UNIVERSITI PUTRA MALAYSIA

FUNCTIONAL PROPERTIES AND CHARACTERIZATION OF YOGURT FORTIFIED WITH *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell AND MICROENCAPSULATED *Lactococcus lactis* Gh1

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By

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FUNCTIONAL PROPERTIES AND CHARACTERIZATION OF YOGURT FORTIFIED WITH *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell AND MICROENCAPSULATED *Lactococcus lactis* Gh1

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Nowadays, functional food market is dominated by dairy based probiotic products mainly yogurt. The nutritional values of yogurt can be further enhanced by the inclusion of *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell extract and the addition of locally isolated probiotic *Lactococcus lactis* Gh1. *S. dulcificum* is a plant that possesses unique characteristics that can be used as a healthy food additive which acts as taste modifier besides providing antioxidant and anti-diabetic effects. The screening results of seed, leaf and pulp of *S. dulcificum* showed that pulp extracts contained significantly ($P<0.05$) higher antioxidant (85.69 ± 0.004) and phenolic content (15.93 ± 0.002 µg GAE Equiv. / g sample) than the other plant parts. More interestingly, *S. dulcificum* pulp also has stronger anti-diabetic properties than the standard drug, acarbose and hence it was chosen to be incorporated into yogurt. *L. lactis* Gh1 was encapsulated via extrusion (with alginate-starch coating agent) and spray drying (with gum Arabic and *S.dulcificum* pulp) methods to increase the cells viability. A total of seven different yogurts (C.plain (plain yogurt), C.pulp (contained pulp extract), F.plain (contained free cell *L. lactis* Gh1), F.pulp (contained free cell *L. lactis* Gh1 and pulp extract), B.plain (contained alginate-starch encapsulated *L. lactis* Gh1), B.pulp (contained pulp extract and alginate-starch encapsulated *L. lactis* Gh1), S.dry (contained spray dried *L. lactis* Gh1 coated with gum Arabic and pulp) were formulated. The yogurts were fermented at 42°C until pH 4.5 prior to storage at 4°C for 21 days. All the yogurts fortified with pulp extract showed a faster rate of pH reduction (~3 hours) than the plain yogurt (6 hours). The total phenolic content (TPC) of all yogurts showed an increasing trend throughout the storage. The presence of *S. dulcificum* pulp extract elevated ($P<0.05$) the TPC in the yogurts whereas the antioxidant activity (DPPH assay) and inhibitory activity towards the key enzymes associated with diabetes (α-amylase and α-glucosidase) showed a gradual increase on the first 7 days but decreased afterward. In comparison, yogurts fortified with pulp extract showed a higher ($P<0.05$) antioxidant and anti-diabetic activity than the plain

yogurt. The viability of *L. lactis* Gh1 in yogurt showed the highest survivability even on the last day (day 21) of refrigerated storage when it was being encapsulated in beads and according to the following order: B.plain (9.43 log CFU/mL)>B.pulp (9.04 log CFU/mL)> F.pulp (8.26 log CFU/mL) > S.dry (8.08 log CFU/mL)> F.plain (6.76 log CFU/mL). Meanwhile, the CFU/mL of the yogurt starter cultures, *Streptococcus thermophilus* and *Lactobacillus dulbrueckii* of all yogurts were drastically increased for the first week of storage especially for yogurts added with a non-microencapsulated *L. lactis* Gh1 (F.plain and F.pulp) but started to decrease after day 7. The flow behaviour of all yogurt samples had exhibited shear thinning behaviour with n<1. In general, the incorporation of *S. dulcificum* and microencapsulated *L. lactis* Gh1 had greatly enhanced the quality and potential benefits of the functional yogurt products.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

SIFAT FUNGSIAN DAN PENCIRIAN YOGURT YANG DIPERKAYA DENGAN *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell DAN MIKROKAPSUL *Lactococcus lactis* Gh1

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Pada masa kini, pasaran makanan berfungsi didominasi oleh produk probiotik berasaskan tenusu terutamanya yogurt. Nilai nutrisi yogurt boleh dipertingkatkan lagi dengan penambahan ekstrak dari *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell dan penambahan probiotik terpencil tempatan, *Lactococcus lactis* Gh1. *S. dulcificum* adalah tumbuhan yang mempunyai ciri-ciri unik yang boleh digunakan sebagai aditif makanan yang sihat yang bertindak sebagai pengubah selera selain memberikan kesan antioksidan dan anti-diabetes. Hasil saringan terhadap biji, daun dan isi buah *S. dulcificum* dengan ketara ($P < 0.05$) menunjukkan ekstrak isi buah mengandungi antioksidan (85.69 ± 0.004) dan kandungan fenolik (15.93 ± 0.002 μg GAE Equiv / g sampel) yang lebih tinggi berbanding bahagian tumbuhan lain. Tambah menarik, isi buah *S. dulcificum* juga mempunyai ciri anti-diabetes yang lebih kuat berbanding ubat yang biasa digunakan, acarbose dan oleh itu ia dipilih untuk dimasukkan ke dalam yogurt. *L. laktis* Gh1 telah dikapsulkan melalui kaedah penyemperitan (dengan ejen salutan alginat-kanji) dan semburan pengeringan (bersama gam Arab dan *S. dulcificum*) untuk meningkatkan kebolehidupan sel. Sejumlah tujuh yogurt yang berbeza (C.plain (yogurt biasa), C.pulp (mengandungi ekstrak isi buah), F.plain (mengandungi sel bebas *L. lactis* Gh1), F.pulp (mengandungi sel bebas *L. lactis* Gh1 dan ekstrak isi buah), B.plain (mengandungi alginat-kanji kapsul *L. lactis* Gh1), B.pulp (mengandungi ekstrak isi buah dan alginate-kanji kapsul *L. lactis* Gh1), S.dry (mengandungi *L. lactis* Gh1 semburan kering yang disaluti dengan gam Arab dan isi buah) telah diformulasikan. Yogurt difermentasi pada suhu 42 °C sehingga mencapai pH 4.5 sebelum disimpan pada 4 °C selama 21 hari. Kesemua yogurt yang diformulasikan dengan ekstrak isi buah telah menunjukkan kadar pengurangan pH (~ 3 jam) yang lebih cepat berbanding dengan yogurt biasa (6 jam). Kandungan fenolik (TPC) semua yogurt tersebut menunjukkan trend peningkatan sepanjang dalam masa penyimpanan. Kehadiran ekstrak isi buah *S.*

dulcificum telah meningkatkan (P <0.05) nilai TPC dalam semua yogurt tersebut manakala aktiviti antioksidan (assay DPPH) dan aktiviti perencat enzim utama penyebab kencing manis (α-amilase dan α-glucosidase) menunjukkan peningkatan beransur-ansur pada 7 hari pertama tetapi menurun selepas itu. Sebagai perbandingan, yogurt diperkaya dengan ekstrak isi buah menunjukkan aktiviti antioksidan dan anti-diabetes yang lebih tinggi (P <0.05) daripada yogurt biasa. Kebolehidupan *L. lactis* Gh1 dalam yogurt telah menunjukkan kemandirian yang tertinggi sehingga hari terakhir (hari 21) penyimpanan sejuk apabila ia dikapsulkan dalam manik dan mengikut turutan berikut: B.plain (9.43 log CFU/mL)> B.pulp (9.04 log CFU/mL)> F.pulp (8.26 log CFU/mL)> S.dry (8.08 log CFU/mL)> F.plain (6.76 log CFU/mL). Sementara itu, CFU/mL dari kultur permulaan yogurt, *Streptococcus thermophilus* dan *Lactobacillus dulbrueckii* dalam semua yogurt telah meningkat secara drastik untuk minggu pertama penyimpanan terutamanya bagi yogurt yang ditambah dengan *L. lactis* Gh1 yang tidak dikapsulkan (F.plain dan F.pulp) tetapi mengalami penurunan selepas 7 hari. Kelakuan aliran semua sampel yogurt telah mempamerkan kelakuan penipisan ricih dengan n <1. Secara umumnya, penggabungan *S. dulcificum* dan mikrokapsul *L. lactis* Gh1 telah meningkatkan kualiti dan potensi manfaat produk yogurt fungsian.

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I certify that a Thesis Examination Committee has met on 15 May 2019 to conduct the final examination of Nurul Farhana binti Fazilah on her thesis entitled "Functional Properties and Characterization of Yogurt Fortified with *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell and Microencapsulated *Lactococcus lactis* Gh1" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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%	Percentage
α	Alpha
a_w	Water activity
°C	Degree Celsius
µL	Microliter
µm	Micrometer
µg	Microgram
CFU/mL	Colony forming unit per millilitre
$CaCl_2$	Calcium chloride
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
GAE	Gallic acid equivalent
g	Gram
HCl	Hydrochloric acid
K	Flow consistency index
KH_2PO_4	Monopotassium phosphate
LAB	Lactic acid bacteria
M	Molarity
MRS	de Man Rogosa Sharpe broth and agar
mg	Milligram
mL	Millilitre
mm	Millimetre
mM	Millimolar
N	Normality
NaCl	Sodium chloride
NaOH	Sodium hydroxide
Na_2CO_3	Sodium carbonate
n	Flow behaviour index
nm	Nanometer
OD	Optical Density
OPA	O-phthalaldehyde
pNPG	p-nitropheynyl glucopyranoside
QR	Quercetin equivalent
rpm	Revolutions per minute
TTA	Titratable acidity
U/mL	Unit per mililitre
v/v	Volume per volume
w/v	Weight per volume

CHAPTER 1

INTRODUCTION

Todays, probiotic products have begun to gain worldwide interest for promoting health care apart from its good taste. Among dairy probiotic products, yogurt has the sharpest growth trends between consumers because it meets consumer's dietary needs. It is a ready to eat food which is also rich in nutritional components. Yogurt eating has been associated with several health benefits including improvement of lactose metabolism, anti-mutagenic properties, anti-carcinogenic properties, management of hypertension, anti-diarrheal properties, immune system stimulation and improvement in inflammatory bowel disease (Shakerian., 2015; Unal and Akalin., 2012). Attempts to make the fermented milk such as yogurt more palatable and nutritious have recently extended with the inclusion of rare fruits, natural extracts from plants, specific nutrients, collagen, as well as the additional probiotics and prebiotics**.**

Generally, yogurt is fermented milk using lactic acid bacteria as starter culture. Mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* are commonly used as starter culture for yogurt, but both of them cannot survive in sufficient amount in the digestive tract (Wihansah *et al*., 2018). To confer health benefit, probiotic bacteria must arrive in intestines and remain viable in sufficient numbers at 6-7 log CFU/g of products (Fuller 1989; Kailasapathy and Chin., 2000; Krasaekoopt *et al*., 2003). Therefore, in formulating probiotic yogurts, the addition of another probiotic strain that capable of surviving passage through digestive tract is essential in ensuring the minimum number of viable probiotic is sustained. In addition, cell microencapsulation technique is an alternative in protecting the cells to further enhance the survivability of probiotic strains. For successful encapsulation of viable cells, it is important to preserve the bacterial viability under different handling processes along with the type of encapsulation material that is compatible with the food material (Haffner *et al*., 2016).

In general, different types of probiotic strains offered different nutritional benefits such as improvement in inflammatory bowel disease, anti-diarrhoeal properties, lactose metabolism, anti-mutagenic properties and immune system simulation (Shah, 2000). *Lactococcus lactis* species forms an important part of the group of lactic acid bacteria (LAB) and having GRAS (Generally Recognized as Safe) status. Strains belonging to this species have been featured in dairy industry for decades due to its specific biochemical traits such as lipolysis, proteolysis and citrate breakdown, hence contributing typical taste and flavor to a variety of fermented dairy foods. Furthermore, the production of bacteriocins by some of the *L. lactis* strains is well documented (Hwanhlem *et al*., 2017; Benkerroum *et al*., 2000). Hence, it is interesting to further explore on this strain especially its possible application in food products.

Yogurt has been associated with various therapeutic properties, including its potency as an anti-diabetic food through the mechanism of α-amylase and α-glucosidase inhibition (Apostolidis *et al*., 2006). These functional properties could be enhanced with the addition of natural agents such as fruits, plant and herbs. Recent studies have shown that plant-based foods containing high phenolic and flavonoids compounds are linked to intestinal α-amylase and α-glucosidase inhibitory activities (Abou-arab *et al*., 2011). To date, many plant and herbal extracts have been reported to efficiently inhibit the enzymatic activity of α-amylase and α-glucosidase (Amirdivani, 2015; Kazeem *et al*., 2013).

Synsepalum dulcificum (Schumach. & Thonn.) Daniell also known as miracle fruit or miracle berry is an indigenous tropical plant growing in West Africa. The plant can be harvested at least twice a year making the yield stable (He *et al*., 2016). Miracle fruit is about an inch in length with a bright red colour and known for its ability in converting sour tasting foods to sweet taste. Glycoprotein known as miraculin found in the pulp of this fruit is the compound responsible for this unique taste modifying function (Du *et al*., 2014; He *et al*., 2015; He *et al*., 2016). The binding of miraculin to the receptor cells of the tongue suppresses the response of a sour taste in the central nervous system. The effect would last until the miraculin is diluted and eliminated by saliva. The taste modification function gives a great potential for this fruit to be exploited in the food industry especially as an alternative sweetener or additive to mask the undesirable sour taste in food products such as yogurt (Wong and Kern, 2011; Wilken and Satiroff, 2012). Moreover, miracle fruit's pulp and seed contained high nutrient contents which can be used for dietary supplement in food (Agblekpe *et al*., 2016). Stem of miracle fruit also contained antioxidant and antityrosinase effects which can be potential applications in food supplementation and medical cosmetology (Wang *et al*., 2011). Hence, it is interesting for miracle fruit to be formulated into functional foods such as yogurt to improve the nutritional and therapeutic properties.

To date, very little is known about the anti-diabetic properties and health benefits of *S. dulcificum* plant extract. The ability of this plant to inhibit the enzymatic activity of α-amylase and α-glucosidase when incorporated into food product also have yet to be studied and explored. Keeping in view the potential of anti-diabetic properties of *S. dulcificum* plant extracts (Chen *et al.*, 2006), the present study is endeavored to develop functional yogurts fortified with *S. dulcificum* extract and potential probiotic strain, *L. lactis* Gh1. In this regard, the inclusion of the plant extracts and microencapsulated probiotic is expected to enhance the nutritional values of the yogurt and as such functional food that contained sufficient viable probiotic strains and components which are able to inhibit α-amylase and α-glucosidase could be developed.

This study embarks on the following specific objectives:

1. To evaluate the antioxidant and anti-diabetic properties of *Synsepalum dulcificum* via *in-vitro* studies.
2. To access the viability and characteristics of microencapsulated *Lactococcus lactis* Gh1 via extrusion and spray drying methods.
3. To examine the influence of microencapsulated *Lactococcus lactis* Gh1 and *Synsepalum dulcificum* on functional properties of the formulated yogurt.

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LIST OF PUBLICATIONS

Publications

Fazilah, N.F.; Ariff, A.B.; Khayat, M.E.; Rios-Solis, L. and Halim, M*. (2018). Influence of probiotics, prebiotics, synbiotics and bioactive phytochemicals on the formulation of functional yogurt. *Journal of Functional Foods* 48: 387-399. doi:10.1016/j.jff.2018.07.039 (Q1, IF:4.165).

Fazilah, N.F., Hamidon, N.H., Ariff, A.B., Khayat, M.E., Wasoh, H. and Halim*, M. (2019). Microencapsulation of *Lactococcus lactis* Gh1 with gum Arabic and *Synsepalum dulcificum* via spray drying for potential inclusion in functional yogurt. *Molecules* 24: 1422 (Q2, IF:3.098).

Conference Proceeding

Nurul Farhana Fazilah, Arbakariya B. Ariff, Mohd Ezuan Khayat, Murni Halim * (2019). Influence of Synsepalum dulcificum and microencapsulated *Lactococcus lactis* Gh1 on the characteristics of functional yogurt. Bioprocessing and Biomanufacturing Symposium. Swiss Garden Beach Resort, Perak, Malaysia. 9 – 10 April 2019 (Poster Presentation)

Awards

Gold Medal, 3rd Educational Project of Innovation (EPIC) 2018, Functional yogurt fortified with *Synsepalum dulcificum* and microencapsulated probiotic *Lactococcus lactis.*

Silver Medal, International EUREKA Innovation Exhibition 2018, Functional yogurt fortified with spray dried *Lactococcus lactis* and miracle fruit.

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