

UNIVERSITI PUTRA MALAYSIA

THE EFFECTS OF DIFFERENT LEAVES ON MICROFLORA, PHYTOCHEMISTRY AND ITS SENSORY ACCEPTABILITY OF GULAI TEMPOYAK PASTE

MOHD HAFIZ ABDUL ARIS

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By

MOHD HAFIZ ABDUL ARIS

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

May 2015



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

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May 2015

Chairman Faculty : Assoc. Prof. Nor Ainy Mahyudin, PhD : Food Science and Technology

Antimicrobial activity of Vietnamese coriander, turmeric, and asam gelugor leaves were determined via *in vitro* using agar well diffusion test and minimum inhibitory concentration (MIC). The leaves of these plants have continuously applied in the food system, and in this case, gulai tempoyak paste (GTP) had been chosen as the food subject to evaluate the effectiveness of these leaves in controlling microbial growth through the study of storage condition. Apart from that, the phytochemistry and the sensory properties of GTP were determined to identify the differences between nil addition of plant leaves (control sample) and with addition of Vietnamese coriander, turmeric, and *asam gelugor* leaves. Based on agar well diffusion test result, a concentration of 2 mg/mL of Vietnamese coriander leaves extract in distilled water showed strong antibacterial effect with $(17.20 \pm 5.42 \text{ mm})$ at inhibition zone. As for antifungal activity, extract of turmeric leaves in distilled water resulted strong activity $(15.13 \pm 0.17 \text{ mm})$ at inhibition zone compared to other solvents. Meanwhile, as for MIC, 2 mg/mL was the lowest concentration needed from all extracts of leaves to inhibit growth of microbial, as shown in Vietnamese coriander, turmeric, and asam gelugor leaves extracts towards selected spoilage microorganisms. On the other hand, GTP without addition of leaves was treated as control and all the prepared GTPs were stored at 30 °C for 2 days before analyses had been carried out using total plate count (TPC) and yeast and mould count (YMC). The addition of asam gelugor leaves to GTP for 5 minutes of the cooking period significantly (p<0.05) reduced TPC ($\log_{10} 3.54$ CFU/g) compared to Vietnamese coriander ($\log_{10} 4.67$ CFU/g) and turmeric leaves ($\log_{10} 4.70$ CFU/g). Apart from that, this study also demonstrated that TPC and YMC for GTP with plant leaves reduced significantly within 14 days when stored at 4 °C, and up to 8 days when stored at 30 °C, thus, emphasize the microbiological evaluation of GTP. Moreover, the phytochemistry properties of the GTP samples demonstrated

significant differences (p<0.05) in pH, moisture, ash, crude fat, crude fibre and carbohydrate between the control sample and the treated samples. Higher level of crude fibre content was demonstrated in GTP added with Vietnamese coriander $(4.42 \pm 0.23\%)$. Based on sensory evaluation test, the addition of plant leaves slightly affected the acceptability of eating and the physical quality scores of GTP. insignificant difference (p>0.05) in Nevertheless, there was colour. viscosity/consistency, spiciness, and aftertaste. GTP added with Vietnamese coriander leaves resulted in better aroma mean score, and at once produced significantly higher result for overall acceptability. In conclusion, these three types of plant leaves exhibited antimicrobial activity of against selected spoilage bacteria and the addition of plant leaves in GTP possibly improved the microbial safety by inhibiting microbial growth. Furthermore, there were significant effects on microbiological safety of GTP when added with Vietnamese coriander, turmeric, and asam gelugor leaves at different exposure time based on TPC and YMC. Besides, the phytochemistry and the sensory properties of the GTP samples with addition of plant leaves also improved in terms of crude fibre content as well as enhanced sensory attributes for colour, aroma, taste, and overall acceptability of the product.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

KESAN DAUN BERBEZA KE ATAS MIKROFLORA, FITOKIMIA DAN PENERIMAAN SENSORI PES GULAI TEMPOYAK

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Aktiviti antimikrobial daun kesum, kunyit dan asam gelugor ditentukan secara in vitro menggunakan agar ujian penyebaran keseluruhan dan kepekatan perencatan minimum. Daun tumbuhan ini terus digunakan dalam sistem makanan dalam kes ini, makanan yang dijadikan subjek ialah pes gulai tempoyak (GTP) untuk menilai tahap keberkesanan dalam mengendalikan pertumbuhan mikroorganisma oleh kajian keadaan penyimpanan. Selain itu, sifat fitokimia dan sensori GTP dikaji bagi menentukan perbezaan samaada penambahan daun tumbuhan (kesum, kunyit dan asam gelugor) memberi kesan kepada produk. Berdasarkan hasil ujian penyebaran keseluruhan, pada kepekatan 2 mg/mL ekstrak daun kesum dalam air memberikan anti-bakteria yang kuat dengan (17.20 ± 5.42 mm) zon perencatan. Untuk anti-kulat, ekstrak daun kunyit dalam air memberikan aktiviti yang kuat bagi zon perencatan (15.13 \pm 0.17 mm) berbanding dengan pelarut lain. Bagi MIC, 2 mg/mL adalah kepekatan yang paling rendah yang ekstrak tumbuhan menghambat pertumbuhan yang ditunjukkan oleh ekstrak daun kesum, kunyit dan asam gelugor terhadap mikroorganisma perosak yang dipilih. Sementara itu, GTP tanpa penambahan daun dianggap sebagai kawalan dan semua GTP siap disimpan pada 30 °C selama 2 hari sebelum dianalisis menggunakan pengiraan plat total (TPC) dan pengiraan yis dan kulat (YMC). Penambahan daun asam gelugor untuk GTP yang di masukkan pada 5 minit masa memasak secara signifikan (p<0.05) mengurangkan TPC (log₁₀ 3.54 CFU/g) berbanding daun kesum (log₁₀ 4.67 CFU/g) dan daun kunyit (log₁₀ 4.70 CFU/g). Selain daripada itu, kajian ini juga menunjukkan bahawa TPC dan YMC untuk GTP apabila ditambah dengan daun tumbuhan dapat mengurangkan dengan signifikan dalam masa 14 hari apabila disimpan pada 4 °C dan sehingga 8 hari apabila disimpan pada 30 °C. Selain itu, sifat-sifat fitokimia sampel GTP menghasilkan perbezaan yang signifikan (p<0.05) untuk ujian pH, kadar air, abu, lemak kasar, serat kasar dan karbohidrat antara

sampel kawalan dan sampel rawatan. Kandungan serat kasar yang lebih tinggi ditunjukkan dalam GTP yang ditambah dengan daun kesum ($4.42 \pm 0.23\%$). Berdasarkan ujian penilaian deria, penambahan daun tumbuhan sedikit mempengaruhi penerimaan panel dan skor kualiti fizikal GTP. Tidak terdapat perbezaan yang signifikan (p>0.05) dalam warna, kelikatan/konsistensi, kepedasan, dan after taste. GTP ditambah dengan daun kesum menghasilkan skor min aroma yang lebih tinggi skor sekaligus menghasilkan penerimaan keseluruhan yang lebih tinggi daripada panel. Kesimpulannya, ketiga-tiga jenis daun tumbuhan mempamerkan aktiviti antimikrobial terhadap bakteria perosak makanan yang dipilih dan penambahan daun tumbuhan dalam GTP dapat meningkatkan keselamatan mikrobiologi dengan mengawal pertumbuhan mikroorganisma dan ada kesan signifikan terhadap keselamatan mikrobiologi GTP apabila ditambah dengan daun kesum, kunyit dan asam gelugor pada masa pendedahan semasa dimasak yang berbeza berdasarkan TPC dan YMC. Selain itu, sifat-sifat fitokimia dan sensori sampel GTP yang ditambah dengan daun tumbuhan juga bertambah baik pada kadar kandungan serat kasar selain meningkatkan sifat-sifat deria untuk warna, aroma, rasa dan penerimaan keseluruhan produk.

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DECLARATION

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LIST OF ABBREVIATIONS

AG	asam gelugor
ANOVA	Analysis of Variance
AOAC	Association of Official Agricultural Chemists
BHA	Butylated hydroxanisole
CFU/g	Colony Form Unit per gram
cm	centimeter
CRD	Completely Randomized Design
Cu	copper
DMRT	Duncan's Multiple Range Test
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DW	distilled water
g	gram
GTP	gulai tempoyak paste
Н	hexane
H_2SO_4	sulphuric acid
HCl	hydrochloric acid
HIV	Human Immunodeficiency Virus
ICMSF	International Committee on Microbiological Specification for
101101	Food
MIC	Minimum Inhibitory Concentration
min	minute
mm	milimeter
mL	mililiter
NaCl	sodium chloride
NaOH	sodium hydroxide
RTE	ready-to-eat
RTEC	ready to end-cook
RTC	ready to cook
RTH	ready to heat
TL	turmeric leaf
TPC	total plate count
UKFG	United Kingdom Food Grade
VC	Vietnamese coriander
v/v	volume per volume
WHO	World Health Organization
YMC	yeast and mould count
μg	microgram
µg/mL	microgram per mililiter



CHAPTER 1

INTRODUCTION

Fermented food are food substrates that are invaded or overgrown by edible microorganisms whose enzymes, particularly amylases, protease and lipases hydrolyse polysaccharides, proteins and lipids to non-toxic products with development of unique flavours, aromas and textures pleasant and attractive to the human consumer. Indigenous fermented foods make up an important contribution to the human diet in many developing countries (Murty and Kumar, 1995; Steinkraus, 1996). Some of these foods form the essential diet components of certain populations and are consumed either as main dishes or as condiments (Steinkraus, 1996). Fermented durian pulp or locally known as 'tempoyak' is a popular side dish and a condiment in Malaysia. This product is creamy, yellow whitish in colour, and has a strong distinctive aroma. During fermentation, the texture of the durian pulp changes from solid to a semisolid mass accompanied by a strong flavour, the flavour is a result of a unique combination of various sugars, organic acids as well as various volatile organic compounds (Yuliana and Garcia, 2009). Although, fermented durian pulp is recognized as product of lactic acid fermentation, the microbiological and sensory quality of the product made from *tempoyak* is still undescribed.

Gulai tempoyak is a traditional dish that uses fermented durian pulp as its main ingredient and mixed together with fresh turmeric, chilies, water, salt, and some herbs, such as Vietnamese coriander (Persicaria odorata) cooked with freshwater fish. Besides, versatility of Malay dish makes it very unique, as it is know that Malaysia includes a variety of plants in its vast dishes. The plants that are used in cooking can be of various parts, such as leaves, fruits, bark, stem, seed, flower, root, and tuber. Gulai tempoyak is usually prepared from scratch, but nowadays, ready-made pastes are becoming more popular due to busy lifestyles and convenience purposes. In order to meet consumers' demands for healthy and free of additive food, they plays a major role in the modification of our food supply and their demand is currently driven towards foods that are "natural" and free of additives, yet safe for consumption and convenient for use (Rhodehamel, 1992). Consequently, essential oils and plant extracts are the natural antimicrobial constituents in highest demand (Burt, 2004). In addition, the search for natural antimicrobials has led food scientists to investigate the effectiveness of inhibitory compounds to be apply in processed food such as organic acids, essential oils, bacteriocins, dried fermentation-based products, and bioactive compounds (Lemay et al., 2002).

Furthermore, various synthetic chemical preservatives have been applied to food preservation despite the common low temperature preservation applications. Organic substances like boric acid, sulphite derivatives, sodium bisulphate, sodium metabisulphite, and a variety of chemical additives are

applied considerably to control the growth of spoilage microorganisms in food products (Abu Bakar et al., 2008; Nirmal and Benjakul, 2011). Thus, the safety aspects of chemical or synthetic food additives toward our health have been questioned and argued among consumers. As researchers and consumers have increasing concern about potential health issues associated with chemical and synthetic food additives, they continuously focusing on the utilize of plant products as alternatives to synthetic ones. With that, the demand for natural preservatives has increased worldwide (Pundir and Jain, 2010). The exploration and the investigation of novel antimicrobial agents from natural resources are inclusive of plants or plant based products and others that have been used mainly for food safety and food preservation purposes (Fajimi and Taiwo, 2005; Tagoe et al., 2010). Owing to the antimicrobial and antioxidant properties of fruits and vegetables, their extracts have significant consideration to be used in foods as food additives or natural preservatives (Nanasombat and Lohasupthawee, 2005; Nkambule, 2008; Amrita et al., 2009; Pundir and Jain, 2010).

Application of natural antimicrobial as a natural agent to control microbiological quality of food products have been reported in many country that own their traditional and native plants. In Thailand, green curry and acidic paste are develops to control microbial quality of ready to cook white shrimp (Siripongvutikorn *et al.*, 2012). While in Canada, Lemay *et al.* (2002) studied on effect of natural antimicrobial as a preservative in a cooked and acidified chicken model. There are study done by Gutierrez *et al.* (2008) focusing on antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. Apart from that, comprehensive review has made on natural additives in wheat based pasta and noodle products that using natural antimicrobial. (Li *et al.*, 2014).

On top of that, a large variety of plants is known to contain phytochemicals and phytonutrients that give benefits to mankind. They are abundant in biologically active compounds, including flavonoids, polyphenols, alkaloids, and polysaccharides, which have been well recognized for their pharmacological properties, such as antioxidant, antimicrobial, antifungal, anti-inflammatory, sedative, antimutagenic, antidiabetic, antiaging, hypotensive, anti-stress, and anticancer activities (Mauri *et al.*, 1998). The bioactive compounds that are contained in the plants can be extracted from various parts, including leaf, stem, bark, root, flower, fruit, and seed.

Vietnamese coriander, which is scientifically termed as *Persicaria odorata* is locally known as *kesum* or *laksa* leaves. Meanwhile, turmeric (*Curcuma longa*) leaf is very common in Malay cuisine as it is used for its aroma, colour, and flavour. *Garcinia atroviridis*, locally known as *asam gelugor*, is used for flavouring agent and its leaf and fruit have always been added in cooking practices. These three types of plant leaves are widely used in Malay cooking, as well as for alternative medicine. Additionally, the extracts of

those plant leaves are also known to exhibit antimicrobial properties against various microorganisms. Fresh and dry leaves extracts from Vietnamese coriander essential oil and solvent extraction exhibited antimicrobial activity against several microorganisms (Sasangko *et al.*, 2011; Ridzuan *et al.*, 2013).

In addition, literature has proved the antimicrobial activity of turmeric leaves (Pundir and Jain, 2010; Arutselvi *et al.*, 2012) against several microorganisms, while the crude extracts of *Garcinia atroviridis* from various plant parts have been reported with prominent antimicrobial activity, especially against bacterial microorganisms (Mackeen *et al.*, 2000). Besides, to date, there has been no report on any comparative study concerning leaves of Vietnamese coriander, turmeric, and *asam gelugor* that employed the extraction method to look into antimicrobial activity against food spoilage related microorganisms as these fresh plant leaves are utilized as natural preservatives for *gulai tempoyak* paste (GTP).

Therefore, the objectives of this study are:

- 1. To determine the antimicrobial activity of Vietnamese coriander (*Persicaria odorata*), turmeric (*Curcuma longa*), and *asam gelugor* (*Garcinia atroviridis*) leaves.
- 2. To investigate the microbiological safety of gulai tempoyak paste (GTP) added with Vietnamese coriander (*Persicaria odorata*), turmeric (*Curcuma longa*), and *asam gelugor* (*Garcinia atroviridis*) leaves.
- 3. To determine the phytochemistry and sensory properties of gulai tempoyak paste (GTP) added with Vietnamese coriander (*Persicaria odorata*), turmeric (*Curcuma longa*), and asam gelugor (*Garcinia atroviridis*) leaves.

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APPENDICES

APPENDIX A

Figures



Ingredients needed for preparing GTP and three different leaves used; *asam* gelugor, turmeric, and Vietnamese coriander



Storage of GTP in air tight container

Sensory evaluation of GTP



G

Sample preparation and sensory evaluation being conducted

APPENDIX B



RESPONDENT'S CONSENT

Please read the following information carefully and do not hesitate to discuss any questions you may have with the researcher.

STUDY TITLE : DEVELOPMENT OF *GULAI TEMPOYAK* PASTE ADDED WITH VIETNAMESE CORIANDER, TURMERIC, AND *ASAM GELUGOR* LEAVES AND THEIR EFFECTS ON MICROBIOLOGICAL QUALITY AND ACCEPTABILITY.

CONSENT

I	Identity	Ca	rd No.
address			
	hereby	volunta	rily agree
to take part in the research stated above	7		

recording/ focus group/interview-based/ guestionnaire-based).

I understand that I have the right to withdraw from this research at any time without giving any reason whatsoever. I also understand that this study is confidential and all information provided with regard to my identity will remain private and confidential.

 ${\sf I}^*$ wish / do not wish to know the results related to my participation in the research

I agree/do not agree that the images/photos/video recordings/voice recordings related to me be used in any form of publication or presentation (if applicable)

* delete where necessary

Date :....

No.Tel/Email :....

I confirm that I have explained to the respondent the nature and purpose of the above-mentioned research.

Date	Signature		
	(Res	searcher)	

APPENDIX C Consumer acceptance test score sheet

		Panelist no.:			
		Sex :			
		Age :			
		Race :			
Instructions:					
 2. Place code code samp num code 3. Prior 4. Pleas 5. Evalu with 6. Pleas evalu 7. Wait 8. Do n samp 9. Pleas 	drink the water provided to rinse your patters each sample. It least 30 seconds to start with another sate t eat chewing gum one hour before starts	erall opinion for each need to circle the and put the sample be stir well. pple. then continue to test alate before amples. evaluate the			
Dislike extrem	l l l l l l l l l l l l l l l l l l l	Like extremely			
Viscosity/Consistency(Kepekatan)					
Dislike extrem Aron	-	Like extremely			
Dislike extrem	ly Neither like or dislike	Like extremely			

Taste: Sweetness (Kemanisan)						
1 1	I	I	I	I	I	
Dislike extremely	Neithe	er like or dislike		Like extremely		1
Taste: Salt	tiness (Kemasina	an)				
Diclike ovtromoly	Naitha	· like or dislike		Like ovtromoly		
Dislike extremely	Neithei			Like extremely		
Taste: Sou	ırness (Kemasar	nan)				
1						
Dislike extremely	Neither	like or dislike	•	Like extremely		-
Taste: Her	bal (Rasa herba					
Taste. Her		·				
Dislike extremely	Neither	like or dislike		Like extremely		
Taste: Spie	cy (Kepedasan)					
					I	
Dislike extremely	Neither	like or dislike		Like extremely		
Aftertaste						
Altertaste						
	0	1	2	3	4	5
	Not detected			Strong		
Overall ac	ceptability (Pen	erimaan keseluru	uhan)			
Dislike extremely	Neither	like or dislike	L	ike extremely		
Comments:						
			••••			
	TH	ANK YOU				

APPENDIX D

Sensory evaluation flyers

SENSORY EVALUATION TEST 'The anthentic of Gulai Tempoyak dish' 'Gulai Tempoyak' is the famous traditional Malay dish originated from Temerloh, Pahang which have delicate taste of fermented durian paste (tempoyak). DATE: 11 JUNE 2014 (WEDNESDAY) TIME: 10 a.m -4 p.m VENUE: Sensory Lab, Food 3, FSTM Lets have a try and feel it authentic taste!!! Prize will be given to all participant FOR MORE INFORMATION CONTACT 0199892995 (Hafiz)