UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF POWDERED KOMBUCHA SOURDOUGH STARTER CULTURE AND CHARACTERIZATION OF KOMBUCHA SOURDOUGH BREAD

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By

BIZURA HASIDA BINTI MOHD ROBY

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 dearest husband, Mohamad Hafiz bin Mohamad
In loving memory of my late mother, Rasiah binti Mat Idris,
My father, Mohd Roby bin Jaffar,
My siblings and my whole family,
and all my friends.

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

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

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December 2020

Chair : Anis Shobirin binti Meor Hussin, PhD
Faculty : Food Science and Technology

Sourdough bread has gained consumer interest due to its excellent sensory characteristics and health functions. However, sourdough starter culture preparation for breadmaking is very tedious, and the inconvenience of liquid traditional sourdough starter (LTSS) culture is not preferred in the modern lifestyle. Therefore, this research was conducted to optimise the production of liquid kombucha sourdough starter (LKSS) culture using Response Surface Methodology (RSM), to evaluate the effect of gum Arabic (GA) on the properties of encapsulated kombucha sourdough starter (EKSS) culture powder, and to characterize the properties of sourdough bread prepared using the EKSS culture. RSM was applied to optimise the incubation temperature (31-37^{O}C) and refreshment time (every 8-12 hours) of LKSS culture production, to minimise the ripening time (h) and maximise the leavening rate (mL/h). The LKSS culture produced at its optimum conditions was compared with the LTSS culture for the microbiological properties and metabolomics profiling using 1H-NMR based technique. The LKSS culture was transformed into powder via encapsulation using GA and spray drying. The EKSS culture properties were evaluated compared to the unencapsulated kombucha sourdough starter (UKSS/control) culture. The powders were stored at different storage to investigate the culture viability throughout 3 months of storage. The EKSS culture was used in breadmaking, and the bread properties were compared with LTSS and baker's yeast (BY) bread. RSM optimisation revealed the optimised incubation temperature and refreshment time for the LKSS culture production are at 34^{O}C and every 7 hours, respectively. The LKSS culture showed significantly ($p<0.05$) higher lactic acid bacteria (LAB) (11.07±0.04 log10CFU/g) and yeast (10.37±0.05 log10CFU/g) counts compared to LTSS. The 1H-NMR analyses showed the presence of 15 metabolites in LKSS culture. The major compounds contributing to LKSS culture's differences were alpha-aminobutyric acid, alanine, acetic acid, riboflavin, pyridoxine, anserine, tryptophan, gluconic acid, and trehalose. GA was found adequate to produce EKSS culture with significantly ($p<0.05$) lower moisture content (5.20±0.19%) and water activity

(0.32±0.02), higher LAB (9.93±0.10 log10CFU/g) and yeast (9.40±0.15 log10CFU/g) viability and survival percentage (90.27% for LAB and 89.52% for yeast) after spray drying compared to control. EKSS culture was stable at 27-29^{O}C as high microbial viabilities for 2 months of storage were observed. EKSS culture extract also exhibited high antibacterial activity (>85% inhibition) against pathogenic and spoilage bacteria used in this study. EKSS culture has produced bread with significantly higher loaf volume (976.70±25.2mL) and specific loaf volume (4.38±0.12mL/g), lower pH (5.16±0.02), longer storage life (6 days), and lower crumb firmness (116.07±6.28g) than BY bread. EKSS culture bread inoculated with spoilage fungi delayed the mycelia growth to 5–10 days compared to BY bread. EKSS culture bread extract demonstrated significantly ($p<0.05$) higher antibacterial activity (50±0.07 to 100±0.00%) than BY bread extract. EKSS culture bread had significantly ($p<0.05$) higher taste and overall acceptability scores than BY bread, indicating this bread was well accepted by consumers. The findings showed that the EKSS culture promises to produce functional sourdough bread with extended shelf life and improved quality that could greatly benefit the bread industry.

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

PEMBANGUNAN SERBUK KULTUR PEMULA DOH MASAM KOMBUCHA DAN PENCIRIAN ROTI DOH MASAM KOMBUCHA

Oleh

BIZURA HASIDA MOHD ROBY

Disember 2020

Chair : Anis Shobirin binti Meor Hussin, PhD
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Roti doh masam telah mendapat perhatian pengguna kerana ciri-ciri sensori yang sangat baik dan fungsinya terhadap kesihatan. Walau bagaimanapun, penyediaan kultur pemula doh masam untuk pembuatan roti sangat rumit dan kesulitan kultur pemula doh masam tradisional cecair (LTSS) tidak sesuai untuk gaya hidup moden. Oleh itu, kajian ini telah dijalankan untuk mengoptimumkan penghasilan kultur pemula doh masam kombucha cecair (LKSS) menggunakan Kaedah Respon Permukaan (RSM), untuk menilai kesan gam Arab (GA) terhadap serbuk kultur pemula doh masam kombucha terkapsul (EKSS), dan untuk mencirikan sifat-sifat roti doh masam yang diperbuat menggunakan kultur EKSS. RSM telah digunakan untuk mengoptimumkan suhu pengeraman (31-37^{O}C) dan masa penyegaran (setiap 8-12 jam) bagi penghasilan kultur pemula doh masam kombucha cecair (LKSS) untuk meminimumkan masa pematangan (j) dan memaksimumkan kadar kenaikan (mL/j). Kultur LKSS yang dihasilkan pada keadaan optimum telah dibandingkan dengan kultur LTSS bagi ciri-ciri mikrobiologi dan pemprofilan metabolomik menggunakan teknik berasaskan ^{1}H-NMR. Kultur LKSS telah diolah menjadi serbuk melalui pengkapsulan menggunakan GA dan pengeringan semburan. Ciri-ciri kultur EKSS dinilai secara perbandingan dengan kultur pemula doh masam kombucha takterkapsul (UKSS/kawalan). Serbuk-serbuk ini disimpan pada suhu simpanan yang berbeza untuk mengkaji kebolehidupan kultur ini sepanjang 3 bulan penyimpanan. Kultur EKSS telah digunakan dalam pembuatan roti dan ciri-ciri roti dibandingkan dengan roti yang diperbuat menggunakan kultur LTSS dan yis segera (BY). Pengoptimuman secara RSM mendedahkan suhu pengeraman dan masa penyegaran yang optimum untuk penghasilan kultur LKSS adalah masing-masing pada suhu 34^{O}C dan setiap 7 jam. Kultur LKSS dengan signifikan ($p < 0.05$) menunjukkan kiraan bakteria asid laktik (LAB) (11.07 ± 0.04 $\log_{10}$CFU/g) dan yis (log 10.37 ± 0.05 $\log_{10}$CFU/g) yang lebih tinggi berbanding kultur LTSS. Analisa ^{1}H-NMR menunjukkan kehadiran 15 metabolit dalam kultur LKSS. Sebatian-sebatian utama yang menyumbang kepada perbezaan kultur LKSS adalah asid alfa-aminobutirik, alanina, asid asetik, riboflavin, piridoksina, anserina, triptofan, asid glukonik and trehalosa. Gam Arab

didapati secara signifikan ($p < 0.05$) cukup untuk menghasilkan kultur EKSS dengan kandungan kelembapan (5.20 ± 0.19%) dan aktiviti air (0.32 ± 0.02) yang lebih rendah, kebolehidupan LAB (9.93 ± 0.10 $\log_{10}$CFU/g) dan yis (9.40 ± 0.15 $\log_{10}$CFU/g) serta peratusan kemandirian (90.27% untuk LAB dan 89.52% untuk yis) yang lebih tinggi selepas pengeringan semburan berbanding kawalan. Kultur EKSS stabil pada suhu 27-29^{O}C memandangkan kebolehidupan mikrob yang tinggi selama 2 bulan penyimpanan diperhatikan. Ekstrak kultur EKSS juga menunjukkan aktiviti antibakteria yang tinggi (>85% perencatan) terhadap bakteria patogen dan bakteria perosak yang digunakan dalam kajian ini. Kultur EKSS telah menghasilkan roti secara signifikan mempunyai isipadu (976.70±25.2mL) dan isipadu spesifik (4.38±0.12mL/g) yang lebih tinggi, pH lebih rendah (5.16±0.02), hayat penyimpanan lebih lama (6 hari) dan kepejalan roti yang lebih rendah (116.07±6.28g) berbanding roti BY. Roti kultur EKSS yang diinokulasi dengan kulat perosak dilambatkan pertumbuhan miselium kepada 5-10 hari berbanding roti BY. Ekstrak roti kultur EKSS menunjukkan dengan signifikan ($p<0.05$) aktiviti antibakteria (50±0.07-100±0.00%) yang lebih tinggi berbanding ekstrak roti BY. Roti kultur EKSS dengan signifikan ($p<0.05$) mempunyai skor rasa dan penerimaan keseluruhan yang lebih tinggi berbanding roti BY, menunjukkan roti ini diterima baik oleh pengguna. Penemuan kajian menunjukkan bahawa kultur EKSS menjanjikan penghasilan roti doh masam berfungsi dengan jangka hayat lebih panjang dan kualiti lebih baik, yang boleh memberikan manfaat yang besar kepada industri roti.

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Indeed, with every hardship, there is ease.

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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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
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LIST OF ABBREVIATIONS

AAB	Acetic Acid Bacteria
AACC	American Association for Clinical Chemistry
ANOVA	Analysis of Variance
a_w	Water activity
BY	Baker's Yeast
CCD	Central Composite Design
CFU	Colony Forming Unit
CO_2	Carbon Dioxide
EKSS	Encapsulated Kombucha Sourdough Starter
EPS	Exopolysaccharides
GABA	γ-aminobutyric acid
GI	Glycaemic Index
KSS	Kombucha Sourdough Starter
LAB	Lactic Acid Bacteria
LTSS	Liquid Kombucha Sourdough Starter
NMR	Nuclear Magnetic Resonance spectroscopy
RSM	Response Surface Methodology
UKSS	Unencapsulated Kombucha Sourdough Starter
WSI	Water Solubility Index
SCOBY	Symbiotic Culture of Bacteria and Yeast
UWF	Unbleached Wheat Flour

CHAPTER 1

INTRODUCTION

1.1 Research Background

Sourdough starter culture is a traditional leavening agent used to create leavened bread before the invention of baker's yeast in the 1860s. Then, the commercial baker's yeast promptly became the primary leavening method due to its convenience and consistency (Couch, 2016). However, the tradition of making sourdough bread is still being practiced in some countries such as the USA, Italy, Germany, and France using traditional starter cultures that were handed down from generation to generation (Siepmann et al., 2018). Sourdough bread is gaining increased popularity nowadays due to the health benefits such as higher nutritional properties, lower glycemic index and starch digestibility, and the high quality associated with sourdough fermentation, such as longer shelf life and better sensorial properties in comparison to yeast bread (Catzeddu, 2019; Gobbetti et al., 2019).

Traditional sourdough starter culture preparation involves a spontaneous fermentation of flour and water mixture and propagation by back-slopping (Catzeddu, 2019). Backs-slopping, also known as feeding or refreshment, is a process whereby a new flour and water is added periodically into a fermented mixture of flour and water that was fermented for a certain time at a defined temperature to form a new fermented flour and water (Boyaci-Gunduz & Erten, 2020; Coda et al., 2017). Mixing the flour and water will activate the growth of a mixed community of naturally present lactic acid bacteria (LAB) and yeast (Coda et al., 2017). During the sourdough fermentation, yeast breaks down carbohydrates to produce carbon dioxide and enables the bread to rise. In addition, LAB utilizes maltose and produces lactic acid and acetic acids that create a sour taste in bread (Gobbetti et al., 2014; Ortiz et al., 2013).

Two forms of sourdough starter cultures consisted of traditional liquid (fresh) and powder forms (Kulp & Lorenz, 2003). The sourdough starter cultures were initially distributed in liquid form (Santivarangkna et al., 2007), but due to the dependency for continuous refreshments to keep it active (Reale et al., 2019; De Vuyst et al., 2014), many attempts have been made to develop stable, dried sourdough starter cultures as convenient bakery ingredients. Dried sourdough starter culture is obtained from mature sourdoughs and can be stabilized via drum drying, freeze-drying, fluidized bed drying, and spray drying (Brandt, 2007; Decock & Cappelle, 2005). Spray drying is comparatively an economical process and was reported to be suitable for the continuous production of dried starter cultures (Peighambardoust et al., 2011).

In this study, kombucha was used as a booster to enhance microbial activity and reduce sourdough starter culture preparation time. Like other fermented food, kombucha is also rich in natural microflora as it contains yeasts, lactic acid bacteria, and acetic acid bacteria that live together symbiotically (May et al., 2019; Jayabalan & Waisundara, 2019). Kombucha is a slightly sweet and slightly acidic beverage produced from the fermentation of sugared tea and consumed worldwide for its refreshing and beneficial properties on human health (Teoh et al., 2004). In the previous study, kombucha was used in milk fermentation to enlarge the assortment of the fermented dairy products as a functional food and natural preservative (Spasenija et al., 2012; Iličić et al., 2011).

According to De Vuyst and Vancanneyt (2007), several factors affect sourdough characteristics, such as the type of flour, the sourdough fermentation conditions (pH and temperature), and the selection of starter cultures with specific and desirable metabolic properties. In this study, optimized incubation temperature and refreshment times of kombucha sourdough starter culture production were determined using Response Surface Methodology (RSM) to obtain the minimum ripening time and the highest leavening rate value. In addition, the ripe kombucha sourdough starter culture was developed into powder form via encapsulation using gum Arabic as wall material and spray drying technique. Finally, the powder was rehydrated and activated to determine the ability to make sourdough bread without the addition of baker's yeast. The application of kombucha sourdough starter culture powder was expected to be successfully used for bread making. Therefore, it could be of great benefit to home bakers and the bread industry in Malaysia.

1.2 Problem Statement

Preparation of traditional sourdough starter culture is a very long process that usually requires more than a week to get a ripe culture for bread making as it relies on the naturally occurring lactic acid bacteria and yeasts that are present in the substrate which is usually only flour and water. To accelerate the process, a substrate that naturally rich in beneficial microbes could be incorporated during the sourdough starter culture preparation such as kefir, kimchi, yogurt, fermented juice, etc. as being reported by previous researchers (Gordún et al., 2015; Mantzourani et al., 2014; Choi et al., 2012; Plessas et al., 2011, 2005). The addition of these substrates will increase the microbial diversity of sourdough that might contribute to sourdough's sensory characteristics and shorten the ripening time.

As the fermented substrates were ripen, indicated by double leavening and formation of sweet odor, fresh liquid sourdough starter culture was formed and ready to use for bread making. An unused liquid sourdough starter can be refrigerated for subsequent usage, but continuous refreshment is needed to keep the culture alive. Therefore, the disadvantages of the liquid sourdough starter culture are short shelf life, which requires consistent ingredients of refreshment and appropriate temperature to maintain the microbial bioavailability (De Vuyst et al., 2014). Moreover, the microbial ecosystem of this liquid sourdough starter culture is easily affected by many factors, for e.g. the storage temperature, refreshment numbers, hygienic conditions, etc. (Lattanzi et al., 2014).

Due to these inconvenience factors, a stable, dried sourdough starter culture is highly recommended for the sourdough industry. Drying can decrease the water activity that will hinder microbial growths and unwanted enzymatic reactions during storage, thus prolong the shelf life of the food products. In addition, drying can reduce the weight and volume of products and reduce the cost of packaging, storage, and transportation (Affandi et al., 2017). Reale et al. (2019) recommended a dried sourdough starter culture produced via spray drying technique over traditional starter culture due to their stability and consistency. On the other hand, Boza et al. (2004) reported that the encapsulation technique could protect the high viability of the microbial cells during spray-drying and subsequent storage. Encapsulation of probiotics by spray drying technique has been successfully used in the food industry for the last decades (Gul, 2017; Cano-Higuita et al., 2015; Ilha et al., 2015; Páez et al., 2013). Milk powder, pectin, starch, casein, whey proteins, maltodextrin, gum Arabic, etc., are the examples of wall materials widely used for encapsulation (Sohail et al., 2013; Manojlović et al., 2010). Gum Arabic is extensively used in spray drying because it supplies excellent emulsifying properties, highly soluble, and surface-active (Gul, 2017).

Kombucha, which is an ancient fermented tea beverage rich in a symbiotic culture of acetic acid bacteria, lactic acid bacteria, and yeasts, was used to initiate the fermentation of sourdough starter culture. Then, kombucha sourdough starter culture powder was produced to improve the handling, packaging, storage, transportation, and sourdough bread making. However, to the best of our knowledge, no previous work has been reported examining the properties of powdered kombucha sourdough starter culture and its application in sourdough bread without the addition of baker's yeast.

1.3 Objectives of the Study

The objectives of this study were:

1. To optimize the production of kombucha sourdough starter culture using Response Surface Methodology (RSM)

2. To evaluate the effect of gum Arabic on the properties of encapsulated kombucha sourdough starter culture powder

3. To characterize the properties of sourdough bread prepared using encapsulated kombucha sourdough starter culture powder

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LIST OF PUBLICATIONS

Publication in Referred Journal:

Mohd Roby, B. H., Muhialdin, B. J., Abadl, M. M. T., Mat Nor, N. A., Marzlan, A. A., Lim, S. A. H., Mustapha, N.A., & Meor Hussin, A. S. (2020). Physical properties, storage stability, and consumer acceptability for sourdough bread produced using encapsulated kombucha sourdough starter culture. *Journal of Food Science*, *85*(8), 2286-2295.

Publication in Conference Proceedings:

Mohd Roby, Meor Hussin, A. S., B. H., Muhialdin, B. J., Mat Nor, N. A., Abadl, M. M.T. (2019). Development of encapsulated sourdough starter powder for the production of functional bread with value-added health benefits. Hype-Interdisplinary Conference 2019, Universiti Teknologi Malaysia, Malaysia. (Poster Presentation)