res. Int.* 39(2):203–11.
- Cardenas, A., W.A. Monal, F.M. Goycoolea, I.H. Ciapara, C. Peniche. 2003. Diffusion Through Membranes of The Polyelec-Trolyte Complex of Chitosan and Alginate. *Macromol. Biosci.* 3:535-539.
- Castilla, O.S., C.L. Calleros, H.S.G. Galindo, J.A. Ramirez and E.J.V. Carter. 2010. Textural Properties Alginate-Pectin Beads and Survivability of Entrapped *Lactobacillus Casei* in Simulated Gastrointestinal Conditions and Yoghurt. *Food Res. Int.*, 43: 111-117.
- Chandramouli, V., K. Kailasapathy, P. Peiris, and M.Jones. 2004. An Improved Method of Microencapsulation and Its Evaluation to Protect *Lactobacillus spp.* In Simulated Gastric Condition. *J of Microbiol Methods* 56:27–35.
- Champagne, C.P.; N. Morin; R. Couture; C. Gagnon; P. Pelen. and C. Lacroix. 1992. The Potential of Immobilized Cell Technology to Produce Freeze-Dried, Phage-Protected Cultures of *Lactococcus Lactis*. *Food Research International* 25:419-427.
- Chou, L.Z. and B. Weimer. 1999. Isolation and Characterization of Acid and Bile-Tolerant Isolates From Strains of *Lactobacillus acidophilus*. *J. Dairy Sci.* 82:23-31.
- Claesson, M. J., D. V. Sinderen, and P. W. O'Toole. 2007. The Genus *Lactobacillus*– A Genomic Basis for Understanding Its Diversity. *FEMS Microbiol. Lett.* 269:22-28.
- Collado, M. C., E. Isolauri, S. Salmien, and Y. Sanz. 2009. The Impact of Probiotic on Gut Health. *Curr Drug Metab.* 10(1):68-78.
- Cummings J.H., G.T. Macfarlane, H.N. Englyst. 2001. Prebiotic Digestion and Fermentation. *Am. J. Clin. Nutr* 73, 415S–420S.

- Cui, J., E.H. Holmes, T.G. Greene, P.K. Liu. 2000. Oxidative DNA Damage Precedes DNA Fragmentation after Experimental Stroke in Rat Brain. *FASEBJ* 14:955–967.
- Dommels, Y.E.M., R.A. Kemperman, Y.E.M.P. Zebregs, and R.B. Draaisma. 2009. Survival of *Lactobacillus reuteri* DSM 17938 and *Lactobacillus rhamnosus* GG in the Human gastrointestinal Tract with Daily Consumption of A Low-Fat Probiotic Spread. *Appl. Environ. Microbiol.* 75(19):6198-204.
- Dimantov, A., M. Greenberg, E. Kesselman, and Shimoni. 2003. Study of High Amylase Corn Starch as Food Grade Enteric Coating in A Microcapsule Model Systems. *Innov. Food Sci. Eng. Technol.* 5:93-100.
- Eckles, C.H, W.B Comb and H. Macy. 1951. *Milk and Milk Product*. 4th Edition. New York: Mc Graw-Hill Book Company, Inc.
- Effendi, H. M. S. 2009. *Teknologi Pengolahan dan Pengawetan Pangan*. Bandung: Alfabeta.
- Eikmeier, H., H.J. Rehm. 1987. Stability of Calcium-Alginate During Citric Acid Production of Immobilized *Aspergillus niger*. *Appl Microbiol Biotechnol.* 26:105-111.
- Ellenton, J.C. 1998. *Encapsulation Bifidobacteria*. Master thesis. University of Guelph.
- Evans P.R., C. Piesse C., Y.T. Bak, and J.E. Kellow. 1998. Fructose-Sorbitol Malabsorption and Symptom Provocation in Irritable Bowel Syndrome: Relationship to Enteric Hypersensitivity and Dysmotility. *Scand. J. Gastroenterol* 33: 1158–1163.
- Fahimdanesh, M., N. Mohammadi, H. Ahari, M.A.K. Zanjani, F.Z. Hargalani, K. Behrouznasab. 2012. Effect of Microencapsulation plus Resistant Starch on Survival of *Lactobacillus Casei* and *Bifidobacterium Bifidum* in Mayonnaise Sauce. *Afr. J.Microbiol. Res.* 6: 6853-6858.

- FAO/WHO. 2001. *Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria*. American Cordoba Park Hotel, Cordoba, Argentina.
- FAO/WHO. 2002. *Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food*. London.
- FAO/WHO. 2007. *FAO Technical Meeting on Prebiotics*. Italy.
- Fardiaz, S. 1989. *Mikrobiologi Pangan: Penuntun Praktek Laboratorium*. Bogor: IPB Jurusan Teknologi Pangan dan Gizi.
- Fernandez, B.F., M. E. Pardo, P. Humbert, R.Leon, J.M. Llovet, and M.A. Gassull. 1991. Role of Fructose-Sorbitol Malabsorption in the Irritable Bowel Syndrome. *Gastroenterology* 101: 1453–1454.
- Fraizer WC, Westhoof DC. 1988. *Food Microbiology International*. Singapore: Mc Graw Hill.
- Gee, J.M., D. Cooke, S. Gorick, G.M. Wortley, R.H. Greenwood, A. Zumbe, and I.T. Johnson. 1991. Effects of Conventional Sucrose-Based, Fructose-Based and Isomalt-Based Chocolates on Post Prandial Metabolism in Non-Insulin Dependent Diabetics. *Eur. J. Clin. Nutr.* 45:561–566.
- Gehring, F. and E.J. Karle. 1981. Sweetening Agent, Palatinin under Specific Consideration as to Microbiological and Caries-Prophylactic Aspects. *Z Ernährung swiss* 20:96–106.
- Gilliland SE. 1989. *Acidophilus* Milk Products: A Review of Potential Benefits to Consumers. *J. Dairy Sci.* 72:2483-2494.
- Gilliand, S.E. and M.L. Speck. 1977. Use of the Minitex System for Characterizing *Lactobacilli*. *Appl. Envir. Microbiol.* 33:1289-1292.
- Gouin S (2004). Microencapsulation-Industrial Appraisal Of Existing Technologies And Trend. *Trends Food Sci Technol.* 15: 330-347.

- Gostner, A., M. Blaut, *et al.* 2006. Effect of Isomalt Consumption on Faecal Microflora and Colonic Metabolism in Healthy Volunteers. *Br. J. Nutr.* 95 (1): 40-50.
- Granato, D., G.F. Branco, A. G. Cruz, J.D.A.F. Faria, and N.P. Shah. 2010. Probiotic Dairy Products as Functional Foods. *Comprehensive Reviews in Food Science and Food Safety* 9: 455–470.
- Haralampu, S.G. 2000. *Resistant Starch-A Review of The Physicalproperties And Biological Impact of RS3. Carbohydrate Polymers.* 41: 285-292.
- Hartati,S., E. Harmayani, dan E.S. Rahayu. 2003. Perubahan Kimiawi dan Organoleptik Sari Buah Pepaya Nanas yang Disuplementasi *Lactobacilli* Probiotik selama Penyimpanan. Dalam *Seminar Nasional dan Pertemuan Tahunan Perhimpunan Ahli Teknologi Pangan Indonesia (PATPI)*: 263-271.
- Harti,A.S, R.A. Samsumaharto, dan Hosea. 2012. Efek Penambahan Chito-Oligosakarida Sebagai Prebiotik Terhadap Pertumbuhan *Lactobacillus acidophilus* FNCC 0051 Secara *In Vitro*. Surakarta. *Jurnal Biomedika* Vol. 5(1) : 2302-1306.
- Helferich, W and D . Westhoff. 1980 . All Absut Yoghurt Prentice-Hall, Mc. New Jersey : EngelWood-Cliffs.
- Homayouni, A. 2008. Therapeutical Effects of Functional Probiotic, Prebiotic and Symbiotic Foods. 1st Ed. Tabriz: Tabriz University of Medical Sciences.
- Homayouni, A., A. Azizi, M.R. Ehsani, S.H. Razavi, and M.S. Yarmand. 2008. Effect of Microencapsulation and Resistant Starch on The Probiotic Survival And Sensory Properties of Synbiotic Ice Cream. *Food Chemistry* 111 pp. 50-55.
- Huezo, R. M.E., C.L. Calleros, J.G.R. Ocampo, O.S. Castilla, C. Perez-Alonso, D.J.P. Gonzalez. 2011. Survivability of Entrapped *Lactobacillus rhamnosus* in Liquid and Gel Core Alginate Beads

- during Storage and Simulated Gastrointestinal Conditions. Mexico. *Revista Mexicana de Ingenieria Quimica* 10(3) : 353-361.
- Hutter, R., F. Boswart, and K. Irsigler. 1993. Insulin Verbrauch Von Typ-I-Diabetikern nach Oraler Gabe Von Isomalt. *Akt Ernahr* 18:149–154.
- ISAPP. 2009. *Clarification of the Definition of a Probiotic*. Retrieved Available at :www.isapp.net. [21 Juni 2013].
- ISAPP. 2005. *Establishing Standards for Probiotic Products*. Retrieved Available at :www.isapp.net. [6 November 2013].
- Iyer, C. and K. Kailasapathy. 2006. Effect of Co-encapsulation of Probiotics with Prebiotics on Increasing the Viability of Encapsulated Bacteria under in Vitro Acidic and Bile Salt Conditions and in Yogurt. *Journal of Food Science* 70(1), M18–M23.
- Jankowski, T., M. Zielinska, and W. Wysłowska. 1997. Encapsulation of Lactic and Bacteria with Alginate or Starch Capsules. *Biotechnol Technol.* 11:31-34.
- Kailasapathy, K. 2002. Microencapsulation of Probiotic Bacteria: Technology And Potential Application. *Current Issues in Intestinal Microbiology*, 3: 39-48.
- Kailasapathy, K. 2006. Survival of Free And Encapsulated Probiotic Bacteria and Their Effect on The Sensory Properties of Yogurt. *Lebensm. Wiss. Technol.* 39:1221–1227.
- Kebary, K.M.K., S.A. Hussein, and R.M. Badawi. 1998. Improving Viability of Bifidobacterium and Their Effect on Frozen Ice Milk. *J. Dairy Sci.* 26: 319-337.
- Khalil, A.H., E.H. Mansour. 1998. Alginate Encapsulated *Bifidobacteria* Survival in Mayonnaise. *J. Food Sci.* 63:702-705.

- Khazaeli, P., A. Pardakhty, and F. Hassanzadeh. 2008. Formulation of Ibuprofen Beads by Ionotropic Gelation. *Iranian Journal of Pharmaceutical Research* 7 (3): 163-170.
- Khoriyah, L.K. dan Fatchiyah. 2013. Karakter Biokimia dan Profil Protein Yoghurt Kambing PE Difermentasi Bakteri Asam Laktat (BAL). *J. Exp. Life Sci. Vol.* 3(1): 2338-1655.
- Kim, I.K., Y.J. Baek, and Y.H. Yoon. 1996. Effects of Dehydration Media and Immobilization in Calcium-Alginate on The Survival of *Lactobacillus casei* and *Bifidobacterium bifidum*. *Korean J Dairy Sci.* 18: 193-198.
- Kimestri and Asma Bio. 2013. Pengaruh Sukrosa terhadap Jumlah Bakteri dan Karakteristik Kimia pada Whey Kerbau Fermentasi. (Retrieved available at : <http://repository.unhas.ac.id/handle/123456789/4770?show=full>). [29 Oktober 2013].
- Ki Yong Lee and Tae Ryeon Heo. 1999. Survival of *Bifidobacterium longum* Immobilized in Calcium Alginate Beads in Simulated Gastric Juices and Bile Salt Solution. *Appl. Environ. Microbiol.* 66(2) 869-873.
- Kleessen, B., G. Stoof, J. Proll, D. Schmied, J. Noack, and M. Blaut. 1997. Feeding Resistant Starch Affects Fecal and Microflora and Short Chain Fatty Acid in Rats. *J Animal Sci.* 75:2453-2462.
- Klien, J., J. Stock, K.D. Vorlop. 1983. Pore Size and Properties of Spherical Calcium Alginate Biocatalysts. *Eur. J. Appl. Microbiol. Biotechnol.* 18:86-91.
- Klingeberg, Michael, Kozyanowski, and Gunhild. 2004. *Use of Isomalt (Mixture of 1,6 gps and 1,1 gpm) as Prebiotic for the Production of a Medicament Used for the Treatment of Intestinal Diseases, Among Other Things.* United States Patent Application Publication US 2006/0147500 A1.

- Klikenberg, G., K.Q. Lystad, D.W. Levine, and N. Dyrset. 2001. Cell Release from Alginate Immobilized *Lactococcus lactis* ssp. *Lactis* in Chitosan and Alginate Coated Beads. *J. Dairy Sci.* 84:1118-1127.
- Krasaekoopt W., B. Bhandari, H. Deeth. 2003. Evaluation of Encapsulation Techniques of Probiotics for Yoghurt. *Int. Dairy J.* 13: 3-13.
- Krasaekoopt, W., B. Bhandari, H. Deeth. 2004. The Influence of Coating Materials on Some Properties of Alginate Beads and Survivability of Microencapsulated Probiotic Bacteria. *Int. Dairy J.* 14:737-743.
- Kritchevsky, D. 1995. Epidemiology of Fiber, Resistant Starch and Colorectal Cancer. *Eur J. Cancer Prev.* 4: 345-352.
- Kun Nan Chen, M.J. Chen, J.R. Liu, C.W. Lin, and H.Y. Chiu. 2005. Optimization of Incorporated Prebiotics as Coating Materials for Probiotic Microencapsulation. Taiwan. *J. of Food Sci.* 70 (5) : 261-266.
- Lacroix, C., C. Paquin, J.P. Arnaud. 1990. Batch Fermentation with Entrapped Cells of *Lactobacillus casei*: Optimization The Rheological Properties of The Entrapment Gel Matrix. *Appl. Microbiol. Biotechnol.* 32:403-408.
- Larisch, B.C, D. Poncelet, C.P. Champagne, and R.J. Neufeld. 1994. Microencapsulation of *Lactococcus lactis* subsp. *Creamoris*. *J Microencap.* 11: 189-195.
- Langkilde, A.M., H. Andersson, T.F. Schweizer, and P. Wursch. Digestion and Absorption of Sorbitol, Maltitol and isomalt from The Small Bowel. A Study In Ileostomy Subjects. *Eur. J. Clin. Nutr.* 1994(48):768-775.
- Le Blay, G., C. Michel, H.M. Blottiere, and C. Cherbut. 1999. Enhancement of Butyrate Production in The Rat Caecocolonic Tract by Long-Term Ingestion O Resistant Potato Starch. *Brit. J. Nut.* 82:419-426.

- Lee, K.I. and T.R. Heo T.R. 2000. Survival of *Bifidobacterium Longum* Immobilized in Calcium Alginate Beads in Simulated Gastric Juices and Bile Salt Solution. *Appl. Environ. Microbiol.* 66: 869-973.
- Liong, M.T. 2008. Roles of Probiotics and Prebiotics in Colon Cancer Prevention: Postulated Mechanisms and In-vivo Evidence. *Int. J. Mol. Sci.* 9(5) : 854-863.
- Lisal, J.S. 2005. Konsep Probiotik dan Prebiotik untuk Modulasi Mikrobiota Usus Besar. *Medical Nusantara* 26 : 256-262.
- Livesey, G., 2003. Health Potential of Polyols as Sugar Replacers, with Emphasis on Low Glycaemic Properties. *Nutrition Research Reviews* 16:163-191.
- Macfarlane, G.T., J.H. Gummings. 1991. The Colonic Flora, Fermentation and Large Bowel Digestive Function. In SF Phillips, JH Pemberton And RG Shorter (Eds.). *The Large Intestine: Physiology, Pathophysiology and Disease*. New York: Raven Press.\
- Macfarlane G., Steed H., Macfarlane S. 2008. Bacterial Metabolism and Health Related Effects of Galactooligosaccharides and Other Prebiotics. *J. Appl. Microbiol.* 104: 305–344.
- Maduningsih, G.L. 2008. Stabilitas Bakteri Probiotik *Lactobacillus acidophilus* dan *Bifidobacterium longum* dalam Yogurt Susu Kambing di dalam Saluran Pencernaan Tikus. [Skripsi]. Program Studi Teknologi Hasil Ternak, Fakultas Peternakan, Institut Pertanian Bogor. Bogor.
- Makarova, K., A. Slesarev, Y. Wolf, A. Sorokin, B. Mirkin, E. Koonin, A. Pavlov, N. Pavlova, V. Karamychev, N. Polouchine, V. Shakhova, I. Grigoriev, Y. Lou, D. Rohksar, S. Lucas, K. Huang, D. M. Goodstein, T. Hawkins, V. Plengvidhya, D. Welker, J. Hughes, Y. Goh, A. Benson, K. Baldwin, J.-H. Lee, I. Díaz-Muñiz, B. Dosti, V. Smeianov, W. Wechter, R. Barabote, G. Lorca, E. Altermann, R. Barrangou, B. Ganesan, Y. Xie, H. Rawsthorne, D. Tamir, C. Parker, F. Breidt, J. Broadbent, R. Hutkins, D. O'Sullivan, J. Steele, G. Unlu, M. Saier, T.

- Klaenhammer, P. Richardson, S. Kozyavkin, B. Weimer, and D. Mills. 2006. Comparative Genomics of The Lactic Acid Bacteria. *Proc. Natl. Acad. Sci. USA.* 103(42): 15611–15616.
- Mandal, S., Puniya, A.K. and Singh, K. 2006. Effect of alginate concentration on survival of encapsulated *Lactobacillus casei* NCDC-298. *International Dairy Journal* 16: 1190-1195.
- Marx, J.L. 1989. *A revolution in Biotechnology*. Cambridge: Cambridge University.
- Martinsen, A., C. Skjak-Braek, and Smidsrod. 1989. Alginate as Immobilization Material: 1. Correlation Between Chemical And Physical Properties of Alginate Gel Beads. *Biotechnol Bioeng.* 33:79-89.
- Meydani, S.N. and W.K. Ha. 2000. Immunologic Effects of Yoghurt. *Am. J. Clin. Nutr.* 71(4):861-72.
- Mohammadi, N., h. Ahari, M. Fahimdanesh, M.A.K. Zanjani, A.A. Anvar, and E. Shokri. 2013. Survival of Alginate-Prebiotic Microencapsulated *Lactobacillus acidophilus* in Mayonnaise Sauce. Iran. *Iranian Journal of Veterinary Medicine* 6(4):259-264.
- Monedero V., G. P. Martines, and M. Yebra. 2010. Perspectives of Engineering Lactic Acid Bacteria for Biotechnological Polyol Production. *Appl. Microbiol. Biotechnol.* 86: 1003–1015.
- Mortazavian, A., S.H. Razavi, M.R. Ehsani, and S. Sohrabvandi. 2007. Principles and Methods of Microencapsulation of Probiotic Microorganisms. *Iranian Journal of Biotechnology* 5(1) 1-18.
- Mozzi, F., G. Rollan, G.S. Giori, G, F.G. Valdez. 2001. Effect of Galactose and Glucose on The Exopolysaccharide Production and The Activities of Biosynthetic Enzymes in *Lactobacillus casei* CRL 87. *J. Appl. Microbiol.* 91:160-7.

- Muir, J.G., Z.X. Lu, G.P. Young, D.C. Smith, G.R. Dollier, and D. O'Dea. 1995. Resistant Starch in The Diet Increase Breathe Hydrogen and Serum Acetate in Human Subjects. *American J. Clin. Nutr.* 61:792-799.
- Murtiari, E. 2012. Total Probiotik Susu Kambing Fermentasi Menggunakan Starter Probiotik *Lactobacillus acidophilus* FNCC 0051 Selama Inkubasi. Semarang. *Jurnal Teknologi Pangan dan Hasil Pertanian Vol. 7(1) : 28-37.*
- Naidu, A. S., and R.A. Clemens. 2000. Probiotics. In: Naidu A. S. (ed.) *Natural Food Antimicrobial Systems*. Florida :CRC Press.
- Nazzaro, F., F. Fratianni, R. Coppola, A. Sada, P. Orlando. 2009. Fermentative Ability of Alginate-Prebiotic Encapsulated *Lactobacillus Acidophilus* and Survival under Simulated Gastrointestinal Conditions. *J. Funct. Foods.* 1(3):319-323.
- O'Sullivan, O., J. O'Callaghan, A. S. Vegas, O. McAuliffe, L. Slattery, P. Kaleta, M. Callanan, G. F. Fitzgerald, R. P. Ross, and T. Beresford. 2009. Comparative Genomics of Lactic Acid Bacteria Reveals A Niche-Specific Gene Set. *BMC Microbiol.* 9: 1471-2180.
- Ouwehand, A.C. and Salminen, S.J. 1998. The Health Effects of Viable and Non-Viable cultured Milk. *Intl. Dairy J.* 8: 749–758.
- Petzoldt, R., P. Lauer, M. Spengler, and K. Schoffling. 1982. Palatinite in Type II Diabetics. Effect on Blood Glucose, Serum-insulin, C-Peptide and Free Fatty Acids. *Dtsch. Med. Wochenschr* 107:1910–1913.
- Phillips, J., J.G. Muir, A. Birkett, Z.X. Lu, G.P. Jones, K. O'Dea, and G.P. Young. 1995. Effect of Resistant Starch on Fecal Bulk and Fermentation-Dependent Events in Human. *American J. Clin. Nut.* 62:121-130.
- Picot, A. and C. LACROIX. 2004. Encapsulation of *Bifidobacteria* in Whey Protein Microcapsules and Survival in Simulated

Gastrointestinal Conditions and in Yoghurt. *International Dairy Journal* 14:505-515.

Prangdimurti, E., N.S. Palupi, F.R. Zakaria . 2007. Metode Evaluasi Nilai Biologis Karbohidrat dan Lemak. <http://xa.yimg.com/kq/groups/20875559/932235840/name/modul12.pdf> f diakses tanggal 9 – 10- 2013.

Prevost H and C. Divies. 1992. Cream Fermentation By A Mixed Culture of *Lactococci* Entrapped in Two-Layer Calcium Alginate Gel Beads. *Biotechnol. Let.* 14 583-588.

Priadi, A. dan L. Natalia. 2006. Infeksi *Ornithobacterium rhinotracheale* (ORT) pada Ayam di Indonesia. *JITV* 11:61-68.

Rahayu, K. 1989. *Mikrobiologi Pangan*. Universitas Gadjah Mada Press. Yogyakarta.

Rahayu, E. S. 2008. *Probiotic for Digestive Health. Food Review-Referensi industri dan teknologi pangan Indonesia*. Retrieved Available at: http://www.food_review.biz/login/preview.php?view&id=55932. [2 September 2013].

Rahayu, W.P., F. Kusnandar, and W.E. Prayitno. 2011. Stability of Viable Counts of Lactic Acid Bacteria during Storage of Goat Milk Soft Cheese. Bogor. *Microbiol.* Vol. 5(4) : 149-153.

Rokka, S. and P. Rantamaki, 2010. Protecting Probiotic Bacteria by Microencapsulation: Challenges for Industrial Applications. *Eur. Food Res. Technol.* 231: 1-12.

Roy, D., J. Goulet, A. Leduy. 1987. Continues Production of Lactic Acid from Whey Permeate by Free and Calcium-Alginate Entrapped *Lactobacillus helveticus*. *J. Dairy Sci.* 70: 506-513.

Rumessen J.J., Gudmand-Hoyer E. 1998. Fructans of Chicory: Intestinal Transport and Fermentation of Different Chain Lengths and Relation to Fructose and Sorbitol Malabsorption. *Am J. Clin. Nutr.* 68: 357–364.

- Salminen, S. and A. V. Wright. 1993. *Lactic Acid Bacteria*. Marcel Dekker Inc. pp. 200-201.
- Sarmiento, *et al.* 2007. Alginate or Chitosan Nanoparticles are Effective for Oral Insulin Delivery. *Pharmaceutical research* 24(12): 2198-2206.
- Saunders D.R., Wiggins H.S. 1981. Conservation of Mannitol, Lactulose, and Raffinose by the Human Colon. *Am. J. Physiol.* 241: G397–G402.
- Science Photo Library. 2013. *Lactobacillus achidophilus*. Retrieved available at : <http://sciencephotolibrary.tumblr.com/post/33441648147/lactobacillus-achidophilus-is-a-lactic-acid>. [1 September 2013].
- Senok, A.C. 2009. Probiotics in the Arabian Gulf Region. *Food & Nutrition Research*. Retrieved Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC_2651754/pdf/FNR-53-1842.pdf. [2 September 2013].
- Shah, N. P. 2007. Functional Cultures and Health Benefits. *Int. Dairy J.* 17:1262-1277.
- Shah, N.P. dan R.R. Rarula. 2000. Microencapsulation of Probiotic Bacteria and Their Survival in Frozen Fermented Dairy Desserts. *Aust. J. Dairy Technol.* 55: 139-144.
- Sheu, T.Y., R.T. Marshall. 1991. Improving Culture Viability Infrozen Dairy Desserts by Microencapsulation. *J. Dairy Sci.* 74: 107-111.
- Sheu, T.Y., R.T. Marshall. 1993. Microentrapment of *Lactobacilli* In Calcium Alginate Gel. *J. Food Sci.* 54(3): 557-561.
- Shitandi, A., M. Alfred, and M. Symon. 2007. Probiotic Characteristic Of *Lactococcus* Strain From Local Fermented *Amaranthus Hybrdus* And *Solanum Nigrum*. *African Crop Science Confrence Proceedings* 8:1809-1812.

- Silvester, K.R., H.N. Englyst, and J.H. Gummings. 1995. Recovery of Starch From Whole Diets Containing Resistant Starch Measured in Vitro and Fermentation of Effluent. *American J. Clin. Nut.* 62: 403-411.
- Smidsrod, O. and G. Skjak-Braek. 1990. Alginate for Cell Immobilization. *Trends in Food Sci Technol.*8:71-75.
- Stecchini, M. L., M. D. Torre, M. MunSumo, Sumantri, dan Subono, 1993. *Prinsip Bioteknologi*. Jakarta: PT. Gramedia Pustaka Utama.
- Sujaya, I.N., Y. Ramona, D.N.M. Utami, N.L.P. Suariani, N.P. Widarini, K.A. Nocianitri, and N.W. Nursini. 2008. Isolation and Characterization of Lactic Acid Bacteria from Sumbawa mare milk. *J. Vet.* 9:52-59.
- Sultana, K., G. Godward, N. Reynolds, R. Arumugaswamy, P. Peiris, and K. Kailasapathy. 2000. Encapsulation of Probiotic Bacteria with Alginate-Starch and Evaluation of Survival in Simulated Gastrointestinal Conditions and in Yoghurt. *Int. J. Food Microbiol.* 62: 47-55.
- Sumo, Sumantri, Subono. 1993. *Prinsip Bioteknologi*. Jakarta : PT. Gramedia Pustaka Utama.
- Sun, W. and M.W. Griffiths. 2000. Survival of *Bifidobacteria* In Yogurt And Simulate Gastric Juice Following Immobilization In Gellanxanthan Beads. *Int. J. Food Microbiol.* 61: 17-25.
- Surono, I.S. 2004. *Probiotik Susu Fermentasi dan Kesehatan*. Jakarta : Yayasan Pengusaha Makanan dan Minuman Seluruh Indonesia (YAPMMI).
- Suskovic,J., K.Blazenka, G.Jadranka and M.Srecko. 2001. Role of Lactic Acid Bacteria And *Bifidobacterium* In Symbiotic Effect. *Food Technol.. Bioteclunol.* 39 :227-235.

- Tamime, A. Y. dan R. K. Robinson. 2007. *Tamime and Robinson's Yogurt Science and Technology (third edition)*. Cambridge England : Woodhead Publishing Limited.
- Tanaka H., M. Masatose, I.A. Veleky. 1984. Diffusion Characteristics of Substrates in Calcium-Alginate Beads. *BiotechnolBioeng.* 26: 53-58.
- Thompson, D.B. 2000. Strategies for The Manufacture of Resistant Starch. *Trends in Food Sci. Technol.* 11: 245-253.
- Truelstrup-Hansen, L., P.M. Allan-Wojtas, Y.L. Jin, and A.T. Paulson. 2002. Survival of Free and Calcium-Alginate Microencapsulated *Bifidobacterium spp.* in Simulated Gastro-Intestinal Conditions. *Food Microbiol.* 19: 35-45.
- Weichselbaum, E. 2009. Probiotics and Health: A Review of The Evidence. *Nutrition Bulletin* 34: 340-73.
- Widodo, S. dan E. Wahyuni. 2003. Bioenkapsulasi Probiotik (*Lactobacillus casei*) dengan Pollard dan Tepung Terigu serta Pengaruhnya terhadap Viabilitas dan Laju Pengasaman. *J.Tek. dan Industri Pangan* 14:98-106.
- Winarno, F. G. dan I. E. Fernandez. 2007. *Susu dan Produk Fermentasinya*. Bogor: M-BRIO Press.
- Winarti, S., E. Harmayani, Y. Marsono, and Y. Pranoto. 2013. Effect of Inulin Isolated from Lesser Yam (*Dioscorea esculenta*) on the Growth of Probiotics Bacteria and SCFA Formation during Fermentation. Yogyakarta. *Inter. Research Journ. Of Microbio.* Vol. 4(2):53-63.
- Yusmarini, R. Indrati, T. Utami, dan Y. Marsono. 2010. Aktivitas Proteolitik Bakteri Asam Laktat Dalam Fermentasi Susu Kedelai. Riau. *J. Teknol. dan Industri Pangan* Vol. 21(2).
- Zhou, Y., E. Martins, A. Groboillot, C.P. Champagne, R.J. Neufeld. 1998. Spectrophotometric Quantification of Lactic Acid Bacteria in Alginate and Control of Cell Release with Chitosan Coating. *J. Appl. Microbiol.* 84: 342-348.