



UNIVERSITI PUTRA MALAYSIA

***CHARACTERIZATION AND FUNCTIONAL PROPERTIES OF PECTIN
DERIVED FROM LEAVES, PULP AND SEEDS OF LADY'S FINGER
(Abelmoschus esculentus Moench)***

NUR FARHANA ABD RAHMAN

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By

NUR FARHANA ABD RAHMAN

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

October 2014

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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 of Science

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October 2014

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Institute**

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: Halal Product Research Institute**

In Malaysia, lady's finger also known as okra (*Abelmoschus Esculentus* Moench) can be easily available all year round with low maintenance cost. In order to overcome issues regarding contamination of pork-related gelatin substance in food products, the development of plant based gelling agent was encouraged as an alternative to replace animal gelatin. Based on previous studies, okra plant was indicated as one of the more promising crops which possess unique properties of a gelling agent. Therefore, this study aims to extract, characterize the physicochemical and functional properties of pectin derived from okra leaves, pulp (skin without seeds) and seeds. Pectin was extracted using a sequential extraction with the applications of hot buffer (HB), hot buffer with chelating agents (CH), diluted alkali (DA) and concentrated alkali (CA) soluble solids. The fractions obtained were compared to commercial pectin in terms of functional group identification using Fourier Transform Infrared Spectroscopy (FTIR), nutritional composition, monosaccharide composition, pH, melting point, viscosity, gel strength and functional properties. Optimization of concentration was performed to determine the optimal concentration for gel formation and subjected to melting point, viscosity and gel strength analysis. The highest pectin yield was extracted from seeds with all fractions yield of 86%, followed by the leaves (75%) and pulp (71%). CH extraction gave the highest yield (>40%) compared to HB and DA from okra leaves, pulp and seeds. Nutritional composition showed total dietary fibre was the highest in okra leaves (65.06 ± 1.59) which was significantly different ($p < 0.05$) from okra pulp and seeds. HB fraction of leaves, pulp and seeds had highly purified pectin due to high anhydro uronic acid content and degree of esterification. Gel formation was achieved when okra pectin was blended together with konjac glucomannan (KG) and stored 16 - 18hr at $4^{\circ}\text{C} \pm 1.0$ (5.0% of okra pectin: 1.6% of KG). The presence of major functional groups of pectin lies at $1,300\text{-}800\text{ cm}^{-1}$ in most of the HB, CH and DA fractions of okra leaves, pulp and seeds. Gel formation was identified by analysing gel strength and viscosity which showed HB had lower gel strength (< 15 Bloom grams) than CH fraction, meanwhile DA fraction exceeds 50 (g) which generally failed to form a gel. Galacturonic acid and rhamnose were identified in all fractions of okra leaves, pulp and seeds by using HPLC-RI. Water and oil holding capacities of HB fraction from okra pulp were higher compared to others, meanwhile DA fractions of okra leaves showed a

higher emulsifying ability and better foaming properties than the commercial pectin. Conclusively, characterization and functional properties of HB, CH and DA fractions derived from okra leaves, pulp and seeds showed potential use of the plant as gelling agent.



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**PENCIRIAN DAN CIRI-CIRI FUNGSI PEKTIN YANG DIPEROLEHI
DARIPADA DAUN, PULPA DAN BIJI BENDI (*Abelmoschus esculentus* Moench)**

Oleh

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Oktober 2014

**Pengerusi
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Di Malaysia, bendi dikenali sebagai okra (*Abelmoschus Esculentus* Moench) yang mudah didapati sepanjang tahun dengan kos penjagaan yang rendah. Untuk mengatasi isu berkaitan kontaminasi gelatin-babi dalam produk makanan, pembangunan sumber yang berasaskan tumbuhan adalah digalakkan sebagai alternatif untuk menggantikan gelatin haiwan. Berdasarkan kajian sebelum ini, pokok bendi adalah salah satu tanaman yang mempunyai satu ciri unik iaitu sebagai agen gel. Oleh itu, kajian ini bertujuan untuk mengekstrak, menentukan sifat fizikokimia dan fungsian pektin yang diperolehi daripada daun bendi, pulpa (kulit tanpa biji) dan biji. Pektin telah diekstrak dengan menggunakan pengekstrakan berurutan dengan aplikasi penimbal yang bergerak balas (panas) (HB), penimbal yang bergerak balas (panas) dengan agen pengelat (CH), alkali cair (DA) dan alkali pekat (CA) untuk pepejal terlarut. Bahagian yang diperolehi dibandingkan dengan pektin komersial dari segi identifikasi kumpulan berfungsi dengan menggunakan spektroskopi inframerah transformasi fourier (FTIR), komposisi nutrisi, komposisi monosakarida, pH, takat lebur, kelikatan, kekuatan gel dan sifat kefungsiannya. Pengoptimuman konsentrasi telah dijalankan untuk menentukan kepekatan optimum untuk membentuk gel, takat lebur, kelikatan dan analisis kekuatan gel. Hasil tertinggi pektin diekstrak daripada biji bendi dengan keseluruhan hasil sebanyak 86% diikuti oleh daun bendi (75%) dan pulpa (71%). Pengekstrakan CH memberikan hasil yang paling tinggi (>40%) berbanding dengan HB dan DA daripada daun bendi, pulpa dan biji. Komposisi nutrisi menunjukkan jumlah serat tertinggi dalam daun bendi (65.06 ± 1.59) di mana ia jauh berbeza ($p < 0.05$) dengan pulpa dan biji bendi. Bahagian HB yang telah diekstrak daripada daun, pulpa dan benih bendi menunjukkan pektin yang tulen kerana kandungan anhydro uronic asid dan darjah pengesteran proses yang tinggi. Pembentukan gel telah dicapai apabila pektin dicampurkan dengan konjac glucomannan (KG) dan disimpan selama 16-18 jam, pada $4 \text{ }^{\circ}\text{C} \pm 1.0$ (5.0% pektin bendi : 1.6% KG). Kehadiran kumpulan berfungsi utama pektin terletak di 1,300-800 cm^{-1} di kebanyakan HB, CH dan DA bahagian daun, pulpa dan biji bendi. Pembentukan gel telah dikenal pasti melalui analisis kekuatan dan kelikatan gel. Kekuatan gel HB adalah lebih rendah (< 15) (g) daripada bahagian CH, sementara bahagian DA melebihi 50 (g) dimana ia gagal untuk membentuk gel. Galacturonic asid dan rhamnose adalah komposisi gula yang telah dikenal pasti dalam semua bahagian daun, pulpa dan biji bendi dengan menggunakan HPLC - RI. Bahagian HB daripada

pulpa bendi telah menunjukkan kemampuan mengikat air dan minyak yang lebih tinggi berbanding dengan bahagian lain, sementara bahagian DA daripada daun bendi menunjukkan kemampuan mengemulsi yang lebih tinggi dan sifat berbusar yang lebih baik daripada pektin komersial. Kesimpulannya, pencirian dan sifat fungsian daripada bahagian HB, CH dan DA yang diekstrak daripada daun, pulpa dan biji bendi menunjukkan tumbuhan yang berpotensi untuk digunakan sebagai agen penjelan.



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LIST OF ABBREVIATIONS

AIS	Alcohol insoluble solids
AUA	Anhydro uronic acid
CA	Concentrated alkali
CH	Chelating agent
DA	Diluted alkali
DE	Degree of esterification
EA	Emulsion ability
EAI	Emulsion ability index
FA	Foaming ability
FS	Foaming Stability
FTIR	Fourier Transform Infra-red
HB	Hot buffer
HBSS	Hot buffer soluble solids
HPLC	High performance Liquid Chromatography
MeO	Methoxyl
KG	Konjac glucomannan
LMP	Low methoxyl pectin

CHAPTER 1

INTRODUCTION

1.1 Research background

Food is very important in our life as it is essential for growth, energy production, cell repair and maintenance of proper health. Any issues regarding food eventually will attract public interest and indirectly affect the requirement of the body. Therefore, it is necessary for humans to consume not only ordinary food but also functional food which promotes health. As Muslims, it is obligatory to consume halal food which concerns more about the source of food products and suggested to consume 'tayyib' food, which generally known as 'halal' and 'tayyib' food. Halal is an Arabic word known as permitted or permissible by the Shariah Law. When applied to the halal food and drinks, it means permissible for consumption by Muslims. Meanwhile, 'tayyib' refers to wholesomeness of food which concludes nutritious, healthy, quality and safe to consume. The opposite term of 'halal' is 'haram' which is known as illegal, prohibited, forbidden to consume by people if it involves food and beverages application. For instance, prohibition of pork-based consumption is strictly mentioned in Holy Quran, in Surah Al Ma'idah : verse 3 which stated "You are forbidden (to eat) that which dies of itself (flesh of unslaughtered animals), blood (which is flowing), and the flesh of swine (including all), also any flesh dedicated to any other than Allah, and those killed by strangling, and those beaten or gored to death, and those killed by a fall, and those mangled by beasts of prey, unless (you find it still alive), and slaughter it, and those sacrificed to idols; and (you are also forbidden) to seek division by arrows. That is a vicious practice."

Commonly used food additives such as emulsifier, gelling agent, stabilizer and many more were added in order to obtain desired foodstuff texture. Gelling agent is one of the food additives substantially used in food to obtain desired texture and fulfil satisfaction of consumer. Nowadays, most of the commonly used commercial gelling agent in food industry consists of gelatin from porcine and bovine due to its unique properties. However its source presents problems for religions such as Islam and Judaism. These communities cannot accept any porcine related food products and if bovine gelatin is used, it must be processed in accordance to Islamic Law. Since then, there has been considerable interest in finding and using alternative substitutes.

Therefore, finding an alternative or similarities in functional properties such as gelation that can be acceptable by these religious groups is very recommendable and eventually overcoming halal food issues rising recently. Research focuses on the exploitation of particular plant namely ladies finger (okra) which can be classified as plant based source.

Okra (*Abelmoschus esculentus* (L.) Moench) known as ladies finger had a branched, semi-woody, annual or biennial, and the height of the plant in the range of 1- 3 m. In India, okra is called as 'bhindi' meanwhile in Malaysia it is commonly known as 'bendi' or 'kacang bendi'. The leaves of okra are alternate, long-stemmed, widely spaced up the stem and deeply divided with toothed margins (Morton, 1990). Okra is widely known as the plant which has viscous mucilaginous and contributes to the production of a plasma expander, suspending and emulsifying agents (Emeje et al.,

2011). In addition, parts of the immature fruit of okra can be used in folk medicine as a diuretic agent and treatment for dental disease (Ndjouenkeu et al., 1996). It was also used as fat substitute in chocolate bar cookies (Romanchik-Cerpovikz et al., 2002), egg white substitute (Costantino and Romanchick-Cerpoviez, 2004) and chocolate frozen dairy dessert (Romanchik-Cerpovikz et al., 2006). Lengsfeld et al. (2004) reported that okra has high carbohydrate content which can be used as a food additive that eventually can act against gastric irritative and inflammatory disease. Generally, okra plants possess highly viscous and slimy mucilage, which is composed of mixtures of polysaccharides and other materials such as protein (Woofle et al., 1977). Sequential extraction of okra cell wall material revealed that okra contained different types of polysaccharides, including pectins, xyloglucans, xylans, and celluloses (Sengkhampan et al., 2009).

Pectin is the methylated ester of polygalacturonic acid that contained 1,4-linked α -D-galacturonic acid residues that could be obtained from many types of plant sources such as hetero polysaccharides derived from cell walls of higher terrestrial plants, peels of citrus fruits, guava and apples (Sengkhampan et al., 2009). It is capable of forming gels with the presence of sugar and acid solutions under suitable conditions. It is usually added in jams and jellies as a gelling agent (Mishra et al., 2001). Moreover, pectin is also widely used as a thickener, texturizer and stabilizer in food industry. The suitability of pectin for different purposes depends on its chemical and characteristics of the extracted pectin. Pectin extracted from a variety of sources can be different in molecular weight, degree of esterification and methoxyl content, thus possessing different functional properties. Therefore, it is an inevitable aspect that pectin should be investigated more thoroughly (Madhav and Pushpalata, 2002; Aina et al., 2012; Ismail et al., 2012).

1.2 Problem statements

Nowadays, the halal status of gelatine as a food additive is doubted. Generally, gelatin is from animal source; porcine and bovine based are the most commonly used. Animals such as porcine and those bovine which do not fulfil the requirement of slaughtering process according to Shariah law are not allowed to be consumed especially by Muslim and Jewish society. However, gelling agent is one of the food additives that are substantially used in food products to obtain desired texture and fulfil satisfaction of consumer. Majority of commercial gelling agents applied in food industry is gelatin. Gelatin is a valuable protein derived from animal by-products, obtained through partial hydrolysis of collagen originated from cartilages, bones, tendons and skins of animals, where one of the functions is to increase the viscosity and acts as thickeners in food product.

Most of commercial gelatin mainly derived from porcine and bovine source. Recently porcine gelatin gained increased interest due to issues of bovine spongiform encephalopathy (BSE) products sourced from cow (Baziwane and He, 2003; Gudmundsson, 2002). BSE is widely known as mad cow disease (Morrison et al., 1999). However, its sources present problems for Muslims and Jewish society. These communities cannot consume any porcine related food products and for beef gelatin, it has to be processed in accordance with religious requirements.

In such situation, finding an alternative or similarities to the functional properties of gelation that can be acceptable to these religious groups is highly recommended since there is no study comparing the gelation properties of okra polysaccharides derived from leaves, pulp and seeds with commercial pectin as control. In addition, their unique properties such as “mucilage” that are present naturally, eventually initiate an idea to explore this property in more details. In Asian countries, okra leaves are rarely consumed directly, unlike in most African countries where the leaves are consumed as vegetables as often as the okra fruits are consumed. In Malaysia, the leaves are wasted once the okra fruits are sufficiently grown. Instead of discarding the leaves, they can be effectively disposed by producing useful products from them.

Moreover, okra plants can be obtained any time, everywhere and can be easily grown at a low maintenance cost. According to FAO database, the five highest okra producing countries in 2012 were India with 6 million (tons) followed by Nigeria (1.1 million tons), Sudan (263000 tons), Iraq (160000 tons) and Cote d'Ivoire (134260 tons) over the total production of okra 8 359 944 tons (FAOSTAT, 2012). Okra is widely grown in the tropics, sub-tropics and warmer areas of the temperate zones (Camciuc et al., 1998). Higher yields are obtained with hot weather (temperatures above 26°C), especially in regions with warm nights (>20°C). Production of okra in Malaysia was 26,530 tons in the year 2012, and therefore ranks 14th the most okra yield production in the world (FAOSTAT, 2012). These are also some other reasons why this plant was chosen in this research. In the end, research focuses on the exploitation of particular plant namely, ladies finger (okra), which can be classified as plant based source. Properties of okra leaves, pulp and seeds can be compared directly with gelatin, and determined whether it is suitable or not as an alternative substance for gelatin in terms of physico-chemical, gel formation and functional properties in this research.

1.3 Significance of the study

The parameter of this research will determine whether okra plant pectin is comparable to the animal based gelatin. The importance of the characterization on physicochemical and functional properties of okra plant pectin can be categorized as an alternative to commercial pectin or gelatin. This study is important especially to those who are vegetarian by which they could not consumed animal based food product. Instead of using gelatin which is sourced from animal, pectin is applicable as it is sourced from plants. Moreover, okra plant is available abundantly anywhere regardless of season, a very suitable plant to be used as it can be easily obtained anytime with low maintenance cost for farming. Thus, it could possibly ease the research as the availability of the sources is easily obtained.

Basically, okra fruits were harvested when they were immature and high in mucilage to prepare the dishes with a desired consistency. As for mature okra seeds, can be used as animal feed specifically for poultry feed. Besides, roasted okra seeds also are used as a good coffee substitute in Turkey (Calisir et al. 2005). In addition, okra seeds have been claimed to be a small source of oils that contain an unsaturated fatty acid known as linoleic acid (Andras et al., 2005; Anwar et al., 2010). However, particularly, okra leaves are rarely used for consumption in Malaysia. At the end, after okra fruits are harvested, they are discarded and become waste.

Therefore, to utilize both okra leaves and fruits, this research was performed to identify the preliminary potential of their residues. Besides, limited literature can be obtained regarding gelling agent properties from okra leaves and fruits which directly compared with mammals gelatin. Therefore, in this study, it can be used for preliminary references as one of the alternative gelatin to replace mammals gelatin based. This study promises a financially lucrative process if pectin can be produced from an alternative okra source. Pointedly, it could help reduce agricultural waste production particularly for okra leaves.

1.4 Objectives

1.4.1 General objective

To evaluate the physico-chemical and functional properties of pectin derived from okra leaves, pulp and seeds.

1.4.2 Specific objectives

1. To determines different types of pectin by sequential extraction.
2. To evaluate the effects of different extraction conditions on the yield, chemical composition and functional groups of okra leaves, pulp and seeds.
3. To determine nutritional composition, sugar composition and functional properties of pectin from okra leaves, pulp and seeds.

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APPENDICES

Appendix 1. Foaming ability (FA) and stability (FS) of CP, HB, CH and DA fractions of okra leaves, pulp and seeds at different concentration

Sample	Fractions	Concentration (%)	FA %	FS %	
				30 min	60 min
Leaves	HB	0.5	34.33 ± 4.04	4.67 ± 0.71	nd
		1	56.78 ± 2.79	10.55 ± 0.95	5.93 ± 0.90
		2	51.67 ± 2.89	11.50 ± 2.12	5.67 ± 1.15
		3	43.33 ± 2.89	5.50 ± 0.71	5.33 ± 0.58
	CH	0.5	nd	nd	nd
		1	nd	nd	nd
		2	8.30 ± 0.81	3.11 ± 0.02	3.11 ± 0.02
		3	5.33 ± 0.32	4.85 ± 0.35	2.09 ± 0.12
	DA	0.5	63.29 ± 1.91	61.90 ± 4.13	61.90 ± 4.13
		1	48.33 ± 2.89	45.00 ± 5.00	43.33 ± 2.89
		2	41.05 ± 1.49	39.47 ± 3.72	39.47 ± 3.72
		3	48.67 ± 3.21	48.33 ± 2.89	48.00 ± 3.46
Pulp	HB	0.5	nd	nd	nd
		1	nd	nd	nd
		2	4.92 ± 0.14	4.50 ± 0.71	nd
		3	7.52 ± 0.78	4.54 ± 0.65	1.70 ± 0.56
	CH	0.5	nd	nd	nd
		1	nd	nd	nd
		2	6.63 ± 0.72	6.52 ± 1.09	5.84 ± 1.13
		3	6.20 ± 1.33	5.69 ± 0.61	1.55 ± 0.70
	DA	0.5	nd	nd	nd
		1	6.32 ± 1.49	nd	nd
		2	10.26 ± 0.37	1.70 ± 0.61	nd
		3	16.23 ± 0.62	nd	nd

Appendix 2. Foaming stability (FS) of CP, HB, CH and DA fractions of okra leaves, pulp and seeds at different concentration

Sample	Fractions	Concentration (%)	FA %	FS %	
				30 min	60 min
Seeds	HB	0.5	nd	nd	nd
		1	5.50 ± 0.71	1.25 ± 0.35	nd
		2	14.00 ± 1.41	6.00 ± 1.41	2.50 ± 0.70
		3	11.29 ± 1.01	4.40 ± 0.57	2.94 ± 0.08
	CH	0.5	nd	nd	nd
		1	nd	nd	nd
		2	4.46 ± 0.47	3.08 ± 0.95	nd
		3	5.13 ± 0.19	2.02 ± 0.97	1.02 ± 0.03
	DA	0.5	14.50 ± 0.71	4.00 ± 1.41	4.00 ± 1.41
		1	4.37 ± 0.75	3.89 ± 1.43	3.17 ± 2.45
		2	13.43 ± 0.61	8.89 ± 3.87	7.25 ± 2.02
		3	15.94 ± 0.79	14.02 ± 3.51	5.87 ± 4.06
Pectin		0.5	33.16 ± 2.23	28.95 ± 3.72	26.84 ± 0.74
		1	6.73 ± 0.58	6.28 ± 1.43	4.71 ± 0.78
		2	nd	nd	nd
		3	nd	nd	nd

Values are given as mean ± standard deviation from triplicate determinations.

nd: not determined; HB : hot buffer; CH: chelating agent, DA: Diluted alkali

LIST OF PUBLICATIONS

Publication in Journal

Abd Rahman, N. F., Shamsudin, R., Ismail, A., & Karim Shah, N. N. A. (2016). Effects of post-drying methods on pomelo fruit peels. Food Science and Biotechnology, 25(S1), 85–90. doi:10.1007/s10068-016-0102-y

Publication in International Proceeding

Nur Farhana A. R., Sadeq H. A.S., Amin I. and Shuhaimi M. (2014). Okra (*Abelmoschus esculentus* L. Moench) pectin: Extraction yield and chemical composition. Malaysia International Halal Research and Education Conference (MIHREC 2014). Mariott Putrajaya Hotel, Putrajaya, Wilayah Persekutuan Putrajaya, Malaysia, 2nd - 4th Disember 2014