UNIVERSITI PUTRA MALAYSIA

PULSED ULTRASOUND-ASSISTED EXTRACTION OF POMEGRANATE (Punica granatum L. var. Malas) PEEL FOR PRODUCTION OF VALUE-ADDED PARBOILED WHEAT NOODLES

MILAD KAZEMI

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By

MILAD KAZEMI

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

I sincerely present this thesis to:

- **My parents who have devoted all their time and energy all during my education and have supported me in hardships. They have always motivated me by their sincere love**

- **My brothers and my sister whose encouragements and supports have always been with me during this period**

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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

PULSED ULTRASOUND-ASSISTED EXTRACTION OF POMEGRANATE (*Punica granatum* L. var. Malas) PEEL FOR PRODUCTION OF VALUE-ADDED PARBOILED WHEAT NOODLES

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July 2015

Chairman: **Associate Professor. Roselina Karim, PhD**
Faculty: **Food Science and Technology**

In the current study, an attempt was made to improve the functional properties of parboiled wheat noodles by using pomegranate (*Punica granatum* L. var. Malas) peel extracts. Pomegranate peel is known to be rich in bioactive compounds including phenolic compounds that are beneficial for human health. Although pomegranate peel contains a notable content of phenolic compounds, it is not currently being utilized commercially and simply thrown away as waste or used for feeding of cattle (521 kg/ ton). On the other hand, noodles are one of the popular foods in the Asian region because of their low cost, ease of preparation, palatable taste and being a rich source of carbohydrate. However, they lack in functional properties. Therefore, extracting the polyphenols from the pomegranate peel using new extraction techniques such as pulsed ultrasound-assisted extraction (PUAE) and incorporating them into wheat noodles not only improve the functional properties of the noodles but also will add value to the by-product of the pomegranate industry. Moreover, applying PUAE can improve the shortcomings related to the conventional extraction methods such as time and energy consumption, low efficiency and relatively high solvents consumption. Since no study has been done on the optimization of PUAE from pomegranate peel of the Malas variety, this study is undertaken with the following objectives: (i) to optimize the operating conditions of pulsed ultrasound-assisted extraction (PUAE) for obtaining the highest yield of crude extract with the maximum antioxidant properties from pomegranate peel, and (ii) to determine the effect of incorporation of pomegranate peel extract (PPE) on the physicochemical and textural properties, and microstructural and sensory characteristics of white salted and yellow alkaline noodles. In the first part of the study, the effects of three extraction variables including extraction time (min), duty cycle (%) and intensity level (W/cm^2) on the yield of crude extract, total phenolic content (TPC), DPPH free radical scavenging activity, ferric reducing antioxidant power (FRAP), punicalagin, ellagic acid (EA) and gallic acid (GA) contents, and chroma (color saturation) of PPE were investigated using response surface methodology in an attempt to find the optimum PUAE condition. The results

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revealed that all the ultrasound variables except for duty cycle significantly ($p \leq 0.05$) affected the antioxidant activity and phenolic content of the crude extract from pomegranate peel. Among the extraction variables studied, the extraction time was the most significant ($p \leq 0.05$) variable which positively affected all the response variables. The intensity level of ultrasonic waves also had positive significant ($p \leq 0.05$) effect on the majority of the response variables except for FRAP. Based on the observation, the highest and lowest extraction yields were 41.63% and 26.82%, respectively. The results showed that TPC of the crude extract varied from 273.05 to 320.26 mg GA equivalent/g of dry weight extract. The FRAP ranged from 6.21 to 7.17 mmol Fe^{2+}/ g dry weight and DPPH free radical scavenging activity (IC_{50}) of the crude extract varied from 220.25 µg/ml to 266.74 µg/ml depending on the extraction conditions. The quantities of punicalagin, EA and GA varied from 128.02 to 146.61 mg punicalagin/g dry weight, 10.12 to 22.53 mg EA/g dry weight and 2.60 to 5.11 mg GA/100 g dry weight, respectively. The current study demonstrated that the optimum extract was achieved by extraction for 10 min using 50% duty cycle and intensity level of 105 W/cm^2. The desirable extraction yield (41.14 %), TPC (318.71 mg GAE/g), DPPH free radical scavenging activity (220.03 µg/ml), FRAP (7.02 mmol $FeSO_4$/g), punicalagin content (146.58 mg/g), EA content (20.66 mg/g), GA content (5.34 mg/100g) and the chroma (32.84) were obtained under the optimum extraction conditions. Therefore, the optimization of PUAE conditions led to obtaining high yield of the crude extract with the highest antioxidant activity in a very short extraction time (i.e. 10 min). In the second part of the work, a full factorial design was applied to investigate the effects of different concentrations of the pomegranate peel extract (0%, 0.75%, and 1.50%) and the noodle type (white salted and yellow alkaline wheat noodles) on the antioxidant activity (TPC, DPPH, and FRAP), physicochemical properties (color, pH and cooking quality), textural properties (firmness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience), microstructural features and sensory characteristics of the noodles. The results indicated that the optimum cooking time of fortified white salted noodles (WSN) was shorter than yellow alkaline noodles (YAN). Percentages of cooking losses were within the acceptable range i.e. from 4.44% to 6.04%. The addition of pomegranate peel extract (PPE) to the noodle formulation did not significantly ($p > 0.05$) affect the cooking yield of wheat noodles; even though it led to a reduction in pH of noodles. The total phenolic contents, DPPH radical scavenging activity, and FRAP values varied from 4.07 to 8.61 mg GAE/g, 23.20 to 95.16% and 32.56 to 114.48 µmol $FeSO_4$/g, respectively. The fortified white salted and yellow alkaline noodles showed significant ($p \leq 0.05$) differences in color and textural properties. The fortified white salted noodles tend to have a softer texture, whereas the fortified yellow alkaline noodles were substantially harder compared to the control YAN. Microstructural observation using scanning electron microscopy (SEM) revealed that the fortified noodles had a more homogeneous and compact microstructure. Based on hedonic test, no significant difference ($p > 0.05$) was observed in the overall acceptability of these products with average scores ranging from 5.72 to 6.45; indicating that the degree of liking of panelists was between slightly like to moderately like. Thus, it can be concluded that the addition of pomegranate peel extract to the parboiled wheat noodles could improve their antioxidant properties without affecting the taste and overall acceptability of the noodles.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai 1memenuhi keperluan untuk ijazah Master Sains

PERAHAN DENYUTAN PADA ULTRABUNYI PADA EKSTRAK KULIT BUAH DELIMA (PUNICA GRANATUM L. VAR. MALAS) UNTUK PENGELUARAN NILAI TAMBAH PADA MI SEPARA MASAK

Oleh

MILAD KAZEMI

Julai 2015

Pengerusi: **Profesor Madya Roselina Karim, PhD**
Fakulti: **Sains dan Teknologi Makanan**

Mi adalah salah 1satu makanan yang popular di rantau Asia kerana kosnya yang rendah, mudah disediakan, rasanya yang sedap dan merupakan sumber yang kaya dengan karbohidrat. Walau bagaimanapun, ia tidak kaya dengan sebatian bioaktif, seperti antioksidan. Satu percubaan telah dibuat untuk meningkatkan sifat-sifat fungsian mi gandum separa masak dengan menggunakan ekstrak kulit buah delima (*Punica granatum* L. var. Malas). Kulit buah delima telah terkenal kaya dengan sebatian bioaktif termasuk sebatian fenolik yang memberi manfaat kepada kesihatan manusia. Oleh itu, pengekstrakan polifenol daripada kulit buah delima dan memasukkannya ke dalam mi gandum bukan sahaja menambahbaik baik sifat-sifat fungsian mi tetapi akan menambah nilai kepada hasil sampingan industri buah delima. Teknik pengekstrakan dibantu puls ultrabunyi (PUAE) merupakan satu kaedah baru yang muncul sedekad yang lalu. Oleh kerana tiada kajian yang telah dilakukan terhadap pengoptimuman PUAE dari kulit buah delima daripada varieti Malas, maka kajian ini dijalankan dengan objektif seperti berikut: (i) untuk mengoptimumkan keadaan operasi pengekstrakan dibantu puls ultrabunyi (PUAE) bagi mendapatkan hasil ekstrak kasar dengan sifat-sifat antioksidan yang maksimum daripada kulit buah delima, dan (ii) untuk menentukan kesan penambahan ekstrak kulit buah delima kulit (PPE) ke atas sifat fizikokimia dan tekstur, serta ciri-ciri mikrostruktur dan deria mi gandum putih bergaram dan mi kuning beralkali. Dalam bahagian pertama kajian ini, kesan tiga pembolehubah pengekstrakan termasuk masa pengekstrakan (min), getaran kerja (%) dan tahap keamatan (W/cm^2) terhadap hasil ekstrak kasar, kandungan jumlah fenol (TPC), aktiviti pemerangkap radikal bebas DPPH, kuasa antioksidan ferik terturun (FRAP), kandungan punikalagin, asid elagik (EA) dan asid galik (GA), dan kroma (ketepuan warna) PPE telah disiasat. Keputusan menunjukkan bahawa semua pembolehubah ultrabunyi kecuali getaran kerja menjejaskan aktiviti antioksidan dan kandungan fenolik ekstrak kasar kulit buah delima kulit dengan ketara ($p \leq 0.05$). Di antara pembolehubah pengekstrakan yang dikaji, masa pengekstrakan adalah pembolehubah yang paling ketara ($p \leq 0.05$) mempengaruhi kesemua respons pembolehubah secara positif. Tahap intensiti

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gelombang ultrasonik juga mempunyai kesan positif yang signifikan ($p \leq 0.05$) ke atas majoriti respons pembolehubah kecuali FRAP dan kandungan EA. Berdasarkan pencerapan, hasil perahan yang tertinggi dan terendah adalah masing-masing, 41.63% dan 26.82%. Hasil kajian menunjukkan bahawa TPC ekstrak kasar berubah daripada 273.05 sehingga 320.26 mg bersamaan GA/g berat kering ekstrak. Julat FRAP adalah di antara 6.21 sehingga 7.17 mmol Fe^{2+}/g berat kering dan aktiviti pemerangkap radikal bebas DPPH (IC_{50}) ekstrak kasar berubah daripada 220.25 µg/ml kepada 266.74 µg/ml bergantung kepada keadaan pengekstrakan. Kuantiti punikalagin, EA dan GA masing-masing berubah daripada 128.02 kepada 146.61 mg punicalagin/g berat kering, 10.12 kepada 22.53 mg EA/g berat kering dan daripada 2.60 kepada 5.11 mg GA/100g berat kering. Kajian ini menunjukkan bahawa pengoptimuman ekstrak telah dicapai melalui pengekstrakan selama 10 min menggunakan getaran kerja 50% dan tahap keamatan 105 W/cm^2. Hasil ekstraksi yang sewajarnya (41.14%), TPC (318.71 mg GAE / g), aktiviti pemerangkap radikal bebas DPPH (220.03 µg/ ml), FRAP (7.02 mmol $FeSO_4$/g), kandungan punikalagin (146.58 mg/g), EA kandungan (20.66 mg/g), kandungan GA (5.34 mg/100 g) dan kroma (32.84) telah diperolehi pada keadaan pengeluaran yang optimum. Kebolehpercayaan keadaan PUAE optimum telah disahkan oleh pengesahan empirikal. Keputusan penyelidikan semasa menunjukkan bahawa ada kemungkinan dengan menggunakan masa pengekstrakan yang sangat pendek (iaitu 10 min) pemulihan ekstrak kasar dengan aktiviti antioksidan yang tertinggi dapat diperolehi. Dalam bahagian kedua kerja ini, kesan kepekatan (0%, 0.75% dan 1.50%) ekstrak kulit buah delima (PPE) yang berbeza keatas sifat-sifat antioksidan dan fizikokimia, kualiti memasak, sifat-sifat tekstur, ciri-ciri mikrostruktur dan deria mi gandum putih bergaram dan mi kuning (beralkali) telah disiasat. Keputusan menunjukkan bahawa masa memasak yang optimum bagi mi putih bergaram (WSN) yang diperkaya adalah lebih pendek daripada mi kuning beralkali (YAN). Peratus kehilangan memasak berada pada dalam julat yang boleh diterima iaitu daripada 4.44% sehingga 6.04%. Penambahan PPE kepada formulasi mi tidak mempengaruhi hasil memasak mi gandum dengan ketara ($p > 0.05$); walaupun ia membawa kepada pengurangan pH mi. Jumlah kandungan fenolik, nilai-nilai aktiviti pemerangkap radikal bebas DPPH dan FRAP masing-masing, berubah daripada 4.07 kepada 8.61 mgGAE/g, 23.20 kepada 95.16% dan 32.56 kepada 114.48 µmol$FeSO_4$/g. Jumlah kandungan fenolik, aktiviti pemerangkap radikal bebas DPPH dan FRAP menunjukkan nilai tertinggi apabila 1.50% ekstrak kulit buah delima ditambah ke dalam mi. Mi putih bergaram dan mi kuning beralkali yang diperkaya menunjukkan perbezaan yang signifikan ($p \leq 0.05$) ke atas warna dan ciri-ciri tekstur. Mi putih bergaram yang diperkaya cenderung untuk mempunyai tekstur yang lebih lembut, manakala mi kuning beralkali yang diperkaya pula mempunyai tekstur yang agak keras berbanding dengan mi kawalan YAN. Pencerapan mikrostruktur menggunakan mikroskopi imbasan elektron (SEM) menunjukkan bahawa mi yang diperkaya mempunyai mikrostruktur yang lebih homogen dan padat. Berdasarkan ujian hedonik, tidak ada perbezaan yang signifikan ($p > 0.05$) dicerap terhadap penerimaan keseluruhan produk-produk ini dengan skor purata di antara 5.72 sehingga 6.45; menunjukkan bahawa darjah kesukaan ahli panel adalah di antara suka sedikit sehingga suka sederhana. Dengan ini dapat disimpulkan bahawa penambahan ekstrak kulit buah delima kulit ke dalam mi gandum separa masak boleh memperbaiki sifat fungsian tanpa menjejaskan atribut deria mi tersebut.

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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

ANOVA	Analysis of variance
CUAE	Continuous ultrasound-assisted extraction
DPPH	2,2-diphenyl-1-picrylhydrazyl
EA	Ellagic acid
Fe^{2+}	Iron (II)
FRAP	Ferric reducing antioxidant power
G	Gram
GA	Gallic acid
GAE	Gallic acid equivalent
H	Hour
h°	Hue angle
HCl	Hydrochloric acid
HPLC	High-performance liquid chromatography
IC_{50}	Inhibitory concentration
kg	Kilogram
kHz	Kilohertz
kV	Kilovolt
L*	Lightness
M	Meter
MAE	Microwave assisted extraction
MeOH	Methanol
mg	Milligram
mg/ml	Milligram per milliliter
MHz	Megahertz
min	Minute

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ml	Milliliter
mm	Millimeter
mm/s	Millimeter per second
mM	Millimolar
mmol	Millimole
mol/ml	Mole per milliliter
MPa	Megapascal
nm	Nanometer
N.s	Newton-second
PPE	Pomegranate peel extract
PUAE	Pulsed ultrasound-assisted extraction
R	Correlation coefficient
RSM	Response surface methodology
s	Scond
SEM	Scanning electron microscopy
SFE	Supercritical fluid extraction
TPC	Total phenolic content
UAE	Ultrasound-assisted extraction
UK	United Kingdom
USA	United States of America
UV	Ultraviolet
v/v	Volume per volume
W/cm^2	Watt /Square centimeter
WSN	White salted noodle
YAN	Yellow alkaline noodle
°C	Degree centigrade

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µg	Microgram
µl	Microliter
µm	Micrometer
µmol	Micromole

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CHAPTER 1

INTRODUCTION

Over the last few years, an increasing trend towards healthy, nutritious and natural diet with nutraceutical properties in order to decrease the risk of chronic mental and physical diseases has been reported (Foschia, Peressini, Sensidoni, & Brennan, 2013). In this respect the concept of functional food provide a close relationship between food manufacturers and consumers health (Balestra, Cocci, Pinnavaia, & Romani, 2011; Kendall, Batterham, Prenzler, Ryan, & Robards, 2008). In order to improve the nutritional value of food products, bioactive compounds are added to fortify them. Some researchers have studied and proven the health benefits and favourable effects of plant based bioactive compounds on the human body (Kris-Etherton, Hecker, Bonanome, Coval, Binkoski, Hilpert, Griel, & Etherton, 2002; Krishnan & Prabhasankar, 2010). All researchers and food manufacturers are encouraged to exploit the benefits of polyphenols for production of functional foods with acceptable sensory features (Dominguez-Perles, Moreno, Carvajal, & Garcia-Viguera, 2011; Ferreira, Santos, Moro, Basto, Andrade, & Gonçalves, 2013). It has been proven that the consumption of suitable amount of antioxidants provide advantages to human health through different mechanisms such as disruption of chain oxidation reactions through electron capture and/or reducing free radicals, (Aviram, Dornfeld, Rosenblat, Volkova, Kaplan, Coleman, Hayek, Presser, & Fuhrman, 2000; Lampe, 1999; Pandey & Rizvi, 2009; Scalbert, Manach, Morand, Rémésy, & Jiménez, 2005) and also inhibition of pro-oxidative metals (e.g. iron) activity (Sugihara, Arakawa, Ohnishi, & Furuno, 1999). Furthermore, the consumption of natural antioxidants is highly preferred over synthetic ones due to the following reasons: (i) natural antioxidants are cheaper and (ii) they are considered as a safer additive by the consumers (Bera, Lahiri, & Nag, 2006; Nanditha, Jena, & Prabhasankar, 2009; Pokorný, 1991).

Pomegranate (*Punica granatum* L.) tree is a medical and ancient plant. It is one of the important cultivated fruits of Iran (Elfalleh, Nasri, Marzougui, Thabti, M'rabet, Yahya, Lachiheb, Guasmi, & Ferchichi, 2009). Pomegranate fruit is an abundant source of functional bioactive compounds such as antioxidants (Guo, Yang, Wei, Li, Xu, & Jiang, 2003; Mirdehghan & Rahemi, 2007). Pomegranate peel comprises about 50% of the total fruit weight. Pomegranate peel contains numerous nutraceutical components which can provide anticarcinogenic, antimutagenic, antitumoral, and antidiabetic properties. Furthermore, these bioactive compounds can reduce the risk of cardiovascular diseases (Caliskan & Bayazit, 2012; Viuda-Martos, Fernández-López, & Pérez-Álvarez, 2010). Despite the fact that pomegranate peel (PP) contains a notable content of phenolics, it is simply thrown away as waste or used for feeding of cattle (Çam & Hışıl, 2010; Hasnaoui, Wathelet, & Jiménez-Araujo, 2014). Based on statistics about 521 kg of pomegranate peel is generated from each ton of fresh fruit (Moorthy, Maran, Muneeswari, Naganyashree, & Shivamathi, 2015; Qu, Pan, & Ma, 2010).

Different methods of extraction and identification of bioactive compounds from pomegranate peel have been reported by several researchers (Fischer, Carle, & Kammerer, 2011; Panichayupakarananta, Issuriya, Sirikatitham, & Wang, 2010;

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Seeram, Lee, Hardy, & Heber, 2005b). However, the conventional extraction methods were applied for extraction in most cases. The major disadvantages of using conventional extraction are as follows: low yield, poor efficiency, long extraction, high energy consumption and low quality extract due to thermal and/or chemical degradation induced by the extraction process (Chemat & Khan, 2011; Chemat, Lagha, AitAmar, Bartels, & Chemat, 2004).

Research on the application of ultrasound-assisted technique for extraction of polyphenols has recently attracted great interest among researchers and manufacturers in the food industry. The ultrasound-assisted extraction (UAE) is considered as an environmental friendly technique due to the decrease of chemicals usage and reduction in operation time, (Khan, Abert-Vian, Fabiano-Tixier, Dangles, & Chemat, 2010; Tabaraki, Heidarizadi, & Benvidi, 2012). The extraction of antioxidants from pomegranate peel by using different types of solvents such as ethyl acetate, acetone, methanol, and ethanol-water have been reported by previous researchers (Li, Guo, Yang, Wei, Xu, & Cheng, 2006; Negi & Jayaprakasha, 2003; Singh, Chidambara Murthy, & Jayaprakasha, 2002; Tabaraki et al., 2012; Viuda-Martos, Ruiz-Navajas, Fernández-López, Sendra, Sayas-Barberá, & Pérez-Álvarez, 2011). In most cases, these findings recommended the usage of methanol and acetone due to the high extraction efficiency. However, the USA Food and Drug Administration (FDA) recommended non-toxic food grade solvents like ethanol for extraction purpose. Pan, Qu, Ma, Atungulu, and McHugh (2011) investigated the effect of different UAE parameters such as treatment time, intensity level and duty cycle on the quality of the crude extract from pomegranate peel.

Noodle is one of the commonly consumed foods in Asian countries due to its cheap price and desirable flavor (Fu, 2008). It has been reported that more than 40% of the total wheat flour in Asia is applied for producing noodles (Chang & Wu, 2008). Approximately 105.59 billion packets (bags and cups) of instant noodles were sold in the world market within one year from 2013 to 2014. In this regard, Malaysians with the total use of 1.35 billion packets was ranked as thirteenth in terms of global consumption of noodles (WINA, 2014). Yellow alkaline noodles (YAN) and white salted noodles (WSN) are two major types of Asian noodles. They are made from basic ingredients including wheat flour, water, and salt (Inglett, Peterson, Carriere, & Maneepun, 2005). These noodles are rich sources of carbohydrates, but they contain insufficient contents of bioactive compounds such as antioxidants, dietary fibers, vitamins, and minerals (Silva, Sagis, van der Linden, & Scholten, 2013).

The main goal of this study was to fortify yellow alkaline and white salted noodles using pomegranate peel extracts in an attempt to produce fortified noodles rich in antioxidant properties. The specific objectives of the present study were as follows:

1. To optimize the pulsed ultrasound-assisted extraction (PUAE) conditions for obtaining the crude extract with the highest antioxidant properties from pomegranate peel; and

2. To investigate the effect of adding pomegranate peel extract on physicochemical and textural properties, microstructural changes and sensory attributes of fresh wheat noodles.

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