UNIVERSITI SAINS MALAYSIA

UNIVERSITY RESEARCH GRANT
FINAL REPORT
Geran Penyelidikan Universiti
Laporan Akhir

A.	**TITLE OF RESEARCH:** *Tajuk penyelidikan:* Evaluation of the bioconversion of isoflavones by probiotics in soymilk and characterization of the fermented soymilk during storage
B.	**PERSONAL PARTICULARS OF RESEARCHER** / *MAKLUMAT PENYELIDIK:*
(i)	**Name of Research Leader:** *Nama Ketua Penyelidik:* LIONG MIN TZE
	Name of Co-Researcher *Nama Penyelidik Bersama:* ROSMA AHMAD, WAN NADIAH WAN ABDULLAH
(ii)	**School/Institute/Centre/Unit :** *Pusat Pengajian /Institut/Pusat/Unit :* P. P. TEKNOLOGI INDUSTRI

C. **Research Platform (Please tick (/) the appropriate box):**
Pelantar Penyelidikan (Sila tanda (/) kotak berkenaan):

[ ]	**A. Life Sciences** *Sains Hayat*
[X]	**B. Fundamental** *Fundamental*
[ ]	**C. Engineering & Technology** *Kejuruteraan & Teknologi*
[ ]	**D. Social Transformation** *Transformasi Sosial*
[ ]	**E. Information & Communications Technology (ICT)** *Teknologi Maklumat & Komunikasi*
[ ]	**F. Clinical Sciences** *Sains Klinikal*
[ ]	**G. Biomedical & Health Sciences** *Bioperubatan Sains Kesihatan*

E. **ABSTRACT OF RESEARCH**

(An abstract of between 100 and 200 words must be prepared in **Bahasa Malaysia and in English**. This abstract will be included in the Annual Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

The main objective of this project was to evaluate the growth properties and bioconversion of isoflavones of probiotics in soymilk and the characterization of the fermented soymilk over storage. Probiotics from the genera of lactobacilli and bifidobacteria were evaluated for their viability and growth characteristic in soymilk. Two types of soymilk were evaluated; soymilk supplemented with prebiotics and with B-vitamins. Unsupplemented soymilk was used as the control. In the presence of prebiotics and B-vitamins, all of the probiotic strains studied showed viability exceeding 7 $\log_{10}$ CFU mL^{-1} over 24h. Additionally, the supplementation of prebiotics and B-vitamins also increased the production of lactic acid, and increased activity of α-galactosidase which led to enhanced hydrolysis and utilization of soy oligosaccharides. Probiotic fermented soymilk also showed enhanced ACE-inhibitory activity and lower IC_{50} values, indicating in-vitro antihypertensive properties. The β-glucosidase specific activity of probiotics was also enhanced upon supplementation of prebiotics and B-vitamins, leading to increased bioconversion of isoflavones in soymilk. The concentration of genistin was decreased, accompanied by an increased concentration of genistein upon fermentation by probiotics. Additionally, lower concentrations of malonyl daidzin was also detected, indicating increased hydrolysis of malonyl daidzin to daidzin. Results from our present study indicated that soymilk could act as a good carrier for probiotic microorganisms, with various bioactive properties.

Abstrak Penyelidikan

(Perlu disediakan di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan juga Bahasa Ingge** Abstrak ini akan dimuatkan dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu ca untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & masyarakat luar).

Objektif utama projek ini adalah untuk mengkaji pertumbuhan dan biokonversi isoflavon oleh probiotik di dalam susu soya, dan pencirian susu soya terfermen tersebut semasa penstoran. Probiotik daripada genera laktobasili dan bifidobakteria telah dikaji untuk keterusan hidup dan ciri-ciri pertumbuhan di dalam susu soya. Dua jenis susu soya digunakan; susu soya yang ditambah dengan prebiotik dan yang ditambah dengan vitamin-B. Susu soya tanpa penambahan digunakan sebagai kawalan. Dengan penambahan prebiotik dan vitamin-B, semua probiotik yang dikaji memaparkan bilangan sel melebihi 7 $\log_{10}$ CFU mL^{-1} pada 24j. Penambahan prebiotik dan vitamin-B turut meningkatkan penghasilan asid laktik oleh probiotik, dan meningkatkan aktiviti α-galaktosidase yang meningkatkan hidrolisis dan penggunaan oligosakarida soya. Susu soya terfermen probiotik juga menunjukkan peningkatan aktiviti penindasan ACE dan penurunan kesan IC_{50}, yang menunjukkan ciri antihipertensi in-vitro. Aktiviti spesifik β-glukosidase probiotic turut meningkat selepas penambahan prebiotik dan vitamin-B, menyebabkan peningkatan dalan biokonversi isoflavon dalam susu soya. Kepekatan genistin telah menurun, manakala kepekatan genistein meningkat selepas fermentasi oleh probiotic. Tambahan, kepekatan malonyl daidzin turut berkurangan, menunjukkan peningkatan hidrolisis malonyl daidzin kepada daidzin. Data daripada projek ini menunjukkan bahawa susu soya dapat bertindak sebagai pembawa yang baik untuk mikroorganisma probiotik, dengan tambahan kesan-kesan bioaktif.

H. **a) Results/Benefits of this research**
Hasil Penyelidikan

No. *Bil:*	Category/Number: *Kategori/ Bilangan:*	Promised	Achieved
1.	**Research Publications (Specify target journals)** *Penerbitan Penyelidikan (Nyatakan sasaran jurnal)* 1. Ng, KH, Lye, HS, Easa, AM and Liong, MT. 2008. Growth characteristics and bioactivity of probiotics in tofu-based medium during storage. **Annal Microbiol** 58: 477-487. **2.** Fung, WY, Woo, YP, and Liong, MT. 2008. Optimization of Growth of *Lactobacillus acidophilus* FTCC 0291 and evaluation of growth characteristics in soy whey medium: a response surface methodology approach. **J Agri Food Chem** 56: 7910-7918. 3. Liong, MT, Easa, AM, Lim, PT and Kang, JY. 2009. Survival, growth characteristics and bioactive potential of *Lactobacillus acidophilus* in a soy-based cream cheese. **J Sci Food Agri** 89: 1382-1391. **4.** Woo, YYP, Fung, WY, Easa, AM and Liong, MT. 2009. Probiotic-fermented soyfoods: Benefits and enhanced bioactivities. **Acta Alimentaria Hung** 38: 381-391. **5.** Lye, HS, Kuan, CY, Ewe, JA, Fung, WY and Liong, MT. 2009. The improvement of hypertension by probiotics: effects on cholesterol, diabetes, renin and phytoestrogens. **Int J Mol Sci** 10: 3755-3775 6. Zulkurnain, M, Goh, MH, Karim, AA and Liong, MT. 2008. Development of a soy-based cream cheese. **J Texture Stud** 39: 635-654. 7. Teh, SS, Rosma, A, Wan Nadiah, WA and Liong, MT. 2009. Evaluations of Agrowastes as Immobilizers for Probiotics in Soymilk. **J Agri Food Chem** 57: 10187-10198 **8.** Fung, WY and Liong, MT. 2009. A Response Surface Analysis of In vitro Antihypertensive Property of *L. acidophilus*-fermented Soy Whey. **LWT Food Sci Technol** 43: 563-567. **9.** Yeo, SK and Liong, MT. 2010. Effect of Prebiotics on the Viability and Growth Characteristics of Probiotics in Soymilk. **J Sci Food Agri** 90: 267-275. 10. Ewe, JA, Wan Nadiah, WA and Liong, MT. 2010. Viability and Growth Characteristics of *Lactobacillus* and *Bifidobacterium* in Soymilk Supplemented with B-vitamins. **Int J Food Sci Nutri** 61: 87-107 **11.** Teh, SS, Rosma, A, Wan Nadiah, WA and Liong, MT. 2010. Enhanced Growth of Lactobacilli in Soymilk at Room Temperature upon Immobilization on Agrowastes. **J Food Sci** 75: M155-M164(1) **12.** Teh, SS, Rosma, A, Wan Nadiah, WA and Liong, MT. 2010. Growth Characteristics of Agrowastes-Immobilized Lactobacilli in Soymilk during Refrigerated Storage. **Int J Food Sci Tech** (Revision submitted)	**9**	**12**

I. **BUDGET** / *BAJET*

Perbelanjaan :Expenditure

Project Account No.	: 1001 / PTEKIND / 811020
Total Approved Budget	: RM 181,479
Total Additional Budget	: RM 50,000
Grand Total of Approved Budget	: RM 231,479

Yearly Budget Distributed

Year 1	: RM 107,143
Year 2	: RM 38,168
Year 3	: RM 36,168

Additional Budget Approved

Year 1	: RM -
Year 2	: RM 40,000
Year 3	: RM 10,000

Total Expenditure	: RM 229,990.40
Balance	: RM 1488.60

- **Please attach final account statement from Treasury**

Signature of Researcher
Tandatangan Penyelidik

DR. LIONG MIN TZE
Ph.D. (Melb) Australia
School of Industrial Technology
Universiti Sains Malaysia. (USM)
11800 Penang, Malaysia

22/6/10

Date
Tarikh

Annals of Microbiology, **58 (3)** 477-487 (2008)

Growth characteristics and bioactivity of probiotics in tofu-based medium during storage

Keat-Hui NG, Huey-Shi LYE, Azhar-Mat EASA, Min-Tze LIONG*

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Received 21 March 2008 / Accepted 26 June 2008

Abstract - *Lactobacillus acidophilus* FTCC 0291, *Lactobacillus bulgaricus* FTCC 0411, *Lactobacillus casei* FTCC 0442, *Lactobacillus fermentum* FTD 13 and *Bifidobacterium bifidum* BB 12 were screened for their α-galactosidase activity over 24 h. *Lactobacillus bulgaricus* FTCC 0411 and *L. fermentum* FTD 13 showed highest α-galactosidase specific activity and were selected to be incorporated into tofufa for a storage study of 9 days at 25 and 4 °C. The viability of both probiotics in tofufa exceeded 10^6 CFU/g and was maintained over storage, mainly contributed by their ability to hydrolyse oligosaccharides and to utilise the reducing sugars produced. The presence of probiotics in tofufa showed an increase in the concentrations of organic acids which led to a decrease in pH levels. This exhibited a preservative effect, where total aerobes and total anaerobes were 2 $\log_{10}$ CFU/g lower than the control. Probiotics in tofufa also liberated peptides with angiotensin-converting enzyme (ACE) inhibitory properties. Consistent viability and high proteolytic activity of probiotics in tofufa during storage suggested that tofufa is a suitable carrier for live probiotics with bioactive potential.

Key words: *Lactobacillus*, soy, antibacterial, ACE inhibition, oligosaccharides.

INTRODUCTION

Probiotics are 'live microorganisms that are used as dietary supplements with the aim of benefiting the health of consumers by positively influencing the intestinal microbial balance' (Crittenden *et al.*, 2001). Viable probiotic organisms in foods have several scientifically established and/or clinically proven health effects, such as reduction and prevention of diarrhoeas of different origins, improvement of the intestinal microbial balance, alleviation of lactose intolerance, enhancement of immune potency, antitumorigenic activities and antihypertensive properties (Liong, 2007).

Tofufa is a soft form of tofu that is generally consumed as an Asian dessert and served with sugar syrup. It is also known as tofu-pudding and made via coagulation of soymilk. Soy products have an excellent nutritional status based on their high protein content and all the essential amino acids to meet biological requirements when consumed at the recommended level of protein intake (Naganagouda and Mulimani, 2006). In addition, it contains no cholesterol or lactose and only a small quantity of saturated fatty acid, and is suitable for those who are lactase-deficient (Wang *et al.*, 2002). Moreover, soy-based foods also provide a range of health benefits to consumers due to their antihypertensive, hypocholesterolemic and antiatherogenic properties, and reduced allergenicity (Donkor *et al.*, 2007).

Various food products have been developed as carriers for probiotics, mainly of dairy origins because consumers commonly associate fermented dairy products with live cultures and perceive a benefit in the presence of these cultures (Sanders, 2000). The benefits of soy have attracted much attention recently and numerous other soy products have been evaluated as possible probiotic vehicles. Donkor *et al.* (2005) found that the protein in fermented soymilk could encourage the growth of probiotic strains such as *Lactobacillus acidophilus, Lactobacillus casei* and *Streptococcus thermophilus*. In addition, the strains reportedly could release bioactive peptides with angiotensin-converting enzyme (ACE) inhibitory activities ranging from 17% and 43%. Hsieh and Chou (2006) found that probiotic in soymilk produced α-galactosidase enzyme (that could not be produced by human) which reduced the concentration of flatulence-causing oligosaccharides. Moreover, *Lactobacillus* ssp. and bifidobacteria have been found to act as active ingredients in probiotic food such as bioyoghurt, dietary adjuncts and health-related products. Shimakawa *et al.* (2003) reported that *Bifidobacterium breve* strain Yakult was capable of growing in soymilk and reached a growth of 10^9 CFU/ml with unchanged viability during storage at 10 °C for 20 days. In another study, Farnworth *et al.* (2007) showed that the growths of lactobacilli and bifidobacteria in soymilk and soy yogurts produced a faster drop in pH during fermentation than in cows' milk, as a result of their ability to use different sugars in soy media. However, there has yet to be any published data to our knowledge that utilises tofufa as a carrier, and the viability of probiotics in such a medium remains unknown. In addition, tofufa is normally preservative-free and thus, highly perishable due to its high microbial loads. The antimicrobial property of probiotics could shed some light at prolonging the shelf-life of tofufa in the absence of preservatives.

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JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Optimization of Growth of *Lactobacillus acidophilus* FTCC 0291 and Evaluation of Growth Characteristics in Soy Whey Medium: A Response Surface Methodology Approach

WAI-YEE FUNG, YUH-PING WOO, AND MIN-TZE LIONG*

School of Industrial Technology, Food Technology Division, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia

Four strains of probiotics were evaluated for their α-galactosidase activity. *Lactobacillus acidophilus* FTCC 0291 displayed the highest specific α-galactosidase activity and was thus selected to be optimized in soy whey medium supplemented with seven nitrogen sources. The first-order model showed that meat extract, vegetable extract, and peptone significantly ($P < 0.05$) influenced the growth of *L. acidophilus*. The second-order polynomial regression estimated that maximum growth was obtained from the combination of 7.25% (w/v) meat extract, 4.7% (w/v) vegetable extract, and 6.85% (w/v) peptone. The validation experiment showed that response surface methodology was reliable with a variation of only 1.14% from the actual experimental data. Increased utilization of oligosaccharides and reducing sugars contributed to increased growth of *L. acidophilus* in the soy whey medium. This was accompanied by increased production of short-chain fatty acids and a decrease in pH.

KEYWORDS: ***Lactobacillus*; growth; soy whey; RSM; optimization**

INTRODUCTION

Probiotics are defined as viable microorganisms that when administered in adequate amounts promote or support a beneficial balance of the autochthonous microbial population of the gastrointestinal tract (*1*). *Lactobacillus* and *Bifidobacterium* are the most common probiotic bacteria used as food adjuncts. A number of health benefits have been reported upon consumption of probiotic organisms including antimicrobial activity, alleviation of lactose intolerance, antidiarrheal properties, anticarcinogenic properties, and modulation of immune system and hypocholesterolemia (*2*). However, commercially available probiotic supplements are often costly, mainly due to their expensive production processes and media.

Soy is rich in protein and has been associated with hypocholesterolemia, reduced risks of atherosclerosis, cancer, hypertension, osteoporosis, and alleviation of postmenopausal symptoms. Strains of probiotics have been shown to grow well in soy milk, being able to utilize soy oligosaccharides and producing lower amounts of short-chain fatty acids (SCFA).

Approximately 225.6 million metric tons of soybeans is produced annually worldwide (*3*). Disposal of soybean wastes is often costly and conjures environmental issues. Soy whey is the liquid waste from pressing of coagulated soy milk, produced abundantly during the manufacture of tofu. Soy whey has a rich nutritive content comprising protein, fat, starch, and sugars. Most of the sugars comprise stachyose (6.4 g/L), raffinose (1.6 g/L), sucrose (11.3 g/L), fructose (1.1 g/L), and glucose (1.2 g/L) (*5*). Much of the oligosaccharides are carried in the whey, whereas the protein content is lower due to partial precipitation during the coagulation of soy milk. The total nitrogen was reported to be 0.82 g/L, suggesting a protein content of 5.1 g/L (*4*). Thus, with proper evaluation and development, soy whey could be utilized as a high-quality microbial fermentation medium to harvest various nutrients, enzymes, and microorganisms.

Response surface methodology (RSM) has become increasingly favorable in optimizing compositions of microbiological media, parameters for food processes, and enzyme hydrolysis. RSM is robust and effective in analyzing responses that are affected by many factors and their interactions. In optimization processes, RSM is less time-consuming and tedious compared to the conventional one-factor-at-a-time method (*5*).

Thus, the aim of this study was to optimize the growth of a probiotic in soy whey medium supplemented with nitrogen sources using RSM. In addition, the growth properties of the probiotic in soy whey medium such as pH, production of acids, and utilization of sugars within the optimized region for maximum growth were also evaluated.

MATERIALS AND METHODS

Bacteria and Medium Preparation. Strains of *Lactobacillus acidophilus* FTCC 0291, *Lactobacillus casei* FTCC 0442, *Lactobacillus fermentum* FTD 13, and *Bifidobacterium bifidum* BB12 were obtained

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Research Article

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Survival, growth characteristics and bioactive potential of *Lactobacillus acidophilus* in a soy-based cream cheese

Min-Tze Liong,* Azhar Mat Easa, Poh-Tuan Lim and June-Yan Kang

Abstract

BACKGROUND: Soy-based products have received much attention lately as dairy replacers and carriers for probiotics, without the cholesterol and lactose intolerance factors. We have previously developed a soy cream cheese product and would like to evaluate its suitability as a carrier for probiotic microorganisms. Soy cream cheese is commercially uncommon, while a probiotic soy cream cheese is yet to be available in the market.

RESULTS: Five strains of probiotics were screened for their α-galactosidase activity. *Lactobacillus acidophilus* FTCC 0291 showed the highest α-galactosidase-specific activity and was incorporated into soy cream cheese for a storage study of 20 days at 25 and 4 °C. *L. acidophilus* FTCC 0291 in soy cream cheese at both storage temperatures maintained a viability exceeding 10^7 CFU g^{-1} over storage. Oligosaccharide and reducing sugar analyses indicated that *L. acidophilus* FTCC 0291 was capable of utilizing the existing reducing sugars in soymilk and concurrently hydrolyzing the oligosaccharides into simpler sugars for growth. *L. acidophilus* FTCC 0291 also produced organic acids, leading to decreased pH. Under low pH and high organic acid concentration, the growth of total aerobes and anaerobes was significantly ($P < 0.05$) suppressed compared to the control. The hydrolysis of protein in soymilk produced essential growth factors such as peptides and amino acids that may have promoted the growth of *L. acidophilus* FTCC 0291 and the release of bioactive peptides with *in vitro* angiotensin I-converting enzyme inhibitory activity.

CONCLUSION: This study showed that soy cream cheese could be used as a carrier for probiotic bacteria, with potential antihypertensive property.

Keywords: *Lactobacillus*; growth; soy; cream cheese; ACE

INTRODUCTION

Probiotic bacteria are defined as 'live microorganisms that when administered in adequate amounts could confer a health benefit on the host'.[1] Dairy products containing probiotic cultures such as bifidobacteria, *Lactobacillus acidophilus* and *Lactobacillus casei* have been found to alleviate lactose intolerance, inhibit pathogenic microorganisms and viruses, produce vitamins, reduce cholesterol levels, inhibit tumors, improve immune response, stabilize the gut mucosal barrier and prevent diarrhea.[2] To perform their claimed benefits, these bacteria must be present beyond 10^6 log CFU g^{-1} at the time of consumption and remain viable in the distal part of the gastrointestinal tract.[2]

Most of the commercially available probiotic carriers are dairy-based products, such as yoghurt, fermented milk, ice cream and cheeses. One of the main setbacks of consuming dairy products by those with deficiency in the enzyme β-galactosidase is lactose intolerance, in which lactose cannot be digested and absorbed, leading to abdominal cramping, bloating, diarrhea and nausea.[3] Infants who are not breast-fed but dependent on cow's milk formula have also been found to develop allergy towards cow's milk proteins. Recently, the production of soy-based products, such as soy yoghurt and soy ice creams, as an alternative to dairy-based products has attracted much attention.[4] In addition to their lactose-free and reduced fat content, these soy-based products are also a good source of protein and have been reported to exert health benefits due to their hypolipidemic, reduced allergenicity, anticholesterolemic and antiatherogenic properties.[5] However, considerable amounts of flatulence-causing raffinose oligosaccharides (ROs) in soy foods such as raffinose and stachyose limit their biological value and acceptability.[6] The hydrolysis of these oligosaccharides requires α-galactosidase, an enzyme that hydrolyzes the α-galactoside bond found in these indigestible sugars.

Fermentation of soy products with organisms that possess high α-galactosidase activity has been found to minimize the content of flatulence-causing oligosaccharides in soy products.[7] Bifidobacteria and lactobacilli such as *Lactobacillus fermentum*[8] and *Lactobacillus acidophilus*[9] have been reported to produce varying levels of α-galactosidase. In addition, probiotics have been reported to exhibit proteolytic activity to produce shorter-chain peptides and amino acids to meet their requirement

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PROBIOTIC-FERMENTED SOYFOODS: BENEFITS AND ENHANCED BIOACTIVITIES

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(Received: 16 June 2008; accepted: 17 November 2008)

The consumption of soy has long been associated with various health-enhancing effects including hypocholesterolemia, antihypertension, alleviation of post-menopausal symptoms, and reducing risks of ovarian cancer and cardiovascular diseases. Past studies have indicated that these effects are mediated by bioactive components of soy such as isoflavones and bioactive peptides. However, some of these bioactive components could only confer health benefits upon bioconversion by gastrointestinal microorganisms in the intestines. This has led to increased interest to evaluate the possibility of utilizing probiotics to enhance the bioactivities of soyfoods. Probiotics are live microorganisms that could exert health benefits on the host when administered in adequate amounts. Probiotic-fermented soyfoods have been shown to have increased antihypertensive properties and could better alleviate menopausal associated disorders. However, this area is relatively new and although seemed promising, most of the recent findings have emphasized on in vitro evaluations, while the lack of in-vivo evidence and/or incompatible outcomes between in vitro experiments and in-vivo trials has led to the need to better understand the exact mechanisms involved. This present review highlights some of the benefits of soy and addresses the currently investigated bioactivities of probiotic-fermented soyfoods.

Keywords: soy, isoflavones, probiotic, antihypertension, post-menopausal symptoms, hypercholesterolemia, bioactive peptides

Soy (*Glycine max*) is a legume indigenous to East Asia, although the main cultivation areas are now North America. The world produces an average of 225.6 million metric tons of soybeans annually (DORFF, 2007). The USA produced 86.77 million metric tons of soybeans that accounted for 42% of the world's soybean trade annually. Other top producing nations include Canada, Brazil, Argentina, China, India, Paraguay, Bolivia,

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Review

The Improvement of Hypertension by Probiotics: Effects on Cholesterol, Diabetes, Renin, and Phytoestrogens

Huey-Shi Lye, Chiu-Yin Kuan, Joo-Ann Ewe, Wai-Yee Fung and Min-Tze Liong *

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Received: 28 June 2009; in revised form: 30 July 2009 / Accepted: 10 August 2009 / Published: 27 August 2009

Abstract: Probiotics are live organisms that are primarily used to improve gastrointestinal disorders such as diarrhea, irritable bowel syndrome, constipation, lactose intolerance, and to inhibit the excessive proliferation of pathogenic intestinal bacteria. However, recent studies have suggested that probiotics could have beneficial effects beyond gastrointestinal health, as they were found to improve certain metabolic disorders such as hypertension. Hypertension is caused by various factors and the predominant causes include an increase in cholesterol levels, incidence of diabetes, inconsistent modulation of renin and imbalanced sexual hormones. This review discusses the antihypertensive roles of probiotics via the improvement and/or treatment of lipid profiles, modulation of insulin resistance and sensitivity, the modulation of renin levels and also the conversion of bioactive phytoestrogens as an alternative replacement of sexual hormones such as estrogen and progesterone.

Keywords: probiotic; antihypertension; cholesterol; diabetes; renin; phytoestrogen

1. Introduction

Probiotics are viable microorganisms that confer health benefits to the host once consumed in adequate amounts, primarily by promoting the proliferation of beneficial gastrointestinal indigenous microflora. Various microorganisms have been found to posses such properties, although *Lactobacillus* and *Bifidobacterium* are the most common probiotic bacteria used as food adjuvants. A

DEVELOPMENT OF A SOY-BASED CREAM CHEESE

MUSFIRAH ZULKURNAIN, MEI-HUANG GOH, ABDUL ALIAS KARIM and MIN-TZE LIONG[1]

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Accepted for Publication May 16, 2008

ABSTRACT

Various amounts of blended tofu, oil, salt, carrageenan, pectin and maltodextrin were used to develop soy cream cheese products that were trans fat free, with texture properties similar to those of commercial dairy cream cheese. Response surface methodology predicted three formulations with similar spreadability value of that obtained from commercial cheese. Carrageenan and salt were useful to impart the desired firmness, while maltodextrin provided body to the product. Pectin introduced viscous behavior to the final texture but was proven useful in preventing syneresis. Rheological study at 25 and 4C manifested that, although the developed products mimicked the texture of the commercial sample, they had less elasticity. Chemical analysis revealed that the soy cream cheeses had lower fat and higher protein and fiber contents than the commercial sample. They were also stable against syneresis and oil separation over storage of 20 days.

PRACTICAL APPLICATIONS

The production of soy-based products as alternatives to dairy products has attracted much attention recently. In addition to their lactose-free and reduced fat content, soy-based products are also a good source of protein. There has yet to be any published data on the development of a soy-derivative cream cheese. This paper emphasizes the rheological development of soy cream cheeses in the presence of food hydrocolloids. Their main and interaction effects on texture properties were assessed to mimic those of dairy cream cheese. A comprehensive and systematic approach of formulating the soy cream cheeses was applied by using response surface methodology.

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JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 10187–10198 10187
DOI:10.1021/jf902003a

Evaluation of Agrowastes as Immobilizers for Probiotics in Soy Milk

SUE-SIANG TEH, ROSMA AHMAD, WAN-NADIAH WAN-ABDULLAH, AND MIN-TZE LIONG*

School of Industrial Technology, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia

The objective of this study was to evaluate agricultural wastes as immobilizers for probiotics in liquid foods, such as soy milk. Probiotic strains were initially evaluated for acid and bile tolerance and the ability to produce α-galactosidase. Rinds of durian, mangosteen, and jackfruit were dried, ground, and sterilized prior to immobilization of selected strains (*Lactobacillus acidophilus* FTDC 1331, *L. acidophilus* FTDC 2631, *L. acidophilus* FTDC 2333, *L. acidophilus* FTDC 1733, and *Lactobacillus bulgaricus* FTCC 0411). Immobilized cells were inoculated into soy milk, and growth properties were evaluated over 168 h at 37 °C. Soy milk containing free cells without agrowastes was used as the control. Immobilized probiotics showed increased growth, greater reduction of stachyose, sucrose, and glucose, higher production of lactic and acetic acids, and lower pH in soy milk compared to the control. The results illustrated that agrowastes could be used for the immobilization of probiotics with enhanced growth, utilization of substrates, and production of organic acids.

KEYWORDS: Soy milk; probiotic; agrowastes; immobilization; durian; cempedak; mangosteen

INTRODUCTION

Probiotics are microorganisms that possess various benefits to the host upon consumption and have been reported to eliminate the growth of pathogens, alleviate lactose tolerance, decrease serum cholesterol levels, and reduce the risks of cancer (*1*).

Agrowastes can be defined as wastes generated from animals and plants agriculturally such as leaves, roots, hulls, manures, and plant fibers. Although agrowastes are not classified as hazardous wastes, they are produced abundantly from crops each year, leading to environmental and economical issues. For example, 880 million tons of cereals are produced worldwide annually, of which 550 million tons are wheat straw, whereas approximately 700,000 tons of okara are produced annually from the production of tofu in Japan (*2*). More than 6 million hectares of land in Malaysia is utilized for major crops such as oil palm, rubber, paddy, coconut, and cocoa, yet only 24.5% of the total agricultural biomass is used for energy consumption and the rest is left as wastes. There are approximately 17000 ha of land in Malaysia that are utilized for the cultivation of fruit, producing approximately 0.25 million tonnes of fruits (*3*). However, only 20% of the whole fruit is edible, whereas the skin, core, base, and rind are normally discarded as wastes. Due to increased economical and environmental concerns, agrowastes are used as bedding for animals and livestock feeding or added into soil as green fertilizer. Panthapulakkal and Sain have previously documented other uses of agrowastes such as soil conditioners or fertilizers, biofuels, thermoplastics, activated charcoal, and components of other composite materials (*4*). Fruit agrowastes are often rich in dietary fiber and sugars and could be used as substrates for microorganisms. Wastes from the pineapple fruit have been used as fermentation broth for the cultivation of *Saccharomyces cerevisiae* and *Candida utilis* (*3*), whereas sago waste and palm oil sludge have been used for the cultivation of *Myceliophthora thermophila* and *Trichoderma harzianum*, respectively, in submerged fermentation (*5*). However, limited information is available on the use of agrowastes in solid state fermentation, whereas their potential as immobilizers for probiotics has not been evaluated. To our knowledge, this is the first study to evaluate such a property.

The viability of probiotics should exceed 10^7 CFU/g of product to exhibit therapeutic effects in the host and maintained at a minimum level of 10^6 CFU/g to be recognized as a probiotic food. Stresses to organisms begin in the stomach, in the presence of acids and with pH between 1.5 and 3.0, and in the upper intestines that contain bile (*6*). The time from entrance to release from the stomach has been estimated to be approximately 90 min, with further digestive processes requiring longer residence time (*7*). Additionally, it has been found that free cells of probiotics without protection in food matrices such as yogurt do not have a long shelf life containing the required level of total viable counts (*8*). Thus, many methods have been developed to enhance the viability of probiotics, such as microencapsulation (*9*), stress adaptation, mutation, alginate coating (*10*), and lyophilization. However, some of these techniques, such as microencapsulation, are less suitable as they involve packing of cells in sealed capsules, which may hinder their release in specific sites in the lower intestines (*11*), whereas lyophilization reduces cell counts during harsh temperature changes. Immobilization has been utilized and was found to increase the growth on cells, enhance their storage stability, and prolong the shelf life of

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Evaluation of proteolytic and ACE-inhibitory activity of *Lactobacillus acidophilus* in soy whey growth medium via response surface methodology

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ABSTRACT

Soy whey is a rich by-product of tofu-manufacturing industries. We have previously optimized the growth of *Lactobacillus acidophilus* FTCC 0291 in soy whey upon supplementation of meat extract, vegetable extract and peptone using response surface methodology (RSM). The present study evaluated the proteolytic and angiotensin-I converting enzyme (ACE)-inhibitory activities of *L. acidophilus* FTCC 0291 in the optimized soy whey medium. The probiotic-fermented soy whey exhibited growth-associated proteolysis and ACE-inhibitory activity. Proteolysis was highly correlated with ACE-inhibitory activity, indicating that peptides liberated via fermentation may have exerted in vitro antihypertensive properties. Of the three nitrogen sources studied, peptone was found to have the highest influence on growth performance and ACE-inhibitory activity. Our results strongly indicated that probiotic-fermented soy whey produced in vitro antihypertensive bioactivity, and hence could be further developed into a carrier for probiotics with enhanced functional properties.

1. Background and objectives

Soy is a versatile crop which has found applications in a myriad of industries including food, textile, printing, and fuel. The annual soybean production in the U.S., the world's leading producer, have increased more than 30 million metric tons from 1986 to 2006 (Soy Stats, 2008) due to increasing demands. Increasing demand also means increasing generation of its wastes. Soybean waste comprises of okara and soy whey, most abundantly generated from soymilk production and the pressing of tofu. With the naturally high content of protein and sugars in soy, including probiotic-fermentable oligosaccharides such as raffinose and stachyose, soy whey could be a good growth medium for cultivation of beneficial probiotics possessing the α-galactosidase enzyme (Donkor, Henriksson, Vasiljevic, & Shah, 2006).

Probiotics are defined as "live organisms, which when consumed in adequate amounts, confer a health effect on the host" (Liong, 2007). Probiotics proliferate well in soymilk as it produces low amounts of acidic metabolites which inhibit growth (Liu 1997). Many strains of probiotics possess α-galactosidase and thus could proliferate well in soymilk. *Lactobacillus paracasei* has been found to proliferate better in soy whey compared to cow's milk (Thi, Champagne, Lee, & Goulet, 2003). Probiotics are generally recognized for its role in maintaining a healthy gut which then influences the health of the host (Ishibashi, Yaeshima, & Hayasawa, 1997). In soymilk, fermentation activity of probiotics reportedly led to the production of bioactive substances including isoflavones and bioactive peptides. The release of bioactive peptides from the proteolysis action of probiotics have been postulated to result in antihypertensive effect by inhibiting the key enzyme responsible for hypertension, angiotensin-I converting enzyme (ACE) (Sentandreu & Toldra, 2007).

Bioactive peptides such as valyl-prolyl-proline (Val-Pro-Pro), isoleucyl-prolyl-proline (Ile-Pro-Pro) and (Tyr-Pro) exhibited ACE-inhibitory activity and blood pressure lowering effect in spontaneously hypertensive rats (Seppo, Jauhiainen, Poussa, & Korpela, 2003). Proteolytic strains of probiotic bacteria were found to release ACE-inhibitor peptides that would further improve the health benefits of probiotic foods (Donkor, Henriksson, Vasiljevic, & Shah, 2005). Proteolytic activity of probiotics on proteins in soymilk or soy yoghurt liberated peptides leading to in vitro ACE-inhibitory effect (Gobbetti, Ferranti, Smacchi, Goffredi, & Addeo, 2000; Donkor et al., 2007; Tsai, Chen, Pan, Gong, & Chung, 2008). *Lactobacillus helveticus* fermented milk containing bioactive peptides have been associated with antihypertensive effect (Seppo et al., 2003), while an earlier study using fractionated whey from soymilk fermented with probiotics, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Bifidobacterium longum* also suggested similarly promising antihypertensive property (Tsai, Lin, Pan, & Chen, 2006).

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Effect of prebiotics on viability and growth characteristics of probiotics in soymilk

Siok-Koon Yeo and Min-Tze Liong*

Abstract

BACKGROUND: Soy products have attracted much attention lately as carriers for probiotics. This study was aimed at enhancing the growth of probiotics in soymilk via supplementation with prebiotics.

RESULTS: *Lactobacillus* sp. FTDC 2113, *Lactobacillus acidophilus* FTDC 8033, *Lactobacillus acidophilus* ATCC 4356, *Lactobacillus casei* ATCC 393, *Bifidobacterium* FTDC 8943 and *Bifidobacterium longum* FTDC 8643 were evaluated for their viability and growth characteristics in prebiotic-supplemented soymilk. In the presence of fructooligosaccharides (FOS), inulin, mannitol, maltodextrin and pectin, all strains showed viability exceeding 7 $\log_{10}$ colony-forming units mL^{-1} after 24 h. Their growth was significantly ($P < 0.05$) increased on supplementation with maltodextrin, pectin, mannitol and FOS. Additionally, supplementation with FOS, mannitol and maltodextrin increased ($P < 0.05$) the production of lactic acid. Supplementation with FOS and maltodextrin also increased the α-galactosidase activity of probiotics, leading to enhanced hydrolysis and utilisation of soy oligosaccharides. Finally, prebiotic supplementation enhanced the utilisation of simpler sugars such as fructose and glucose in soymilk.

CONCLUSION: Supplementation with prebiotics enhances the potential of soymilk as a carrier for probiotics.

Keywords: prebiotics; *Lactobacillus*; *Bifidobacterium*; soymilk; growth

INTRODUCTION

Lactobacillus acidophilus, Lactobacillus casei and *Bifidobacterium* are the major microflora in the human intestinal tract and are common strains of probiotics. Probiotics are defined as 'live micro-organisms which when administered in adequate amount confer a health benefit on the host'.[1] These bacteria are thought to confer health-beneficial effects such as activation of the immune system, anticarcinogenicity, antihypertensivity and regulation of microbial balance in the gut. Owing to their potential health benefits, they are often added to dairy preparations to produce healthy functional products.[2–4] However, the demand for alternatives to dairy products is growing because of problems with allergenicity, cholesterol levels and lactose intolerance and the desire for vegetarian alternatives.[5]

Soybean, a vegetarian food, is well known as an inexpensive, cholesterol- and lactose-free source of protein and calories for human consumption.[6] In addition, soy has been reported to reduce the risk of postmenopausal symptoms, osteoporosis and prostate cancer.[7] Considering the nutritional content of soy, soy products could be substituted for dairy products, and various attempts have been made to develop soy-based products as probiotic dietary adjuncts in recent years. However, the consumption of soy has been limited owing to its undesirable beany flavour and the presence of oligosaccharides (stachyose and raffinose) that often lead to flatulence and stomach discomfort. Oligosaccharides can be hydrolysed by α-galactosidase, an enzyme that is usually deficient in the human intestinal tract. Past studies have demonstrated that probiotics possess varying levels of α-galactosidase. Fung *et al.*[8] demonstrated that probiotics could hydrolyse α-galactosyl oligosaccharides in soy whey, while Liong *et al.*[9] reported that incorporation of probiotics with α-galactosidase activity could increase the hydrolysis of soybean oligosaccharides and consequently reduce their antinutritive properties in soy cream cheese.

Prebiotics are defined as 'non-digestible carbohydrates that beneficially affect the host by selectively stimulating the growth and/or activity of colonic microflora'.[10] Fructooligosaccharides (FOS) and inulin have attracted much attention lately as prebiotics. FOS contains 2–10 fructose units linked by glycosidic bonds, while inulin includes a broad range of fructans with chains of 3–60 units.[11] Another oligosaccharide with prebiotic properties is maltodextrin. Maltodextrins are maltooligosaccharides with a degree of polymerisation ranging from 3 to 9 and often act as flavour enhancers, fat replacers and bulking agents in foods.[12] Recently, polyols such as mannitol have been included in the prebiotic group owing to their indigestibility properties.[13] Pectin, an essential additive in beverages and yogurts, has also been found to exert prebiotic effects.[14]

In order for a probiotic dietary adjunct to confer beneficial effects, the viability of the probiotic organisms should be a minimum concentration of 10^6 colony-forming units (CFU) g^{-1}. It has been suggested that the minimum theurapeutic dose is

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Viability and growth characteristics of *Lactobacillus* in soymilk supplemented with B-vitamins

JOO-ANN EWE, WAN-NADIAH WAN-ABDULLAH & MIN-TZE LIONG

Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, Malaysia

Abstract

Ten strains of *Lactobacillus* were evaluated for their viability in soymilk. *Lactobacillus acidophilus* ATCC 314, *L. acidophilus* FTDC 8833, *L. acidophilus* FTDC 8633 and *L. gasseri* FTDC 8131 displayed higher viability in soymilk and were thus selected to be evaluated for viability and growth characteristics in soymilk supplemented with B-vitamins. Pour plate analyses showed that the supplementation of all B-vitamins studied promoted the growth of lactobacilli to a viable count exceeding 7 log CFU/ml. α-Galactosidase specific activity of lactobacilli as determined spectrophotometrically showed an increase upon supplementation of B-vitamins. High-performance liquid chromatography analyses revealed that this led to increased hydrolysis of soy oligosaccharides and subsequently higher utilization of simple sugars. Production of organic acids as determined via high-performance liquid chromatography also showed an increase, accompanied by a decrease in pH of soymilk. Additionally, the supplementation of B-vitamins also promoted the synthesis of riboflavin and folic acid by lactobacilli in soymilk. Our results indicated that B-vitamin-supplemented soymilk is a good proliferation medium for strains of lactobacilli.

Keywords: *B-vitamins, Lactobacillus, probiotics, soymilk, growth*

Introduction

Strains of lactobacilli such as *L. acidophilus*, *L. casei* and *L. gasseri* are predominant members of the intestinal microflora and have been associated with probiotic properties. Probiotics are defined as 'live organisms that able to confer health effects on the host when consumed in sufficient amounts' (Liong 2007, pp 316–328.). Probiotics are always recognized for their roles in inhibition of intestinal pathogens, prevention and reduction of antibiotic-associated diarrhea, alleviation of lactose intolerance. (Liong 2007; Vasiljevic and Shah 2008).

Soy has been claimed to exhibit health benefits to consumers, and numerous soy-based foods have been evaluated as possible probiotic vehicles (Ng et al. 2008). Soymilk, the water extract of soybean, is a rich source of high-quality protein and amino acid. It contains no cholesterol or lactose and very small amounts of saturated fatty acid (Wang et al. 2002). In addition to these superior nutritional characteristics, it is also considered to have a potential role in the prevention of chronic diseases such as atherosclerosis, cancer, osteoporosis and menopausal disorders (Liu et al. 2002). Soymilk has been used as the culture media for the growth and biochemical activities

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Enhanced Growth of Lactobacilli in Soymilk upon Immobilization on Agrowastes

SUE-SIANG TEH, ROSMA AHMAD, WAN-NADIAH WAN-ABDULLAH, AND MIN-TZE LIONG

ABSTRACT: Cell immobilization is an alternative to microencapsulation for the maintenance of cells in a liquid medium. The objective of this study was to evaluate the effects of agrowastes from durian (*Durio zibethinus*), cempedak (*Artocarpus champeden*), and mangosteen (*Garcinia mangostana*) as immobilizers for lactobacilli grown in soymilk. Rinds from the agrowastes were separated from the skin, dried, and ground (150 μm) to form powders and used as immobilizers. Scanning electron microscopy revealed that lactobacilli cells were attached and bound to the surface of the immobilizers. Immobilized cells of *Lactobacillus acidophilus* FTDC 1331, *L. acidophilus* FTDC 2631, *L. acidophilus* FTDC 2333, *L. acidophilus* FTDC 1733, and *L. bulgaricus* FTCC 0411 were inoculated into soymilk, stored at room temperature (25 °C) and growth properties were evaluated over 168 h. Soymilk inoculated with nonimmobilized cells was used as the control. Utilization of substrates, concentrations of lactic and acetic acids, and changes in pH were evaluated in soymilk over 186 h. Immobilized lactobacilli showed significantly better growth ($P < 0.05$) compared to the control, accompanied by higher production of lactic and acetic acids in soymilk. Soymilk containing immobilized cells showed greater reduction of soy sugars such as stachyose, raffinose, sucrose, fructose, and glucose compared to the control ($P < 0.05$).

Keywords: agrowastes, cempedak, durian, immobilization, lactobacilli, mangosteen, soymilk

Introduction

Lactobacilli are lactic acid bacteria that have been identified for exerting health promoting effects. Previous studies have reported that lactobacilli could provide health enhancing effects to the host such as antimutagenic (Hsieh and Chou 2006), alleviation of lactose intolerance, reduction of cancer risk, decreasing of serum cholesterol level, and antihypertension (Gomes and Xavier 1999).

The Intl. Dairy Federation has stated that lactobacilli should be present at least 10^7 CFU/g in food products to the minimum durability date to provide therapeutic effect to host (Ouwehand and Salminen 1998). However, some studies have documented that total viable count of lactobacilli in some commercial yoghurts does not exceed desired level specified for therapeutic effect (Kailaspathy and Chin 2000). Hence, much efforts have emphasized on increasing the survivability of lactobacilli in food products including the addition of prebiotics such as frutooligosaccharides (FOS) and inulin into food products (Rossi and others 2005), improving the properties of packaging condition (Tanaka and Hatanaka 1992), and buffering capacity effect by adding wheat protein into food products (Ravula and Shah 1998). Although cellular survivability has been found to improve, the effects remain controversial and in some instances, minimal especially in a liquid medium.

Cell immobilization has been used to increase the growth of microorganisms and is defined as entrapment of biomass within various gel matrices such as alginate, agar, gelatin and polyacrylates (Walsh and Malone 1995). Various materials have been used as carriers for cell immobilization such as cryo-polyvinyl-alcohol gels (cryoPVAGs) to immobilize *Saccharomyces cerevisiae* (Lozinsky 1994), natural fibers and artificial fibers as immobilizers for *E. coli* (Chu and others 2009) and polyethyleneimine for baker's yeast cell immobilization (D'Souza and Melo 2001). However, the production of the above carriers required high cost (Lozinsky and Plieva 1998).

Agrowastes are unwanted residues such as roots, pulps, husks, and peels left from agricultural plantation. Agrowastes generated world widely had lead to serious environmental issues (Rodriguez-Couto 2008). Hence, the main objective of this study was to develop immobilizers from agrowastes for lactobacilli in soymilk. Additionally, agrowastes have been reported to contain sugars, fibers, and other nutritional compounds that are easily assimilated by microorganisms for growth (Rodriguez-Couto 2008). Thus, growth characteristics of lactobacilli in soymilk were also evaluated. Most commercially available pasteurized soymilk is stored at room temperature and remains as such unless the package has been unsealed upon consumption. Thus, growth characteristics were evaluated at room temperature (25 °C).

Materials and Methods

Bacterial cultures

Strains of *L. bulgaricus* FTCC 0411, *L. acidophilus* FTDC 2333, *L. acidophilus* FTDC 1733, *L. acidophilus* FTDC 2631, *L. acidophilus* FTDC 1331 were obtained from the Culture Collection Centre of School of Industrial Technology, Univ. Sains Malaysia, Penang, Malaysia. Stock cultures were stored at −20 °C in 40% (v/v) sterile glycerol. Each strain was sub-cultured in sterile de Mann, Rogosa, Sharpe (MRS) broth (Himedia®, Mumbai, India) supplemented with 0.05% (w/v) filter-sterilized (0.20 μm) L-cysteine·HCl (Bioshop®, Burlington, Canada) solution for 3 times and incubated at 37 °C for 20 h prior to use.

Preparation of agrowaste immobilizers

Durian (*Durio zibethinus*), cempedak (*Artocarpus champeden*), and mangosteen (*Garcinia mangostana*) were obtained from local orchards (Penang, Malaysia). The rind portions were cut into

MS 20091052 Submitted 10/23/2009, Accepted 1/7/2010. *Authors are with School of Industrial Technology, Univ. Sains Malaysia, 11800 Penang, Malaysia. Direct inquiries to author Liong (E-mail: mintze.liong@usm.my).*

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WOO YUH PING

Penang, Malaysia

Date: 6/5/2008

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FUNG WAI YEE

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6. Pusat Pengajian Ketua Projek: Teknologi Industri Keputusan Peperiksaan: SPM (sila nyuk sijil)
(Lampirkan 1 salinan sijil)
7. (a) Jika Siswazah Lanjutan nyatakan taraf di Institut Pengajian Siswazah: ~~*Penuhmasa/Sambilan*~~
(b) Jika pelajar Penuhmasa, adakah itu merupakan projek tahun akhir: ~~***YA/TIDAK***~~
8. (a) Tajuk Projek: Bioconversion of isoflavone in soymilk by lactobacilli & Bifidobacteria
(b) Ketua Projek: Dr. Liong Min Tze
(c) Sumber Pembiayaan Projek: ***USM JANGKA PENDEK / FUNDAMENTAL (FRGS)/ FUNDAMENTAL (TOP DOWN) / IRPA KATEGORI EA/PR/SR / GERAN LUAR*** (Rm-USM)
(d) No. Akaun Projek: 1001/PTEKIND/811070
9. Gaji Harian: RM 25 x 30 hari = RM 750 + (Imbuhan Tetap Khidmat Awam) = RM _____
Gaji Bulan : RM _____ + (Imbuhan Tetap Khidmat Awam) = RM _____
10. No. Akaun Bank (BCB/Muamalat) Kakitangan Projek: 1243006724352O
11. Faedah Persaraan/KWSP 11% : 12% No. KWSP: _____
*(Sila Lihat Nota**)*
12. No. PERKESO: _____
(Sila sertakan 2 salinan Kad Pengenalan bersama)

BORANG KG 1-3

UNIVERSITI SAINS MALAYSIA

Have to cancel.

KENYATAAN GAJI KAKITANGAN PROJEK SAMBILAN
(HENDAKLAH DIISI DALAM *5 SALINAN* DAN KEMBALIKAN KE PEJABAT PENGURUSAN & KREATIVITI PENYELIDIKAN, BANGUNAN CANSELORI, ARAS 6)

Bil: 1221 - 2008 Tarikh: 19/8/08

Kepada : **BENDAHARI**

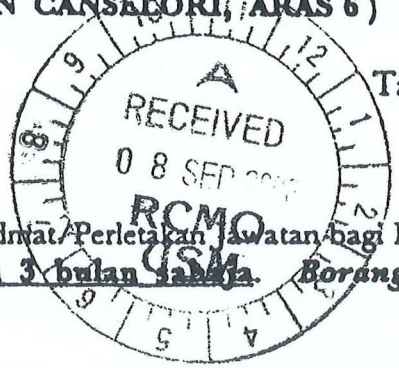
Perlantikan Baru/Perubahan Semasa Berkhidmat/Perletakan Jawatan bagi Kakitangan-Kakitangan Projek Sambilan Untuk Jangkamasa **Maksimum 3 bulan sahaja**. ***Borang ini hanya untuk calon warganegara Malaysia sahaja.***

1. Nama: KUAN YAU HOONG (HURUF BESAR) Jantina:L/P:- L
2. Jawatan: STUDENT ASSISTANT *No.Matrik Pelajar:
3. Alamat Tetap: 47, JALAN RAJA GOPAL, PULAU TIKUS, 10350 GEORGETOWN, PULAU PINANG.
4. Tarikh Lahir: 16/10/1984 No.K/P (Baru): 841016-07-5485
5. Jangkamasa Perlantikan: 25/8/08 Hingga: 15/9/08
6. Pusat Pengajian Ketua Projek: TEKNOLOGI INDUSTRI Keputusan Peperiksaan: BSc. *(Lampirkan 1 salinan sijil)*
7. (a) Jika Siswazah Lanjutan nyatakan taraf di Institut Pengajian Siswazah: *Penuhmasa/Sambilan*
   (b) Jika pelajar Penuhmasa, adakah itu merupakan projek tahun akhir: ***YA/TIDAK***
8. (a) Tajuk Projek: Effects of a synbiotic product on serum lipid profiles and morphology of red blood cells in human.
   (b) Ketua Projek: Dr. Liong Min Tze
   (c) Sumber Pembiayaan Projek: ***USM JANGKA PENDEK / FUNDAMENTAL (FRGS)/ FUNDAMENTAL (TOP DOWN) / IRPA KATEGORI EA/PR/SR / GERAN LUAR***
   (d) No. Akaun Projek: 305/PTEKIND/613218
9. Gaji Harian: RM 25/~~x to hour~~ = ~~RM400~~ + (Imbuhan Tetap Khidmat Awam) = RM —
   Gaji Bulan : RM — + (Imbuhan Tetap Khidmat Awam) = RM —
10. No. Akaun Bank (BCB/CIMB Muamalat) Kakitangan Projek: 0713-0065356-52-6
11. Faedah Persaraan/KWSP 11% : 12% No. KWSP: 16618612 *(Sila Lihat Nota**)*
12. No. PERKESO: —
    (Sila sertakan 2 salinan Kad Pengenalan bersama)

BORANG KG 1-3

UNIVERSITI SAINS MALAYSIA

KENYATAAN GAJI KAKITANGAN PROJEK SAMBILAN
(HENDAKLAH DIISI DALAM *5 SALINAN* DAN KEMBALIKAN KE PEJABAT PENGURUSAN & KREATIVITI PENYELIDIKAN, BANGUNAN CANSELORI, ARAS 6)



Bil: 1325-2008 Tarikh: 3/9/08

Kepada : **BENDAHARI**

Perlantikan Baru/Perubahan Semasa Berkhidmat/Perletakan Jawatan bagi Kakitangan-Kakitangan Projek Sambilan Untuk Jangkamasa **Maksimum 3 bulan sahaja**. ***Borang ini hanya untuk calon warganegara Malaysia sahaja.***

1. Nama: LIM TING JIN (HURUF BESAR) Jantina: L/~~P~~ L
2. Jawatan: Pembantu Tadbir Rendah (N11) *No.Matrik Pelajar: 86307
3. Alamat Tetap: NO. 11, LORONG SEKILAU 48, TAMAN MAHKOTA, BUKIT SEKILAU, 25300 KUANTAN, PAHANG DARUL MAKMUR.
4. Tarikh Lahir: 27/01/1985 No.K/P (Baru): 850127-06-5575
5. Jangkamasa Perlantikan: 02/09/2008 Hingga: ~~2/11/2008~~ 1/12/08
6. Pusat Pengajian Ketua Projek: TEKNOLOGI INDUSTRI Keputusan Peperiksaan: SPM
   (Lampirkan 1 salinan sijil)
7. (a) Jika Siswazah Lanjutan nyatakan taraf di Institut Pengajian Siswazah: *Penuhmasa/~~Sambilan~~*
   (b) Jika pelajar Penuhmasa, adakah itu merupakan projek tahun akhir: ***~~YA~~/TIDAK***
8. (a) Tajuk Projek: Evaluation of the bioconversion of isoflavones by probiotics soymilk and characterization of the fermented soymilk during storage
   (b) Ketua Projek: Dr. Liong Min Tze
   (c) Sumber Pembiayaan Projek: ***USM JANGKA PENDEK / FUNDAMENTAL (FRGS)/ FUNDAMENTAL (TOP DOWN) / IRPA KATEGORI EA/PR/SR / GERAN LUAR***
   (d) No. Akaun Projek: 1001/PTEKIND/811020
9. Gaji Harian: RM ~~1,104.59~~ 709.59 + (Imbuhan Tetap Khidmat Awam) = RM 95/-
   Gaji Bulan : RM ~~1,104.59~~ + (Imbuhan Tetap Khidmat Awam) = RM 300/-
10. No. Akaun Bank (BCB/~~Muamalat~~) Kakitangan Projek: 0709006877522
11. Faedah Persaraan/KWSP 11% : 12% No. KWSP: 1818026
    *(Sila Lihat Nota**)*
12. No. PERKESO: —
    (Sila sertakan 2 salinan Kad Pengenalan bersama)

MEETING and VISIT

jitbiotechnology jitbiotechnology [jitbiotechnology@gmail.com]

Sent: Sunday, May 23, 2010 2:32 PM

To: Liong Min Tze

Cc: Prof Dr Peh [kkpehken@gmail.com]

Dr Liong Min Tze
Senior Lecturer
School of Industrial Technology
Universiti Sains Malaysia
11800 Penang
Malaysia

Dear Dr Liong

Due to our frutiful meeting earlier with YBhg Datuk Johari Ghani, CEO of CIHB / Permanis Sdn Bhd, I would like to invite you for subsequent meetings and plant site visits to better understand the processes for the probiotic product development.

Details of the meetings are as follows:

Date/Time:
May 25, 2:30 pm (meeting; JIT Biotech)

Date/Time:
June 7, 2:30 pm (plant visit: Twister)

Date/Time:
June 18, 3:00 pm (plant visit: cincau and soymilk)

Date/Time:
June 24, 3:00 pm (meeting; CI Holding Berhad)

I appreciate if you can confirm your attendance soonest.

Thank you.

Regards
Shamsul Bahari
Managing Director
JIT Biotechnology Sdn Bhd

UNIVERSITI SAINS M
JABATAN BENDA
SUB KUMP WANG UNIV PEN
PENYATA PERBELANJAAN P

NAMA PROJEK :

EVALUATION OF THE BIOCONVERSION OF ISOFLAVONES BY PROBIOTICS SOYMILK AND CHARACTERIZATION OF THE FERMENTED SOYMILK DURING STORAGE
TEMPOH :
KETUA PROJEK : DR. LIONG MIN TZE
PUSAT PENGAJIAN TEKNOLOGI INDUSTRI

AKAUN	PTJ	PROJEK	DONOR	PERUNTUKAN PROJEK	PERBELANJAAN TERKUMPUL SEHINGGA THN LALU
111	PTEKIND	811020		59,004.00	23,650.49
221	PTEKIND	811020		11,000.00	9,554.16
222	PTEKIND	811020		0.00	346.00
223	PTEKIND	811020		1,500.00	69.10
226	PTEKIND	811020		3,000.00	0.00
227	PTEKIND	811020		45,500.00	58,312.87
228	PTEKIND	811020		6,000.00	0.00
229	PTEKIND	811020		3,000.00	15,654.07
335	PTEKIND	811020		102,475.00	109,045.00
552	PTEKIND	811020		0.00	209.97
				231,479.00	216,841.66

SENARAI JUMLAH-JUMLAH KECIL :

110	EMOLUMEN	59,004.00	23,650.49
220	PERKHIDMATAN DAN BEKALAI	70,000.00	83,936.20
330	ASET	102,475.00	109,045.00
550	PERBELANJAAN LAIN	0.00	209.97
		231,479.00	216,841.66

Dokumen ini adalah cetakan komputer dan tiada tandatangan diperlukan

LAYSIA"

ARI

LIDIKAN (1001)

DA 31 MEI 2010

Tarikh Laporan : 01/06/2010

ERUNTUKAN SEMASA	TANGUNGAN SEMASA	BAYARAN SEMASA	BELANJA SEMASA	BAKI PROJEK
35,353.51	0.00	0.00	0.00	35,353.51
1,445.84	0.00	0.00	0.00	1,445.84
-346.00	0.00	0.00	0.00	-346.00
1,430.90	0.00	0.00	0.00	1,430.90
3,000.00	0.00	0.00	0.00	3,000.00
-12,812.87	612.50	4,504.00	5,116.50	-17,929.37
6,000.00	0.00	0.00	0.00	6,000.00
-12,654.07	0.00	8,032.24	8,032.24	-20,686.31
-6,570.00	0.00	0.00	0.00	-6,570.00
-209.97	0.00	0.00	0.00	-209.97
14,637.34	612.50	12,536.24	13,148.74	1,488.60

35,353.51	0.00	0.00	0.00	35,353.51
-13,936.20	612.50	12,536.24	13,148.74	-27,084.94
-6,570.00	0.00	0.00	0.00	-6,570.00
-209.97	0.00	0.00	0.00	-209.97
14,637.34	612.50	12,536.24	13,148.74	1,488.60