PROBIOTIC VIABILITY AND SENSORY PROPERTIES OF ICE-CREAM SUPPLEMENTED WITH ENCAPSULATED *Lactobacillus bulgaricus*

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ABSTRACT

The aim of the research is to study the encapsulation method that being used to protect probiotic bacteria *Lactobacillus bulgaricus (Lb)* in ice cream for a period of 90 days of storage at -20 ˚C. Three different formulations have been developed i.e. Formulation A (FA, mixtures with gelatin 0.1%), Formulation B (FB, mixtures with gelatin 0.3%) and Formulation C (FC, mixtures with gelatin 0.5%). All statistical analysis has been reported at $p<0.05$. It was found that the microencapsulation or coating of *L.bulgaricus* significantly influenced their survivality compared to uncoated probiotic. Formulation B with coated *Lb* (FB.3) showed the highest viability which achieved $10^{8.34}$ cfu/g. FB.3 showed the best outcome in terms of the quality factor measurements i.e. dry matter, fat, moisture content, viscosity which are 34.50 ± 0.89%, 7.66 ± 0.07%, 65.50 ± 0.89%, 125,000 ± 0.00 mPa.s respectively, and the size range of beads from 0.8 to 1.0 mm within the accepted level. At the end of 90 days storage at -20˚C, the viability of the coated cells in formulation B are still within the accepted level with 2.2 x 10^8 cfu/g population which remained above 10^6 cfu/g. There are significant increase in viscosity with increased gelatin levels similarly with moisture content. Meanwhile dry matter significantly reduced in coated probiotic compared to uncoated in all of the ice-cream samples but protein showed remarkable reduction in FA and FB only. Sample FB showed significant reduction ($p<0.05$) on melting rate and viscosity. The highest fat content in FB gave the highest viability as compared to FA and FC. From the sensory evaluation towards probiotic flavour and acidic flavour, the overall panel rank for the highest acceptance is in Formulation A with coated *Lb* (FA.3) as compared to uncoated probiotic ice-cream i.e Formulation A with uncoated *Lb* (FA.2); Formulation B with uncoated *Lb* (FB.2) and Formulation B with coated *Lb* (FB.3). Furthermore, there are significant differences ($p<0.05$) between non probiotic ice-cream and probiotic ice-cream for all sample in terms of probiotic flavour and acidic flavour. Based on defined cooked flavour, probiotic flavour, and acidic flavour, there was a remarkable overall acceptance on the ice cream with coated probiotic. Meanwhile, acceptance upon uniformity of the ice cream based on colour and appearance showed more variation among the sensory panels. It can be concluded that the microencapsulation had good effect on sensorial acceptability ($p>0.05$) of probiotic ice cream. The results indicate that dairy ice cream can be considered a suitable vehicle for incorporating coated probiotic microorganisms for higher survival and better sensorial acceptance.

ABSTRAK

Tujuan penyelidikan ini adalah untuk membangun kaedah pengkapsulan untuk melindungi bakteria probiotik *Lactobacillus bulgaricus (Lb)* dalam ais krim sepanjang tempoh 90 hari penyimpanan pada suhu -20 ˚C. Terdapat tiga formulasi yang dibangunkan iaitu Formulasi A (FA, campuran gelatin 0.1%), Formulasi B (FB, campuran gelatin 0.3%) dan Formulasi C (FC, campuran gelatin 0.5%). Keseluruhan analisis statistik diperolehi adalah pada $p<0.05$. Didapati kebolehidupan *L.bulgaricus* yang dikapsulkan adalah signifikan berbanding dengan yang tidak dikapsulkan. Formulasi B yang mengandungi *Lb* yang dikapsulkan (FB.3) menunjukkan kebolehidupan paling tinggi dengan mencapai $10^{8.34}$ cfu/g. FB.3 menunjukkan hasil yang terbaik terhadap pengukuran faktor kualitinya iaitu bahan kering, lemak, kandungan kelembapan dan kelikatan masing-masing adalah 34.50 ± 0.89%, 7.66 ± 0.07%, 65.50 ± 0.89%, dan 125,000 ± 0.00 mPa.s, manakala julat saiz butiran kapsul pada anggaran 0.8 hingga 1.0 mm dalam lingkungan nilai yang boleh diterima. Pada akhir tempoh penyimpanan selama 90 hari pada suhu -20˚C, kebolehidupan sel formulasi B yang dikapsulkan masih dalam tahap yang diterima pada 2.2 x 10^8cfu/g sel iaitu melebihi 10^6 cfu/g. Peningkatan yang signifikan berlaku ke atas kelikatan dengan penambahan kuantiti gelatin, begitu juga terhadap kandungan kelembapan. Sementara itu, peratus bahan kering menurun secara ketara dalam probiotik yang dikapsulkan berbanding dengan tanpa-kapsul di dalam semua sampel ais krim manakala nilai protein menunjukkan penurunan dalam FA dan FB sahaja. Sampel FB pula menunjukkan penurunan yang signifikan ($p<0.05$) ke atas kadar kecairan dan kelikatan. Kandungan lemak yang paling tinggi dalam FB memberikan kebolehidupan paling tinggi berbanding dengan FA dan FC. Hasil penilaian sensori terhadap perisa probiotik dan rasa masam mendapati penerimaan tertinggi keseluruhan panel pada Formulasi A yang mengandungi *Lb* yang dikapsulkan (FA.3) berbanding dengan ais krim probiotik yang tidak dikapsulkan iaitu Formulasi A yang mengandungi *Lb* yang tidak dikapsulkan (FA.2); Formulasi B yang mengandungi *Lb* yang tidak dikapsulkan (FB.2) dan Formulasi B yang mengandungi *Lb* yang dikapsulkan (FB.3). Tambahan pula terdapat perbezaan yang signifikan ($p<0.05$) terhadap perisa probiotik dan rasa masam bagi semua sampel di antara ais krim tanpa probiotik dengan ais krim berprobiotik. Berdasarkan ketentuan rasa-dimasak, perisa probiotik dan rasa masam, ais krim mengandungi probiotik yang dikapsulkan mendapat penerimaan yang tinggi secara keseluruhannya. Sementara itu, penerimaan terhadap keseragaman ais krim bagi sifat warna dan rupa luar menunjukkan pelbagai variasi di kalangan panel. Kesimpulannya, mikrokapsulan memberi kesan yang baik terhadap penerimaan penilaian sensori ($p>0.05$) ke atas ais krim berprobiotik. Hasil kajian menunjukkan ais krim sesuai digunakan sebagai sumber untuk bakteria probiotik yang dikapsulkan memperolehi kadar kebolehidupan yang tinggi dan penerimaan sensori yang lebih baik.

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LIST OF SYMBOLS

%	-	Percentage
g	-	Gram
mg	-	milligram
ml	-	millilitre
L	-	Litre
CFU/ml		Colony forming unit per millilitre
cm	-	centimeter
µm	-	micrometer
h	-	hour
m	-	Metre
min	-	Minutes
mm	-	millimetre
°C	-	Celcius
g/l	-	Gram per liter
F.A	-	Formulation A
F.B	-	Formulation B
F.C	-	Formulation C
HCl	-	Hydrochloric acid
rpm	-	revolutions per minute
SEM	-	Scanning Electron Microscopic
DF	-	degree of freedom
F	-	F value

LIST OF EQUATIONS

NOMENCLATURES

ANOVA	-	Analysis of Variance
Gel	-	Gelatine
NaCl	-	Sodium chloride
SEM	-	Scanning Electron Microscopy
W	-	Weight
cm^2	-	centimeter square
cm^3	-	Centimeter cubic
eqn	-	Equation
F	-	Formulation
F3	-	Formulation 3
kg	-	Kilogram
kgf/h	-	Kilogram force per hour
m	-	Meter
mg	-	Milligram
mg/ml	-	Miligram per Mililitre
min	-	Minute
min^{-1}	-	Per minute
mL	-	Milliliter
mL/min	-	MilliLiter per minute
mm	-	Millimeter
v/v	-	Volume per volume
w/v	-	Weight per volume
w/w	-	Weight per weight
rpm	-	Revolution per minute
s	-	second
R&D	-	Research and development
µm	-	Micrometer
µL	-	Microliter
g	-	Gram
°	-	Degree
ºC	-	Degree celcius
%	-	Percent
<	-	Less than
>	-	More than

CHAPTER 1

INTRODUCTION

1.1 Research Background

Probiotics are defined as live microorganisms in which, when administered in adequate amounts can confer health benefits to the consumers. Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) define probiotics as ‘‘live microorganisms (bacteria or yeasts), whereby when ingested or locally applied in sufficient numbers will confer one or more specified demonstrated health benefits for the host’’ (FAO/WHO, 2001). The prebiotic compounds are consumed by probiotics as carbon or energy source in colon (Homayouni et al., 2008).

Probiotic ice-creams with culture or fermented frozen desserts are gaining popularity among dairy products nowadays (Akın et al., 2007). It was reported that ice cream is a good carrier in delivering probiotic bacteria to consumers (Homayouni et al., 2008). Probiotic bacteria have been increasingly used in fermented dairy products which include yoghurts, soft, semi-hard, and hard cheeses, ice cream, and frozen fermented dairy desserts because of their perceived health benefits (Anil Kumar et al., 2007). The pH of non-fermented ice cream is close to 7 and this provides the possibility for satisfactory survival of probiotic bacteria (Homayouni et al., 2008).

Among probiotic dairy products, probiotic ice-cream is gaining high popularity because it can be stored for a long period of time without changes in its attributes and it is a very popular product in the worldwide (Akin et al., 2007; Haynes and Playne 2002; Hekmat and Mcmahon 1992; Kailasapathy and Sultana 2003).

The increasing trend in health awareness from all over the world has prompted the consumers and health professionals to adopting health promotion and disease preventive strategies. Antibiotic therapy is currently the most commonly used therapy to treat bacterial infections; it is an end-of-pipe approach to the health maintenance, curative, and is always associated with unpleasant effects. Thus, the awareness and popularity of probiotics among the global population is increasing by leaps and bound. Probiotics, on the other hand, promote a desirable gastro-intestinal microflora which is known to enhance the overall wellness (Gomes, 1999; Reid, 1999; Tannock, 2004).

Viability of probiotic bacteria must be maintained throughout the product's shelf-life and the gut environment (Kailasapathy & Chin, 2000). Some authors have proven that the freezing process affects the number of live probiotic cells dramatically. International Dairy Federation (IDF) has suggested that a minimum of 10^7 probiotic bacterial cells should be alive at the time of consumption per gram of the product (Kailasapathy & Sultana, 2003).

The survival of probiotic bacteria in fermented frozen desserts improves with encapsulation. Encapsulation helps to isolate the bacterial cells from the effects of the hostile environment and gastrointestinal tract, thus potentially preventing cell loss. Encapsulation thus may enhance the shelf-life of probiotic cultures in frozen dairy products (Homayouni et al., 2008).

In order to achieve successful promotion of functional probiotic products, the food industry will have to satisfy the demands and expectations of the consumers. Thus, all of the probiotic foods should be safe and have good sensory properties.

The sensory characteristics of fermented milks play an important role in product acceptance of consumers (Patrignani, 2006).

This research reports an attempt to study viability of encapsulated probiotic bacteria, determination of the physical and chemical characteristics and an evaluation of the effect towards sensory properties in frozen ice-cream condition.

1.2 Research Objective

The objective of this research is to study the viability of microencapsulated probiotic bacteria *Lactobacillus bulgaricus* in formulated ice-cream and to evaluate its effects on the sensorial qualities of ice-cream product.

1.3 Research Scopes

The scopes of this research include:

1) Pretreatment study on formulation of probiotic ice-cream.

2) To study the viability of probiotic bacteria under frozen temperature (-20°C) in formulated ice-cream during 90 days storage.

3) To investigate the relation between probiotic viability and quality factor measurements of probiotic ice-cream.

4) To evaluate the sensory properties of the probiotic ice-cream.

1.4 Scopes of Thesis

Ice-cream is an ideal matrix for delivery of probiotic organisms to human body as compared to fermented dairy products (Akin et al., 2007; Haynes and Playne 2002; Hekmat and Mcmahon 1992; Kailasapathy and Sultana 2003). Therefore, the viability of probiotic microorganism in the food and drink product has been identified as an important part of the research as well as the subject of this thesis. The remainder of this thesis is organised into four chapters as follows:

Chapter 2 reviews on ice-cream as functional food products showing potential for use as probiotic vehicles. The ice-cream matrix might be a good vehicle for probiotic cultures due to its composition which includes milk proteins, fat and lactose, as well as other compounds (Cruz et al, 2009).

Meanwhile, chapter 3 describes on the materials and methods used in the experimental work. The steps involved in this study are preparation of bacterial strain *Lactobacillus bulgaricus* (*LB*) and culture conditions for fermentation and harvesting to used in free cell (uncoated probiotic) and encapsulated probiotics (coated probiotic) process; evaluation of physical and chemical quality of probiotic ice-cream and sensory evaluation of probiotic ice-cream.

Lastly, chapter 4 discusses on the result obtained from the experiment work which had been done in chapter 3. All of the data collected were analyzed and the results for the analysis were discussed. Encapsulation allows live probiotic bacteria to be protected from its environment, thereby maximizing the probiotic bacteria viability during processing and storage.

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