UNIVERSITI PUTRA MALAYSIA

MICROBIAL QUALITY AND ANTIOXIDANT ACTIVITY OF AN IMPROVED BITTER GOURD (Momordica charantia L.) DRINK

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FSTM 2018 15

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

September 2017

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

MICROBIAL QUALITY AND ANTIOXIDANT ACTIVITY OF AN IMPROVED BITTER GOURD (*Momordica charantia* L.) DRINK

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Chair: Nor Ainy Mahyudin, Associate Professor, PhD
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The bitter gourd (*Momordica charantia* L.) (MC) is being consumed in various countries due to its nutritional value and is high in antioxidants. In this study, the (MC) is used in the preparation of a ready-to-serve (RTS) drink with antioxidant properties. The objectives of this research are to determine the physicochemical and antioxidant properties of a RTS MC drink with different concentrations of the MC, to conduct a sensory evaluation on the MC drink for acceptability and finally to determine the effect of water quality of the MC drink stored at different temperatures. The MC drink was prepared by water extraction of its juice, added with dissolved brown sugar, artificial honey, low fat yogurt, lime juice and mint extract. A proximate analysis was carried out followed by DPPH radical scavenging assay. A sensory evaluation was then carried out among 40 untrained panelists on a Hedonic scale of 1 to 7. The microbial quality was determined using the aerobic plate count, enumeration of moulds and yeasts, presumptive test for coliforms and detection of *Escherichia coli*. Five concentrations (0.58, 0.70, 0.80, 0.90 and 1.00 g/mL) of the MC drink was developed. The proximate analyses carried out showed that the RTS drink consist of high moisture content from 92.94 – 94.65%. The pH and ˚Brix of the MC drink increased as the concentration increased, whereby the pH was 4.30 – 4.54, and the ˚Brix was 5.0 – 5.6. The increase in concentration of MC has led to an increase in the radical scavenging activity. At the original concentration of 0.58 g/mL, the inhibition was at 71.51% which increased to 87.22% at the final concentration of 1.00 g/mL. The most accepted concentration of the MC drink according to the sensory evaluation was 0.80 g/mL with a mean score of 4.09 (like slightly), while the least accepted concentration was 1.00 g/mL and obtained the lowest mean score of 3.80 (dislike slightly). At the selected concentration (0.80 g/mL), the amount of bacterial growth exceeded 6 log CFU/mL after 24 hours for the MC drink stored at room temperature (28±2˚C). When stored at refrigerated

temperature (4±2˚C) it took 72 hours to reach 6 log CFU/mL. The total coliform count for all juice samples were between 93-210 MPN/g. *E. coli* was detected in the MC drink but not in the water used for its preparation. This study demonstrated MC has the potential to be used as an ingredient in preparing RTS drinks containing antioxidant properties.

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

KUALITI MIKROB DAN KEGIATAN ANTIOKSIDAN MINUMAN PERIA (*Momordica charantia* L.) SEDIA DIHIDANG YANG DITAMBAH BAIK

Oleh

CHERN PEI ERN

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Pengerusi: Nor Ainy Mahyudin, Profesor Madya, PhD
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Buah peria (Momordica charantia L.) (MC) sering dihidang dalam pemakanan di banyak negara disebabkan nilai nutrien dan kandungan antioksidan yang tinggi. Dalam kajian ini, MC telah digunakan dalam penyediaan minuman sedia dihidang (RTS) dengan sifat-sifat antioksidan. Objektif penyelidikan ini adalah untuk menilai sifat fizikokimia dan antioksidan RTS MC yang berkepekatan berbeza, untuk membuat penilaian deria pada minuman MC untuk penerimaan pengguna, dan akhirnya untuk menentukan kesan kualiti air terhadap minuman MC yang disimpan pada suhu yang berbeza. Minuman MC telah disediakan melalui pengekstrakan air jus, ditambah dengan gula perang, madu tiruan, yogurt lemak rendah, jus limau dan ekstrak pudina. Analisis proksimat telah dijalankan dan seterusnya pemeriksaan aktiviti antioksidan. Satu penilaian deria telah dijalankan oleh 40 ahli panel tidak terlatih menggunakan skala Hedonik 1 hingga 7. Kiraan plat aerobik, penghitungan kulat dan yis, ujian penandaian koliform dan pengesanan *Escherichia coli* dijalankan untuk penentuan kualiti mikrob. Lima kepekatan (0.58, 0.70, 0.80, 0.90 dan 1.00 g/mL) minuman MC telah diwujudkan. Analisis proksimat yang dijalankan menunjukkan bahawa minuman ini terdiri daripada kandungan air yang tinggi iaitu 92.94 – 94.65%. pH dan Brix minuman semakin meningkat mengikut kepekatan, di mana pH adalah 4.30 – 4.54, dan Brix adalah 5.0 – 5.6. Peningkatan kepekatan MC telah meningkatkan kegiatan antioksidan. Pada kepekatan asal 0.58 g/mL perencatannya adalah 71.51% yang meningkat kepada 87.22% pada kepekatan akhir 1.00 g / mL. Mengikut keputusan penilaian deria, kepekatan paling diterima adalah 0.80 g/mL dengan skor purata 4.09 (menyukai sedikit), manakala kepekatan yang paling kurang diterima adalah 1.00 g/mL dan mendapat purata mata terendah sebanyak 3.80 (tidak menyukai sedikit). Pada kepekatan yang dipilih (0.80 g/mL), jumlah pertumbuhan mikrob telah melebihi 6 log CFU/mL selepas 24 jam untuk minuman MC yang disimpan pada suhu bilik (28±2˚C). Jumlah pertumbuhan mikrob dalam minuman MC yang disimpan pada suhu sejuk (4±2˚C) melebihi 6 log CFU/mL selepas 72 jam. Jumlah kiraan koliform bagi semua sampel jus adalah 93-210 MPN/g. *E. coli* boleh didapati di minuman RTS tetapi tidak

wujud dalam air yang digunakan untuk penyediaan minuman. Kajian ini menunjukkan bahawa MC mempunyai potensi untuk digunakan sebagai ramuan dalam menyediakan minuman RTS yang mengandungi antioksidan.

ACKNOWLEDGEMENTS

First and foremost, I would like to offer my utmost gratitude to the chairman of my supervisory committee, Dr. Nor Ainy Mahyudin, who offered advice and guidance throughout the process of completing this study. Also not forgetting my co-supervisors Dr. Hazrina Ghazali and Dr Nor Khaizura Mahmud @ Ab Rashid for their advices and encouragement.

I would also like to thank all the laboratory assistants from the Biochemical Laboratory and the Microbiology Laboratory for their professional support. Thanks also to all supporting staffs from the department of Postgraduates, Research and Innovation.

Besides, I would like to sincerely thank my colleagues Faez Nur Syafiqah, Nur Amira, Selvaneswary, Zuraidah, Salahaldin and Shu'aibu for their help and support. It was a great time working with all of you and it had made our time in the laboratory more enjoyable. Not forgetting all other UPM friends that offered encouragement.

Special thanks to Putra Grant - Putra Graduate Initiative (IPS) and Graduate Research Fellowship (GRF) for providing financial support.

Last but not least, I would want to thank my family for their unending support throughout my studies in university.

I certify that a Thesis Examination Committee has met on 21 December 2017 to conduct the final examination of Nadiah Husseini binti Zainol Abidin on her thesis entitled "Hybrid Raman-Erbium Random Fiber Laser" 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 Doctor of Philosophy.

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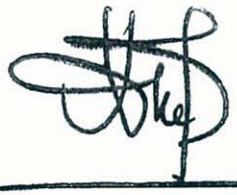

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LIST OF ABBREVIATIONS

˚C	Degree in Celcius
µL	Microliter
Abs	Absorbance
AOAC	Association of Official Agricultural Chemists
A_w	Water activity
BAM	Bacteriological Analytical Manual
CFU	Colony-forming units
E. coli	*Escherichia coli*
g	grams
IC_{50}	Half maximal inhibitory concentration
L	Liter
LAB	Lactic acid bacteria
LST	Lauryl tryptose/lauryl sulphate broth
L-EMB	Eosin methylene blue, Levine's formulation
MC	*Momordica charantia* L.
mg	milligrams
mL	milliliter
N	Amount of substance in moles, mol.
RTS	Ready-to-serve
TFTC	Too Few to Count
TNTC	Too Numerous to Count
TSS	Total soluble solids
USFDA	US Food and Drug Administration

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The consumption of natural fruit and vegetable juices has increased in recent years as opposed to caffeine containing beverages like coffee, tea and carbonated soft drinks (Waghray et al., 2012). Juices are the liquid products, purees or concentrates extracted from one or a mixture of fruits or vegetables (Fraternale et al., 2011). The addition of sugars or acids can be permitted but has to be in accordance with standards and regulations (Aneja et al., 2014). As fresh juices can be too acidic or too strongly flavoured, diluting or blending is carried out for a more pleasant consumption (Bagde and Tumane, 2011). Besides providing refreshing satisfaction, functional juices are able to provide necessary nutrients. It is known to have low sodium, cholesterol and fat content, high concentration of vitamin C, polyphenols and antioxidants that are able to prevent nutrient-related diseases such as heart diseases, cancer and diabetes (Aneja et al., 2014).

Momordica charantia L. (MC), which is also known as bitter gourd or bitter melon grows at tropical and subtropical areas of Asia, Africa and the Caribbean (Efird et al., 2014). It is often grown for its consumption as a vegetable, and also used for its medicinal properties in developing countries such as Brazil, China, Colombia, Cuba, Ghana, Haiti, India, Mexico, Malaya, New Zealand, Panama and Peru (Grover and Yadav, 2004). More than 200 different compounds that are potentially possessing medicinal properties are isolated from different parts of the bitter gourd including its fruit, seeds, leaves and stems. Some of the most studied constituents that showed evidence in improving glycemic control are charantin, polypeptide-p, vicine, momordin and other derivatives (Efird et al., 2014). It is also high in phenolic compounds such as gallic acid, gentisic acid (2, 5-dihydroxyl benzoic acid), catechins, chlorogenic acid and epicatechin (Din et al, 2011). Studies for the past few decades showed that MC possess various beneficial properties such as antidiabetic, antiviral, antitumor, antileukemic, antibacterial, anthelmintic, antimutagenic, antimycobacterial, antioxidant, antiulcer, anti-inflammatory, hypocholesterolemic, hypotriglyceridemic, hypotensive, immunostimulant, and insecticidal properties (Grover and Yadav, 2004).

Antioxidants in natural foods are gaining attention due to their function as chemopreventive agents against oxidative damage (Wu and Ng, 2007). The MC is shown to possess potent antioxidant and free radical scavenging activities, which explained the therapeutic benefits suggested by traditional claims (Wu and Ng, 2007). Various parts of MC including the leaf, stem, the green fruit and the ripe fruit are known to have antioxidant (Kubola and Siriamornpun, 2008). The seeds of the MC also shown potent antioxidant activity, and is regarded as directly or indirectly responsible to its hypoglycemic activity (Sathishsekar, and Subramanian, 2005).

There is a growing concern in the microbial safety in raw juices as microbial contamination has become a major health problem. Raw juices are among one of the street foods and are sold at public places and at the roadside (Reddi et al., 2015). The sanitary condition of water used to prepare juices are also a concern. Water sources for washing fruits are mostly obtained from wells and taps, at the same time there are also vendors not washing fruits before juicing and water used for dilution of juices are not boiled (Nonga, Simforian, and Ndabikunze, 2014). These water sources including environmental exposure, improper washing, prolonged preservation without refrigeration and use of unhygienic water may lead to the increase in foodborne illnesses due to pathogens (Tambekar et al., 2009). Juices can become a transmission path for pathogens such as *Escherichia coli* O157, *Salmonella* and *Cryptosporidium*. Juices are also more susceptible to spoilage due to high water content, raw ingredients in contact with air and microorganisms in the environment during handling processes (Aneja et al., 2014).

In this study, the bitter gourd is used to prepare a ready-to-serve drink with minimal amount of processing techniques. The drink was tested for its antioxidant properties as well as the overall proximate analysis to identify its physicochemical properties. Due to the strong taste of the bitter gourd, it is a challenge to improve on the original recipe by increasing the concentration of MC, but is easily accepted by most consumers. Therefore, a sensory evaluation was carried out to determine the acceptability of the ready-to-serve drink. As the ready-to-serve drink only undergo minimal amount of processing, it is succumb to high perishability. Microbiological quality tests were carried out to observe the shelf life of the drink.

1.2 Problem Statement

The bitter gourd (*Momordica charantia* L.) (MC) is consumed in many ways due to its nutritional contents including vitamin A and C, phosphorous and iron (Raman & Lau, 1996). It is developed into drinks and beverages due to its convenience. It is necessary to conduct proximate analyses in order to determine if a certain RTS drink adhere to local laws and regulations before it can be sold in the market. The level of antioxidant activity in the drink has to be determined and in relation to study the influence of MC concentration against the antioxidant activity level.

The MC is a good option for preparing healthy ready-to-serve drinks as it contain antioxidant properties (Kubola and Siriamornpun, 2008) but the taste is not prefered by most people and it needs to be processed to increase palatability (Satkar, Kulthe and Chalke, 2013). The bitter flavour of MC is due to presence of the alkaloid momordicin produced in the fruit and leaves (Thakur and Sharma, 2016; Din et al., 2011).Therefore, there is a need for developing a palatable recipe for the bitter gourd drink. A sensory evaluation of the ready-to-serve drink is necessary in order to determine the most palatable formulation among consumers.

Juices are very susceptible to spoilage and a transmitting vehicle of foodborne pathogens (Aneja et al., 2014) and is also not handled properly by food handlers (Nonga, Simforian, and Ndabikunze, 2014; Tambekar et al., 2009). A study on the microbial shelf life of this drink formulation is needed so that food handlers have the knowledge on how long the drink can be kept after preparation before serving in order to reduce wastage due to spoilage and for it to be safe for consumption.

1.3 Objectives of Study

Based on the stated problems, the following objectives of the study are formed:

1. To determine the physicochemical and antioxidant properties of a ready-to-serve bitter gourd (*Momordica charantia* L.) drink with different concentrations of the bitter gourd (*Momordica charantia* L.)
2. To conduct a sensory evaluation on the ready-to-serve bitter gourd (*Momordica charantia* L.) drink for acceptability
3. To determine the effect of water quality of the ready-to-serve bitter gourd (*Momordica charantia* L.) drink stored at different temperatures against the microbial shelf life.

1.4 Hypotheses

The following hypotheses were developed and will be answered in the study:

1. A higher concentration of the bitter gourd (*Momordica charantia* L.) in the ready-to-serve drink will enhance the level of antioxidant radical scavenging activity
2. The increase in concentration of bitter gourd (*Momordica charantia* L.) in the ready-to-serve drink will reduce its palatability and overall acceptability
3. The microbial quality of the ready-to-serve bitter gourd (*Momordica charantia* L.) drink can be enhanced using boiled water and stored at low storage temperatures

1.5 Significance of Study

An improved ready-to-serve drink using the bitter gourd (MC) with antioxidant properties will be developed from the original recipe (Ghazali et al., 201612270829). This study is able to provide the developed recipe with basic physicochemical information such as moisture, protein, fat, fibre, ash and carbohydrate content. The antioxidant activity inhibition level will be determined using the DPPH radical scavenging assay and the relationship between the concentrations of MC in the RTS drink against the antioxidant activity can be studied. By conducting a sensory evaluation, the most accepted concentration of MC in the drink can be determined and it can provide with a general level of acceptability of the MC taste among consumers. The study on microbial quality can evaluate the current sanitary conditions of water used in the preparation of fresh RTS drinks. The effects of water quality and storage

temperatures of fresh RTS drinks on its microbial safety will also be demonstrated through this study.

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