BAB IV
BAHAN DAN METODE PENELITIAN

4.1. Bahan untuk Proses

Bahan-bahan yang digunakan dalam pembuatan sel imobil adalah kultur bakteri *L. acidophilus* FNCC 0051 diperoleh dari Universitas Gadjah Mada Yogyakarta, Na-alginat murni (merk "Zigma A2033-100G"), larutan $CaCl_2$ 1%, isomalt, larutan NaCl 0,85% (merk "Riedel-de Haën 31434"), *oxgall* (merk "Pronadisa Cat. 1612.00"), larutan HCl 37% (merk "MERCK 1.00317") diperoleh dari Laboratorium Analisa Pangan, Fakultas Teknologi Pangan, Universitas Katolik Widya Mandala Surabaya, larutan Na-sitrat 0,1 M teknis dan susu UHT (*Ultra High Temperature*) "Ultra Milk" yang dibeli di supermarket "Alfaexpress".

4.1.2. Bahan untuk Analisa

Bahan yang digunakan untuk penelitian ini adalah MRS *Broth* (merk "Pronadisa Cat. 1215.00"), Agar "*Bacto Agar*"(merk "MERCK 214010"), Pepton *from meat* (merk "Merck 1.07224"). Spesifikasi MRS *Broth*, Agar "*Bacto Agar*", dan Pepton *from meat* terdapat pada Lampiran 1. Bahan pembantu yang digunakan untuk analisa adalah akuades, 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.

4.2. Alat

4.2.1. Alat untuk Proses

Alat yang digunakan pada pembuatan sel imobil adalah syrink (merk “Termuno”), spuit injeksi (merk “Terumo Needle” single use (1,20x38mm)), *water jug* 1000mL, bunsen, kakitiga, kassa asbes, penangas air, enkast, batang pengaduk, *beaker glass* 600 mL (merk “Schott Duran”), *beaker glass* 250 mL (merk “Schott Duran”), *beaker glass* 100 mL (merk “Schott Duran”), gelas ukur 100 mL (merk ”RRC), pipet ukur steril 1 mL (merk ”HBG”), pipet ukur steril 5 mL (merk ”HBG”), pipet ukur steril 10 mL (merk ”HBG”), erlenmeyer 250 mL (merk “Schott Duran”), *cup* plastik 45mL (merk “Lion Star”) yang terbuat dari plastik jenis *Polyprophylene* (PP) yang disterilkan terlebih dahulu dengan sinar UV selama 2 jam, autoklaf (merk “Geared Gauge” dan “All American” Model no. 25 X), inkubator (merk “WTC Binder”), oven (merk “WTC Binder”), *laminar flow* “Telstar AV-100”, timbangan digital (merk “Mettler Toledo”), lemari es “Rotary Compressor Mitsubishi” .

4.2.2. Alat untuk Analisa

Alat-alat yang digunakan untuk analisa adalah pH meter (merk “Trans Instrument” TI-2100), sendok porselen, sendok plastik, cawan petri, pipet tetes, erlenmeyer 250mL (merk “Schott Duran”), pipet ukur steril 1 mL (merk ”HBG”), pipet ukur steril 5 mL (merk ”HBG”), pipet ukur steril 10 mL (merk ”HBG”), gelas ukur 100mL (merk “Pyrex”), timbangan digital merk “Mettler Toledo GB 1302”, mikroskop “Nikon”, tabung reaksi dan rak tabung reaksi, mikrometer sekrup dan tekstur analyzer (merk “Stable Micro Systems Texturometer model TA-XT2i”).

4.3. Waktu dan Tempat Penelitian

4.3.1. Waktu Penelitian

Penelitian pendahuluan dilaksanakan pada bulan Juni 2013 sampai dengan September 2013. Penelitian utama akan dilaksanakan pada bulan Desember sampai dengan Januari 2013.

4.3.2. Tempat Penelitian

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

4.4. Rancangan Penelitian

Rancangan penelitian yang digunakan adalah RAK dengan dua faktor, yaitu konsentrasi Isomalt (1%, 2%, 3%, 4% dan 5%) dan lama penyimpanan (0 hari dan 21 hari) dengan model matematis $Yijk = \mu + \alpha i + \beta j(i) + Kk + \varepsilon ijk$. Taraf-taraf tersebut terdapat 10 kombinasi perlakuan dengan 3 kali pengulangan sehingga akan diperoleh total 30 unit eksperimen. 10 Kombinasi perlakuan tersebut dapat dilihat pada table 4.1. Parameter yang diuji meliputi diameter, tekstur, dan ketahanan sel imobil.

Data yang diperoleh dari hasil pengamatan dan pengujian dianalisa secara statistik menggunakan uji ANOVA (Analysis of Varians) pada $\alpha = 5\%$, untuk mengetahui apakah perlakuan memberikan pengaruh nyata terhadap parameter pengujian. Apabila hasil uji ANOVA menunjukkan ada perbedaan nyata, maka dilanjutkan dengan uji pembedaan untuk menentukan taraf perlakuan

yang memberikan perbedaan yang nyata. Uji pembedaan dilakukan dengan Uji Beda Jarak Nyata Duncan (Duncan's Multiple Range Test/DMRT) dengan $\alpha = 5\%$.

Tabel 4.1. Rancangan Penelitian Kombinasi Perlakuan Konsentrasi Isomalt (I) dan Lama Penyimpanan (L)

Perlakuan		Konsentrasi Isomalt (I)				
		I_1	I_2	I_3	I_4	I_5
Lama penyimpanan (L)	L_1	$L_1 I_1(1)$ $L_1 I_1(2)$ $L_1 I_1(3)$	$L_1 I_2(1)$ $L_1 I_2(2)$ $L_1 I_2(3)$	$L_1 I_3(1)$ $L_1 I_3(2)$ $L_1 I_3(3)$	$L_1 I_4(1)$ $L_1 I_4(2)$ $L_1 I_4(3)$	$L_1 I_5(1)$ $L_1 I_5(2)$ $L_1 I_5(3)$
	L_2	$L_2 I_1(1)$ $L_2 I_1(2)$ $L_2 I_1(3)$	$L_2 I_2(1)$ $L_2 I_2(2)$ $L_2 I_2(3)$	$L_2 I_3(1)$ $L_2 I_3(2)$ $L_2 I_3(3)$	$L_2 I_4(1)$ $L_2 I_4(2)$ $L_2 I_4(3)$	$L_2 I_5(1)$ $L_2 I_5(2)$ $L_2 I_5(3)$

Keterangan:

L_1 : Lama Penyimpanan 0 hari

L_2 : Lama Penyimpanan 21 hari

I_1 : Larutan Isomalt 1%

I_2 : Larutan Isomalt 2%

I_3 : Larutan Isomalt 3%

I_4 : Larutan Isomalt 4%

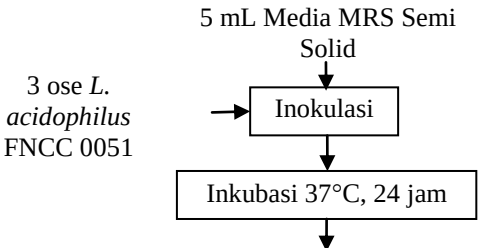
I_5 : Larutan Isomalt 5%

4.5. Pelaksanaan Penelitian

4.5.1 Peremajaan Kultur *L. acidophilus* FNCC 0051

Kultur *L. acidophilus* FNCC 0051 ditumbuhkan pada media MRS Broth dan diinkubasi pada suhu 37^{o}C selama 24 jam untuk mendapatkan kultur cair *L. acidophilus* FNCC 0051 pada fase pertumbuhan logaritma. Peremajaan dilakukan setiap minggu dengan tujuan sebagai persediaan kultur yang selalu dalam kondisi

sehat/optimal. Skema kerja peremajaan kultur *L. acidophilus* FNCC 0051 terdapat pada Gambar 4.1.



Gambar 4.1. Diagram Peremajaan Kultur Stok *L. Acidophilus* FNCC 0051
Sumber: Fardiaz (1989)

Penjelasan proses:

1. Inokulasi

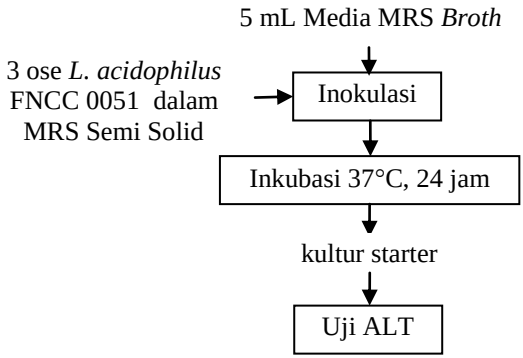
   Tahapan ini bertujuan untuk menginokulasikan starter LA ke dalam masing-masing media de Man, Rogosa and Sharpe (MRS) Brothagar dengan menggunakan ose berkolong sebanyak 3 ose. Proses inokulasi dilakukan secara aseptis yaitu dengan dilakukan di dekat nyala api.

2. Inkubasi

   Tujuan dari tahapan ini adalah untuk memberi kesempatan bagi LA 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).

4.5.2 Pembuatan Kultur *L. acidophilus* FNCC 0051

Tahapan pembuatan kultur *starter L. acidophilus* FNCC 0051dapat dilihat pada Gambar 4.2.



Gambar 4.2. Diagram Pembuatan Kultur Starter *L. acidophilus* FNCC 0051
Sumber: Fardiaz (1989)

Penjelasan proses:

1. Inokulasi Starter

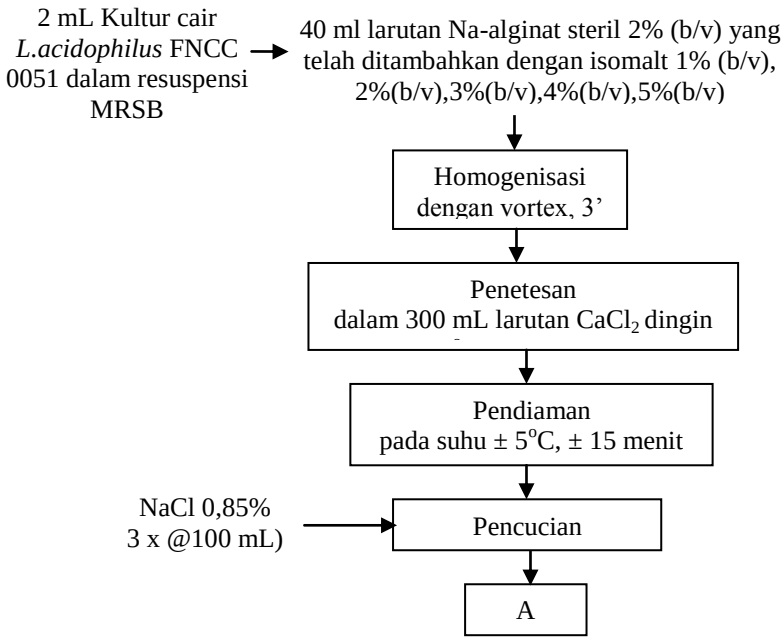
   Tahapan ini bertujuan untuk menginokulasikan starter LA ke dalam masing-masing media de Man, Rogosa and Sharpe (MRS) *broth* dengan menggunakan ose berkolong sebanyak 3 ose. Proses inokulasi dilakukan secara aseptis yaitu dengan dilakukan di dekat nyala api.

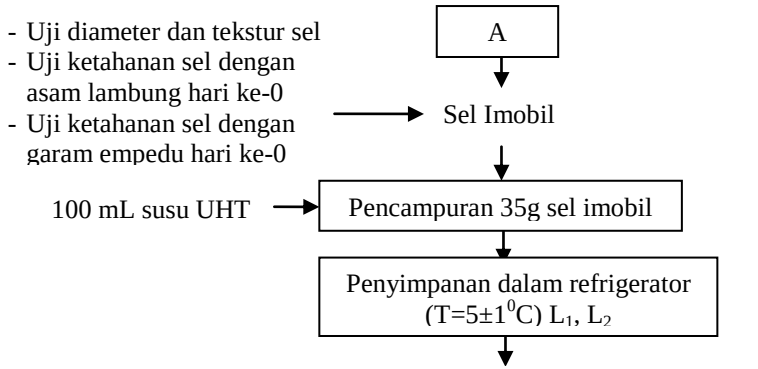
2. Inkubasi

   Tujuan dari tahapan ini adalah untuk memberi kesempatan bagi LA 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).

4.5.3 Pembuatan Sel Imobil

Kultur *L. acidophilus* FNCC 0051 dimasukkan ke dalam larutan Na-alginat steril dan dihomogenkan agar tercampur merata. Campuran tersebut dimasukan dalam syrink dan diteteskan dalam larutan $CaCl_2$ 1% dingin (T-4-7^{0}C) untuk mempercepat pembentukan gel Ca-Alginat. Manik-manik yang terbentuk didiamkan selama ± 15 menit untuk memperkokoh struktur gel sehingga manik-manik tidak mudah berubah bentuk. Setelah itu, manik-manik tersebut dicuci dengan larutan garam NaCl 0,85% sebanyak 3 kali. Fungsi larutan garam fisiologis ini adalah untuk menghilangkan sel-sel yang berada di permukaan sel imobil





Gambar 4.3. Skema Pembuatan Sel Imobil dalam Na-Alginat
Sumber : Sheu and Marshall (1993); Lee and Heo (2000); Klinkenberg (2001)

Penjelasan proses:

1. Homogenisasi

   Tahapan ini bertujuan agar bakteri tercampur merata didalam larutan Na-alginat, homogenisasi menggunakan vortex.

2. Penetasan pada $CaCl_2$

   Tahapan ini bertujuan untuk penaut silang antar molekul alginat yang menyebabkan terjadinya gelatinisasi dan akan membentuk gel matriks kalsium alginat.

3. Pendiaman

   Tahapan ini bertujuan untuk memberikan waktu kontak pada alginat dan kation Ca^{2+} membentuk gel matriks kalsium alginat.

4. Pencucian

   Tahapan ini bertujuan untuk menghilangkan sisa-sisa $CaCl_2$ yang masih menempel pada *beads* dan menghilangkan sel-sel yang berada dipermukaan *beads*.

4.6. Pengamatan dan Pengujian

4.6.1. Pengujian Ketahanan terhadap Asam Lambung

a. Sel imobil (3 gram) dimasukkan dalam media MRS broth yang telah diatur pada pH 2,5 (HCl 0,08 M) kemudian diinkubasi selama 30 menit, 37^0C .

b. 3 gram sel imobil yang telah dikondisikan pada pH 2,5 diambil dengan menggunakan sendok porselen steril secara aseptis kemudian dilarutkan dalam 27 mL larutan Na-sitrat 0,1 M steril pada suhu kamar dan dikocok sampai sel imobil terlarut semua (kurang lebih 10 menit). Setelah itu, dilakukan seri pengenceran, penuangan MRS agar yang telah dicairkan, dan dilanjutkan dengan inkubasi 37^0C selama 48 jam dan perhitungan koloni yang tumbuh. (Lee and Heo, 2000, dengan modifikasi). Prosedur pengenceran dapat dilihat pada Lampiran 4.

c. Jumlah sel yang tahan dinyatakan sebagai ALT BAL (sel/gram).

   Ketahanan *Lactobacillus acidophilus FNCC* 0051 terimobil terhadap asam lambung diperoleh dengan menggunakan rumus :

   Ketahanan asam lambung = log (ALT tabung a) – (ALT tabung b)

   Diasumsikan semakin kecil selisih log, semakin besar ketahanan *Lactobacillus acidophilus* FNCC 0051 terhadap asam lambung.

4.6.2. Pengujian Ketahanan terhadap Garam Empedu

a. Sel imobil (3 gram) yang telah dikondisikan pada pH 2,5 selama 30 menit dimasukkan dalam media yang telah ditambah dengan *oxgall* (garam empedu) sebanyak 1% (b/v) kemudian diinkubasi 37^0C selama 3 jam.

b. Sebanyak 3 gram sel imobil yang telah dikondisikan pada konsentrasi *oxgall* 1% diambil dengan menggunakan sendok porselen steril secara aseptis. Kemudian, sel imobil tersebut dilarutkan dalam 27 mL larutan Na-sitrat 0,1 M steril pada suhu kamar dan dikocok sampai terlarut semua (kurang lebih 10 menit). Setelah itu, dilakukan seri pengenceran, penuangan MRS agar yang telah dicairkan, dan dilanjutkan dengan inkubasi 37^0C selama 48 jam untuk perhitungan koloni yang tumbuh (Lee and4 Heo, 2000, dengan modifikasi). Prosedur pengenceran dapat dilihat pada Lampiran 5.

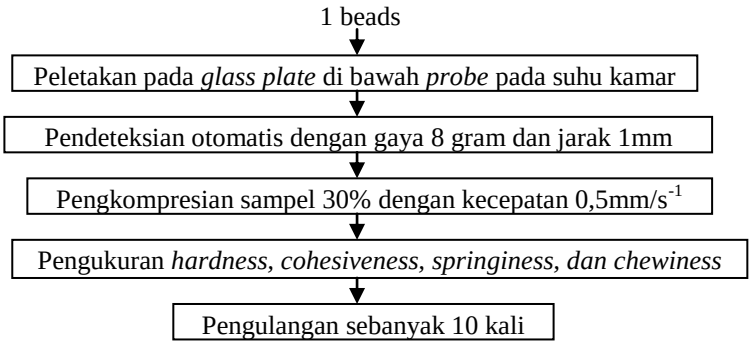
c. Sebagai kontrol, dilakukan prosedur seperti di atas tetapi dalam media MRS-*broth* tanpa penambahan *oxgall.*

d. Ketahanan *Lactobacillus acidophilus* FNCC 0051 terimobil terhadap garam empedu dinyatakan sebagai ketahanan relatif yang diperoleh dengan:

   Ketahanan relatif terhadap garam empedu = log (ALT tabung c – ALT tabung b) – log (ALT tabung d – ALT tabung b)

   Diasumsikan semakin kecil selisih log, semakin besar ketahanan *Lactobacillus acidophilus* FNCC 0051 terhadap *oxgall.*

4.6.3. Pengujian Tekstur (Data bersama)



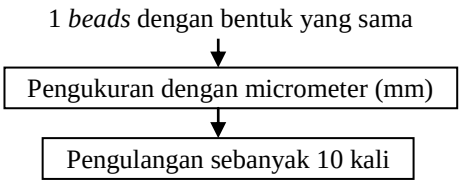
Gambar 4.4. Skema Pengujian Tekstur
Sumber : Rodriguez-Huezo *et al*., (2011) dengan modifikasi

Keterangan :

Karakteristik	Definisi Sensorial	Definisi Instrumental
Kekerasan	Gaya yang diberikan hingga terjadi perubahan bentuk (deformasi) pada objek	
Kerapuhan / *fracturability*	Titik dimana besarnya gaya yang diberikan membuat objek menjadi patah	
Springiness	Panjang dari kompresi kedua dari puncak	
Kohesivitas	Kekuatan dari ikatan-ikatan yang berada di dalam objek yang menuyusun bentuk objek	
Chewiness	Hardness x cohesiveness x springiness	

Sumber: DeMan (1985); Rosenthal (1999)

4.6.4. Pengujian Diameter *Beads* (Data bersama)



Gambar 4.5. Skema Pengujian Diameter *Beads*
Sumber : Rodriguez-Huezo *et al*., (2011) dengan modifikasi

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