
**UNIVERSITI PUTRA MALAYSIA**

# *PROBIOTIFICATION AND ITS EFFECT ON THE ANTIOXIDANT PROPERTIES OF HERBAL TEAS*

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**MASTERS OF SCIENCE**
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# PROBIOTIFICATION AND ITS EFFECT ON THE ANTIOXIDANT PROPERTIES OF HERBAL TEAS

**By**

**NUR ATHIRAH BINTI IBRAHIM**

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**September 2014**

**Chairman: Prof. Shuhaimi Bin Mustafa, PhD**
**Institute: Halal Products Research Institute**

This study investigated the antioxidant properties of freshly prepared and lactic fermented herbal teas. Herbal teas were known to have powerful antioxidant properties. There has been an increased interest in the exploration of enhancing the effectiveness of naturally occurring antioxidant. Brewing time which could affect the nutritive values of teas are often neglected and ignored. Reports on the effect of lactic fermentation on antioxidant properties of herbal teas are limited. Furthermore, many probiotic drinks available in the market are dairy-based. This represents inconvenience for consumers who are on low cholesterol diet and lactose intolerant. Hence, the objectives of this study were to examine the effect of brewing time on the yield of phenolic contents of herbal teas, to determine the suitability of herbal teas as raw materials for the production of probiotic herbal teas and to study the effect of lactic fermentation on antioxidant properties of herbal teas. Four local commercial herbal teas namely “Allspice”, “Scaphium”, “Gora”, and “Cinnamon” were purchased from the local store to be subjected to lactic fermentation. The bacteria used was *Lactobacillus casei* Shirota strain. The antioxidant capacity of freshly prepared and lactic fermented herbal teas was measured using three different assays namely DPPH assay, FRAP assay, and βeta-carotene linoleate bleaching assay. Phenolic contents (TPC) was analyzed using Folin-Ciocalteu reagent and flavonoid contents (TFC) was measured using the aluminium chloride method. The probiotic herbal teas were inoculated with 24 h-old lactic culture and incubated at 37 °C overnight. Changes in pH, acidity, sugar content, and viable cell counts during fermentation under controlled conditions were monitored. It was observed that herbal teas brewed for 3 min yielded the highest phenolic contents. *Lactobacillus casei* retained its viability in all herbal teas after 24 hour of fermentation. Majority of pH and total sugar of probiotic herbal teas were significantly reduced ($p< 0.05$) while total acidity of probiotic herbal teas were significantly increased ($p< 0.05$) with fermentation.

TPC and TFC of probiotic herbal teas were significantly higher ($p< 0.05$) than the freshly prepared herbal teas. The antioxidant activity of fermented herbal teas were higher than freshly prepared herbal teas in all three assays. However, significant increase ($p< 0.05$) of antioxidant activity was exhibited only in β-carotene linoleate bleaching assay for each probiotic herbal teas. The viability of lactic cultures in fermented herbal teas declined significantly ($p< 0.05$) with time and lost viability completely after 1 week of cold storage 4 °C. Generally, it can be concluded that 3 minutes of brewing is the best brewing time to yield more phenolic compounds from herbal teas. Moreover, the selected herbal teas are not the best but can considered to be average substrates for the cultivation of *Lactobacillus casei.* Furthermore, lactic fermentation also has a positive effect on the antioxidant activity of herbal teas and this can be implemented by the food industry to improve the nutritive value of food.

Abstrak tesis yang dikemukan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

**PENAMBAHAN BAKTERIA PROBIOTIK DAN KESANNYA TERHADAP NILAI ANTIOKSIDA TEH HERBA**

Oleh

**NUR ATHIRAH BINTI IBRAHIM**

**September 2014**

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Kajian ini mengupas tentang sifat antioksidan teh herba yang baru disediakan dan teh herba yang diperam dengan bakteria asid laktik. Teh herba terkenal dengan nilai antioxidannya yang tinggi. Didapati bahawa terdapat minat yang tinggi daripada pelbagai pihak untuk mengkaji tentang peningkatkan keberkesanan antioxidan semulajadi. Masa penyediaan teh yang berkemungkinan besar memberi kesan terhadap nilai nutrisi teh sering kali dipandang enteng oleh kebanyakan orang. Penerbitan mengenai kesan pemerapkan teh herba dengan bakteria asid laktik terhadap nilai antioksidannya juga adalah sedikit. Tambahan, kebanyakan minuman probiotik yang berada di pasaran adalah berasaskan susu. Hal ini mendatangkan masalah kepada pengguna yang sedang menjalani diet penjagaan kolesterol dan kepada pengguna yang alah terhadap laktosa. Oleh itu, objektif-objekti penyelidikan ini adalah untuk mengkaji kesan masa penyediaan teh herba terhadap pembebasan kuantiti fenoliknya, menentukan kesesuaian teh herba sebagai material untuk penghasilan teh probiotik dan akhir sekali, mengkaji kesan pemerapan teh herba terhadap sifat antioksidannya. Empat teh herba tempatan yang telah dikomersilkan seperti “Allspice”, “Scaphium”, “Gora”, dan “Cinnamon” dibeli untuk diperam dengan LAB. Bakteria yang digunakan ialah dari strain *Lactobacillus casei* Shirota. Kapasiti antioksidan bagi teh yang baru disediakan dan teh yang diperam oleh LAB telah dinilai menggunakan tiga kaedah yang berlainan iaitu kaedah DPPH, FRAP, dan kaedah pelunturan linoleate βeta-carotene. Kuantiti fenolik (TPC) telah dianalisa menggunakan reagen Folin-Ciocalteu manakala kuantiti flavonoid dinilai menggunakan kaedah aluminium klorid. Teh herba probiotik telah disuntik dengan asid laktik bakteria yang telah dikulturkan selama 24 jam dibawah suhu 37 °C semalaman. Perubahan dalam pH, keasidan, kuantiti gula, dan bilangan sel bakteria yang masih hidup semasa proses pemerapan di dalam keadaan terkawal diperhatikan. Telah didapati bahawa teh-teh herba yang mengambil masa selama 3 minit untuk disediakan membebaskan kandungan fenolik yang paling tinggi. Bakteria *Lactobacillus casei*

juga kekal hidup selepas 24 jam proses pemerapan. Majoriti nilai pH dan kuantiti gula teh-teh herba probiotik memberikan penurunan yang besar ($p< 0.05$) manakala nilai keasidannya pula menunjukkan peningkatan yang besar ($p< 0.05$). TPC dan TFC teh-teh herba probiotic juga memberikan peningkatan yang besar ($p< 0.05$) berbanding teh herba yang baru disediakan. Aktiviti antioksidan teh herba yang diperap adalah lebih tinggi berbanding teh herba yang baru disediakan berdasarkan ketiga-tiga kaedah. Akan tetapi, peningkatan aktiviti aktiosidan yang besar ($p<0.05$) bagi setiap teh herba probiotik hanya diperlihatkan di dalam kaedah pelunturan linoleate β-carotene. Bagi kesan simpan sejuk teh herba probiotik didapati bahawa sel hidup bakteria asid laktik berkurangan secara besar ($p< 0.05$) dengan masa dan mati sepenuhnya selepas 1 minggu penyimpanan sejuk 4 °C. Oleh itu dapat disimpulkan bahawa menyediakan teh herba selama 3 minit menghasilkan pembebasan kandungan fenolik yang tinggi. Selain itu, teh-teh herba yang digunakan dalam pengkajian ini bukanlah substrat yang terbaik tetapi sekadar memadai bagi penghasilan teh herba probiotik. Tambahan, proses pemerapan teh herba dengan bakteria asid laktik mampu meningkatkan kuasa antioksidannya dan proses pemerapan ini juga boleh digunakan oleh industri makanan sebagai teknik bagi menambah baik kandungan nutrisi dalam makanan.

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| | |
|---|---|
| ROS | Reactive Oxygen Species |
| RNS | Reactive Nitrogen Species |
| DNA | Deoxyribonucleic acid |
| ADRs | Adverse Drug Reactions |
| GRAs | Generally Recognized As Safe |
| NO | Nitric Oxide |
| LBS | Lactobacilli Selective Agar |
| LAB | Lactic Acid Bacteria |
| DPPH | 2,2-diphenyl-1-picrylhydrazyl 1,1-diphenyl-2-picrylhydrazyl |
| FRAP | Ferric Reducing Antioxidant Power |
| MRS | Man Rogosa Sharpe |
| CFU | Colony Forming Unit |

# CHAPTER 1

## GENERAL INTRODUCTION

In normal aerobic metabolism, reactive oxygen or nitrogen species (ROS or RNS) is being produced in the living systems. These intermediates are involved in several metabolic processes such as the production of energy, cell signaling, gene transcription, followed by immune defense and many more (Seifried, 2007). Nonetheless, it is the deterioration of antioxidant defense mechanism that leads to the increasing ROS or RNS production, causing oxidative stress. Oxidative stress, has been associated with a number of diseases involving cancer, diabetes, heart disease, neurological disorder, ageing and many more (Valko, 2007). Antioxidant is a substance that could delay or prevent oxidation of a particular substrate when available at lower concentrations than that oxidizable substrate (Wanasundara and Shahidi, 2005). Although there has been a widespread intake of dietary supplements, there are still a number of communities that prefer to practice herbalism. Traditionally, herbs are consumed via infusion of plant parts in warm or hot water (Oh, 2013). However, brewing time of tea is often neglected by many which led to the limited publications and less understanding about its effect on the liberation and stability of phenolic compounds.

Modern people are showing keen interest in functional foods nowadays that more emphasis is given on probiotic products. Previous works have affirmed that the inclusion of probiotics in foods promotes various health benefits. For instance, initial antioxidant activity in cabbage increased when subjected to lactic fermentation due to the chemical process by lactic acid bacteria (LAB) (Kusznierewicz, 2008). Probiotics are widely utilized in dairy-based food products and this posed a major threat to consumers who are on low cholesterol diet and lactose intolerant. Furthermore, considering the wide acceptance and frequent intake of herbal teas worldwide, it is essential to use herbal teas as substrates for the production of non-dairy probiotic beverages. Herbal teas are also free of dairy allergen. With the increasing interest in the intake of dietary antioxidants, herbs have also been investigated for their antioxidant properties and how processes affect the content of antioxidant compounds and antioxidant capacity. Some studies showed a decrease in antioxidant compounds in plants upon lactic fermentation and depending on several factors could increase in the antioxidant capacity. Nevertheless, no reports about the effect lactic fermentation on the antioxidant activity of herbal teas have been found. Hence, it is imperative to investigate the effect probiotification has on the antioxidant potential of herbal teas. The general objective of this study was to develop non-dairy probiotic drinks from herbal teas. The specific objectives include:

1. To demonstrate the best tea brewing time that yields the highest phenolic contents

2. To determine the suitability of herbal teas as substrates for lactic fermentation
3. To examine the effect of lactic fermentation on antioxidant properties of herbal teas

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