UNIVERSITI PUTRA MALAYSIA

DEVELOPMENTS OF SPRAY-DRIED KOMBUCHA TEA BEVERAGE

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DEVELOPMENTS OF SPRAY DRIED KOMBUCHA TEA BEVERAGE

By

NOR ARIFAH BINTI MAT NOR

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

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DEDICATION

This thesis is dedicated to

My murabbi, Dr Mohd Hizul Azri B. Hj. Md. Noor
My father, Mat Nor B. Bidin,
My mother, Radziah Bt Sulaiman,
My siblings and my whole family,
My sister from another parents, Dr Atifah Ahmad Yusoff
My friends of ikhwahs and akhwats.

With love, respect and a bunch of memories
Indeed, we belong to Allah and indeed to Him we will return.

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

DEVELOPMENTS OF SPRAY DRIED KOMBUCHA TEA BEVERAGE

By

NOR ARIFAH BINTI MAT NOR

JANUARY 2020

Chairman :Assoc. Prof. Anis Shobirin binti Meor Hussin
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Kombucha tea (KT) is a healthy and refreshing beverage obtained from the fermentation of sugared tea by symbiotic cultures of acetic acid bacteria and fungi. However, KT will eventually turn too sour to drink and will become vinegar if over fermented. Therefore, this study aimed to evaluate the effects of spray drying on the physicochemical, antioxidant and antibacterial properties of KT. Two wall materials including maltodextrin (MD) and Arabic gum (AG) were used. The physiochemical properties of the spray dried kombucha were 16.33±0.58 Brix° for total soluble solid, 3.48±0.13% for moisture content, 0.25±0.34 for water activity, 3.28±0.03 for pH, 0.42±0.004 g/cm3 for bulk density and 47.27±2.50% for wettability. The antibacterial activity significantly ($p<0.05$) after the kombucha fermentation, while no significant effects observed for the spray drying process on the activity. Spray dried kombucha tea powder produced using AG as wall material showed good physical properties, antioxidant properties and strong antibacterial activity compared to MD. Moreover, second objective is to optimize the condition of production of dried kombucha tea using selected wall materials. Optimization for the spray dried conditions was carried out using surface methodology surface (RSM) with box Behnken design (BBD) for the factors inlet temperature (140 – 180ºC), feed flow rate (25-35 rpm) and aspirator rate (80-100). The optimal conditions estimated for the process were inlet temperature at 178°C, feed flow rate at 25 rpm, and aspirator rate at 80%. The RSM model was significant and inlet temperature was the variable with the greatest impact on most of the responses. The third objective is to characterize the properties of optimized powdered kombucha tea. Metabolomics profiling was performed using ^{1}H NMR based technique. Major metabolites changes were observed for the significant decline of sucrose (17.8626 mmol/L) in unfermented tea and increase ethanol (1.8393 mmol/L) and glucose (2.6751 mmol/L) in the fresh kombucha tea. The acetic acid was significantly reduced (0.053 mmol/L) and the ethanol was totally degraded in the spray dry kombucha tea. The results indicated that the spray drying process using 10% AG can improve the physiochemical properties of kombucha and sustain it is antioxidant and antibacterial activities. Moreover, the spray drying process caused degradation of ethanol making kombucha safe for alcohol allergic and Halal concerned consumers.

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

PEMBANGUNAN MINUMAN BERFUNGSI TEH KOMBUCHA MELALUI PROSES SEMBURAN KERING

Oleh

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Januari 2020

Pengerusi : Prof. Madya Anis Shobirin binti Meor Hussin, PhD
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Teh Kombucha (KT) adalah minuman sihat dan menyegarkan yang diperoleh dari fermentasi teh gula oleh kultur simbiotik bakteria dan kulat asid asetik. Walau bagaimanapun, KT akhirnya akan menjadi terlalu masam untuk diminum dan akan menjadi cuka jika ditapai terlalu lama. Oleh itu, kajian ini bertujuan untuk menilai kesan pengeringan semburan pada sifat fizikokimia, antioksidan dan antibakteria KT. Dua bahan dinding termasuk maltodextrin (MD) dan gum Arab (AG) digunakan. Sifat fisiokimia kombucha yang dikeringkan dengan semburan adalah 16.33 ± 0.58 Brix ° untuk jumlah pepejal larut, 3.48 ± 0.13% untuk kandungan lembapan, 0.25 ± 0.34 untuk aktiviti air, 3.28 ± 0.03 untuk pH, 0.42 ± 0.004 g / cm3 untuk ketumpatan pukal dan 47.27 ± 2.50% untuk kebasahan. Aktiviti antibakteria menunjukkan perbezaan signifikan ($p < 0,05$) selepas penapaian kombucha, sementara tidak ada kesan yang signifikan untuk proses pengeringan semburan pada aktiviti tersebut. Serbuk teh kombucha kering semburan yang dihasilkan menggunakan AG sebagai bahan dinding menunjukkan sifat fizikal yang baik, sifat antioksidan dan aktiviti antibakteria yang kuat berbanding dengan MD. Selain itu, objektif kedua adalah untuk mengoptimumkan keadaan pengeluaran teh kombucha kering menggunakan bahan dinding terpilih. Pengoptimuman untuk keadaan pengeringan semburan dilakukan dengan menggunakan permukaan metodologi permukaan (RSM) dengan reka bentuk kotak Behnken (BBD) untuk faktor suhu masuk (140 - 180ºC), laju aliran umpan (25-35 rpm) dan laju aspirator (80-100). Keadaan optimum yang diperkirakan untuk proses tersebut adalah suhu masuk pada 178 ° C, laju aliran umpan pada 25 rpm, dan kadar aspirator pada 80%. Model RSM adalah signifikan dan suhu masuk adalah pemboleh ubah dengan kesan terbesar pada kebanyakan tindak balas. Objektif ketiga adalah mencirikan sifat teh kombucha serbuk yang dioptimumkan. Profil metabolik dilakukan menggunakan teknik berasaskan NMR ^{1}H. Dari pencirian, khususnya terdapat perbezaan yang signifikan yang ditunjukkan antara teh yang tidak ditapai, teh kombucha yang ditapai dan teh kombucha yang telah melaui proses semburan kering. Perubahan metabolit utama diperhatikan untuk penurunan sukrosa yang ketara (17.8626 mmol / L) dalam teh yang tidak ditapai dan peningkatan etanol (1.8393 mmol / L) dan glukosa (2.6751 mmol / L) dalam kombucha yang telah ditapai. Asid asetik dikurangkan dengan ketara (0,053 mmol / L) dan etanol benar-benar terdegradasi dalam the kombucha yang telah melalui proses kering semburan. Hasilnya,

menunjukkan bahawa proses pengeringan semburan menggunakan 10% AG dapat meningkatkan sifat fisiokimia kombucha dan mempertahankannya adalah aktiviti antioksidan dan antibakteri. Selain itu, proses pengeringan semburan menyebabkan penurunan etanol menjadikan kombucha selamat bagi pengguna alkohol alkohol dan Halal.

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With love, respect and a bunch of memories.

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I certify that a Thesis Examination Committee has met on 13th January 2020 to conduct the final examination of Nor Arifah binti Mat Nor on her thesis entitled "Developments of Spray-Dried Kombucha Tea Beverage" 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 is 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 fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

KT	Kombucha tea
MD	Maltodextrin
AG	Arabic gum
BBD	Box Behnken Design
RSM	Response Surface Methodology
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric Reducing Ability of Plasma assay
WSI	Water Solubility Index
WA	Water Activity
PKT	Pure Kombucha Tea
SDKT	Spray Dried Powder Kombucha Tea
NMR	Nuclear Magnetic Resonance spectroscopy
SCOBY	Symbiotic Culture of Bacteria and Yeast
LAB	Lactic Acid Bacteria
ACETOBACTOR	Acetic Acid Bacteria
SA	Staphylococcus Aureus
BS	Bacillus Subtilis
EC	E. Coli
V	Vibrio
L	Listeria Monocytopenia
ST	Salmonella Typhi

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CHAPTER 1

INTRODUCTION

1.1 Research background

Functional foods and beverages have been classified as fortified, enriched, or enhanced products which have a potentially advantages effect on health when consumed as part of a varied diet on a daily basis, at effective levels. According to (Riley, 2015), fermented foods are one of the top 10 food trends in 2016, continuing the trend over the last few years. Food companies are responding to this growing trend either by commercializing traditional fermented foods (e.g., kefir and kombucha, whose market value in North America alone were $130 million and $480 million, respectively, in 2014) or developing novel fermented foods based on the traditional ones (e.g., Bionade, flavoured malt based beverages fermented using the starter culture of kombucha, and Rythem, coconut milk based and fruit juice based beverages fermented using kefir grains).

As consumer consciousness on functional food escalates, the enthusiasm towards conducting scientific research in this field has also proportionately increased (Watawana et al., 2015). There have been a number of longitudinal studies involving the effects of tea and its major constituents on human health. Tea is the oldest known medicine. It was taken in China 5000 years ago for its stimulating and detoxifying properties in the elimination of alcohol and toxins, to improve blood and urine flow, to relieve joint pains, and to improve resistance to diseases (Balentine et al., 1997).

Tea grew rapidly in importance and was incorporated into many social rituals notably in China, Japan and England. Today, tea is the second most popular beverage in the world after water (Yang et al., 1993). However, one of the most significant current discussions in food science, function and technology is another beverage known as kombucha tea (Banerjee et al., 2010; Bhattacharya, Gachhui and Sil, 2013a; Jayabalan et al., 2014).

Kombucha is a popular probiotic drink among many traditional fermented foods. It is an energizing beverage obtained by the fermentation of sugared tea with a symbiotic colony of acetic bacteria and yeast, consumed for its valuable and favourable effects on human health (Teoh et al., 2004). Research conducted in Russia at the beginning of the century and testimony indicate that kombucha can improve resistance against cancer, prevent cardiovascular diseases, promote digestive functions, stimulate the immune system, reduce inflammatory problems, and can have many other benefits (Dufresne et al., 2000). Although the previous study of the beneficial and/or unfavourable effects of kombucha on human have not been scientifically determined yet, there are certain reasons to believe that kombucha may have desirable positive effects on human health.

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Furthermore, the metabolic and health effects of several probiotic food or drink associated an increasing momentum in recent years (Aloulou et al., 2012).Moreover, it has been discussed that the kombucha beverage enhances immunity, helps digestion, gives relief from arthritis, acts as a laxative, prevents microbial infections, helps in combating stress and cancer and vitalizes the physical body, etc (Watawana et al., 2015). Kombucha tea fermentation normally ranges from 7 to 60 days and the biological activities may increase during this process; however, the best results have been obtained in an average of 15 days (Chu et al., 2006) and best consumed immediately after 15 days of fermentation.

However, for most consumers, this may not be convenient to wait for 15 days whenever they want to drink kombucha. Despite its beneficial effects on human health, there is an increasing concern that if kombucha is over fermented, it will turn to vinegar hence, become too sour too drink. The kombucha must be refrigerated to inactivate the fermentation process but it will gradually lose its effervescence. Thus, kombucha tea requires storage to ensure longer shelf life.

In addition, kombucha must be refrigerated. In the refrigerator it can be kept for a month or even two, but gradually loses its effervescence. In addition, making kombucha available in powder form is a better preservation method. Production of powder kombucha can be carried out using range of processes such as freeze drying, vacuum drying and spray drying (Liu et al., 2016). Spray drying is recognized as one of the most convenient in terms process yield, cost and application (Yonekura et al., 2014a)

Thus, kombucha tea requires storage to ensure longer shelf life. In addition, for pasteurized fresh kombucha and kombucha reconstituted, making kombucha available in powder form is a better preservation method. Because, dry formulation is convenient for transportation and storage, a range of processes have been developed to obtain dry formulations with prolonged shelf life such as freeze drying, vacuum drying and spray drying (Liu et al., 2016).

Most of food stuffs commercialized in powder form use spray dry technique to protect their quality and functionality strictly correspond with the energy efficiency and low cost (Bhandari et al., 2013). The process to produce powder kombucha should have minimal effects on the physical properties, sensory, biological activity, bioactive compounds and stability. To the best of our knowledge, there is no previous work has been reported the production of kombucha powder using spray dried technique and determined the effects on the properties.

Therefore, this study was carried out to produce kombucha tea powder through spray drying using different wall materials such as maltodextrin and Arabic gum. In addition, optimize the condition of production of dried kombucha tea with RSM analysis and characterize the physicochemical properties, biological activity and bioactive compounds of optimized kombucha tea powder. The proposed research is expected to

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develop new powdered kombucha tea at low cost, longer storage life, and convenient for transportation and storage.

1.2 Problem statement

The overall demand for probiotic powder beverage has increased in the context of a rapidly growing market, evidencing the need for their larger scale production. The spray-drying of probiotic bacteria enables a larger production scale than the freeze-drying currently used, energy costs are lower and the process is sustainable (Huang et al., 2017). This is also a promising way to microencapsulate bacteria within various protective matrices to ensure their improved resistance during storage, technological processes and digestive stresses.

Drying is a globally used process for food and beverage preservation, ensuring a stable and longer shelf-life, minimize the cost of transportation and facilitating trade. Freeze-drying always being the chosen technique to preserve probiotic bacteria, but it is a time-consuming and expensive process. Among the drying techniques that are possible, spray-drying is one of the most predominant in the dairy industry (Schuck et al., 2016).

It consists in spraying the liquid feed in fine droplets (10 to 150 μm) that are directed into a flow of hot and dry air (usually 150°C to 250°C). The increase in the air-liquid interface area subsequent to spraying dramatically increases the drying kinetics, and it is generally admitted that drying occurs within a few seconds. When compared to freeze-drying, spray-drying represents a lower specific energy cost and higher productivity (Michalska et al., 2016; Shishir and Chen, 2017; Zoric et al., 2017)Furthermore, spray drying can reduce the weight and volume of product, and this lessen the product's packaging, storage, and transportation costs (Affandi et al., 2017).

In order to obtain probiotic powders with high bacteria viability and good powder quality, the process should be optimized in order to simultaneously achieve a reduction in drying temperature and an acceptable water activity in the powders (Chávez et al., 2007; Muller et al., 2010). Moreover, *in vitro* studies have proved that during digestion, a proper spray-drying wall material can shield probiotics against stress. For instance, a loss of viability was narrow by AG as the wall material during spray-drying since the viability of AG treated bacteria was 100-fold higher than the control following exposure for 120 min to porcine gastric juice at 37°C (Desmond et al., 2002).

Moreover, kombucha tea will eventually turn to vinegar if over fermented. To stop fermentation, kombucha tea must be refrigerated. In the refrigerator, at 40 degrees, it will keep for a month or even two but gradually loses effervescence. Although most of the antioxidant activities that have been obtained have increased with the incubation time, prolonged fermentation is not recommended due to the accumulation of organic acids, which could reach damaging levels for direct consumption (Chu et al., 2006).

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Furthermore, the CO_2 generated can start to be accumulated at the interface between the biofilm and the broth and may block the transfer of nutrients creating a starved environment (Chu et al., 2006). Therefore, in order to avoid kombucha tea from becoming too sour to drink due to over fermented the production of kombucha tea in powder form by spray drying will be produced. In the previous study, we observed that pure kombucha tea has excellent antioxidants, antibacterial activity and many more (Dufresne et al., 2000; Watawana et al., 2015; Soto et al., 2018). But, up until now there is no previous work has been reported examining the powder properties of kombucha tea by spray drying process.

1.3 Objectives of the study

The major aim for this project is to produce kombucha tea in powder form using spray dry with optimized conditions and determine the effects on the physicochemical properties, biological activity and bioactive compounds

1. To evaluate the effect of wall materials on physicochemical properties of spray dried powdered kombucha tea.
2. To optimize the production condition of spray dried kombucha tea using selected wall materials.
3. To determine the physical properties, biological activity and bioactive compounds of optimized powdered kombucha tea.

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DEVELOPMENTS OF SPRAY-DRIED KOMBUCHA TEA BEVERAGE

NAME OF STUDENT: NOR ARIFAH BINTI MAT NOR

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