FEASIBILITY STUDY OF LYCOPENE EXTRACTION FROM TOMATOES USING NON-TOXIC SOLVENT

By

MISS Naphaphan KUNTHAKUDEE

A Thesis Submitted in Partial Fulfillment of the Requirements
for Doctor of Engineering (CHEMICAL ENGINEERING)
Department of CHEMICAL ENGINEERING
Graduate School, Silpakorn University
Academic Year 2017

Copyright of Graduate School, Silpakorn University

การศึกษาความเป็นไปได้ในการสกัดไลโคปีนออกจากมะเขือเทศด้วยตัวทำละลายที่ไม่มีพิษ

โดย
นางสาวนภาพรรณ คันธกุฎี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรวิศวกรรมศาสตรดุษฎีบัณฑิต
สาขาวิชาวิศวกรรมเคมี แบบ 1.1 ระดับปริญญาดุษฎีบัณฑิต
ภาควิชาวิศวกรรมเคมี
บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร
ปีการศึกษา 2560
ลิขสิทธิ์ของบัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร

FEASIBILITY STUDY OF LYCOPENE EXTRACTION FROM TOMATOES USING NON-TOXIC SOLVENT

By

MISS Naphaphan KUNTHAKUDEE

A Thesis Submitted in Partial Fulfillment of the Requirements
for Doctor of Engineering (CHEMICAL ENGINEERING)
Department of CHEMICAL ENGINEERING
Graduate School, Silpakorn University
Academic Year 2017

Copyright of Graduate School, Silpakorn University

Title	Feasibility study of lycopene extraction from tomatoes using non-toxic solvent
By	Naphaphan KUNTHAKUDEE
Field of Study	(CHEMICAL ENGINEERING)
Advisor	Prakorn Ramakul

Graduate School Silpakorn University in Partial Fulfillment of the Requirements for the Doctor of Engineering

.... Dean of graduate school
(Associate Professor Jurairat Nunthanid, Ph.D.)

Approved by

.... Chair person
(Assistant Professor Tarawipa Puangpetch , Ph.D.)

.... Advisor
(Associate Professor Prakorn Ramakul , D.Eng.)

.... Co Advisor
(Professor Ura Pancharoen , D.Eng.Sc.)

.... Examiner
(Assistant Professor Worapon Kiatkitipong , D.Eng.)

.... External Examiner
(Niti Sunsandee , D.Eng.)

57404801 : Major (CHEMICAL ENGINEERING)
Keyword : LYCOPENE, TOMATO, SOLVENT EXTRACTION, VEGETABLE OIL, OPTIMIZATION

MISS NAPHAPHAN KUNTHAKUDEE : FEASIBILITY STUDY OF LYCOPENE EXTRACTION FROM TOMATOES USING NON-TOXIC SOLVENT THESIS ADVISOR : ASSOCIATE PROFESSOR PRAKORN RAMAKUL, D.Eng.

This research is to study the extraction of lycopene from tomatoes using several vegetable oils as non-toxic solvents namely, sunflower oil, soybean oil, olive oil, coconut oil and palm oil. Ethanol was used as the additional solvent to improve extraction efficiency. Tomato paste and tomato peel waste were used as the raw material. The effect of various parameters on extraction yield including solvent to dry peel ratio, particle size, extraction temperature, extraction time and ethanol concentration were investigate.

In case of tomato paste, pure coconut oil brings the highest yield of lycopene. The addition of ethanol can improve the yield of lycopene. A Box-Behnken Design (BBD) of response surface methodology was applied to optimize the process conditions. The optimum conditions were a solvent to paste ratio of 50 mL/g, temperature of 46°C and extraction time of 42 minutes, which achieved a maximum lycopene yield of up to 78 % or namely 8.01 mg/g paste.

In case of tomato peel waste, sunflower oil was the solvent that provided the highest yield of lycopene. The optimum conditions from BBD were as follows: solvent to dry peel ratio of 40 mL/g, particle size of 0.3 mm, ethanol concentration 56 % and extraction time of 90 min. which resulted in a maximum yield of lycopene of 96 % or namely 4.36 mg/g dry peel.

The high purity of lycopene more than 90% of total carotenoids was confirmed by HPLC analysis. The results from kinetic study showed that extraction of lycopene from tomatoes using vegetable oils is the pseudo-second order process. The activation energy was in the range 14.519-18.822 kJ/mol indicating the extraction of lycopene in the investigated system is controlled by diffusion process.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my advisor, Associate Professor Prakorn Ramakul and co-advisor, Professor Ura Pancharoen for giving valuable knowledge, providing guidance and valuable advice throughout this research. I also would like to thank you Professor Milan Hronec for valuable advices, great knowledge about separation and experiences at Slovak University of Technology in Bratislava, Slovakia throughout one year. In particular, I would also be grateful to Assistant Professor Tarawipa Puangpetch, Assistant Professor Worapon Kiatkittipong and Dr. Niti Sunsandee as the chairman and members of the defense committee.

I would like to acknowledge the Thailand Research Fund for the Royal Golden Jubilee Ph.D. program (Grant No. PHD/0065/2557) and Silpakorn University Research and Development Institute (SURDI) for financial support. I also would like to thank the Faculty of Pharmacy, Srinakharinwirot University for the instrument supports.

Most of all, I would like to express my highest gratitude to my family who always pay attention to us all the time for suggestions, supports and encouragements. I cannot achieve this successfulness without them. Finally, I would like to thank my friends, a member of Department of Chemical Engineering, Silpakorn University, for encouragements and useful help.

Naphaphan KUNTHAKUDEE

TABLE OF CONTENTS

Page

ABSTRACT .... D

ACKNOWLEDGEMENTS .... E

TABLE OF CONTENTS .... F

LIST OF TABLES .... J

LIST OF FIGURES L

Chapter 1 Introduction 16

1.1 Background of the study 16

1.2 Objective of the study 19

1.3 Scope of the study 19

Chapter2 20

Theory 20

2.1 Properties of lycopene 20

2.2 Sources of lycopene 23

2.2.1 Synthetic lycopene 24

2.2.2 Natural lycopene source 25

2.2.3 Plant by-products as Natural Lycopene Source 27

2.3 Benefits of lycopene 27

2.3.1 Role of lycopene in the human body 29

2.3.1.1 Antioxidant activity 29

2.3.1.2 Reducing prostate cancer 30

2.3.1.3 Inhibiting cancer cells 30

2.3.1.4 Reducing of blindness 31
2.3.1.5 Reducing of atherosclerosis and heart disease 31
2.3.1.6 Preventing skin damage 31
2.3.2 Functional uses of lycopene in food 32
2.4 Lycopene analytical methods 33
2.5 Extraction methods 34
2.5.1 Solvent extraction 34
2.5.2 Supercritical fluid extraction (SFE) 35
2.5.3 Enzyme-assisted extraction (EAE) 36
2.5.4 Microwave-assisted extraction (MAE) 37
2.5.5 Ultrasound Assisted Extraction (UAE) 37
2.6 Optimization using response surface methodology 40
Chapter 3 Literature reviews 43
3.1 Conventional solvent extraction using organic solvents 43
3.2 Conventional solvent extraction using non-toxic solvents 46
3.3 Effect of extraction parameters on carotenoids yield 48
3.3.1 Effect of solvent 48
3.3.2 Effect of temperature 49
3.3.3 Effect of extraction time 50
3.3.4 Effect of solvent to solid ratio 51
3.3.5 Effect of particle size 52
Chapter 4 Materials and Methods 53
4.1 Materials and chemicals 53
4.2 Extraction of lycopene 53

มหาวิทยาลัยศิลปากร

4.3 Determination of total lycopene content and percentage yield (% Yield) 56
4.4 Analysis of extracted lycopene 56
4.4.1 Ultraviolet-Visible spectroscopy (UV-VIS) analysis 56
4.4.2 High-performance liquid chromatography (HPLC) analysis 57
4.5 Determination of antioxidant activity 59
4.6 Application with tomato processing waste 60
Chapter 5 62
Results and Discussion 62
5.1 Extraction of lycopene from tomato paste using vegetable oils 62
5.2 HPLC analysis of lycopene extracted from tomato paste 66
5.3 Stability of lycopene in vegetable oils 69
5.4 Antioxidant activity of lycopene extracted from tomato paste using vegetable oils 71
5.5 Kinetic study on extraction of lycopene from tomato paste using vegetable oils 73
5.6 Optimization of extraction of lycopene from tomato paste using vegetable oils 81
5.6.1 Response surface analysis 84
5.6.2 Optimization of extraction conditions 87
5.6.3 Validation of optimized condition 88
5.7 Extraction of lycopene from tomato peel waste using vegetable oils 88
5.8 Optimization of extraction of lycopene from tomato peel waste using vegetable oils 90
5.8.1 Response surface analysis 93
5.8.2 Optimization of extraction conditions 95

5.8.3 Validation of optimized condition 96
5.8.4 HPLC analysis of lycopene extracted from tomato peel waste 97
Chapter 6 98
Conclusions and Recommendations 98
6.1 Conclusions 98
6.2 Recommendations 99
REFERENCES 100
Appendices 114
Appendix A Calibration curve 115
A.1 Calibration curve for standard lycopene using UV-VIS spectrometer 115
A.2 Calibration curve for standard lycopene using HPLC 117
A.3 Calibration curve for standard β-carotene using HPLC 119
Appendix B Calculation for yield of lycopene extract and purity 121
B.1 Calculation for yield of lycopene extracted from tomato paste using UV-VIS spectroscopy 121
B.2 Calculation for purity of lycopene extracted from tomato peel waste using HPLC 122
VITA 125

LIST OF TABLES

Page

Table 1 Physical properties of lycopene ... 21

Table 2 Lycopene content of the fruits and vegetables with high concentrations ... 26

Table 3 Lycopene content in tomato products (Papaioannou et al., 2016) ... 26

Table 4 Advantages and disadvantages of various carotenoid extraction methods (Saini & Keum, 2018) ... 39

Table 5 Summary of the Box-Behnken designs ... 42

Table 6 List of chemicals used in the experiment ... 54

Table 7 Fatty acids composition of vegetable oils (Mawatari, Fukuda, Mori, Mia, & Ohno, 2013) ... 64

Table 8 Viscosity of vegetable oils at 30 °C ... 65

Table 9 The changes of lycopene concentration in vegetable oil during storage ... 70

Table 10 IC50 of value of lycopene extracted from tomato paste using different vegetable oils in DPPH scavenging assay ... 73

Table 11 Models fitted for extraction of lycopene with different vegetable oils ... 76

Table 12 Parameters of second-order kinetic model for extraction of lycopene at different extraction conditions ... 80

Table 13 Actual levels and Box-Benkhen Design levels ... 81

Table 14 Experimental design and response values of lycopene yield in coconut oil ... 83

Table 15 Analysis of variance (ANOVA) for a second order model ... 84

Table 16 Independent variables and their levels for Box-Benhken (BBD) ... 90

Table 17 Experimental and predicted response for different levels of experimental design ... 91

Table 18 Analysis of variance (ANOVA) for the second order polynomial model........ 92

LIST OF FIGURES

Page

Figure 1 Chemical structure of lycopene 20

Figure 2 Molecular structures of lycopene isomers (Kong et al., 2010) 22

Figure 3 Flow chart for lycopene synthesis (Saeid, 2015) 23

Figure 4 Total chemical synthesis of lycopene based on: (i) a condensation between 4,4-dimethoxy-3-methylbutanal 4 and methylenebisphosphonic acid tetraethyl ester 5, leading to the C6-phosphonate 6, followed by (ii) a modified Wittig–Horner reaction between 6 and 6-methyl-5-hepten-2-one 7 producing dimethoxy-3,5,9-triene 8, and (iii) another modified Wittig–Horner reaction between C15-phosphonate 2 and C10-triene dialdehyde 3 producing all-*E*-lycopene (Shen et al., 2011). 24

Figure 5 Biological functions of lycopene (Mein et al., 2008) 28

Figure 6 Geometry of the Box-Behnken design with three factors 41

Figure 7 Solvent extraction of lycopene from tomato using non-toxic solvent; (a) experimental set up (b) lycopene extracted 55

Figure 8 Ultraviolet-Visible (UV-VIS) spectrometer (T92+ UV spectrometer, PG Instruments Ltd., UK) 57

Figure 9 High-performance liquid chromatography (HPLC) instruments 58

Figure 10 The instrument for analyzing of DPPH free radical scavenging capacity assay; (a) Microplate reader (Spectramax® M3, Molecular devices, USA) (b) 96-wells microplate 60

Figure 11 Tomato processing waste from Doi Kham Food Product Co., Ltd., Sakon Nakhon, Thailand; (a) raw tomato processing waste (b) dry peel waste 61

Figure 12 UV-Spectrum of lycopene extracted from tomato paste using various vegetable oils compared with UV-Spectrum of lycopene standard 62

Figure 13 Extraction yield of lycopene from tomato paste using various vegetable oils 63

Figure 14 Yield of lycopene extraction in different kinds of vegetable oils with various ethanol concentrations 66

Figure 15 Chromatogram of standard lycopene 67

Figure 16 Chromatogram of standard β-carotene 68

Figure 17 Chromatogram of lycopene extracted with different of vegetable oils. 68

Figure 18 Stability of lycopene in difference vegetable oils during 8 weeks of storage at room temperature in the dark 70

Figure 19 DPPH radical scavenging activity of lycopene extracted from tomato paste using different vegetable oils 72

Figure 20 Effect of temperature on yield of lycopene extraction from tomato paste with sunflower oil 77

Figure 21 of temperature on yield of lycopene extraction from tomato paste with soybean oil 77

Figur 22 Effect of temperature on yield of lycopene extraction from tomato paste with olive oil 78

Figure 23 Effect of temperature on yield of lycopene extraction from tomato paste with coconut oil 78

Figure 24 Effect of temperature yield of lycopene extraction from tomato paste with palm oil 79

Figure 25 Surface plot showing the effects of solvent to paste ratio (X_1) and extraction temperature (X_2) on lycopene extraction yield (%) 85

Figure 26 Surface plot showing the effects of extraction temperature (X_2) and extraction time (X_3) on lycopene extraction yield (%) 86

Figure 27 Surface plot showing the effects of solvent/material ratio (X_1) and extraction time (X_3) on lycopene extraction yield (%) 86

มหาวิทยาลัยศิลปากร

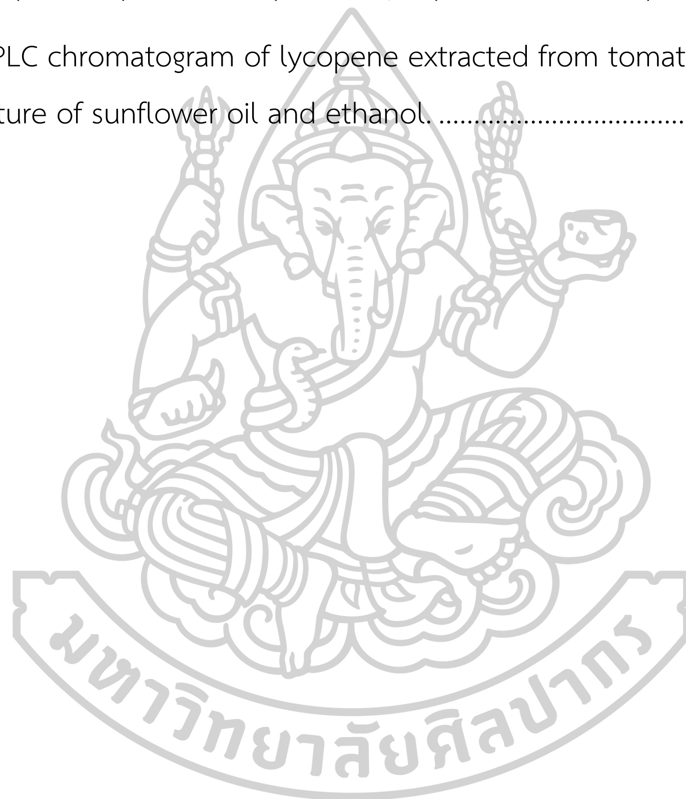
Figure 28 Response optimization plot for lycopene extraction parameters.................. 87

Figure 29 Extraction yield of lycopene from tomato peel waste using various vegetable oils 89

Figure 30 Response surface plots showing the effects of two variables on the response yield of lycopene (mg/g dry peel), with the other two fixed at zero code level... 94

Figure 31 Response optimization plot for lycopene extraction parameters.................. 96

Figure 32 HPLC chromatogram of lycopene extracted from tomato peel waste using solvent mixture of sunflower oil and ethanol. 97



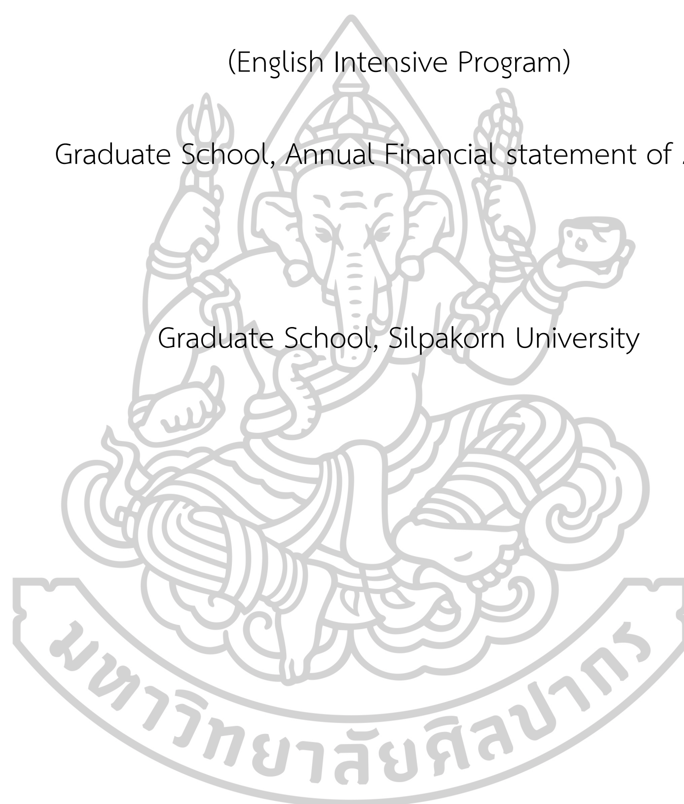
Thesis

The faculty of Community Services has offered a scholarship to undergo a thesis

(English Intensive Program)

Graduate School, Annual Financial statement of 2018

Graduate School, Silpakorn University



Chapter 1

Introduction

1.1 Background of the study

Nowadays, people are turning their attention to health, taking into account the health benefits of prevention and treatment. Obviously, people all over the world have been suffering from dreadful chronic diseases, especially cancer, one of the most killer chronic diseases in human. Carotenoids are important compounds in the human diet as anticarcinogens and cardiovascular disease preventers. Moreover, they can act as antioxidants and precursors of vitamin A (Fiedor & Burda, 2014). They are known to have effect against singlet oxygen, inhibit LDL cholesterol oxidation, control the risk of a range of different cancers and improve cognitive development (Mustafa, Trevino, & Turner, 2012)

Lycopene is one of 600 carotenoid compounds in the class of antioxidants that has a lot of applications in food industries, pharmaceutical and human health. It has been clinically proven that lycopene can prevent cardiovascular and coronary heart diseases and reduce the risk of various cancers especially prostate cancer (Arab & Steck, 2000; Clinton, 1998; Cuevas-Ramos et al., 2013). It can inhibit free radicals, which is an important part of the growth of cancer cells (Shi et al. 2004a). Lycopene can also improve the skin's ability to protect against sunburn and keep the skin looking youthful (Draelos, 2010). In the food industries, lycopene is used as an additive to increase storage stability and nutritional benefits and also as a natural food colorant (Kong et al., 2010; Rizk, El-Kady, & El-Bialy, 2014).

Normally, lycopene, a red carotenoid pigment, was found in fruits and vegetables such as tomatoes, gac, carrots, watermelons, papayas, pink guava, etc.,

มหาวิทยาลัยศิลปากร

especially in tomatoes, lycopene contains about 80-90 % of the total tomato carotenoids (Alda et al., 2009). The human body cannot produce lycopene so it must be obtained from food sources (John Shi & Maguer, 2000; Singh & Goyal, 2008). There is a growing interest in using lycopene as a value-added or functional ingredient in food products. Lycopene as a very potent antioxidant used in many food supplements such as baked goods, breakfast cereals, dairy products including frozen dairy desserts, dairy product analogues, spreads, bottled water, carbonated beverages, fruit and vegetable juices, soybean beverages, candy, soups, salad dressings, and other foods and beverages (Saeid et al., 2016).

Tomato processing waste, a by-product obtained during the processing of tomato juice, is considered as a rich source of lycopene. The quantity of tomato by-product derived from industrial processing is growing annually. Presently, the tomato processing waste is mainly used as animal feed or dumped in landfills (Al-Wandawi, Abdul-Rahman, & Al-Shaikhly, 1985), but high abundance of lycopene in such waste indicates the prospect of utilizing it as a sustainable, alternative and low cost source of lycopene (Papaioannou & Karabelas, 2012).

In all the aforementioned, it seemed promising to extract lycopene from tomato. Recent studies reported about lycopene extraction processes based on supercritical CO_2 (Kassama, Shi, & Mittal, 2008; Machmudah et al., 2012). High yield were achieved without the use of organic solvent but the drawback are large amount of energy consumption and high operating cost. Lycopene can be also extracted using various advanced methods such as ultrasound assisted extraction (Eh & Teoh, 2012; Kumcuoglu, Yilmaz, & Tavman, 2014), microwave assisted extraction (Ho, Ferruzzi, Liceaga, & San Martín-González, 2015) and enzyme assisted extraction (Ranveer, Patil, & Sahoo, 2013; Zuorro, Fidaleo, & Lavecchia, 2011).

Solvent extraction is a conventional method for extraction of lycopene both on a commercial and laboratory scale. The extraction efficiency depend on solvent types and it polarity. The mixtures of both polar and nonpolar solvent are used to enhance extract ability. Usually lycopene could be extracted using various organic solvents including hexane, ethyl acetate or mixtures of acetone/hexane, ethanol/hexane, ethyl acetate/hexane, acetone/ethanol/hexane, etc. However, organic solvent are toxic and not suitable to use as a solvent to extract nutritional compound. A number of long term effects of volatile organic solvents such as hexane and chloroform have been reported including leukemia, renal cancer and neurotoxicity and cardiovascular system disorder (Jin, Hope, Zhai, Smadja-Joffe, & Dick, 2006; Niaz, Bahadar, Maqbool, & Abdollahi, 2015). It is therefore necessary to develop new processes in full correspondence with the green extraction concept for obtaining greener, sustainable and viable processes. One interesting in conventional method for lycopene extraction is the use of edible oils as solvents. Due to the oil solubility of lycopene, vegetable oils are good choice for using as a main solvent instead of organic solvents because they are edible and can be used safely with the human body as well as non-toxic and environmental friendly. The previous studies have reported that the vegetable oils are appropriate solvents for nutritional compounds extraction (Goula, Ververi, Adamopoulou, & Kaderides, 2017; Li, Fabiano-Tixier, Tomao, Cravotto, & Chemat, 2013; Sachindra & Mahendrakar, 2005). Moreover, the oil plays a barrier role against oxygen and consequently retards the oxidation time and degradation rate of carotenoids extract (Pu, Bechtel, & Sathivel, 2010). However, the viscosity of vegetable oil is quite high which results in low diffusivity and consequently low extraction yield. The addition of some solvent would help to solve this problem. Ethanol has been widely use because has a relatively low environmental impact and has a positive net energy balance and is a generally recognized as safe (GRAS) solvent (Das & Bera, 2013).

In this study, green concept extraction was focused to extract lycopene from tomato using vegetable oils as non-toxic solvent.

1.2 Objective of the study

The objectives of this study were to investigate the factors that affect the solvent extraction of lycopene from tomatoes using vegetable oils as non-toxic solvent and to improve the extraction efficiency for application in the extraction of lycopene from tomato processing waste.

1.3 Scope of the study

The scopes of this study include following topics:

1. Extraction of lycopene from tomato paste and tomato processing waste.

2. Optimization using Box-Behnken design in response surface methodology to study the affect the extraction such as type of vegetable oils, extraction temperature, extraction time, solvent/material ratio, composition of solvent mixture.

3. Study the kinetic model for extraction of lycopene from tomatoes using vegetable oils.

4. Study on stability of lycopene extracts in vegetable oils at ambient condition.

5. Analysis of lycopene extracted using ultraviolet-visible spectrophotometry (UV-Vis) and high performance liquid chromatography (HPLC).

6. Determination of antioxidant activity using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity assay.

Chapter2

Theory

2.1 Properties of lycopene

Lycopene is a natural red pigment found in numerous vegetables and fruits i.e. gac fruit, tomato, watermelon, apricot, etc. Lycopene was first discovered in tomato by a French botanist Alexis Millardet in 1876 and it was named later by chemist C.A. Schunck from the scientific name of tomato “Lycopersicon esculentum” (Desai et al., 2018)

Lycopene is a carotenoid hydrocarbon with the molecular formula of $C_{40}H_{56}$ that consists of an acyclic open-chain structure with 11 linearly arranged conjugated double bonds and 2 non-conjugated double bonds. The IUPAC name of lycopene is 2, 6, 10, 14, 19, 23, 27, 31–octamethyl-2, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30-dotriacontatridecaene and common names include Ψ,Ψ-carotene, all-trans-carotene, and (all-E)-lycopene (Olempska-Beer, Merker, Ditto, & DiNovi, 2006).

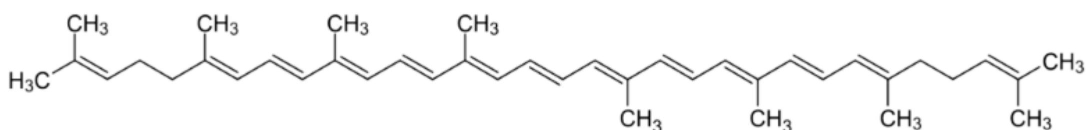


Figure 1 Chemical structure of lycopene

Lycopene is a lipophilic compound with hydrophobic characteristics. Thus, it is soluble in oil and organic solvents i.e. chloroform, hexane, benzene, methylene chloride, acetone and petroleum ether, whereas it is insoluble in water and slightly soluble in methanol and ethanol. The physical properties of lycopene are summarized in **Table 1**.

Table 1 Physical properties of lycopene (Naz, Butt, Sultan, Qayyum, & Niaz, 2014)

Chemical formula	$C_{40}H_{56}$
Molecular weight	536.89
Melting point	172–175 ^{o}C
Powder form	Dark reddish-brown
Solubility	- Soluble in hexane, benzene, chloroform, acetone, petroleum ether - Slightly soluble in ethanol and methanol - Insoluble in water
Sensitivity	Light, oxygen, high temperature and acids

Naturally, most of the carotenoids occur as trans-isomer in plants including lycopene. Nevertheless, lycopene, a highly unsaturated hydrocarbon, can be isomerized from trans-isomer to cis-isomer form due to heat, light, oxygen, acid and metal ions. In the food product, lycopene is mostly in trans-isomer form (approximately 80-96 % of total lycopene content) whereas in serum and tissues it is mostly in cis-isomers (Chakravarthi, 2002; Nguyen & Schwartz, 1998; Stahl, Schwarz, Sundquist, & Sies, 1992). Various geometric isomeric forms such as 15-cis, 13-cis, 11-cis, 9-cis, 7-cis and 5-cis are the commonly identified forms of lycopene as shown in **Figure 2**.

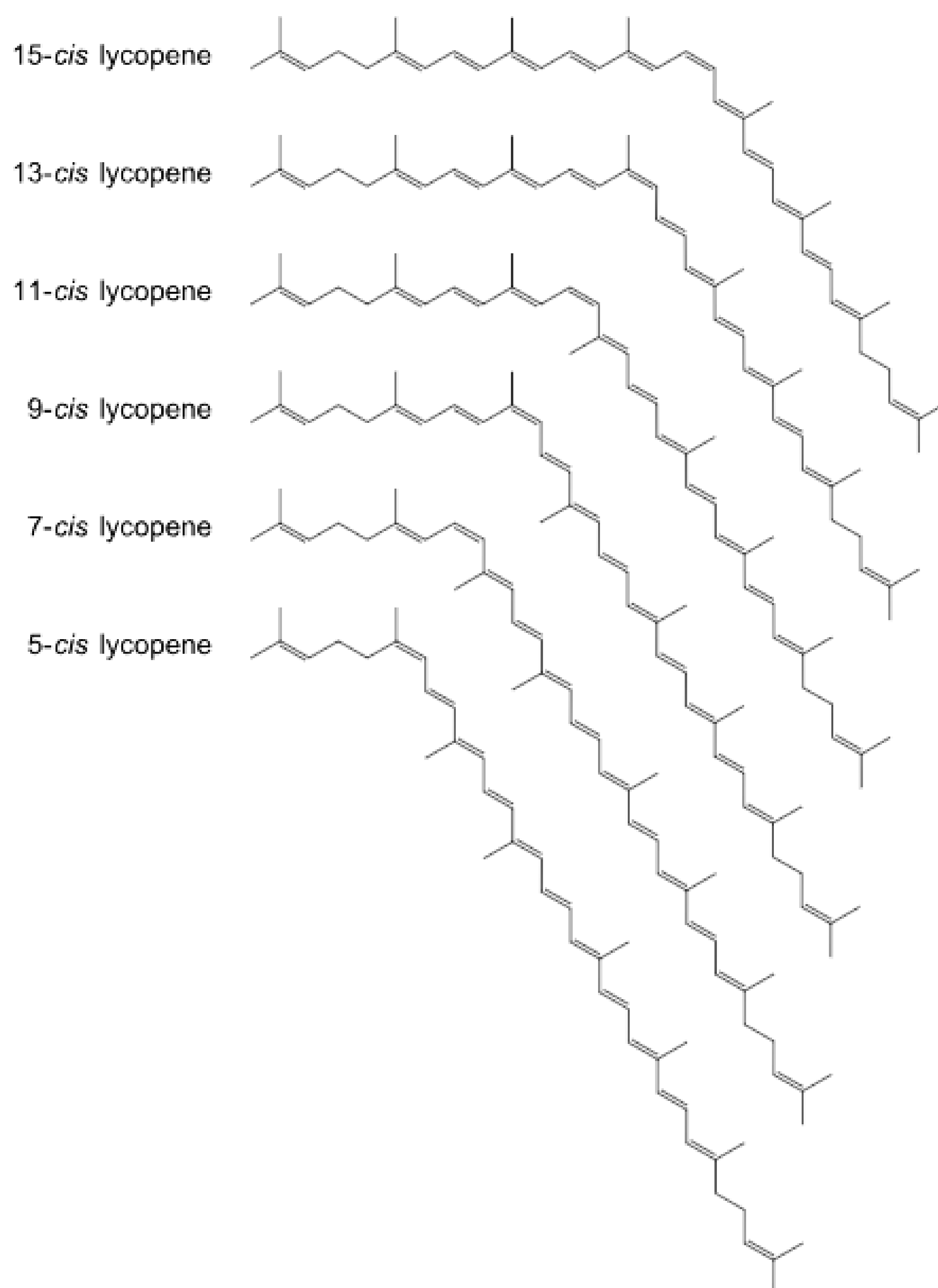


Figure 2 Molecular structures of lycopene isomers (Kong et al., 2010)

2.2 Sources of lycopene

Lycopene can be acquired from natural sources or synthesized chemical by a series of reactions using synthetic reagents and chemical solvents (**Figure 3**). The industrial process of synthetic lycopene has a deleterious environmental impact due to the large amount of chemical solvents consumed. The final product may often contain traces of chemical solvents, impurities and reaction by-products, which could be toxic and these products cannot be included in food.

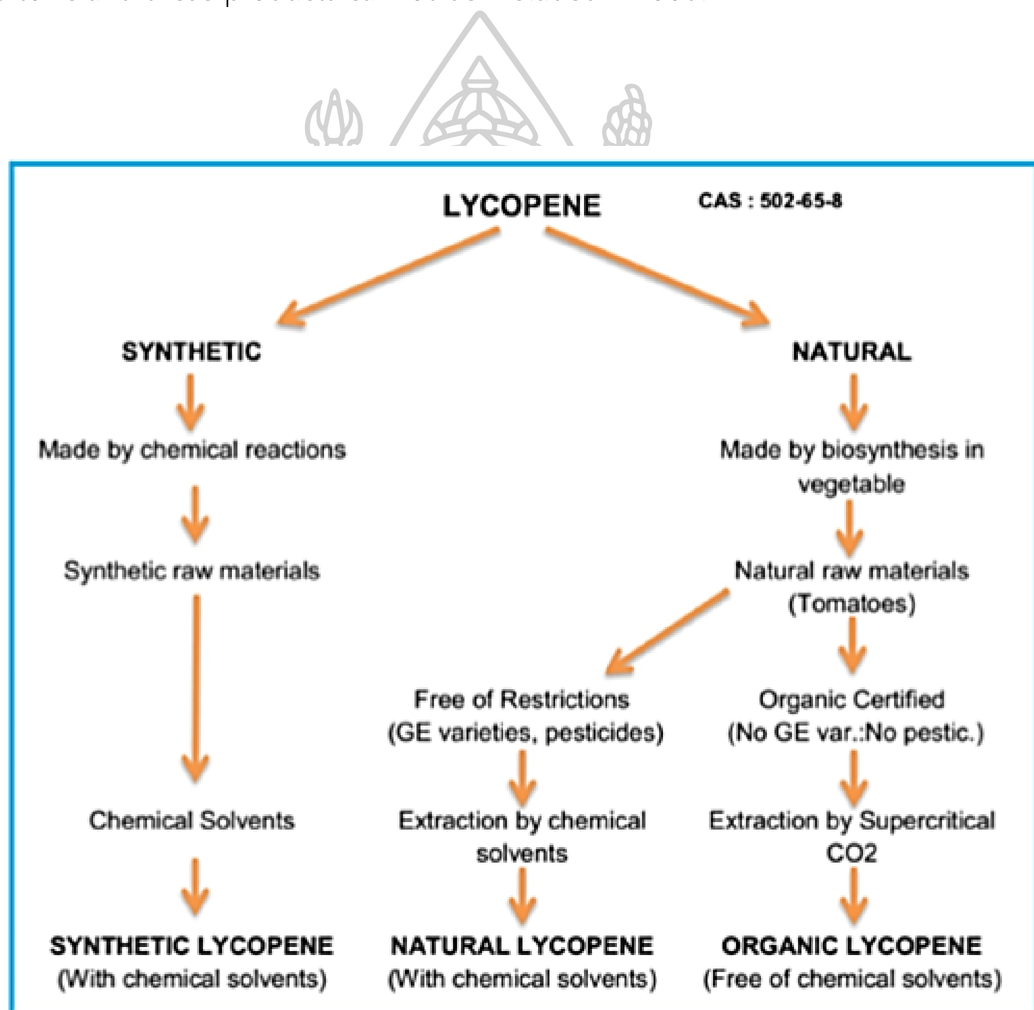


Figure 3 Flow chart for lycopene synthesis (Saeid, 2015)

2.2.1 Synthetic lycopene

Different chemical synthetic pathways for lycopene have been reviewed(Ernst, 2002; Shen et al., 2011), but all cases have their drawback such as low yields, unstable, low quality, and economically unattractive, with sometimes toxic intermediate reagents occurred (Shen et al., 2011). The chemical synthesis of lycopene from smaller molecules generally involves three steps (Shen et al., 2011). In the first phase, C_{15} compound is synthesized and then dissolved in methanol. In the second phase, C_{10} compound is synthesized in crystalline form. In the final phase, these two intermediate compounds are mixed together at a high temperature, in the presence of a catalyst, and lycopene is formed. After cooling, the crude product is filtered out and washed repeatedly with water and methanol. The synthesized material is then further purified by washing with methanol. Following the final filtration, the product is dried using warm nitrogen.

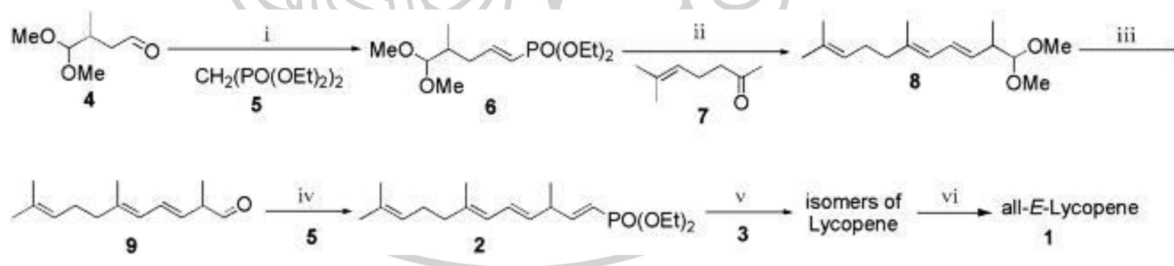


Figure 4 Total chemical synthesis of lycopene based on: (i) a condensation between 4,4-dimethoxy-3-methylbutanal **4** and methylenebisphosphonic acid tetraethyl ester **5**, leading to the C6-phosphonate **6**, followed by (ii) a modified Wittig–Horner reaction between **6** and 6-methyl-5-hepten-2-one **7** producing dimethoxy-3,5,9-triene **8**, and (iii) another modified Wittig–Horner reaction between C15-phosphonate **2** and C10-triene dialdehyde **3** producing all-*E*-lycopene (Shen et al., 2011).

2.2.2 Natural lycopene source

Lycopene brings red color to gac fruit, tomato, watermelon, apricot, papaya, rosehip, passion fruits, guava, pink grapefruit, persimmon etc. (Bao, Yan, Liu, & Xu, 2010; Preedy & Watson, 2009; Tarazona-Díaz, Viegas, Moldao-Martins, & Aguayo, 2011). Tomatoes (especially deep-red fresh tomato fruits) and tomato products are considered the most important source of lycopene in many human diets; therefore, most of the research on lycopene focuses on tomato and tomato-based products (Preedy & Watson, 2009). Lycopene content varies in tomato fruits depending on the type, maturity, geographic location, agronomic conditions and the environmental conditions influencing the ripeness of tomatoes (Kaur, Wani, Oberoi, & Sogi, 2008). Generally, tomato fruit contains 3-5 mg lycopene per 100 grams of raw material. The yellow type has lower lycopene and contains around 0.5 mg per 100 g of β-carotene, while some red types have more than 15 mg per 100 g of raw material (John Shi & Maguer, 2000). Moreover, the tomato peel contains about five times more lycopene than tomato pulp, on wet basis (Machmudah et al., 2012; Naviglio, Pizzolongo, Ferrara, Aragon, & Santini, 2008). Natural lycopene is mostly produced by extraction, concentration and purification from whole tomato fruits that have been grown specifically for this purpose. Concentration of lycopene in tomato vary from 8.8 to 200 µg/g in fresh fruit and from 430 to 2950 µg/g on a dry basis (Aktas & Yildiz, 2011; Bao et al., 2010; Ishida & Bartley, 2009; Machmudah et al., 2012; A. Rao, Ray, & Rao, 2006).

Table 2 Lycopene content of the fruits and vegetables with high concentrations (Papaioannou, Liakopoulou-Kyriakides, & Karabelas, 2016)

Plant source	Lycopene content (µg/g wet weight basis)
Gac fruit	348-6200
Tomato	8.2-200
Watermelon	11.6-112
Guava (pink)	52.3-55
Grapefruit (pink)	3.5-33.6
Papaya	1.1-53
Carrot	0.02-7.8
Pumpkin	3.8-4.6

Table 3 Lycopene content in tomato products (Papaioannou et al., 2016)

Tomato product	Lycopene content (µg/g wet weight basis)
Cooked tomatoes	37
Tomato sauce	62–195
Tomato paste	54–1500
Tomato ketchup	99–414
Tomato juice	50–616
Tomato puree	89–194
Tomato powder	1126–1265

2.2.3 Plant by-products as Natural Lycopene Source

Million tons of tomatoes are annually processed to produce juices, sauces, purees, pastes, and canned tomatoes, resulting in large amounts of tomato peel, pulp, and seed as industrial waste (called in general tomato pomace) that represent 10%– 40% of the fruit weight (Machmudah et al., 2012; I. F. Strati & Oreopoulou, 2011a). A main problem of tomato industry is the accumulation, handling, and disposal of processing wastes. Meanwhile, the prices of raw materials always are growing. Therefore, the recovery of useful compounds from the wastes is interesting. With their transformation to useful products, this trend is in accordance with the environmental balance and sustainable development (Laufenberg, Kunz, & Nystroem, 2003). Food processing by-products from orange, mango, guava, pomegranate, and also vegetables including tomato, and carrot are sources of functional food. Tomato pomace is currently discard as solid waste or animal feed, but the abundance of lycopene in the peel of these wastes suggests the potential of utilizing it as the low-cost source of lycopene(Kaur et al., 2008; I. F. Strati & Oreopoulou, 2011a).

มหาวิทยาลัยศิลปากร

2.3 Benefits of lycopene

In recent years, lycopene has received high attention due to its functional effects in some diseases and a lot of bioactivities. Lycopene is a well-known antioxidant, and exhibit physical quenching rate constant for singlet oxygen almost twice as high as that of β-carotene. According to epidemiological studies, lycopene played an important role in the obstruction of cancer (epithelial cancers, especially), cataracts and ageing diseases (Pyke & Howells, 2002). Lycopene is also the main component for the treatment of prostate cancer and digestive-tract tumors (Stacewicz-Sapuntzakis & Bowen, 2005) and could prevent the formation and the progression of atheromatous plaque (Palozza et al., 2010). Furthermore, lycopene is

also widely applied as a value-added or beneficial ingredient in food products as well as a colorant in cosmetics, beverages and food industries.

The potential health benefits of lycopene in the diet (**Figure 5**) on various conditions is because lycopene having the following attributes: antioxidant, enhancement of cellular gap junction communication, induction of phaseII enzymes by activation of the antioxidant response element (ARE) transcription system, suppression of insulin-like growth factor-1-stimulated cell proliferation by induced insulin-like growth factor binding proteins, anti-angiogenesis and inhibition of cell proliferation associated with malignant tumors, induction of apoptosis (Mein, Lian, & Wang, 2008).

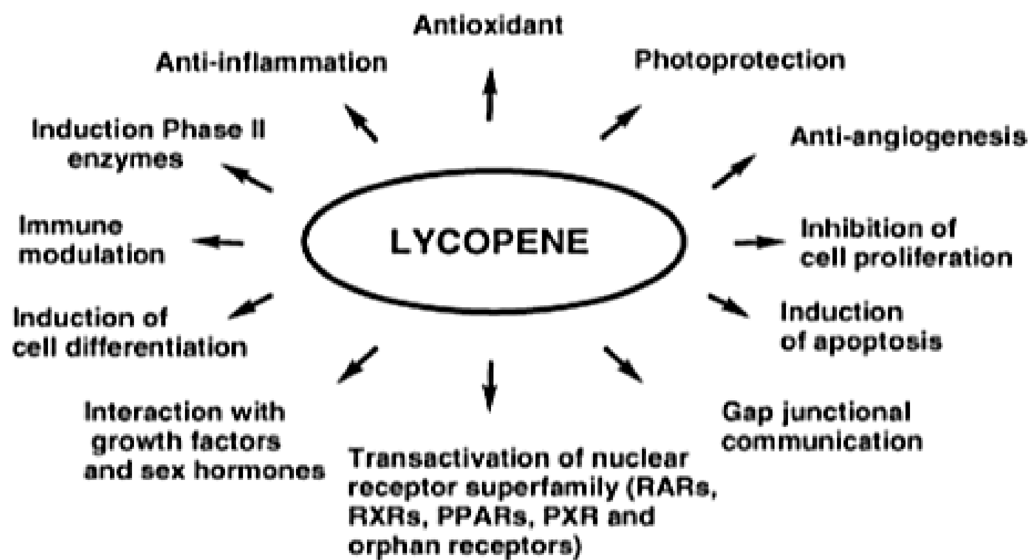


Figure 5 Biological functions of lycopene (Mein et al., 2008)

2.3.1 Role of lycopene in the human body

Lycopene consumption has been famous with many health benefits, including reducing of the risk of cancer and neurodegenerative diseases. It is also functional in reducing urinary tract symptoms (BPH benign prostatic hyperplasia, enlargement of the prostate) and cardiovascular risk associated with type 2 diabetes (Ranveer et al., 2013). An increased lycopene status in the body may regulate gene function, improve inter-cell communication, modulate hormone and immune response and regulate metabolism, thus lowering the risk of chronic disease (Agarwal & Rao, 2000). The main health benefits of lycopene include:

2.3.1.1 Antioxidant activity

Lycopene is one of the most potent antioxidants. Its singlet-oxygen-quenching ability is twice that of β-carotene and ten times higher than that of α-tocopherol (A. V. Rao & Agarwal, 2000). As an antioxidant, it captures reactive oxygen species, increasing the overall antioxidant potential and decreasing the oxidative damage to lipids (lipoproteins, membrane lipids), proteins (important enzymes) and DNA (genetic material), thereby lowering oxidative stress. This reduced oxidative stress leads to reducing of the risk of cancer and cardiovascular heart disease. When the lycopene levels in the blood increase, the levels of oxidized lipoproteins, proteins and DNA compounds decrease. A recently study reported that antioxidant activity of lycopene improved sperm quality (L. Rao & Rao, 2013).

2.3.1.2 Reducing prostate cancer

Many studies showed that taking high amount of lycopene can slow the progression of prostate cancer. The intake of lycopene from various tomato products can reduce the risk of prostate cancer, a result not observed for any other carotenoid. The protective effects were highest for more advanced or aggressive prostate cancer (Agarwal & Rao, 2000).

2.3.1.3 Inhibiting cancer cells

Lycopene can prevent stomach, colon, lung and skin cancers. Free radicals in the body can damage DNA and proteins in the cells and tissues, resulting in inflammation which may lead to cancer. Hence, the antioxidant properties of lycopene in getting rid of free radicals that may reduce the risk of cancer (Hussain, Hofseth, & Harris, 2003). The research in breast, lung and endometrial cancers showed that lycopene is more effective than the other bright vegetable carotenoids α- and β-carotene in delaying the cell cycle progression from one growth phase to the next, thus inhibiting growth of tumor cells. Lycopene also plays a role in modulating intercellular communication by regulating irregular pathways that may be associated with cancer(Singh & Goyal, 2008).

Multiple researches have investigated whether intake of tomatoes or tomato-based products helps prevent digestive tract cancers, including oral, pharyngeal, oesophageal, gastric, colon, and rectal cancer. People with a higher intake of lycopene have been shown to have a reduced risk of growing cervical and breast cancer (Lippi & Targher, 2011).

2.3.1.4 Reducing of blindness

Age-related macular degeneration (ARMD) is the most normal form of blindness in elderly people in the Western world. Lycopene is the only micronutrient whose serum level is shown to be conversely related to the risk of ARMD (Cardinault et al., 2005). Lycopene also reduce the incidence of cancers and cardiovascular diseases which play a role in health of eyes.

2.3.1.5 Reducing of atherosclerosis and heart disease

Lycopene may be helpful in people with high cholesterol, atherosclerosis or coronary heart disease, possibly due to its antioxidant properties. Lycopene prevents oxidation of low density lipoprotein (LDL) cholesterol and slows down the risk of arteries becoming thickened and blocked (A. Rao, 2002). Most published studies in this area suggested tomato juice. Drinking of tomato juice would provide mg lycopene per day recommended for reducing LDL cholesterol (Ried & Fakler, 2011).

2.3.1.6 Preventing skin damage

Lycopene can reduce inflammation and help to protect the skin from damage resulting by UV sun exposure. It is a common ingredient in anti-aging creams and lotions but because it degrades easily, containers must be properly sealed.

2.3.2 Functional uses of lycopene in food

Because of lycopene benefits, there is a growing attention in using lycopene as a value-added or functional ingredient in food products. Lycopene extract from tomatoes can be used as a nutritional supplement in several food categories such as baked goods, breakfast cereals, dairy products including frozen dairy desserts, dairy product analogues, spreads, bottled water, carbonated beverages, fruit and vegetable juices, soybean beverages, candy, soups, salad dressings, and other foods and beverages.

The levels of tomato extract expressed as lycopene levels, added to food depend on the intended function and may vary from 2 mg/L in bottled water to 130 mg/kg in ready to eat cereals. Food and beverage products are formulated to provide about 2 mg lycopene per serving (Rath, Olempska-Beer, & Kuznesof, 2013).

There are initiatives by food scientists to recycle lycopene-rich by-products as food ingredients. Fortifying dry fermented sausage with lycopene can be achieved by adding dried tomato peel to the meat mixture during sausage production (M. Calvo, Garcia, & Selgas, 2008). Extrusion processing allows barley-tomato pomace blends to be formulated into snacks (Altan, McCarthy, & Maskan, 2008).

Lycopene is a natural food coloring, thus eliminating the adverse effects of artificial food colorants. It provides color shades ranging from yellow to red (Choksi & Joshi, 2007). When used as a food colorant, lycopene is stable in food stored at appropriate conditions such as storage at 4°C. Abano, Ma, and Qu (2012) reported that lycopene extract is stable for up to 37 months.

2.4 Lycopene analytical methods

Various analytical methods have been developed to measure and quantify of lycopene content in food and biological samples such as ultraviolet-visible (UV-VIS) spectrophotometry, liquid chromatography (LC), thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) (Fish, Perkins-Veazie, & Collins, 2002; Kopec, Cooperstone, Cichon, & Schwartz, 2012; Luterotti et al., 2013)

HPLC analysis is the popular method to separate, identify, and quantify carotenoids in food and biological samples. Although HPLC analysis provides the accurate quantification of pigments and separation of isomers, it has some disadvantages such as high-cost, time-consuming, and requirement of a high skill level to achieve consistent results (Davis, Fish, & Perkins-Veazie, 2003).

UV-VIS spectrophotometry is more convenient, simple, faster, low cost and using reduced volumes of organic solvents as compare to HPLC. It has been shown to be reliable methods to identify lycopene as the major pigment present in a mixture. The differences of adsorbed spectrum between lycopene and other carotenoids such as β-carotene, α-carotene, or lutein make the quantification of lycopene easy at its characteristic wavelength maximum of 503 nm, with no interference of other compounds, in lycopene-rich samples. However, a main disadvantage of UV-VIS spectrophotometry is that it cannot detect very small quantities of lycopene (less than 1.0 μg) while HPLC can detect low quantities as small as 1.0 ng.

2.5 Extraction methods

2.5.1 Solvent extraction

Solvent extraction is the most widely used method for the separation of biocompounds from abroad range of plant origin matrices. It is a unit operation with the objective to separate desired compounds from a matrix of solid or liquid. The extraction is based on the principle that the solvent penetrate into the matrix and dissolves the soluble compounds. In solid–liquid extraction, the target compound should have a selective solubility in the solvent of choice compared to other components of solid matrix (Aguilera, 2003). The main solvent extraction techniques applied for the extraction of carotenoids from plant origin matrices are Soxhlet extraction and agitation.

Soxhlet extraction is a general and well-established technique, which surpasses in performance other conventional extraction techniques except for, in limited field of applications, the extraction of thermolabile compounds (De Castro & Garcıa-Ayuso, 1998). The efficiency of this method (as also of all other extraction techniques) depends on plant characteristics and particle size, as internal diffusion may be the limiting step during extraction (L. Wang & Weller, 2006). The advantages of soxhlet extraction include the continuous contact of fresh solvent with the solid matrix and the absence of a filtration step after leaching. On the other hand, the extraction time is long, high amount of solvent are consumed, no agitation can be provided, and there is a great possibility of thermal decomposition of carotenoids, as the extraction usually occurs at the boiling point of the solvent for a long time (De Castro & Garcıa-Ayuso, 1998; L. Wang & Weller, 2006). Soxhlet extraction is mostly applied as a laboratory technique, or a micro-scale extraction technique.

Agitation is a simple method which used in industries. This method involves agitating the solvent and the plant (material) together, and leaving them in contact for a period of time. After that the solvent is removed and the residue is usually re-extracted. Agitation involves coupling an agitator to the vessel containing the raw material, while homogenization involves mixing the solvent and the raw material and then leaving them in contact with no further agitation, and shaking involves agitating the tank containing the solvent and the raw material (Prado, Veggi, & Meireles, 2014). The dispersion of the particles in the liquid solvent by the agitation increases the possibility to contact the solid with the solvent. The major disadvantages of this method are the large consumption of solvents, the high energy required to separate the solvent and solute from their mixture, the subsequent clean up and concentration steps. Moreover, it may be occurs the co-extraction of undesired components and the possible degradation of some thermal sensitive compounds at high temperature (L. Wang & Weller, 2006).

2.5.2 Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) is an extraction method which operates above the solvent critical pressure and temperature, enhancing its solvating power (Machmudah et al., 2012). Supercritical fluids combine the physical properties of fluids and gases in an advantageous way, so that they exhibit higher solvent power and higher density than the gases, together with fast diffusion, low surface tension and low viscosity in comparison to liquids. Carbon dioxide (CO2) is a solvent frequently used in the SFE method due to its low critical temperature, no toxicity or flammability, and its availability at low cost and high purity (Machmudah et al., 2012). The application of SFE-CO_2 at production scale is advantageous mainly because CO_2 can be easily removed from the extracts, thus leaving no traces of solvent in the

final product (Tello, Viguera, & Calvo, 2011). The combination of high solubility and low capillary action is especially attractive for extracting compounds from solid raw materials when the target substances are well embedded in the solid matrix. The supercritical fluid extraction is an attractive process alternative, often applied with success; but, of course, the capital expenses necessary for the extraction apparatus are high and this tends to obstruct the application of this technique (Montesano et al., 2008; Naviglio et al., 2008). Therefore, from overall of view, solvent extraction is the general and the first option because of its simplicity and relatively low total processing cost, especially in relatively small capacity plants.

2.5.3 Enzyme-assisted extraction (EAE)

Enzymatic treatment of the plant material may be used prior to conventional solvent extraction process. Enzymes are ideal catalysts that can assist in the extraction, modification and/or synthesis of complex bioactive compounds of natural origin (I. Strati & Oreopoulou, 2014). The enzymatic pretreatment of agro-wastes is an already established approach, with many applications for recovering compounds of biological significance and other high added value products from their compact highly structured plant tissue. The use of cell-wall degrading enzymes that are capable of hydrolyzing the main polysaccharide components of the plant structures, where the natural target compound accumulates, is a mild and efficient means to facilitate its recovery. In particular, enzymes have been used for plant material treatment prior to conventional extraction methods, thus facilitating the extraction. The useful application of enzymes enhances the effectiveness of solvent treatment, either reducing the amount of solvent needed for extraction or increasing the yield of extractable compounds (Puri, Sharma, & Barrow, 2012). Since the plant cell wall comprises of cellulose, hemicellulose and pectins, so, cellulases, hemicellulases,

and pectinases have been used to disrupt the structural integrity of the plant cell wall, thereby enhancing the extraction of bioactive compounds from plants.

2.5.4 Microwave-assisted extraction (MAE)

Microwave-assisted extraction (MAE) has been paid attention in various fields, particularly in medicinal plant research, due to its special heating mechanism, moderate capital cost and well performance under atmospheric conditions. MAE is based on the following principle. The moisture inside the cells was evaporated by the heat which generated from the microwaves, producing a high pressure on the cell wall. The increasing of pressure inside the cell modifies the physical properties of tissues Consequently, it improves the porosity of the biological matrix and, allows better penetration of extracting solvent through the matrix and improved yield of the desired compounds (Routray & Orsat, 2012). The major advantage of MAE is that the matrix is heated internally and externally without a thermal gradient, and the target compounds can be extracted efficiently and protectively using less energy and solvent volume (Joana Gil-Chávez et al., 2013). Some drawbacks associated with MAE are the requirement of additional clean up step to remove solvent from sample matrices and the restriction to only polar solvent application in the system.

2.5.5 Ultrasound Assisted Extraction (UAE)

Ultrasound Assisted Extraction (UAE) was used as an alternative extraction method for natural compounds. The mechanical effect of ultrasound provides a greater penetration of solvent into the cellular materials and result in the disruption of biological cell walls to facilitate the release of its contents (Dolatowski, Stadnik, & Stasiak, 2007). The advantages of sonochemistry in food processing include increase the extraction yield and extraction rate (Chemat & Khan, 2011; Kumcuoglu et al.,

2014; Rastogi, 2011). Sonication can enhance the efficiency of relative lycopene recovery yield with lower the extraction temperature, shorten the total extraction time and smaller amount of solvent and at the same time, minimize the degradation and isomerization of lycopene (Kumcuoglu et al., 2014).

Moreover, enhancement of mass transfer mechanism in extraction can be achieved by ultrasound-microwave assisted extraction (UMAE) that combines microwave and ultrasonic waves providing high momentum and energy to rupture the cell and elute the active compounds to the extraction solvent (Chan, Yusoff, Ngoh, & Kung, 2011; Y. Chen et al., 2010). As a result, extraction proceeds over shorter extraction time and with smaller solvent consumption. UMAE has been used to extract a variety of active compounds such as lycopene from tomatoes (Lianfu & Zelong, 2008), vegetable oil (Cravotto et al., 2008), and polysaccharides (Y. Chen et al., 2010) from various plants. The main drawbacks of UMAE overall appear to be safety limitations and relatively high equipment cost.

Table 4 Advantages and disadvantages of various carotenoid extraction methods (Saini & Keum, 2018)

Method	Advantages	Disadvantages
Soxhlet extraction	- Simple and convenient providing the highest recovery of carotenoids - No complicate apparatus required	- Time-consuming and also uses large amounts of solvents - Can cause thermal degradation and cis-trans isomerization of - Carotenoids
Supercritical fluid extraction (SFE)	- Uses non-flammable, non-toxic and recyclable solvent (CO_2 and ethanol) - Continuous process - Useful for extraction of thermolabile compounds - Provide carotenoids with high purity	- Not suitable for samples containing high amounts of water - Low yield of polar carotenoids - High cost of apparatus
Microwave-assisted extraction (MAE)	- Simple, fast and economical method	- Can cause thermal degradation and cis-trans isomerization
Ultrasound-assisted extraction (UAE)	- Rapid, non-thermal and efficient extraction - Aging of the ultrasonic probe surface can change the extraction efficiency	- Small particle size (≈50 μm) is required to achieve good extraction

Method	Advantages	Disadvantages
Pressurized liquid extraction (PLE)	- Fast (few minutes), requires minimum amount of organic solvent - Highly applicable to a laboratory-scale context	- Difficult to apply to large volumes due to clogging caused by sugars and pectins of plant matrices
Enzyme-assisted extraction (EAE)	- Rapid and efficient extraction with minimal usage of solvents	- High cost of the enzymes

2.6 Optimization using response surface methodology

Optimization of process conditions is one of the most critical stages in the development of an efficient and economic bioprocess (Q. L. P. Tan, Kieu, Kim, & Hong, 2012). Classical and statistical methodologies are available for optimizing process conditions, especially response surface methodology (RSM). RSM is a powerful mathematical method with a collection of statistical techniques wherein, interactions between multiple process variables can be identified with fewer experimental trials (Ashengroph, Nahvi, & Amini, 2013).

There are two main types of response surface designs: central composite designs and Box-Behnken designs. Central Composite designs can fit a full quadratic model. They are often used when the design plan calls for sequential experimentation because these designs can include information from a correctly planned factorial experiment (Gujral, Kapoor, & Jaimini, 2018). Box-Behnken designs usually have fewer design points than central composite designs, thus, they are less expensive to run with the same number of factors. They can efficiently estimate the first- and second-order coefficients; however, they cannot include runs from a

factorial experiment (Ferreira et al., 2007). The Box-Behnken is a good design for response surface methodology because it permits estimation of the parameters of the quadratic model, building of sequential designs and detection of lack of fit of the model.

The Box-Benhken design is an independent quadratic design in that it does not contain an embedded factorial or fractional factorial design. In this design the treatment combinations are at the midpoints of edges of the process space and at the center. These designs are rotatable (or near rotatable) and require 3 levels of each factor. The designs have limited capability for orthogonal blocking compared to the central composite designs (Voehl, Mignosa, Harrington, & Charron, 2016). **Figure 6** showed the geometry of the Box-Behnken design with three factors

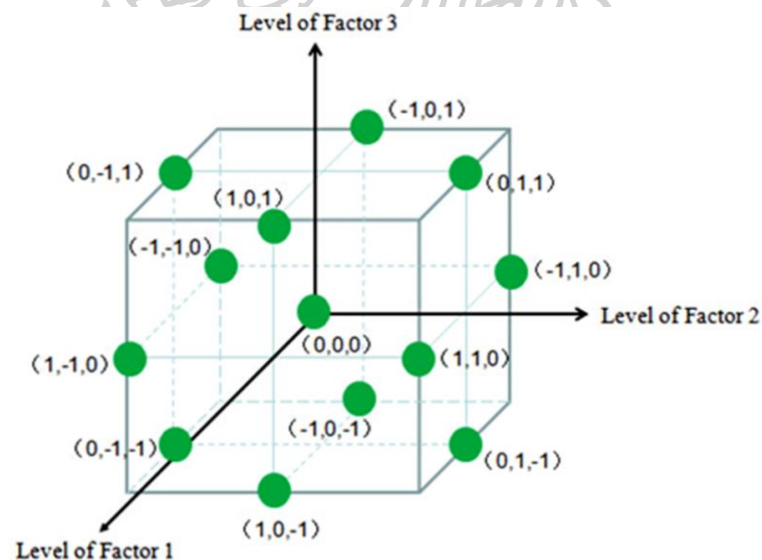


Figure 6 Geometry of the Box-Behnken design with three factors

The number of experiments (N) required for the development of BBD is defined as $N = 2k(k-1) + C_0$, (where k is number of factors and C_o is the number of central points. For comparison, the number of experiments for a central composite design is $N = 2^k + 2k + C_0$ (Ferreira et al., 2007). **Table 5** displays a summary of the Box-Behnken designs that was generated with difference number of factor.

Table 5 Summary of the Box-Behnken designs

Number of factors	Number of runs	Optional number of blocks	Default number of center points
3	15	1	3
4	27	3	3
5	46	2	3
6	54	2	6
7	62	2	6
10	170	2	10

Chapter 3

Literature reviews

This chapter is a collection of research related to carotenoid extraction, especially lycopene. Conventional solvent extraction and its techniques are discussed. Then, the effect of extraction parameters on carotenoids extraction is collected through literature research. Finally, the optimization using response surface methodology was reviewed.

Tomatoes, tomato products and tomato processing are the main source of lycopene compounds, and are also considered an important source of carotenoids in the human diet. Lycopene from tomatoes is always extracted by organic solvents such as hexane, ethanol, acetone, chloroform, petroleum ether, tetrahydrofuran.

3.1 Conventional solvent extraction using organic solvents

I. F. Strati and Oreopoulou (2011a) studied the extractability of tomato waste carotenoids in various organic solvents and to optimize the extraction parameters (type of solvent, extraction time, temperature and extraction steps) for maximum yield. Among other solvents, a new environmentally friendly one, ethyl lactate, was tested which brought the highest carotenoid yield (243.00 mg/kg dry tomato waste) at 70 °C, compared to acetone (51.90 mg/kg), ethyl acetate (46.21 mg/kg), hexane (34.45 mg/kg) and ethanol (17.57 mg/kg). The carotenoid recovery was significantly ($P < 0.05$) affected by the number of extraction cycles and extraction temperature in all solvents. Carotenoid concentration increased with time, a quasi-saturated condition at approximately 30 min of extraction. Consistent with the research of M. M. Calvo, Dado, and Santa-María (2007), they found that the yield of all-trans-

lycopene in ethyl acetate extract was twenty-fold higher than the respective yield obtained in ethanol.

Dewanto, Wu, Adom, and Liu (2002) investigated the effect of thermal processing on lycopene content. Lycopene compound in raw tomatoes was extracted using acetone. The raw tomato had 2.01 mg of trans-lycopene/g of tomato. After 2, 15, and 30 min of heating at 88 °C, the trans-lycopene content had increased to 3.11, 5.45, and 5.32 mg of trans-lycopene/g of tomato, respectively. These results indicate that thermal processing enhanced the nutritional value of tomatoes by increasing the bioaccessible lycopene content.

A solvent mixture of non-polar and polar solvent is often used for lycopene extraction. The combination of polar solvents with the non-polar hexane helps to increase the solubility of the non-polar carotenoids (lycopene and β-carotene). In I. F. Strati and Oreopoulou (2011b) research, the use of a mixture of acetate and hexane proved to extract lycopene and β-carotene in satisfy percentages (96% of total extracted carotenoids), as well as the polar lutein (4% of total extracted carotenoids). In addition, it has been found that lycopene which is extracted by a mixture of solvent e.g. hexane/acetone or hexane/ethanol is more stable than that extracted by other organic solvents.

Periago, Rincon, Agüera, and Ros (2004) investigate the mixture approach for optimizing lycopene extraction from tomato and tomato products. A simple mixture process involving hexane/acetone/ethanol as a solution for extracting lycopene from raw tomato, tomato sauce, and tomato paste was carried out to insist the hypothesis that lycopene extraction rates are relate to the type of solvent. Although the major solvent used in lycopene extraction was hexane, there was a positive secondary synergistic interaction of hexane with ethanol (all sample types) and with acetone

(tomato paste samples); this suggests that a mixture of three components is necessary for optimizing the extraction process. The lycopene contents found in the confirmatory experiments were 4.72, 14.71, and 53.78 mg/100 g for raw tomato, tomato sauce, and tomato paste, respectively, using a mixture solvent of hexane, actone and ethanol (1:1:1).

Kaur et al. (2008) studied the effect of extraction conditions on lycopene extractions from tomato processing waste skin using a mixture solvent of hexane, acetone, and ethanol (2:1:1). A central composite design with five independent variables, namely solvent/meal ratio; number of extractions; temperature; particle size; extraction time was used to study their effects on lycopene extraction. The experimental values of lycopene ranged between 0.639 and 1.98 mg/100 g. Maximum lycopene (1.98 mg/100 g) was extracted when the solvent/meal ratio, number of extractions, temperature, particle size and extraction time were 30:1 v/w, 4, 50 C, 0.15 mm and 8 min, respectively.

Poojary and Passamonti (2015b) investigated the optimization the extraction of pure all- trans-lycopene from the tomato pulp waste. A full factorial design (FFD) consisting of four independent variables including extraction temperature (30–50 °C), time (1–60 min), percentage of acetone in n-hexane (25–75 %, v/v) and solvent volume (10–30 ml) was used to study the effects of process variables on the extraction. The amount of lycopene in the pulp waste was found to be 0.038 mg/g. The optimal conditions for extraction were as follows: extraction temperature 20 °C, time 40 min, a solvent composition of 25 % acetone in n-hexane (v/v) and solvent volume 40 ml. Under these conditions, the maximum recovery of lycopene was 94.7%. The HPLC–DAD analysis demonstrated that, lycopene was obtained in the all-trans-lycopene at a very high purity grade of 98.3 % while the amount of cis-isomers and other carotenoids were limited.

3.2 Conventional solvent extraction using non-toxic solvents

Because of the oil solubility of carotenoids, using vegetable oils as solvents is a promising alternative to conventional methods for carotenoids extraction. Vegetable oils is edible and can be used safely with the human body as well as being non-toxic and environmental friendly.

Extraction of carotenoids from shrimp waste using different vegetable oils was studied by Sachindra and Mahendrakar (2005). The highest yield was achieved by extraction using refined sunflower oil compared to groundnut oil, gingerly oil, soy oil, mustard oil, rice bran oil and coconut oil. The oil to waste ratio ($p < 0.05$), time ($p < 0.01$) and temperature ($p < 0.001$) of heating waste with oil before centrifugation to separate pigmented oil were significantly affected on the extraction yield of carotenoids in sunflower oil. A regression equation was derived for carotenoid yield as a function of time of heating, temperature of heating and oil level to waste. The optimum conditions for extraction of shrimp waste carotenoids in sunflower oil were evaluated to be oil to waste ratio of 2, temperature of 70 °C and heating time of 150 min to achieve a maximum carotenoid yield of 27.56 g/g waste

Extraction carotenoids from pumpkin (Cucurbita moschata) by using virgin coconut oil were investigated by Norshazila et al. (2017). The influence of different extraction conditions; temperature of extraction, duration of extraction and solid to solvent ratio on yield of carotenoids were also evaluated for optimization. The carotenoids from pumpkin had been α-carotene, β-carotene and lutein were identified by using high performance liquid chromatography (HPLC). It found that lutein have the highest concentration in pumpkin. Furthermore, the yield of carotenoids of 76.64 β-carotene μg/g dry matter was achieved in the sample extracted with coconut oil. The extract of carotenoids was found to be optimized at the following extraction conditions; 1:150 solid to solvent ratio and extracted by

using virgin coconut oil for 12 h at 30 °C and the highest carotenoid yield of 171.96 β-carotene μg/g dry matter was obtained.

Extraction using edible oils can be considered as environmental friendly process e.g. applying environmentally friendly solvents, reducing the energy consumption, and producing the non-denatured extract without contaminated compounds. Further, the oil plays a barrier role against oxygen and consequently slows degradation rate of the carotenoids extract and the oxidation time. However, a major problem is the high oil viscosity of oil, which results in low diffusivity and consequently low extraction yield even at high temperatures. In recent years, ultrasound-assisted extraction (UAE) of compounds with antioxidant activities has been widely applied to reduce this problem.

A non-toxic, cheap and easy-to-use process to extract carotenoids from fresh carrots assisted by ultrasound was designed by Li et al. (2013). Sunflower oil was applied as a alternative solvents in this ultrasound-assisted extraction (UAE): a process which is in line with green extraction and bio-refinery concepts. The results showed that the UAE using sunflower as solvent has obtained its highest β-carotene yield of 334.75 mg/L under optimal extraction conditions as follows: carrot to oil ratio of 2:10 w/v, ultrasonic intensity of 22.5 W/cm^2, extraction temperature of 40 °C and sonication time of 20 min.

Green ultrasound-assisted extraction of carotenoids from pomegranate wastes using vegetable oils was investigated by Goula et al. (2017). Sunflower oil and soy oil were used as alternative solvents and the effects of various parameters (extraction temperature, solid/oil ratio, amplitude level, and extraction time) on extraction yield were studied. Sunflower oil showed higher extraction yield than soy oil. The optimum operating conditions were found to be: extraction temperature, 51.5 °C;

peels to solvent ratio of 0.10 and amplitude level of 58.8 % and extraction time of 30 min. The optimum extraction yield was about 0.3255 mg carotenoids/100 g of dry peels. A second-order kinetic model was successfully developed for describing the mechanism of ultrasound extraction under different processing parameters.

3.3 Effect of extraction parameters on carotenoids yield

3.3.1 Effect of solvent

The type of solvent is usually considered as the most important factor for extraction. Lycopene, as most tomato carotenoids, are fat-soluble, organic solvents have been tested for lycopene extraction, including hexane, acetone, ethanol, ethyl acetate, petroleum ether and chloroform, as well as mixtures of polar and non-polar solvents in different ratios (Periago et al., 2004; J Shi, Maguiere, & Bryan, 2002; Taungbodhitham, Jones, Wahlqvist, & Briggs, 1998). However, solvents such as diethyl ether and tetrahydrofuran are not considered because they probably contain peroxides that react with carotenoids.

The oxygenated derivatives (xanthophylls) are more soluble in hydrophilic solvents, whereas carotenes possess a more hydrophobic nature and limited solubility in water and are more soluble in non-polar solvents. (Vági et al., 2007) compared the yields of several carotenoids by soxhlet extraction using hexane or ethanol and found an almost ten-fold increase of the lycopene yield in hexane extract, whereas the yield of β-carotene was almost the same by using ethanol. The ethanolic extract contained mainly polar xanthophylls. A similar trend was observed in the study of M. M. Calvo et al. (2007) who evaluated the extraction yield by using the food grade solvents, ethanol and ethyl acetate, and found that the yield of all-trans lycopene in ethyl acetate extract was twenty-fold higher than the respective

yield obtained in ethanol. Also, I. F. Strati and Oreopoulou (2011b) reported the following order in the yield of lycopene from dried tomato waste extracted with conventional organic solvents: acetone > ethyl acetate > hexane > ethanol. Chloroform was also used in soxhlet extraction of dried tomato skins (Topal, Sasaki, Goto, & Hayakawa, 2006) and dried tomato by-product (Machmudah et al., 2012) with satisfying yields of lycopene. Mixtures of polar and non-polar solvents (ethyl acetate/hexane, acetone/hexane, ethanol/hexane, hexane/acetone/absolute alcohol/toluene and acetone/ethanol/hexane) have also been used in solvent extraction (Kaur et al., 2008; Periago et al., 2004; Phinney, Frelka, Cooperstone, Schwartz, & Heldman, 2017)

3.3.2 Effect of temperature

Several studies demonstrate the effect of temperature. Generally, in all the extraction techniques reviewed, the rise of temperature affects positively the mass transfer process and, consequently, the extraction yield of lycopene and other carotenoids. The increase in extraction temperature generally implies the increase of solvent capacity to solubilize the target compounds and the increase in diffusion rates (Kettle, 2013; Mottaleb & Sarker, 2012). Moreover it helps to better disruption of bonds between the target compound and the matrix and the decrease of solvent viscosity as well as the decrease in surface tension. However, the boiling point of solvents is the limiting factor for the choice of extraction temperature in order to avoid undesirable reactions such as isomerization and / or oxidation of carotenoids (I. F. Strati & Oreopoulou, 2011a). In a study evaluating the extraction yield of lycopene, β-carotene, phytoene and phytofluene from tomato peel powder at varying temperatures for different time periods, the temperature increase resulted in an increase in the carotenoid concentrations; however in the extractions performed with

ethanol at 60 °C, the yield of all-trans-lycopene and their cis-isomers was lower than at 50 °C, indicating that both isomerization and oxidative degradation occurred in the high temperature extractions with ethanol (M. M. Calvo et al., 2007). John Shi, Dai, Kakuda, Mittal, and Xue (2008) found that isomerization may proceed even at 60 °C; however, they obtained an improved yield of lycopene by heating a tomato puree matrix at 100 or 120 °C. The raw material, solvent and extraction conditions seem to affect the isomerization and/or degradation of carotenoids as temperature increases.

3.3.3 Effect of extraction time

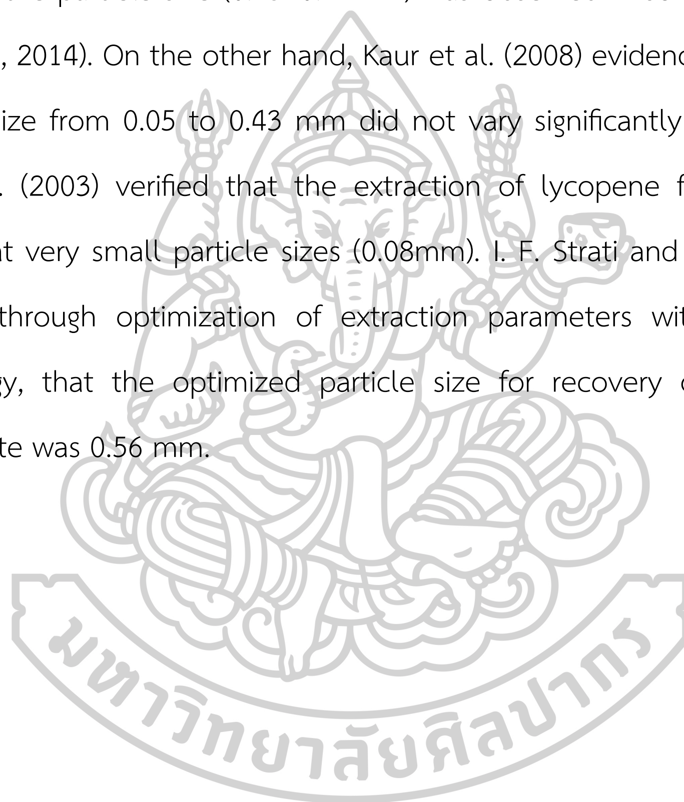
Kaur et al. (2008) showed that lycopene recovery increased with increase in extraction time and number of extraction cycles. A similar trend of increase of the carotenoid recovery with the number of extractions was observed by I. F. Strati and Oreopoulou (2011a). Moreover, they found that the carotenoids extraction is controlled by diffusion step and the extraction rate is lower with the extraction time to approach equilibrium at 30 min that was enough for each extraction step. X. F. Shi, Xu, Li, Zeng, and Sun (2012) investigate the effect of extraction time on the content of lycopene from watermelon. They found that lycopene content rised with the increase of extraction time from 0.5 to 2 h, reaching a maximum of 9.29 mg/kg. However, when the extraction time continued to increase, the lycopene content decreased because the longer extraction times might have a negative effect by degradation or isomerization.

3.3.4 Effect of solvent to solid ratio

Solvent to solid ratio is another factor which affects the extraction of carotenoids. Equilibrium between the uses of high and low solvent to solid ratio has to be found to obtain an optimized value (Pinelo, Rubilar, Jerez, Sineiro, & Núñez, 2005). A high solvent to solid ratio increases concentration gradient, and therefore, increases rate of diffusion. This allows greater extraction of solids by solvent. These results are, in fact, consistent with the mass transfer theory; the concentration gradient between solid and solvent is the driving force for mass transfer. In addition, (Q. Wang, Pagan, & Shi, 2002) showed that a higher solvent to solid ratio dilutes the concentration of dissolved carotenoids at the surface of the particles and results a higher concentration gradient between the internal and the external surfaces of the particles. This phenomenon accelerates the rate of extraction. Furthermore, increasing solvent to solid ratio increases contacting possibility between carotenoid and extracting solvent. Most researchers used a high value of solvent to solid ratio, e.g. 20:1 (v/w) (Naviglio et al., 2008; John Shi et al., 2009) or 30:1 (v/w) (Perretti et al., 2013) to obtain high extraction yields. Kaur et al. (2008) attempted to optimize the extraction conditions of lycopene from dehydrated tomato waste skin and found the optimum solvent to waste of 30:1(v/w) achieving a maximum lycopene yield 19.8 mg/kg. However, after the mass transfer process reached its equilibrium, further increase of solvent to solid ratio prolonged the distance of diffusion from solvent to interior matrix and could hardly enhance any more lycopene yield.

3.3.5 Effect of particle size

Particle size is another factor that effect carotenoids extraction. The yield increases with particle size decrease due to the higher contacting interfacial area when particles are smaller (Landbo & Meyer, 2001). Nevertheless, too small particles can cause packing of the extraction bed, which can result in channeling effects (Sabio et al., 2003) . A trend for an increase in the recovery of trans-lycopene with a decrease in the particle size (0.15–0.72mm) was observed in some studies (I. Strati & Oreopoulou, 2014). On the other hand, Kaur et al. (2008) evidenced that the increase in particle size from 0.05 to 0.43 mm did not vary significantly the lycopene yield. Sabio et al. (2003) verified that the extraction of lycopene from tomato wastes decreased at very small particle sizes (0.08mm). I. F. Strati and Oreopoulou (2011b) suggested, through optimization of extraction parameters with response surface methodology, that the optimized particle size for recovery of carotenoids from tomato waste was 0.56 mm.



Chapter 4
Materials and Methods

4.1 Materials and chemicals

Tomato paste and vegetable oils (coconut oil, olive oil, palm oil, soybean oil, and sunflower oil) were purchased from the local market, Bangkok, Thailand. Tomato processing waste was obtained from Doi Kham Food Product Co., Ltd., Sakon Nakhon, Thailand. All chemicals were used without further purification and shown in **Table 6**.

4.2 Extraction of lycopene

Conventional solvent extraction (CSE) was performed to extracted lycopene from raw materials (tomato paste and tomato processing waste) using vegetable oil as non-toxic solvent. Desired amount of raw material was mixed with the designed volume of solvent under continuous stirring for defined time. The effects of different oils (sunflower oil, soybean oil, olive oil, coconut oil, and palm oil), temperature (30-50 °C), solvent/material ratio (30-100 mL/g) and ethanol concentration (0-100 %) were investigated. After extraction, the mixture was centrifuged to separate the residue from oil and the supernatant oil was further analyzed to determine the lycopene content and purity of lycopene in the extracts with UV-Vis spectrophotometer and HPLC, respectively.

Table 6 List of chemicals used in the experiment

Chemical name	CAS no.	Purity (%)	Manufacturers
Acetonitrile	75-05-8	≥99.9	RCI Labscan Ltd., Thailand
Ethanol	64-17-5	≥99.9	RCI Labscan Ltd., Thailand
2,2-Diphenyl-1-picrylhydrazyl (DPPH)	1898-66-4	90.0	Sigma-Aldrich Co., Ltd., USA
n-Hexane	110-54-3	99.0	QReC Chemical Co., Ltd., New Zealand
6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox)	53188-07-7	97.0	Sigma-Aldrich Co., Ltd., USA
Lycopene	502-68-8	≥85.0	Sigma-Aldrich Co., Ltd., USA
Methanol	67-56-1	≥99.9	RCI Labscan Ltd., Thailand
2-Propanol	67-63-0	99.7	QReC Chemical Co., Ltd., New Zealand
Tetrahydrofuran (THF)	109-99-9	≥99.9	Sigma-Aldrich Co., Ltd., USA

มหาวิทยาลัยศิลปากร

(a)

(b)

Figure 7 Solvent extraction of lycopene from tomato using non-toxic solvent; (a) experimental set up (b) lycopene extracted

4.3 Determination of total lycopene content and percentage yield (% Yield)

To determine the total lycopene content in raw material, 0.5 g of tomatoes were extracted several times repeatedly with 30 ml of acetone in n-hexane (25%v/v) until absorbance of the extract at 503 nm was lower than the instrumental noise which means that the lycopene in the solvent was very close to zero. Finally, total lycopene content was determined by combining lycopene content in the solvent from every repeated sample. The percentage yield (%Yield) was calculated using the following equation:

$$\%Yield = \frac{Amount\ of\ lycopene\ extracted\ in\ a\ single\ extraction\ (mg/g)}{Total\ amount\ of\ lycopene\ content\ in\ raw\ material\ (mg/g)} \times 100 \quad (1)$$

4.4 Analysis of extracted lycopene

The extracted lycopene samples were analyzed using ultraviolet-visible spectrophotometry (UV-Vis) and high performance liquid chromatography (HPLC). To avoid the degradation and isomerzation, standard stock solutions of lycopene were wrapped by aluminium foil and stored in the freezer at 5°C. All analysis was done in duplicate and in the absence of direct sunlight.

4.4.1 Ultraviolet-Visible spectroscopy (UV-VIS) analysis

The lycopene content in the extracts was determined using UV-VIS spectrophotometer (T92+ UV spectrometer, PG Instruments Ltd., UK) with n-hexane as the blank at 503 nm to minimize the interference from other carotenoids (Fish et al., 2002). For calibration, a stock solution of commercially standard lycopene was prepared by dissolving 4.3 mg of standard lycopene in 100 mL n-hexane (43.0 µg/

mL of stock standard solution). The stock solution was diluted with n-hexane to obtain concentration between 4.3-43.0 μg/mL. The calibration curve was plotted between absorbance and concentration with linearity of 0.9996. The linearity of the calibration curve was in accordance with the AOAC specification of R^2 over 0.99.

Figure 8 Ultraviolet-Visible (UV-VIS) spectrometer (T92+ UV spectrometer, PG Instruments Ltd., UK)

มหาวิทยาลัยศิลปากร

4.4.2 High-performance liquid chromatography (HPLC) analysis

The purity of lycopene in the extracts was determined using HPLC instrument (YL9150 HPLC, YL Instruments Co., LTD., KOREA) equipped with a C18 column (250 mm × 4.6 mm × 5 μm, Phenomenex, USA) including C18 guard column (10 mm × 4.6 mm × 5 μm). The mobile phase was composed of acetonitrile and methanol (50:50, v/v). The mobile phase was filtered through a 0.45 **μ**m nylon membrane filter. The mobile phase flow rate was 2.0 mL/min. To prepare the sample, 200 μL of lycopene-enriched oil was dissolved in 4.8 ml of 2-propanol. The final solution was filtered

through a 0.45 μm nylon membrane filter and 20 μL of sample solution was injected for 15 minutes. All measurements were performed at room temperature and a detection wavelength at 472 nm. Peak spectra were collected and analyzed with YL-Clarity Chromatography software

Figure 9 High-performance liquid chromatography (HPLC) instruments

มหาวิทยาลัยศิลปากร

For the calibration, a stock solution of commercially standard lycopene (Sigma Aldrich) was prepared by dissolving 4.36 mg of standard lycopene in 100 mL tetrahydrofuran (43.6 μg/mL of stock standard solution). 200 μL of pure vegetable oil was spiked in the stock solution and the spiked oil sample was diluted with 2-propanol to obtain concentration between 0.218-4.360 μg/mL. The calibration curve was plotted between peak area and concentration with the linearity more than 0.999. The linearity of the calibration curve was in accordance with the AOAC specification of R^2 over 0.99.

4.5 Determination of antioxidant activity

The antioxidant activity was determined using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The experiment was modified from the method of Farrukh (2006). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and butylated hydroxytoluene (BHT) were used as antioxidant standards. The DPPH solution was prepared by dissolving 2.4 mg of DPPH free radical in 100 mL ethanol (24 μg/ mL of DPPH solution). An extracted lycopene sample of 1 mL was dissolved with 4 mL of 2-propanol (200 μL/mL) and then the dissolved solution was diluted with 2-propanol to obtain concentration between 200-6.25 μg/mL. The sample solution was completely mixed using a vortex mixer. 100 μL of each dilution sample was reached with 100 μL of DPPH solution in the 96-wells microplate. After 30 min of incubation at room temperature, the reduction in the number of DPPH free radical was measurement the absorbance at 520 nm using Microplate reader (Spectramax® M3, Molecular devices, USA). The control solution contained 100 μL of 2-propanol and 100 μL of DPPH solution. The blank sample solution contained 100 μL of sample solution and 100 μL of 2-propanol. The percent inhibition (%Inhibition) was calculated using the following equation:

มหาวิทยาลัยศิลปากร

$$\%Inhibition = \frac{A_{control} - (A_{sample} - A_{blank})}{A_{control}} \times 100 \quad (2)$$

where $A_{control}$ is the absorbance of the control solution, A_{sample} is the absorbance of the sample solution and A_{blank} is the absorbance of the blank sample solution.

(a) (b)

Figure 10 The instrument for analyzing of DPPH free radical scavenging capacity assay; (a) Microplate reader (Spectramax® M3, Molecular devices, USA) (b) 96-wells microplate

4.6 Application with tomato processing waste

The optimum condition from the experiments was applied to extract lycopene from tomato waste in concentrated tomato juice production process. The water content of sample was about 77 %. Prior extractions, part of seeds were removed and the sample was dried in an oven at 50 °C until the sample reaches constant weight. The dry sample was ground using a blender and sieved to obtain the various particle sizes.

(a) (b)

Figure 11 Tomato processing waste from Doi Kham Food Product Co., Ltd., Sakon Nakhon, Thailand; (a) raw tomato processing waste (b) dry peel waste

Chapter 5

Results and Discussion

5.1 Extraction of lycopene from tomato paste using vegetable oils

It is well known that the type of solvent has a dominant influence on the extraction process, and it is highly possible that a particular kind of vegetable oil has a significant effect on the extraction of lycopene from tomato paste. In this part, five kinds of environmentally benign solvents, coconut oil, olive oil, soybean oil and palm oil, were tested at extraction temperature of 30°C, a solvent to paste ratio of 60 mL/g and extraction time of 30 minutes.

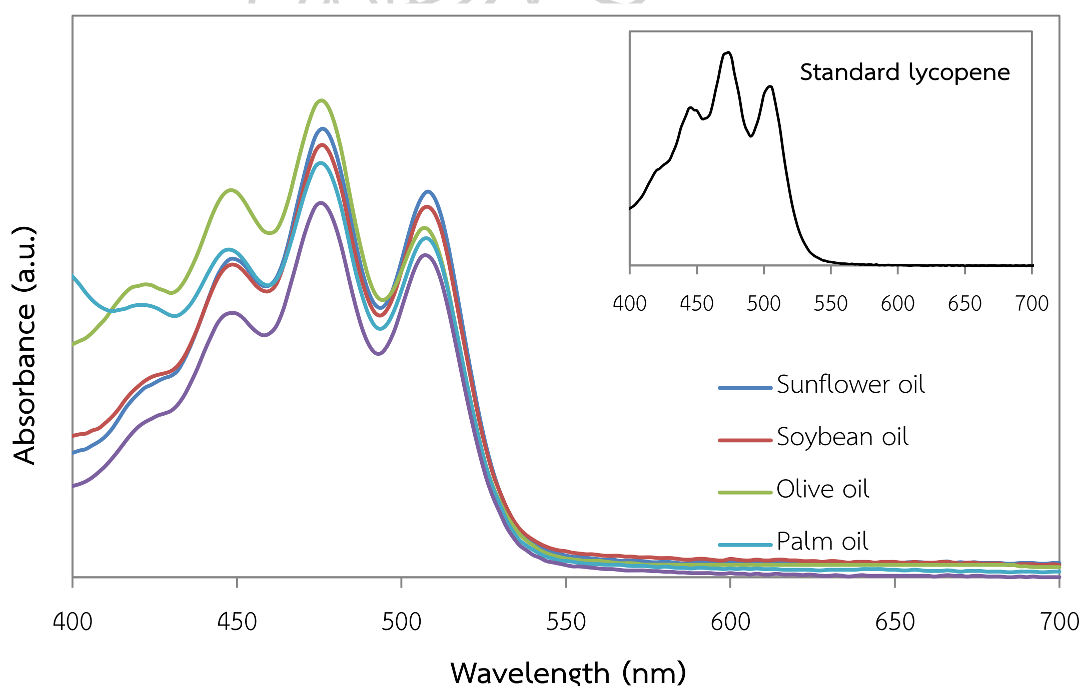


Figure 12 UV-Spectrum of lycopene extracted from tomato paste using various vegetable oils compared with UV-Spectrum of lycopene standard

UV-VIS spectrum of lycopene extracted with different oils was represents **Figure 12**. It shows the visible absorption spectrum of the extract which is similar to the standard lycopene containing three characteristic peaks for lycopene located at 444 nm, 472 nm and 505 nm. The results show that lycopene in tomato waste can be extracted using vegetable oils giving a yield of lycopene in the range of 0.80 to 1.66 mg/g paste. The highest yield of 1.66 mg/g paste was achieved using coconut oil while olive oil, soybean oil, palm oil, and sunflower oil obtained 1.25, 1.05, 0.97 and 0.80 mg/g paste, respectively as shown in **Figure 13**.

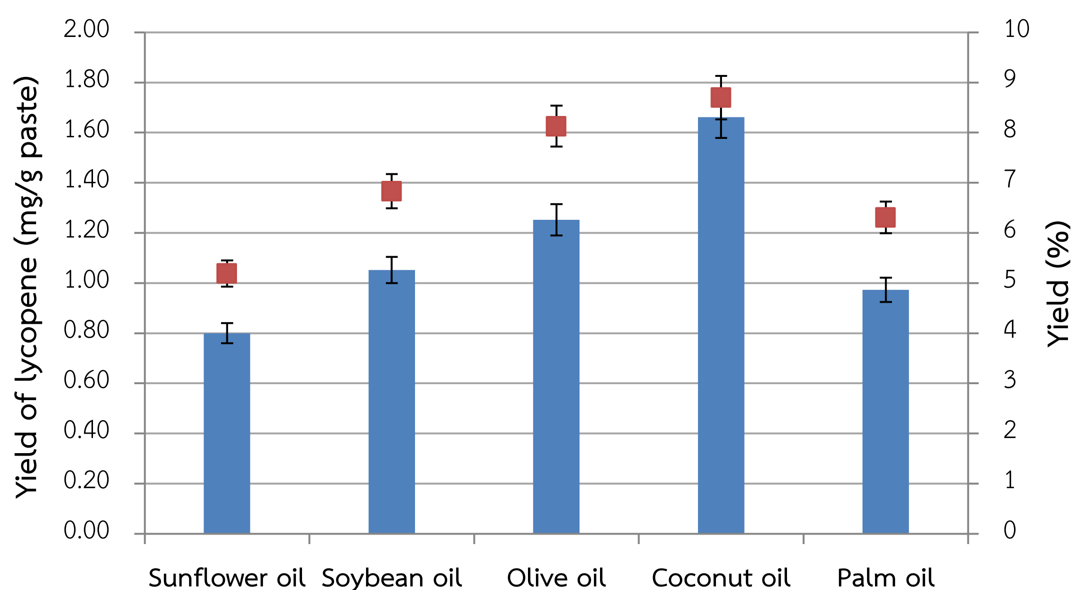


Figure 13 Extraction yield of lycopene from tomato paste using various vegetable oils

Table 7 presented the fatty acids composition of vegetable oils. It showed that coconut oil has the highest content saturated fatty acids followed by palm oil, soybean oil, olive oil and sunflower oil. However, it still not clears about the effect of fatty acids composition of vegetable oil on lycopene extraction.

Table 7 Fatty acids composition of vegetable oils (Mawatari, Fukuda, Mori, Mia, & Ohno, 2013)

Type	Saturated fatty acid				Unsaturated fatty acid		
	Lauric acid C12	Myristic acid C14	Palmatic acid C16	Stearic acid C18	Oleic acid C18:1	Linoleic acid C18:2	Linolenic acid C18:3
Sunflower oil	-	-	6.7	3.7	19.0	69.9	0.7
Soybean oil	-	-	10.3	3.8	24.3	52.7	7.9
Olive oil	-	-	9.9	3.2	75.0	10.4	0.8
Coconut oil	47.0	18.0	9.0	3.0	7.0	2.0	-
Palm oil	-	-	44.2	4.5	39.3	9.6	0.3

From the results in **Figure 13**, it was found that lycopene could be extracted from the tomato paste by vegetable oil, but coconut oil seemed to be the best. The higher extraction yield obtained from the coconut oil may be attributed to its lower viscosity. A low solvent viscosity is usually associated with an improved solvent migration through the matrix to increase extraction efficiency during the extraction process (Goula et al., 2017). However, the extraction efficiency does not follow the same trend as the viscosity sequence (**Table 8**).

มหาวิทยาลัยศิลปากร

Table 8 Viscosity of vegetable oils at 30 °C

Oil	Viscosity[a], η(mPa·s)
Sunflower	37.5, 32.3[b]
Soybean	38.9, 40.5[b]
Olive	47.5, 43.6[c]
Coconut	34.0,
Palm	49.0,

[a] Measured by LVDV2D Brookfield viscometer; Standard uncertainties are u(T) = 0.1 K, and u(η) = 0.0001 mPa·s, [b] From literature at 38 °C (Diamante & Lan, 2014), [c] From literature at 25.5 °C (Sagiroglu, Ozcan, Isbilir, Paluzar, & Toprakkiran, 2013)

According to literature reviews (I. Strati & Oreopoulou, 2014), a mixture of polar and non-polar solvents (acetone/hexane, ethanol/hexane, ethyl acetate/hexane, acetone/ethanol/hexane and hexane/acetone/alcohol/toluene) have also been used in solvent extraction to improve carotenoids yield. Ethanol was chosen to mix with vegetable oil because it is environmentally friendly solvents and a food grade solvent.

Figure 14 shows yield of lycopene extraction in different kinds of vegetable oils with various ethanol concentrations. The yield of the lycopene was noticeably increased by adding ethanol into the vegetable oil, and the yield increased with the increase of the content of ethanol in the vegetable oil. This can be explained by the fact that the lycopene was enclosed within the cells of the tomato. Ethanol can break down the cell walls, thus enabling a higher contact area between the solvent and the solids, and the vegetable oil can easily access to leach the lycopene out and dissolve it (M. M. Calvo et al., 2007). Moreover, adding ethanol in the extraction

with the vegetable oil could reduce the viscosity, so it could improve the solvent movement and enhance the extraction process. The highest lycopene yield of 75 % was achieved by using coconut oil as the solvent while soybean oil, olive oil, palm oil, and sunflower oil resulted in 71 %, 70% percent, 68 % and 59 %, respectively, when use the ethanol concentration of 50 %. So, this ratio was used for further experiments.

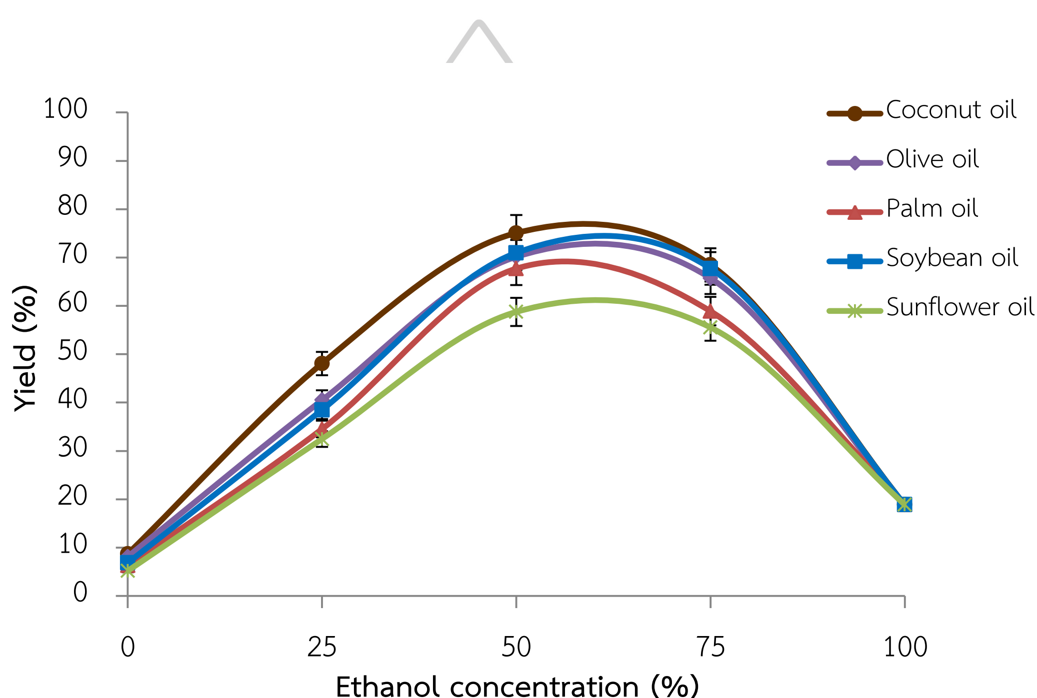


Figure 14 Yield of lycopene extraction in different kinds of vegetable oils with various ethanol concentrations

5.2 HPLC analysis of lycopene extracted from tomato paste

The purity of lycopene in the extracts was determined using HPLC analysis. Identification of lycopene and β-carotene in the extract are made by comparing its retention time with the retention time of standard lycopene and β-carotene as shown in **Figures 15** and **16**, respectively. The chromatogram shown in **Figure 17**

corresponds to lycopene extracted using vegetable oils as the main solvent. Only one peak with retention times 6.80-6.85 min was identified as lycopene. The purity of lycopene was found to be more than 99 % of total carotenoids. β-carotene has not found only a small amount while a small amount of unidentified peaks were detected. The variation in content of lycopene and other compounds may be due to varietal, agricultural, ripening stage, environmental factors and processing conditions (Javanmardi & Kubota, 2006).

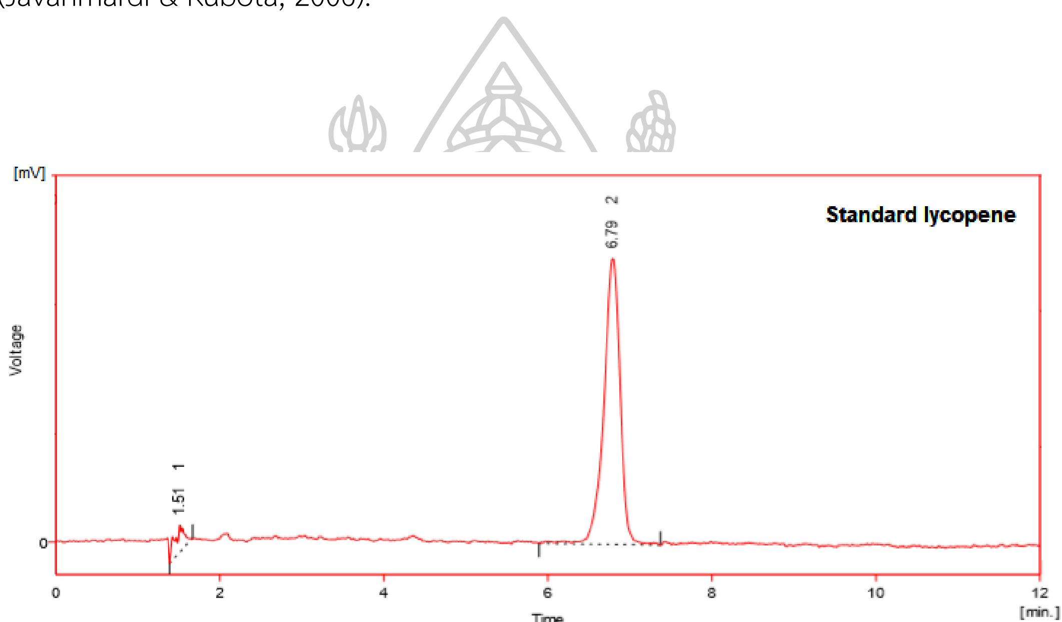


Figure 15 Chromatogram of standard lycopene

มหาวิทยาลัยศิลปากร

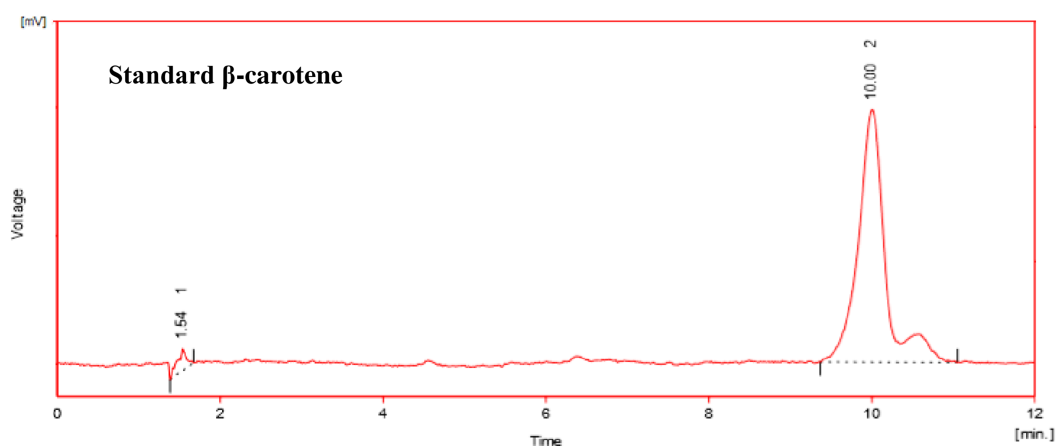


Figure 16 Chromatogram of standard β-carotene

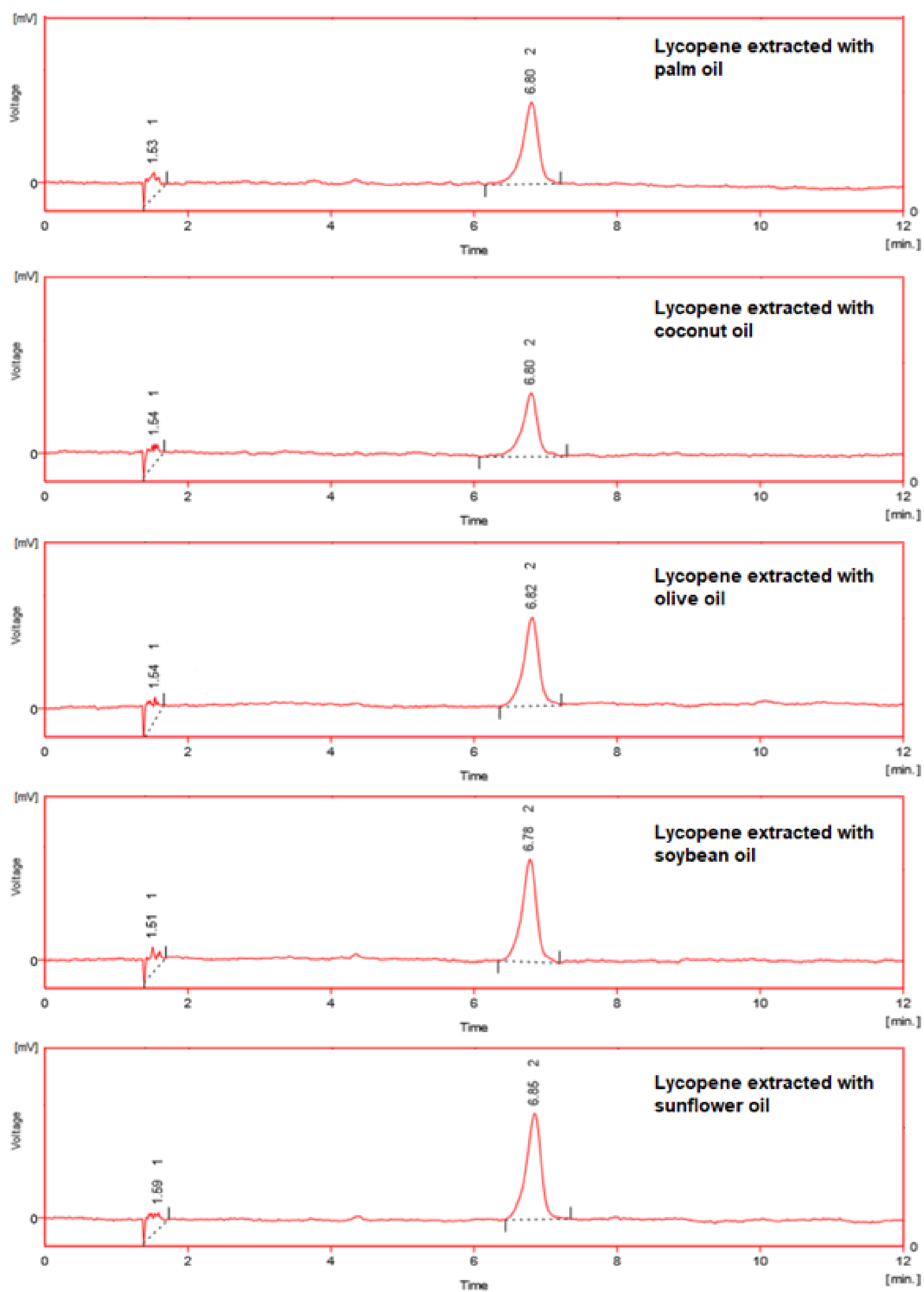


Figure 17 Chromatogram of lycopene extracted with different of vegetable oils.

5.3 Stability of lycopene in vegetable oils

In nature, lycopene in red tomatoes typically contain 94–96% all-trans configuration. The main cause of lycopene degradation in tomato is isomerization and oxidation during processing and storage in extracts and in food products from tomato. It is well known that light, heat, acids, etc. promote isomerization of all-trans lycopene to cis-isomer (John Shi, Le Maguer, Kakuda, Liptay, & Niekamp, 1999). J. Chen, Shi, Xue, and Ma (2009) reported that higher temperatures greatly influenced the cis-isomer contents, and cis-isomer contents increased with elevated temperatures. Increasing temperature increases significantly degradation, which occurs mainly through isomerization at temperatures in the range 75-150 °C and oxidation without isomerization in the range 25-50 °C. Oxidation may occur by autoxidation, which is a spontaneous free-radical chain reaction in the present of oxygen, or photo oxidation, produced by oxygen in the presence of light (W¹sowicz et al., 2004).

In this work, stability of lycopene was estimated from the changes of the lycopene concentration in vegetable oil during 8 weeks of storage at room temperature in the dark (**Table 9**). It found that the lycopene concentration in vegetable oils decreased about 16-21%. At these storage conditions, the decreasing of lycopene stability was probably due to oxidation rather than to isomerization. The higher stability of lycopene was found in coconut oil and palm oil. This may be due to both types of oil are the main components of saturated fatty acids, which are more stable to oxidation than unsaturated fatty acids.

Table 9 The changes of lycopene concentration in vegetable oil during storage

Sample oil	%Remaining					
	1st week	2nd week	3rd week	4th week	6th week	8th week
Sunflower	94.98	92.81	91.33	86.51	81.58	78.83
Soybean	93.77	90.24	89.89	86.36	84.12	78.72
Olive	97.23	94.96	93.28	90.29	87.91	80.97
Coconut	98.70	97.55	94.82	91.79	89.78	83.74
Palm	98.24	96.32	94.48	91.11	89.19	83.59

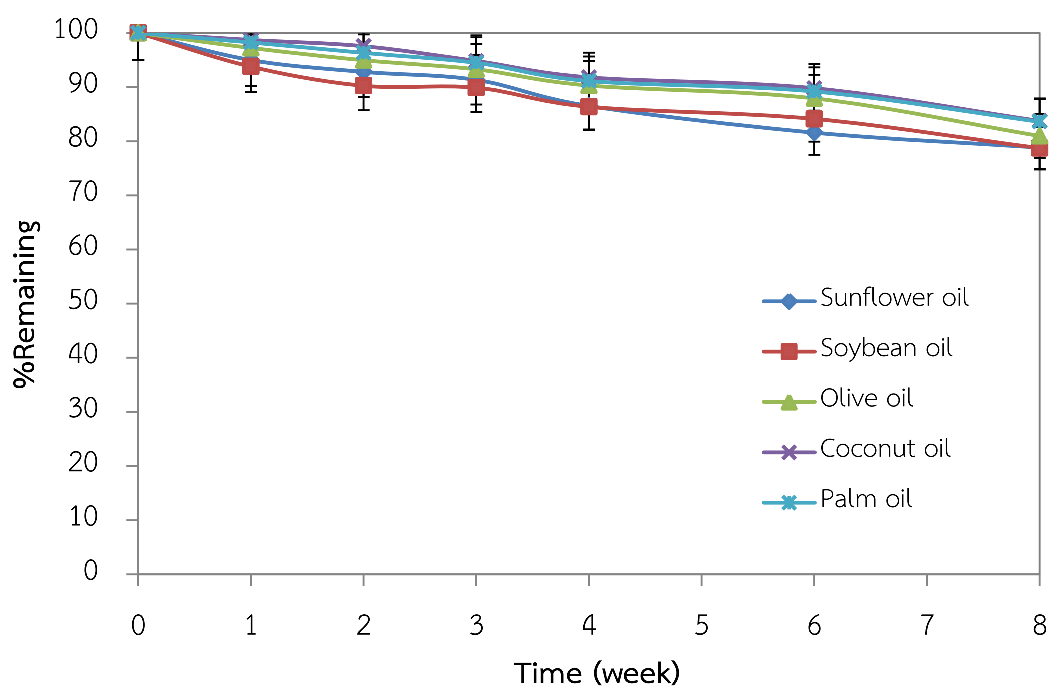


Figure 18 Stability of lycopene in difference vegetable oils during 8 weeks of storage at room temperature in the dark

5.4 Antioxidant activity of lycopene extracted from tomato paste using vegetable oils

α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging method offers for determining the antioxidant potential of a compound, an extract or other biological sources. This is the easiest method, which the extract is mixed with DPPH solution and absorbance is measured after a defined period (Kedare & Singh, 2011). The decrease in absorbance of DPPH radical scavenging was due to the scavenging of the radical by hydrogen donation. It is visually noticeable as a color change from purple to yellow (Biswas, Haldar, & Ghosh, 2010). The DPPH free radical scavenging test is a rapid method for determining antioxidant activity. **Figure 19** show the plotted graph between % inhibition and logarithm concentration of sample to determine the DPPH radical scavenging activity of lycopene extracted from tomato paste using different vegetable oils. In this work, the antioxidant activity was expressed in terms of IC50, which the concentration of extracted lycopene required to reduce the initial DPPH concentration by 50%. In **Table 10**, the IC50 values were as follows: 3.95, 3.99, 6.66, 6.97 and 34.52 μg/mL for palm oil, sunflower oil, olive oil, soybean oil and coconut oil, respectively. However, pure vegetable oil has the antioxidant activity in itself. In this work, the antioxidant activity of pure vegetable oils was not measured. Therefore, the presented IC50 values are derived from both the extracted lycopene and pure oil activity. Comparing with standard Trolox, an antioxidant used in biological or biochemical apllications, lycopene extracts with vegetable oils showed a good antioxidant activity.

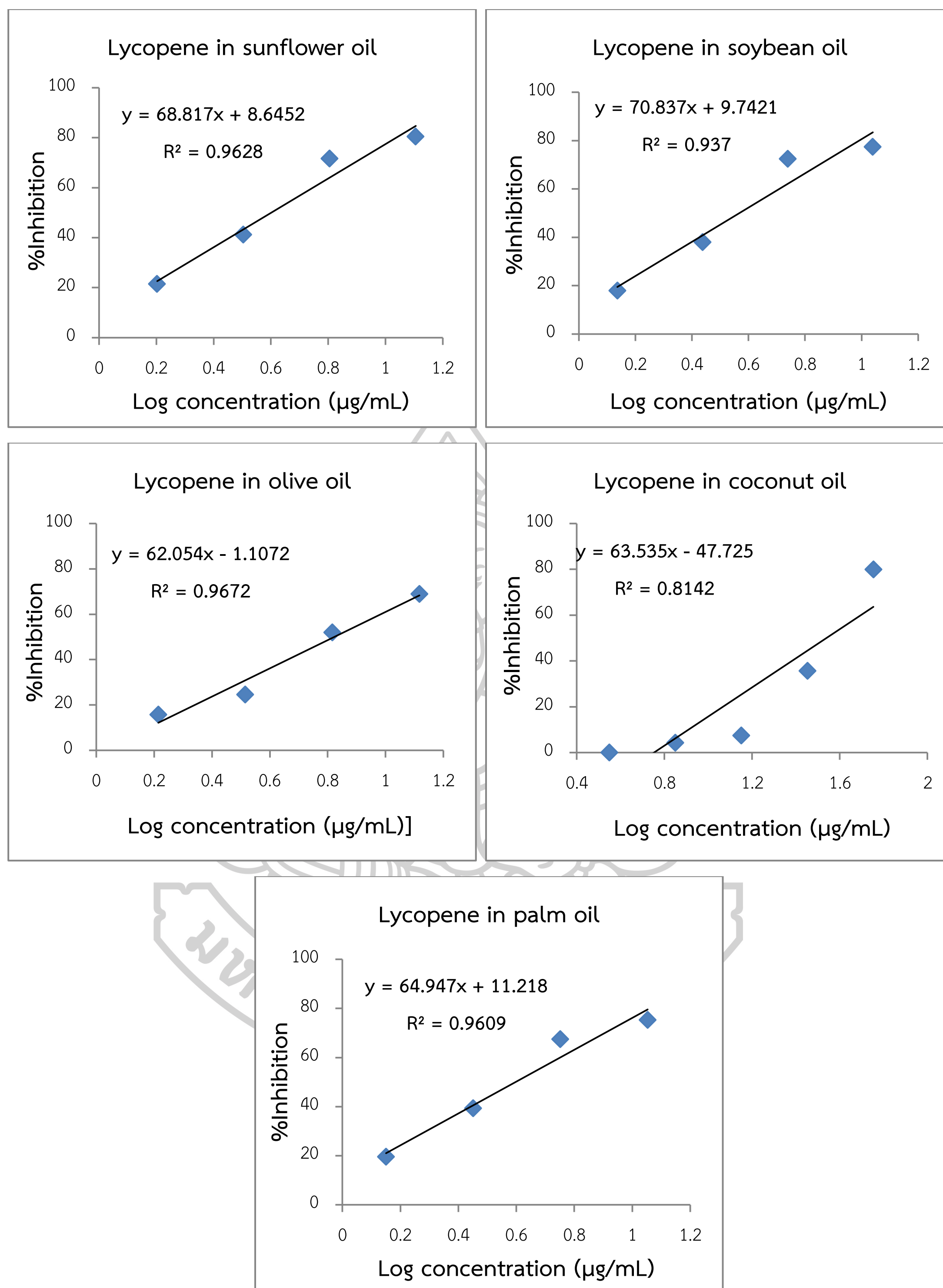


Figure 19 DPPH radical scavenging activity of lycopene extracted from tomato paste using different vegetable oils

Table 10 IC50 of value of lycopene extracted from tomato paste using different vegetable oils in DPPH scavenging assay

Sample oil	IC50 (μg/mL)
Sunflower	3.99
Soybean	6.97
Olive	6.66
Coconut	34.52
Palm	3.95
Trolox	5.24

5.5 Kinetic study on extraction of lycopene from tomato paste using vegetable oils

The kinetic description of solid–liquid extraction helps to design, optimise and simulate the extraction processes and to manage time and energy. The extraction of lycopene was simply assumed to proceed as the lycopene move from solid matrix, $[Lycopene]_m$ to solution, $[Lycopene]_s$ (Eq. (3)). This condition continues until the completion or until the steady state is reached.

$$[Lycopene]_m \rightarrow [lycopene]_s \quad (3)$$

The kinetic models of lycopene extraction were investigated by pseudo-first-order (Bai, Wu, & Grigg, 2009), pseudo-second-order (Qu, Pan, & Ma, 2010) and Peleg's model (Poojary & Passamonti, 2015a), as shown in Equation (4), (5) and (6), respectively.

pseudo-first-order model: $$ln\left(\frac{C_e-C_t}{C_e}\right) = -k_1 t \quad (4)$$

pseudo-second-order model : $$\frac{t}{C_t} = \frac{1}{k_2 C_e^2} + \frac{t}{C_e}$$

(5)

Peleg's model: $$C_t = \frac{t}{K_1 + K_2 t} \quad (6)$$

where k_1 and k_2 is the first-order and second-order extraction rate constant, respectively. C_e is the equilibrium concentration of lycopene in the liquid extract (mg/g), and C_t is the concentration of lycopene in the liquid extract at a given extraction time t (mg/g). K_1 is Peleg's rate constant (min·g/mg) and K_2 is Peleg's capacity constant (g/mg).

The relationship between extraction rate constant (*k*) and temperature (*T*) can be described by the Arrhenius equation (Qu et al., 2010), which is written as Eq. 7:

$$k = k_0 \exp\left(-\frac{E_a}{RT}\right) \quad (7)$$

The transformation of this equation allows obtaining a linear relationship between the extraction rate constant and the reverse of temperature as show in Eq. 8:

$$lnk = lnk_0 + \left(\frac{-E_a}{R}\right)\frac{1}{T} \quad (8)$$

where k_0 is the temperature-independent factor (g/mg·min), E_a is the activation energy of extraction (J/mol), R is the gas constant (8.314 J/mol·K), and T is extraction temperature (K). The k_0 and E were determined from the slope and intercept by plotting ln(k) against 1/T.

The graph of the extraction of lycopene was plotted as a relation of time for different solvent at various temperatures (30, 40 and 50 °C). In all experiments, the extraction yield was significantly time dependent and the profile of the graphs as shown in **Figures 20-24.** The results clearly show that the yield of lycopene rapidly increases with time at first period, after that, less quickly as the progress of extraction continues. This results can be described by the fact that during the initial stage of extraction, when the solvent penetrates into the solid, an extremely high concentration gradient is conducted, resulting in high rates of mass transfer into the liquid phase (Poojary & Passamonti, 2015a). When the extraction time increases, the mass transfer of solutes from the solid phase to the liquid becomes more difficult, because of the decrease in concentration driving force between the solid and liquid phases (Hatami, Cavalcanti, Takeuchi, & Meireles, 2012).

The results of the coefficient of determination (R^2) Models fitted for extraction of lycopene with different vegetable oils are presented in **Table 11**. The pseudo-second-order model appears to offer the best fit for the experimental data, signifying the highest R^2. Parameters of second-order kinetic model for extraction of lycopene at different extraction conditions were presented in **Table 12.** The results showed that the extraction rate constant was significantly increased with the increased extraction temperature. The activation energy (E_a) in all cases which calculated by Arrhenius equation were in the range 14.519-18.822 kJ/mol. This indicated that the extraction of lycopene in the investigated system is controlled by diffusion process since the E_a value was found to be less than 20.9 kJ/mol (El-Hefny, 2007; Javanshir, Abdollahy, Abolghasemi, & Darban, 2011). In addition, the process with E_a value

between 20 and 40 kJ/mol is controlled by both chemical reaction and diffusion process (Lazar, Talmaciu, Volf, & Popa, 2016) while in the case of the process with E_a value more than 40 kJ/mol is controlled by the chemical reaction (González-Centeno, Comas-Serra, Femenia, Rosselló, & Simal, 2015).

Table 11 Models fitted for extraction of lycopene with different vegetable oils

Oil	Model	Average R^2
Sunflower	Pseudo-first-order	0.9274
	Pseudo-second-order	0.9981
	Peleg	0.8704
Soybean	Pseudo-first-order	0.9336
	Pseudo-second-order	0.9981
	Peleg	0.8712
Olive	Pseudo-first-order	0.9054
	Pseudo-second-order	0.9972
	Peleg	0.8630
Coconut	Pseudo-first-order	0.8719
	Pseudo-second-order	0.9971
	Peleg	0.8603
Palm	Pseudo-first-order	0.8940
	Pseudo-second-order	0.9922
	Peleg	0.8922

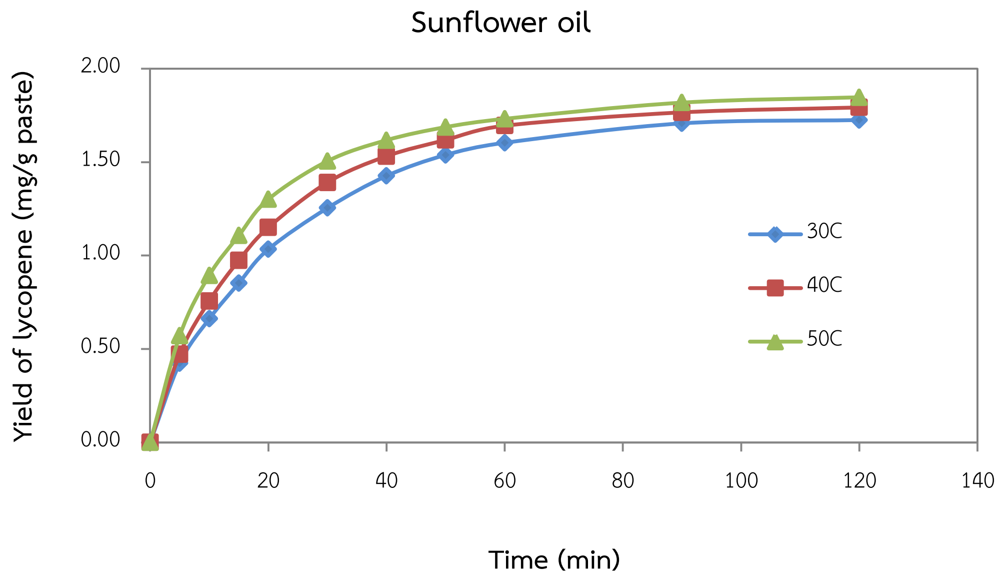


Figure 20 Effect of temperature on yield of lycopene extraction from tomato paste with sunflower oil

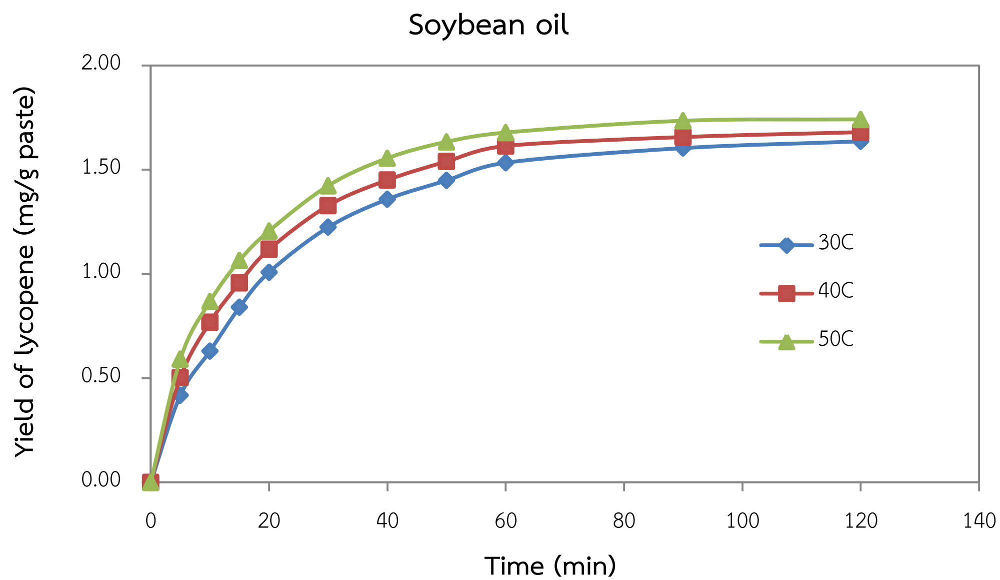
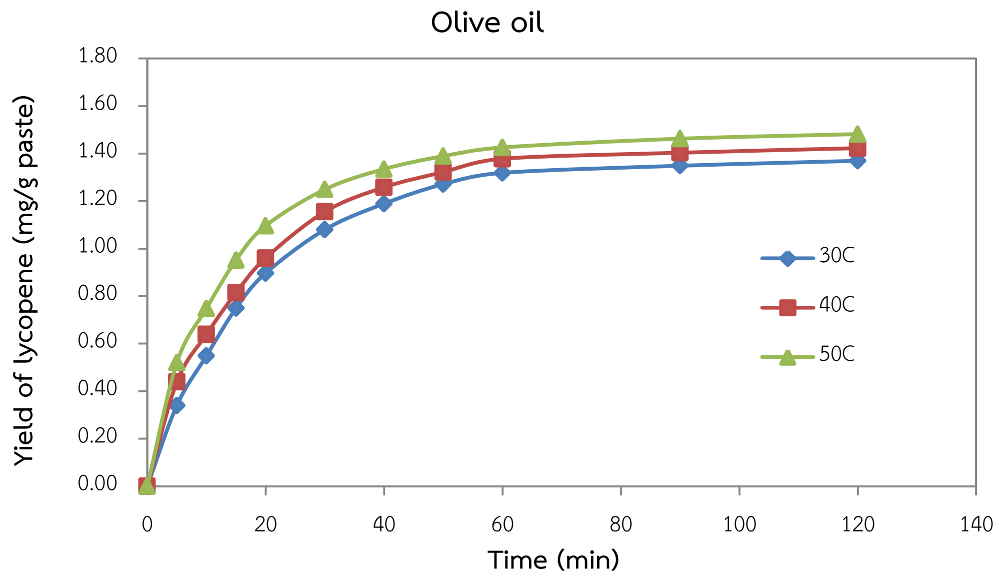


Figure 21 of temperature on yield of lycopene extraction from tomato paste with soybean oil



Figur 22 Effect of temperature on yield of lycopene extraction from tomato paste with olive oil

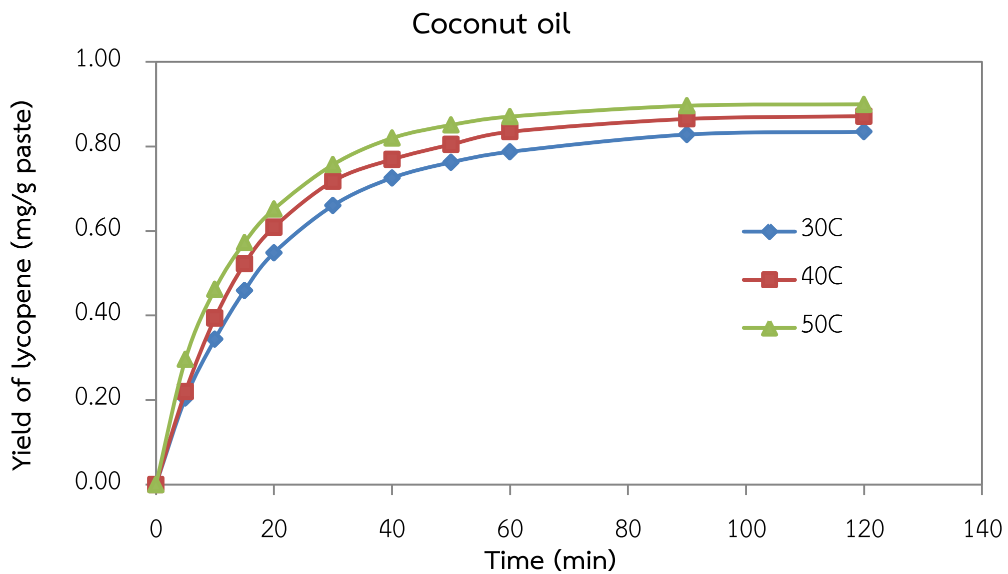


Figure 23 Effect of temperature on yield of lycopene extraction from tomato paste with coconut oil

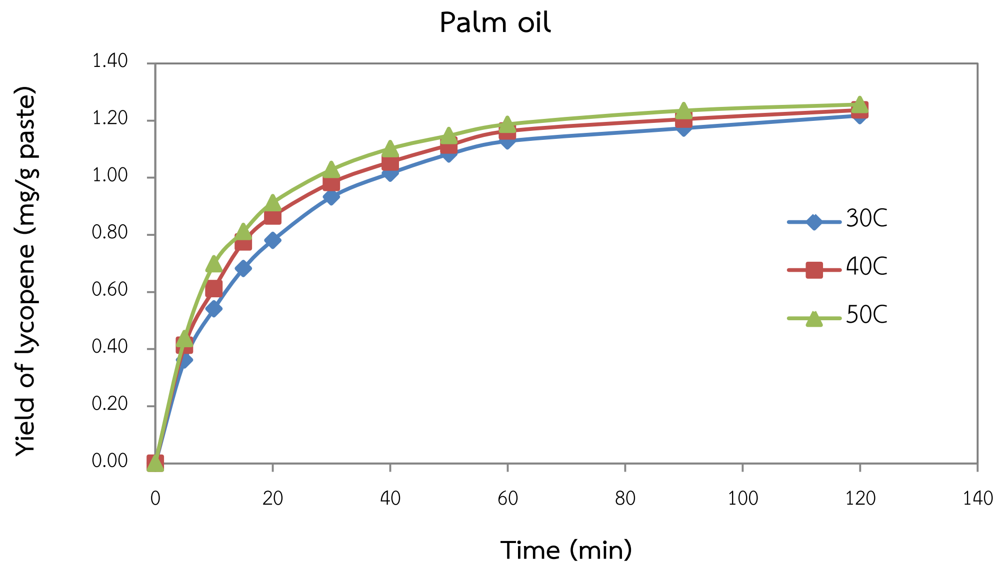


Figure 24 Effect of temperature yield of lycopene extraction from tomato paste with palm oil

มหาวิทยาลัยศิลปากร

Table 12 Parameters of second-order kinetic model for extraction of lycopene at different extraction conditions

Oil type	Extraction temp. (C)	C_e (mg/g_{paste})	k_2 (g_{paste}/mg·min)	E_a (kJ/mol)	R^2
Sunflower	30	2.045	0.026	18.822	0.9852
	40	2.064	0.031		
	50	2.053	0.041		
Soybean	30	1.913	0.029	18.778	0.9832
	40	1.900	0.039		
	50	1.936	0.047		
Olive	30	1.589	0.040	18.198.	0.9916
	40	1.605	0.049		
	50	1.624	0.063		
Coconut	30	0.967	0.066	16.513	0.9582
	40	0.991	0.075		
	50	0.993	0.099		
Palm	30	1.317	0.098	14.519	0.9977
	40	1.338	0.083		
	50	1.317	0.069		

5.6 Optimization of extraction of lycopene from tomato paste using vegetable oils

From previous sections, coconut oil would be seen as the most suitable environmentally benign solvent. It brings the highest yield of lycopene and has a high degree of saturation and good stability. In nutritional concerns, coconut oil has been used for edible and non-edible purposes all over the world. It is the one of the most desirable vegetable oils for confectionery, bakery, frying, etc. Therefore, coconut oil was chosen as the main solvent with the ethanol concentration of 50 % for optimization of extraction of lycopene from tomato paste.

Three factors and three levels of the Box-Behnken Design were used to investigate and optimize the effect of the extraction process variables as the solvent to paste ratio (40-80, X_1), the extraction temperature (30-50°C, X_2) and extraction time (15-45 min, X_4) on the maximum of the lycopene. The coded and actual variables' values were shown in **Table 13.** It should be noted that the process variables and their ranges were chosen from the preliminary experimental results. A total of 15 experiments were required for the estimation of the three process variables.

Table 13 Actual levels and Box-Benkhen Design levels

Factors	Code	Levels		
		-1	0	1
Solvent to paste ratio (mL/g)	X_1	40	60	80
Extraction temperature (°C)	X_2	30	40	50
Extraction time (min)	X_3	15	30	45

The regression equation of the second order polynomial model is presented in Eq. (9)

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum_{j=1}^{k} \beta_j X_j + \sum_{i=1}^{k} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j \qquad (9)$$

where Y is the response, X_i and X_j are the variables (i and j from 1 to k), β_j, β_{jj}, and β_{ij} are the coefficients of the linear, quadratic and the second order terms, respectively, and k is the number of the independent variables (k = 3 in this study). A Minitab V.17.0 program was used to calculate these coefficients. The significant factors and their interactions were determined by the analysis of variance (ANOVA). Optimization was performed by using the response surface and regression equation, which correlated with the significant factors. The second order regression equation for the process variables is as follows:

$$\begin{aligned} \%Yield = {} & 74.132 + 1.814X_1 + 3.714X_2 + 5.179X_3 - 2.096X_1^2 - 6.627X_2^2 \\ & - 0.495X_3^2 - 8.111X_1X_2 + 0.859X_1X_3 - 0.486X_2X_3 \end{aligned} \qquad (10)$$

มหาวิทยาลัยศิลปากร

The values of the lycopene yield were calculated by using the predicted regression model and compared with the experimental results as shown in **Table 14.** The coefficient of determination (R^2) was 0.9839, which indicates the model's accuracy according to the relationship between the response and process variables. The statistical analysis showed that the model, linear component and quadratic component were significant ($p < 0.05$) as shown in the ANOVA in **Table 15**. The analysis of variance also showed that the lack of fit was non significant, which further validated the model.

Table 14 Experimental design and response values of lycopene yield in coconut oil

Run no.	Factors			Yield (mg/g paste)		Yield (%)	
	X_1	X_2	X_3	Exp.	Predicted	Exp.	Predicted
1	-1	-1	0	5.30	5.33	51.47	51.77
2	+1	-1	0	7.44	7.37	72.26	71.62
3	-1	+1	0	7.70	7.67	74.78	75.42
4	+1	+1	0	6.50	6.47	63.13	62.83
5	-1	0	-1	6.90	6.73	67.01	63.69
6	+1	0	-1	7.00	6.93	67.98	69.04
7	-1	0	+1	7.56	7.62	73.38	75.77
8	+1	0	+1	8.01	8.18	77.79	77.68
9	0	-1	-1	5.80	5.93	56.33	57.63
10	0	+1	-1	6.70	6.80	65.07	66.03
11	0	-1	+1	7.20	7.10	69.92	68.96
12	0	+1	+1	7.90	7.77	76.72	75.42
13	0	0	0	7.60	7.63	73.81	74.13
14	0	0	0	7.70	7.63	74.78	74.13
15	0	0	0	7.60	7.63	73.81	74.13

มหาวิทยาลัยศิลปากร

Table 15 Analysis of variance (ANOVA) for a second order model

Source	Degree of freedom	Sum of squares	Mean square	F-value	P-value
Model	9	789.83	87.76	33.99	0.001
-Linear	3	351.24	117.08	45.35	0.000
-Quadratic	3	171.54	57.18	22.15	0.003
-Interaction	3	267.05	89.02	34.48	0.001
Error	5	12.91	2.58		
-Lack of fit	3	12.28	4.09	13.02	0.072*
-Pure error	2	0.63	0.31		
Total	14	802.74			

* not significant

5.6.1 Response surface analysis

In order to study the individual and interaction effect of the independent variables (X_1, X_2, X_3) on the yield of the lycopene *(%Yield)*, response surface plots were constructed from the regression model in Eq. (10). Since there were three factors, two factors were plotted and varied in their range (-1 to +1) while the third factor was fixed at the center (0). The results are shown in **Figures 25 - 27.**

According to the experimental results in **Figure 25**, the lycopene yield increased with the increasing solvent to paste ratio due to high solvent to solid ratio increases concentration gradient of lycopene between raw material and solvent, and hence, increases rate of diffusion (Norshazila et al., 2017). This can be explained by Fick's (first) law of diffusion in Eq. (11):

$$J = k'(C_s - C_t) \tag{11}$$

where J is the flux of lycopene transferred from the solid matrix, k' is the mass transfer coefficient, C_s and C_t are the concentrations of the lycopene in the solid matrix and in the solvent at the time t, respectively. Furthermore, increasing solvent to solid ratio increases the chance of carotenoid components coming into contact with extracting solvent (P. Tan, Tan, & Ho, 2011).

From **Figures 25** and **26**, it is evident that higher temperatures resulted in the increase of the extraction yield of the lycopene. This is probably due to the acceleration of the molecular thermal motion and the increase of the solubility of the lycopene (Xu & Pan, 2013). However, when the temperature was further increased, the lycopene extraction yield decreased due to the degradation of the lycopene molecule (Poojary & Passamonti, 2015b; John Shi & Maguer, 2000).

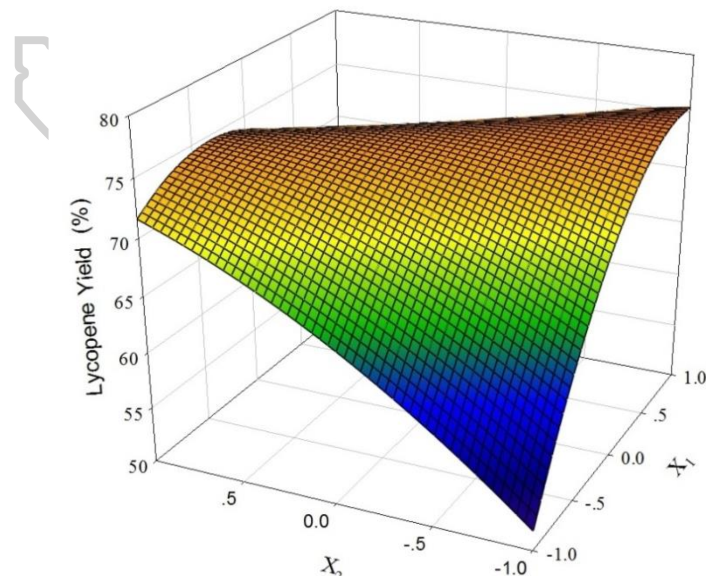


Figure 25 Surface plot showing the effects of solvent to paste ratio (X_1) and extraction temperature (X_2) on lycopene extraction yield (%)

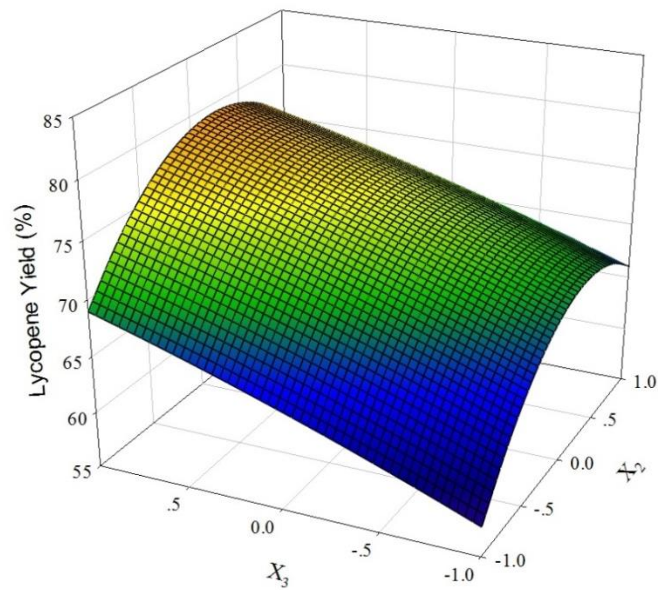


Figure 26 Surface plot showing the effects of extraction temperature (X_2) and extraction time (X_3) on lycopene extraction yield (%)

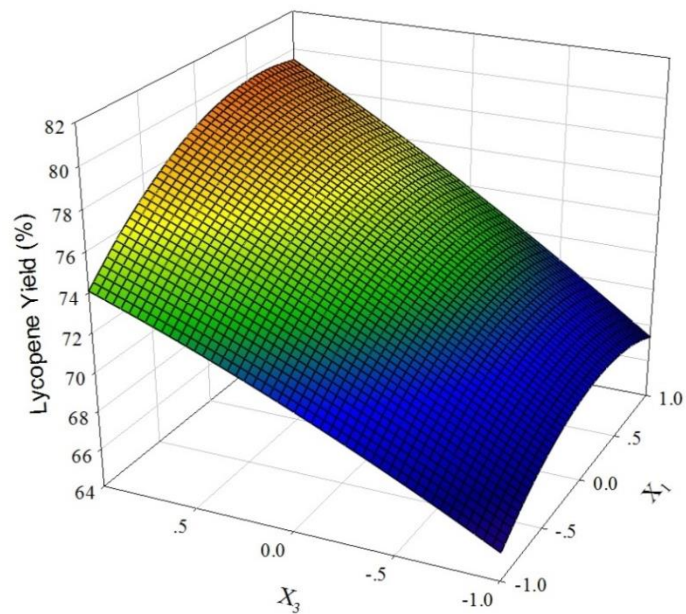


Figure 27 Surface plot showing the effects of solvent/material ratio (X_1) and extraction time (X_3) on lycopene extraction yield (%)

5.6.2 Optimization of extraction conditions

The response optimization plot for the lycopene extraction parameters using the Response Optimizer function of the Minitab Software V.17 was shown in **Figure 28**. The optimized independent variables were found to be X_1 = -0.5, X_2 = 0.60 and X_3 = 0.80. This means that the solvent to paste ratio was 50 mL/g with an extraction temperature of 46 $^{\circ}$C and extraction time of 42 minutes as the optimum condition. The maximum predicted lycopene yield was 78.23 % or namely 8.05 mg/g paste under this condition.

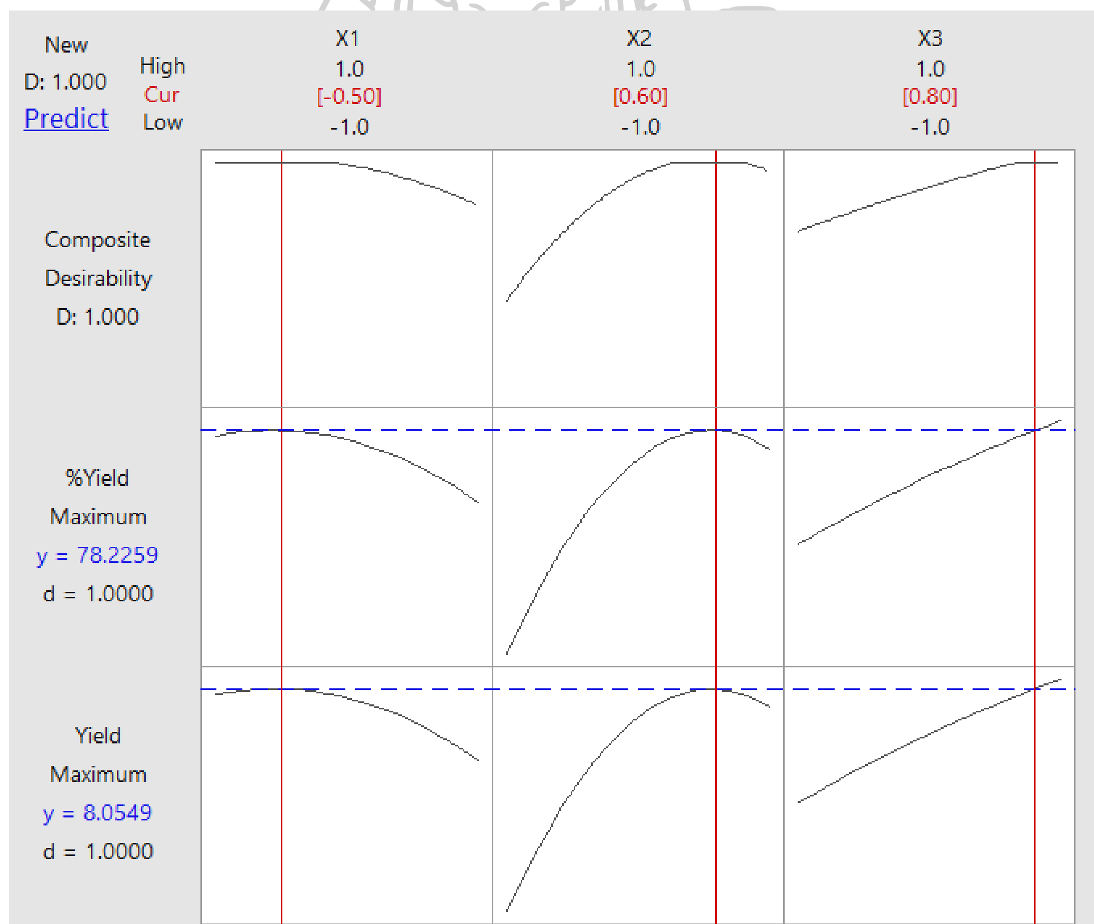


Figure 28 Response optimization plot for lycopene extraction parameters

5.6.3 Validation of optimized condition

The experiment was performed at the predicted optimum condition: solvent to paste ratio of 50 mL/g, extraction temperature of 46 °C and extraction time of 42 minutes to validate the regression model. Under this condition, the predicted lycopene yield was 78.23 % while the experimental value obtained by an equilibrium extraction was 78.02 %. Thus, the optimized value predicted by the proposed model was in good agreement with the experimental value. This indicates that the regression equation model in Eq. (4) was well-suited for the lycopene extraction by using coconut oil and ethanol as a solvent. Moreover, the results showed that, at the optimized condition, coconut oil had more efficiency than organic solvents for the extraction of lycopene from tomato.

5.7 Extraction of lycopene from tomato peel waste using vegetable oils

Tomato is the second most important vegetable plant in the world after potato (Shrestha & Sah, 2014). Around 40 million tons of tomatoes are processed worldwide to produce tomato juice, paste, puree, ketchup, canned tomatoes and many other food products (Nour et al., 2018). During the industrial processing of tomatoes, large quantities of waste are generated about 5-40 % of the raw material (Topal et al., 2006; Zuorro, Lavecchia, Medici, & Piga, 2014). Although this waste has no commercial value, it contains a rich source of lycopene, especially the peel of tomatoes. Utilizing this rich source of tomato waste can be a sustainable, alternative and low cost natural bioactive compound. Improving the value of tomato waste is possible through the development of environment-friendly technologies that can convert the waste into new food ingredients or alternative products.

In this study, tomato peel waste was obtained from Doi Kham Food Product Co., Ltd., Thailand. The water content was about 77 %. Prior extraction, part of seeds was removed and then was dried in an oven at 50 °C until the sample reaches constant weight. The dry sample was ground using a blender and sieved to obtain the particle size varied from 0.2 to 1.0 mm.

The extractability of lycopene from tomato peel waste using vegetable oils such as coconut oil, olive oil, palm oil, soybean oil and sunflower oil was conducted with the ratio of solvent to dry peel 40 mL/g at 30 °C for 60 min. The results show that lycopene in tomato waste can be extracted using vegetable oils giving a yield of lycopene in the range of 0.49 to 0.61 mg/g dry peel. The highest yield of 0.61 mg/g dry peel was achieved using sunflower oil while soybean oil, olive oil, palm oil, and coconut oil obtained 0.59, 0.56, 0.53 and 0.49 mg/g dry peel, respectively as shown in **Figure 29.** Therefore, sunflower oil proved to be the most suitable environmentally benign solvent and it was chosen as the main solvent for further studies.

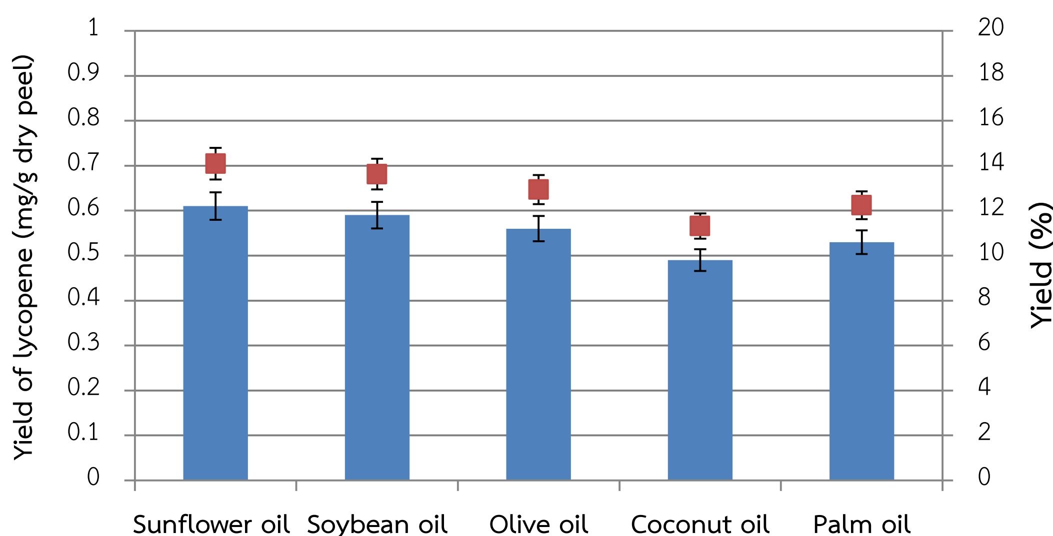


Figure 29 Extraction yield of lycopene from tomato peel waste using various vegetable oils

5.8 Optimization of extraction of lycopene from tomato peel waste using vegetable oils

A Box-Behnken Design (BBD) of the response surface methodology was used to investigate the effects of solvent to dry peel ratio (X_1), particle size (X_2), ethanol concentration (X_3) and extraction time (X_4) on the yield of lycopene (Y). According to the experimental design, the values of the independent variables were coded at three levels as -1, 0, +1 as shown in **Table 16.** It should be noted that the process variables and their ranges were chosen from preliminary experimental results.

Table 16 Independent variables and their levels for Box-Benhken (BBD)

Independent variables	Code	Levels		
		-1	**0**	**1**
Solvent to dry peel ratio (mL/g)	X_1	40	70	100
Particle size (mm)	X_2	0.2	0.4	0.6
Ethanol concentration (%v/v)	X_3	25	50	75
Extraction time (min)	X_4	30	60	90

The yield of lycopene from the 27 sets of experiments was fitted into a second-order polynomial regression model as follows:

$$Y = 2.9733 + 0.3675X_1 - 0.6568X_2 + 0.0974X_3 + 0.0070X_4 - 0.0552X_1^2 - 0.1498X_2^2 - 0.6511X_3^2 + 0.0253X_4^2 - 0.1525X_1X_2 + 0.2947X_1X_3 + 0.2149X_1X_4 + 0.4150X_2X_3 - 0.2072X_2X_4 + 0.3317X_3X_4 \quad (12)$$

Table 17 Experimental and predicted response for different levels of experimental design

run no.	Independent variables				Yield (mg/g dry peel)		Yield (%)	
	X_1	X_2	X_3	X_4	Exp.	Predicted	Exp.	Predicted
1	0	-1	0	-1	2.89	2.91	62.96	63.29
2	0	-1	0	+1	3.94	3.95	85.84	85.95
3	0	+1	0	-1	1.90	1.90	41.39	41.32
4	0	+1	0	+1	2.34	2.33	50.98	50.69
5	-1	0	-1	0	1.94	2.51	55.99	84.47
6	-1	0	+1	0	2.79	2.04	45.53	45.89
7	+1	0	-1	0	2.57	1.99	42.27	70.32
8	+1	0	+1	0	2.09	2.85	60.72	60.64
9	-1	0	0	-1	2.39	2.72	61.20	60.65
10	-1	0	0	+1	3.50	3.03	66.67	67.30
11	+1	0	0	-1	2.81	2.43	52.12	51.59
12	+1	0	0	+1	3.06	3.60	76.31	76.97
13	0	-1	-1	0	3.16	3.15	68.85	96.93
14	0	-1	-1	0	0.97	1.00	21.13	50.23
15	0	+1	+1	0	2.54	2.51	55.34	54.72
16	0	+1	+1	0	2.01	2.03	43.79	44.18
17	0	0	-1	-1	2.08	2.10	45.32	74.03
18	0	0	-1	+1	2.27	2.24	49.46	77.24
19	0	0	+1	-1	1.68	1.70	36.63	37.10
20	0	0	+1	+1	3.05	3.03	66.45	65.92
21	-1	-1	0	0	3.70	3.23	71.68	71.71
22	-1	-1	0	0	2.00	2.33	52.69	52.12
23	+1	+1	0	0	3.29	3.78	80.61	81.04
24	+1	+1	0	0	2.42	2.05	43.57	43.39
25	0	0	0	0	2.97	2.97	64.60	64.78
26	0	0	0	0	2.97	2.97	64.78	64.78
27	0	0	0	0	2.98	2.97	64.95	64.78

The values of lycopene yield were calculated using the predicted regression model and compared with the experimental results as shown in **Table 17.** The coefficient of determination (R^2) was 0.9952 which indicates adequacy of the applied model. **Table 18** displayed the ANOVA of the second order model for extraction of lycopene. All linear (X_1, X_2, X_3 and X_4), quadratic (X_1^2, X_2^2, X_3^2 amd X_4^2) and cross-product (X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4, and X_3X_4) terms in the model proved to be significant at 95% confidence level (P-value < 0.05). It also showed that the lack of fit is non significant indicating that the fitted model is accurate enough to predict the response (Hazir, Hüseyin Koc, & Hiziroglu, 2017).

Table 18 Analysis of variance (ANOVA) for the second order polynomial model

Source	DF	SS	P-Value
Model	14	11.5174	0.000
- Linear	4	6.9118	0.000
- Quardratic	4	2.6798	0.000
- Interaction	6	1.9258	0.000
Error	12	0.0096	
- Lack of fit	10	0.0095	0.063*
- Pure error	2	0.0001	
Total	26	11.5271	
R^2	0.9952		

*not significant

5.8.1 Response surface analysis

In order to study the individual and interaction effect of the independent variables (X_1, X_2, X_3 and X_4) on the yield of lycopene (Y), the response surface plots were constructed from the regression model in Eq. (12). Since there are four factors, two factors were plotted and varied in their range (-1 to +1) while the two factors were fixed at zero code level. The results are shown in **Figure 30.**

The effect of dry peel ratio on lycopene extraction revealed that the yield of lycopene generally increased with an increase of solvent to dry peel ratio as shown in **Figures 30(a)-(f)**. This is because a higher solventto solid ratio can promote a greater concentration gradient between the solid and the solvent which results in increasing mass transfer and facilitating the diffusion of lycopene into the solvent leading to greater extraction of solids by solvent (Al-Farsi & Lee, 2008; Wong, Tan, & Ho, 2013). This explanation was in agreement with Zhang, Lv, Jiang, Cheng, and Fan (2015) which expressed that increasing the amount of extracting solvent results in increased the chances of the bioactive compounds contact to the solvent but will not continue to increase when equilibrium is reached.

Variation in particle size showed that the yield of lycopene generally increased with decreasing the particle size of dry tomato peel waste as shown in **Figures 30(d)-(e)** . A smaller particle size intends a shorter mass transfer distance and a larger contacting surface area which ultimately decreases the extraction time and rise the extraction performance. By the same way, Landbo and Meyer (2001) showed that the total phenolic yield significantly increased with decreasing in particle size during the extraction of antioxidants from black currant juice press residues.

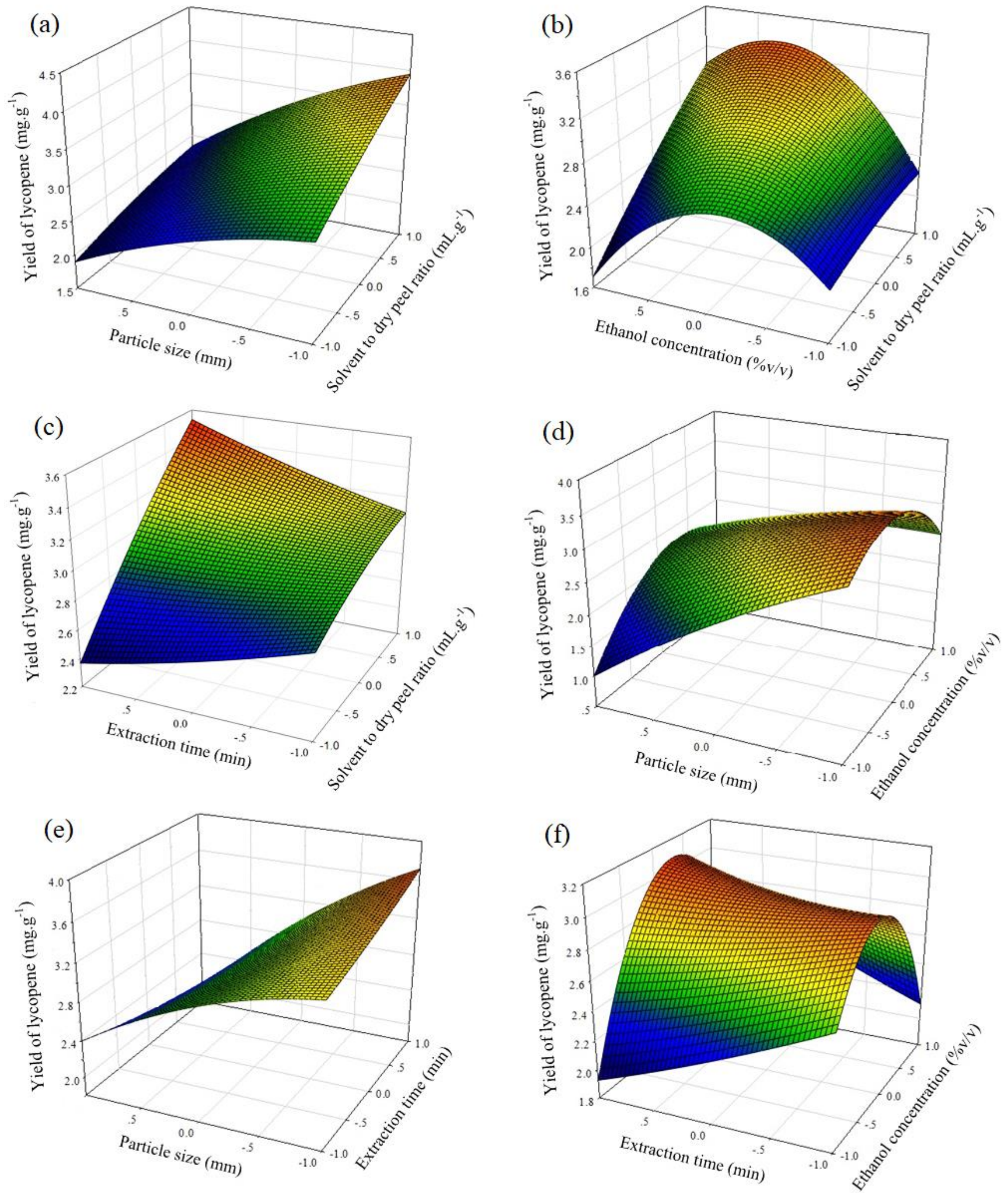


Figure 30 Response surface plots showing the effects of two variables on the response yield of lycopene (mg/g dry peel), with the other two fixed at zero code level.

The effect of ethanol concentration on lycopene extraction revealed that the yield of lycopene increased when the content of ethanol in the vegetable oil increased as shown in **Figures 30(d)** and **30(f).** This can be explained by the fact that lycopene is enclosed within the tomato cells: ethanol can break down the cell walls, thus enabling higher contact area between solvent and solids and the vegetable oil can easily access to leach lycopene out and dissolve it. Moreover, adding ethanol in the extraction process can reduce the viscosity of solvent so it can improve the solvent movement and increase extraction efficiency. However, higher ethanol concentration resulted in a decrease in the yield of lycopene. It is well known that a polar solvent will dissolve polar solutes and a non-polar solvent will dissolve non-polar solutes. Since lycopene is a non-polar compound and soluble in oils, the small volume of oil reduces diffusion and mass transfer, and thus results in a decrease in the yield of lycopene (Poojary & Passamonti, 2015b).

5.8.2 Optimization of extraction conditions

The response optimization plot for lycopene extraction parameters using Response Optimizer function of Minitab Software V.17 is represented in **Figure 31.** The optimized independent variables were found to be X_1 = 1.00, X_2 = -1.00, X_3 = 0.20 and X_4 = 1.00. This means that solvent to dry peel ratio of 40 $mL.g^{-1}$, particle size of 0.3 mm, ethanol concentration 55 %v/v and extraction time of 90 min was the optimum condition with maximum predicted lycopene yield of 97% or namely 4.44 mg/g dry peel.

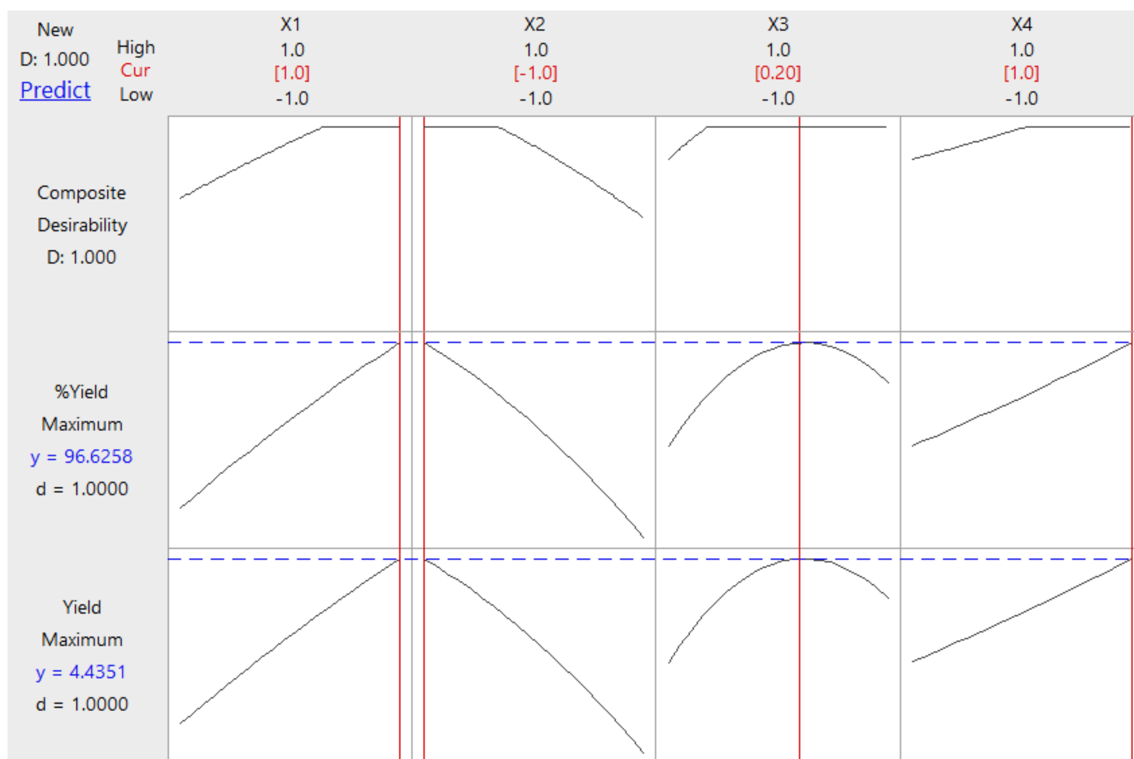


Figure 31 Response optimization plot for lycopene extraction parameters

5.8.3 Validation of optimized condition

The experiment was performed at the predicted optimum condition to validate the regression model. Under this condition, the predicted lycopene yield was 4.44 mg/g dry peel, while the experimental value obtained by an equilibrium extraction was 4.36 mg/g dry peel. Thus, the optimized value predicted by the proposed model is in good agreement with the experimental value. This indicates that the regression equation model in Eq. (12) is well-suited for lycopene extraction using green solvent mixture of sunflower oil and ethanol.

5.8.4 HPLC analysis of lycopene extracted from tomato peel waste

The purity of lycopene in the extracts was determined using HPLC analysis. The isocratic method was obviously able to separate two carotenoids in the tomato peel waste. The chromatogram shown in **Figure 32** corresponds to lycopene extracted using sunflower oil as the main solvent. Peak 1 and 2 with retention times 6.91 and 10.27 min, respectively were identified as lycopene and β-carotene. Identification of lycopene and β-carotene in the extract are made by comparing its retention time with the retention time of standard lycopene and β-carotene. The purity of lycopene was found to be 94%. Lycopene is a main compound detected in tomato peel waste extracts while β-carotene has only a small amount. In addition, a small amount of unidentified compounds were detected at a range of retention time 1.30-4.71 min.

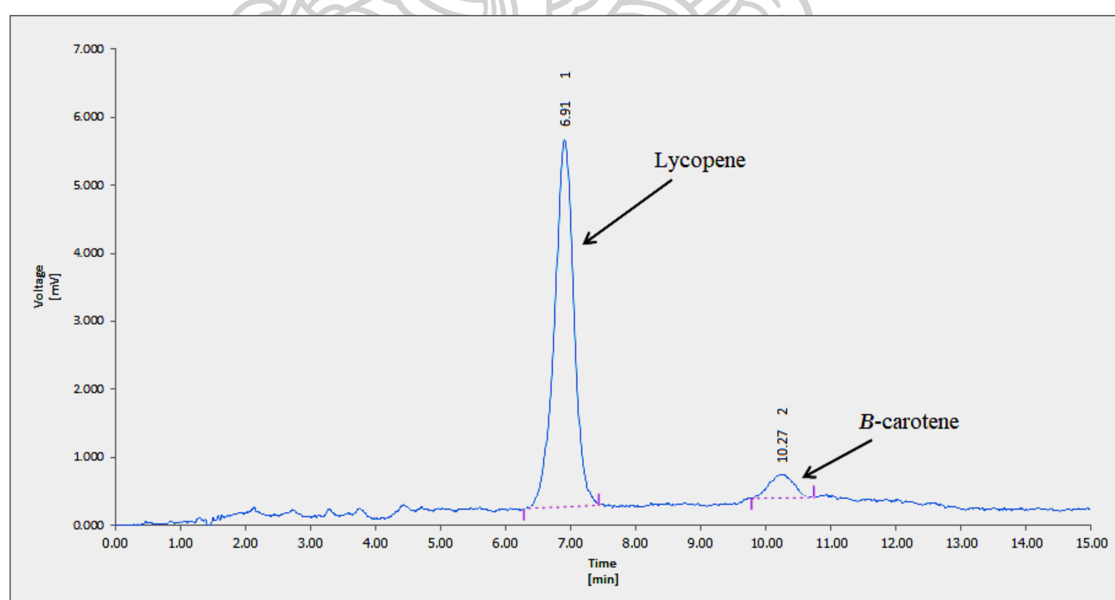


Figure 32 HPLC chromatogram of lycopene extracted from tomato peel waste using solvent mixture of sunflower oil and ethanol.

Chapter 6
Conclusions and Recommendations

6.1 Conclusions

Feasibility study on extraction of lycopene from tomato using vegetable oils was investigated. The conclusion from the investigation could be written as following.

1. Lycopene was successfully extracted from tomatoes sunflower oil, soybean oil, olive oil, coconut oil, and palm oil as non-toxic solvent. Pure coconut oil and sunflower oil provided the highest extraction yield of lycopene from tomato paste and tomato peel waste, respectively. The addition of ethanol could improve the yield of lycopene.

2. The results from kinetic study showed that extraction of lycopene from tomato using non-toxic solvent is the pseudo-second order process and the value of activation energy indicated that the extraction of lycopene in the investigate system is controlled by diffusion process.

3. The optimization of lycopene extraction by Box-Benhken Design (BBD) could be concluded as following.

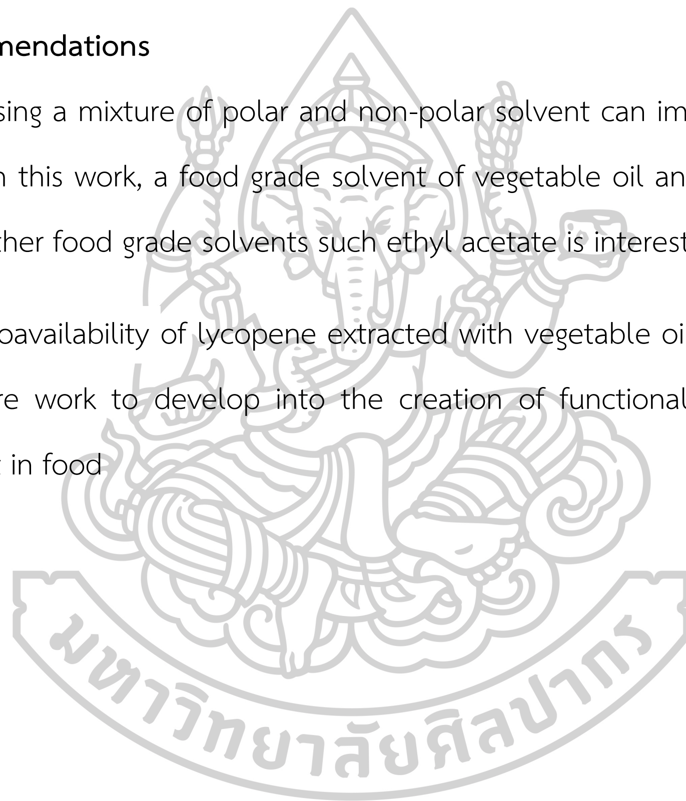
- In case of tomato paste, the extraction of lycopene was performed using the mixture of coconut oil and ethanol (50% v/v). The optimum conditions were solvent/material ratio of 50 mL/g, extraction temperature of 46 °C and extraction time of 42 minutes in which a maximum yield of lycopene 78% or namely 8.01 mg/g paste was achieved.

- In case of tomato peel waste, sunflower oil was chosen as main solvent for extraction. The optimum conditions were as follows: solvent to dry peel ratio of 40 mL/g, particle size of 0.3 mm, ethanol concentration of 55 % and extraction time of 90 min. which resulted in a maximum yield of lycopene 96% or namely 4.36 mg/g dry peel.

6.2 Recommendations

1. Using a mixture of polar and non-polar solvent can improve the extraction efficiency. In this work, a food grade solvent of vegetable oil and ethanol was used. However, other food grade solvents such ethyl acetate is interesting for further study.

2. Bioavailability of lycopene extracted with vegetable oils should be studied in the future work to develop into the creation of functional food or nutritional supplement in food



REFERENCES

Abano, E., Ma, H., & Qu, W. (2012). Influence of combined microwave-vacuum drying on drying kinetics and quality of dried tomato slices. *Journal of Food Quality, 35*(3), 159-168.

Agarwal, S., & Rao, A. V. (2000). Tomato lycopene and its role in human health and chronic diseases. *Canadian Medical Association Journal, 163*(6), 739-744.

Aguilera, J. M. (2003). Solid-liquid extraction *Extraction optimization in food engineering* (pp. 51-70): CRC Press.

Aktas, E. T., & Yildiz, H. (2011). Effects of electroplasmolysis treatment on chlorophyll and carotenoid extraction yield from spinach and tomato. *Journal of Food Engineering, 106*(4), 339-346.

Al-Farsi, M. A., & Lee, C. Y. (2008). Optimization of phenolics and dietary fibre extraction from date seeds. *Food Chemistry, 108*(3), 977-985.

Al-Wandawi, H., Abdul-Rahman, M., & Al-Shaikhly, K. (1985). Tomato processing wastes as essential raw materials source. *Journal of Agricultural and Food Chemistry, 33*(5), 804-807.

Alda, L. M., Gogoasa, I., Bordean, D.-M., Gergen, I., Alda, S., Moldovan, C., & Nita, L. (2009). Lycopene content of tomatoes and tomato products. *Journal of Agroalimentary Processes and technologies, 15*(4), 540-542.

Altan, A., McCarthy, K. L., & Maskan, M. (2008). Evaluation of snack foods from barley–tomato pomace blends by extrusion processing. *Journal of Food Engineering, 84*(2), 231-242.

Arab, L., & Steck, S. (2000). Lycopene and cardiovascular disease–. *The American journal of clinical nutrition, 71*(6), 1691S-1695S.

Ashengroph, M., Nahvi, I., & Amini, J. (2013). Application of Taguchi design and Response surface methodology for improving conversion of isoeugenol into vanillin by resting cells of Psychrobacter sp. CSW4. *Iranian journal of pharmaceutical research: IJPR, 12*(3), 411.

Bai, B., Wu, Y., & Grigg, R. B. (2009). Adsorption and desorption kinetics and equilibrium

of calcium lignosulfonate on dolomite porous media. *The Journal of Physical Chemistry C, 113*(31), 13772-13779.

Bao, Y., Yan, H., Liu, L., & Xu, Q. (2010). Efficient Extraction of Lycopene from Rhodopseudomonas palustris with n-Hexane and Methanol after Alkaline Wash. *Chemical Engineering & Technology, 33*(10), 1665-1671.

Biswas, M., Haldar, P. K., & Ghosh, A. K. (2010). Antioxidant and free-radical-scavenging effects of fruits of Dregea volubilis. *Journal of natural science, biology, and medicine, 1*(1), 29.

Calvo, M., Garcia, M., & Selgas, M. (2008). Dry fermented sausages enriched with lycopene from tomato peel. *Meat science, 80*(2), 167-172.

Calvo, M. M., Dado, D., & Santa-María, G. (2007). Influence of extraction with ethanol or ethyl acetate on the yield of lycopene, β-carotene, phytoene and phytofluene from tomato peel powder. *European Food Research and Technology, 224*(5), 567-571.

Cardinault, N., Abalain, J.-H., Sairafi, B., Coudray, C., Grolier, P., Rambeau, M., . . . Rock, E. (2005). Lycopene but not lutein nor zeaxanthin decreases in serum and lipoproteins in age-related macular degeneration patients. *Clinica chimica acta, 357*(1), 34-42.

Chakravarthi, S. (2002). *The physical and biological factors that influence the isomerization of lycopene.* National Library of Canada= Bibliothèque nationale du Canada.

Chan, C.-H., Yusoff, R., Ngoh, G.-C., & Kung, F. W.-L. (2011). Microwave-assisted extractions of active ingredients from plants. *Journal of Chromatography A, 1218*(37), 6213-6225.

Chemat, F., & Khan, M. K. (2011). Applications of ultrasound in food technology: processing, preservation and extraction. *Ultrasonics Sonochemistry, 18*(4), 813-835.

Chen, J., Shi, J., Xue, S. J., & Ma, Y. (2009). Comparison of lycopene stability in water- and oil-based food model systems under thermal-and light-irradiation treatments. *LWT-Food Science and Technology, 42*(3), 740-747.

มหาวิทยาลัยศิลปากร

Chen, Y., Gu, X., Huang, S.-q., Li, J., Wang, X., & Tang, J. (2010). Optimization of ultrasonic/microwave assisted extraction (UMAE) of polysaccharides from Inonotus obliquus and evaluation of its anti-tumor activities. *International Journal of Biological Macromolecules, 46*(4), 429-435.

Choksi, P. M., & Joshi, V. Y. (2007). A review on lycopene—extraction, purification, stability and applications. *International Journal of Food Properties, 10*(2), 289-298.

Clinton, S. K. (1998). Lycopene: chemistry, biology, and implications for human health and disease. *Nutrition reviews, 56*(2), 35-51.

Cravotto, G., Boffa, L., Mantegna, S., Perego, P., Avogadro, M., & Cintas, P. (2008). Improved extraction of vegetable oils under high-intensity ultrasound and/or microwaves. *Ultrasonics Sonochemistry, 15*(5), 898-902.

Cuevas-Ramos, D., Almeda-Valdés, P., Chávez-Manzanera, E., Meza-Arana, C. E., Brito-Córdova, G., Mehta, R., . . . Gómez-Pérez, F. J. (2013). Effect of tomato consumption on high-density lipoprotein cholesterol level: a randomized, single-blinded, controlled clinical trial. *Diabetes, metabolic syndrome and obesity: targets and therapy, 6*, 263.

Das, S., & Bera, D. (2013). Mathematical model study on solvent extraction of carotene from carrot. *International Journal of Research in Engineering and Technology, 2*(9), 343-349.

Davis, A. R., Fish, W. W., & Perkins-Veazie, P. (2003). A rapid spectrophotometric method for analyzing lycopene content in tomato and tomato products. *Postharvest biology and technology, 28*(3), 425-430.

De Castro, M. L., & Garcıa-Ayuso, L. (1998). Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. *Analytica chimica acta, 369*(1-2), 1-10.

Desai, P. R., Holihosur, P. D., Marathe, S. V., Kulkarni, B. B., Geetanjali, R., Rajeev, R., & Hiremath, S. (2018). Studies on Isolation and Quantification of Lycopene from Tomato and Papaya and its Antioxidant and Antifungal Properties.

Dewanto, V., Wu, X., Adom, K. K., & Liu, R. H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of*

Agricultural and Food Chemistry, 50(10), 3010-3014.

Diamante, L. M., & Lan, T. (2014). Absolute viscosities of vegetable oils at different temperatures and shear rate range of 64.5 to 4835 s– 1. *Journal of food processing, 2014*.

Dolatowski, Z. J., Stadnik, J., & Stasiak, D. (2007). Applications of ultrasound in food technology. *Acta Scientiarum Polonorum Technologia Alimentaria, 6*(3), 88-99.

Draelos, Z. D. (2010). Nutrition and enhancing youthful-appearing skin. *Clinics in dermatology, 28*(4), 400-408.

Eh, A. L.-S., & Teoh, S.-G. (2012). Novel modified ultrasonication technique for the extraction of lycopene from tomatoes. *Ultrasonics Sonochemistry, 19*(1), 151-159.

El-Hefny, N. E. (2007). Kinetics and mechanism of extraction and stripping of neodymium using a Lewis cell. *Chemical Engineering and Processing: Process Intensification, 46*(7), 623-629.

Ernst, H. (2002). Recent advances in industrial carotenoid synthesis. *Pure and applied chemistry, 74*(11), 2213-2226.

Ferreira, S. C., Bruns, R., Ferreira, H., Matos, G., David, J., Brandao, G., . . . Souza, A. (2007). Box-Behnken design: an alternative for the optimization of analytical methods. *Analytica chimica acta, 597*(2), 179-186.

Fiedor, J., & Burda, K. (2014). Potential role of carotenoids as antioxidants in human health and disease. *Nutrients, 6*(2), 466-488.

Fish, W. W., Perkins-Veazie, P., & Collins, J. K. (2002). A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. *Journal of food composition and analysis, 15*(3), 309-317.

González-Centeno, M., Comas-Serra, F., Femenia, A., Rosselló, C., & Simal, S. (2015). Effect of power ultrasound application on aqueous extraction of phenolic compounds and antioxidant capacity from grape pomace (Vitis vinifera L.): experimental kinetics and modeling. *Ultrasonics Sonochemistry, 22*, 506-514.

Goula, A. M., Ververi, M., Adamopoulou, A., & Kaderides, K. (2017). Green ultrasound-assisted extraction of carotenoids from pomegranate wastes using vegetable oils. *Ultrasonics Sonochemistry, 34*, 821-830.

Gujral, G., Kapoor, D., & Jaimini, M. (2018). AN UPDATED REVIEW ON DESIGN OF EXPERIMENT (DOE) IN PHARMACEUTICALS. *Journal of Drug Delivery and Therapeutics, 8*(3), 147-152.

Hatami, T., Cavalcanti, R. N., Takeuchi, T. M., & Meireles, M. A. A. (2012). Supercritical fluid extraction of bioactive compounds from Macela (Achyrocline satureioides) flowers: kinetic, experiments and modeling. *The Journal of Supercritical Fluids, 65*, 71-77.

Hazir, E., Hüseyin Koc, K., & Hiziroglu, S. (2017). Optimization of sanding parameters using response surface methodology. *Maderas. Ciencia y tecnología, 19*(4), 407-416.

Ho, K., Ferruzzi, M., Liceaga, A., & San Martín-González, M. (2015). Microwave-assisted extraction of lycopene in tomato peels: effect of extraction conditions on all-trans and cis-isomer yields. *LWT-Food Science and Technology, 62*(1), 160-168.

Hussain, S. P., Hofseth, L. J., & Harris, C. C. (2003). Radical causes of cancer. *Nature Reviews Cancer, 3*(4), 276.

Ishida, B., & Bartley, G. (2009). Carotenoids: chemistry, sources and physiology. *Guide to nutritional supplements/editor, Benjamin Caballero*.

Javanmardi, J., & Kubota, C. (2006). Variation of lycopene, antioxidant activity, total soluble solids and weight loss of tomato during postharvest storage. *Postharvest biology and technology, 41*(2), 151-155.

Javanshir, S., Abdollahy, M., Abolghasemi, H., & Darban, A. K. (2011). Kinetics of Au (III) extraction by DBC from hydrochloric solution using Lewis cell. *International Journal of Mineral Processing, 98*(1-2), 42-47.

Jin, L., Hope, K. J., Zhai, Q., Smadja-Joffe, F., & Dick, J. E. (2006). Targeting of CD44 eradicates human acute myeloid leukemic stem cells. *Nature medicine, 12*(10), 1167.

Joana Gil‐Chávez, G., Villa, J. A., Fernando Ayala‐Zavala, J., Basilio Heredia, J., Sepulveda, D., Yahia, E. M., & González‐Aguilar, G. A. (2013). Technologies for extraction and production of bioactive compounds to be used as nutraceuticals and food ingredients: an overview. *Comprehensive Reviews in Food Science and*

Food Safety, 12(1), 5-23.

Kassama, L. S., Shi, J., & Mittal, G. S. (2008). Optimization of supercritical fluid extraction of lycopene from tomato skin with central composite rotatable design model. *Separation and Purification Technology, 60*(3), 278-284.

Kaur, D., Wani, A. A., Oberoi, D., & Sogi, D. (2008). Effect of extraction conditions on lycopene extractions from tomato processing waste skin using response surface methodology. *Food Chemistry, 108*(2), 711-718.

Kedare, S. B., & Singh, R. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of food science and technology, 48*(4), 412-422.

Kettle, A. (2013). Use of Accelerated Solvent Extraction to Improve Laboratory Workflow. *Thermo Fisher Scientific*, 5.

Kong, K.-W., Khoo, H.-E., Prasad, K. N., Ismail, A., Tan, C.-P., & Rajab, N. F. (2010). Revealing the power of the natural red pigment lycopene. *Molecules, 15*(2), 959-987.

Kopec, R. E., Cooperstone, J. L., Cichon, M. J., & Schwartz, S. J. (2012). Analysis methods of carotenoids. *Analysis of antioxidant-rich phytochemicals. Hoboken: Chichester: Wiley-Blackwell*, 105-149.

Kumcuoglu, S., Yilmaz, T., & Tavman, S. (2014). Ultrasound assisted extraction of lycopene from tomato processing wastes. *Journal of food science and technology, 51*(12), 4102-4107.

Landbo, A.-K., & Meyer, A. S. (2001). Enzyme-assisted extraction of antioxidative phenols from black currant juice press residues (Ribes nigrum). *Journal of Agricultural and Food Chemistry, 49*(7), 3169-3177.

Laufenberg, G., Kunz, B., & Nystroem, M. (2003). Transformation of vegetable waste into value added products::(A) the upgrading concept;(B) practical implementations. *Bioresource Technology, 87*(2), 167-198.

Lazar, L., Talmaciu, A. I., Volf, I., & Popa, V. I. (2016). Kinetic modeling of the ultrasound-assisted extraction of polyphenols from Picea abies bark. *Ultrasonics Sonochemistry, 32*, 191-197.

Li, Y., Fabiano-Tixier, A. S., Tomao, V., Cravotto, G., & Chemat, F. (2013). Green ultrasound-assisted extraction of carotenoids based on the bio-refinery concept

using sunflower oil as an alternative solvent. *Ultrasonics Sonochemistry, 20*(1), 12-18.

Lianfu, Z., & Zelong, L. (2008). Optimization and comparison of ultrasound/microwave assisted extraction (UMAE) and ultrasonic assisted extraction (UAE) of lycopene from tomatoes. *Ultrasonics Sonochemistry, 15*(5), 731-737.

Lippi, G., & Targher, G. (2011). Tomatoes, lycopene-containing foods and cancer risk. *British journal of cancer, 104*(7), 1234.

Luterotti, S., Marković, K., Franko, M., Bicanic, D., Madžgalj, A., & Kljak, K. (2013). Comparison of spectrophotometric and HPLC methods for determination of carotenoids in foods. *Food Chemistry, 140*(1-2), 390-397.

Machmudah, S., Winardi, S., Sasaki, M., Goto, M., Kusumoto, N., & Hayakawa, K. (2012). Lycopene extraction from tomato peel by-product containing tomato seed using supercritical carbon dioxide. *Journal of Food Engineering, 108*(2), 290-296.

Mawatari, T., Fukuda, R., Mori, H., Mia, S., & Ohno, N. (2013). High pressure rheology of environmentally friendly vegetable oils. *Tribology Letters, 51*(2), 273-280.

Mein, J. R., Lian, F., & Wang, X.-D. (2008). Biological activity of lycopene metabolites: implications for cancer prevention. *Nutrition reviews, 66*(12), 667-683.

Montesano, D., Fallarino, F., Cossignani, L., Bosi, A., Simonetti, M. S., Puccetti, P., & Damiani, P. (2008). Innovative extraction procedure for obtaining high pure lycopene from tomato. *European Food Research and Technology, 226*(3), 327.

Mottaleb, M. A., & Sarker, S. D. (2012). Accelerated solvent extraction for natural products isolation *Natural products isolation* (pp. 75-87): Springer.

Mustafa, A., Trevino, L. M., & Turner, C. (2012). Pressurized hot ethanol extraction of carotenoids from carrot by-products. *Molecules, 17*(2), 1809-1818.

Naviglio, D., Pizzolongo, F., Ferrara, L., Aragon, A., & Santini, A. (2008). Extraction of pure lycopene from industrial tomato by-products in water using a new high-pressure process. *Journal of the Science of Food and Agriculture, 88*(14), 2414-2420.

Naz, A., Butt, M. S., Sultan, M. T., Qayyum, M. M. N., & Niaz, R. S. (2014). Watermelon lycopene and allied health claims. *EXCLI journal, 13*, 650.

Nguyen, M. L., & Schwartz, S. J. (1998). Lycopene stability during food processing. *Proceedings of the Society for Experimental Biology and Medicine, 218*(2), 101-105.

Niaz, K., Bahadar, H., Maqbool, F., & Abdollahi, M. (2015). A review of environmental and occupational exposure to xylene and its health concerns. *EXCLI journal, 14*, 1167.

Norshazila, S., Koy, C., Rashidi, O., HO LH, A. I., NURUL ZAIZULIANA, R., & ZARINAH, Z. (2017). The Effect of Time, Temperature and Solid to Solvent Ratio on Pumpkin Carotenoids Extracted Using Food Grade Solvents. *Sains Malaysiana, 46*(2), 231-237.

Nour, V., Panaite, T. D., Ropota, M., Turcu, R., Trandafir, I., & Corbu, A. R. (2018). Nutritional and bioactive compounds in dried tomato processing waste. *CyTA-Journal of Food, 16*(1), 222-229.

Olempska-Beer, Z. S., Merker, R. I., Ditto, M. D., & DiNovi, M. J. (2006). Food-processing enzymes from recombinant microorganisms—a review. *Regulatory toxicology and Pharmacology, 45*(2), 144-158.

Palozza, P., Simone, R., Catalano, A., Boninsegna, A., Böhm, V., Fröhlich, K., . . . Ranelletti, F. O. (2010). Lycopene prevents 7-ketocholesterol-induced oxidative stress, cell cycle arrest and apoptosis in human macrophages. *The Journal of nutritional biochemistry, 21*(1), 34-46.

Papaioannou, E. H., & Karabelas, A. J. (2012). Lycopene recovery from tomato peel under mild conditions assisted by enzymatic pre-treatment and non-ionic surfactants. *Acta Biochimica Polonica, 59*(1).

Papaioannou, E. H., Liakopoulou-Kyriakides, M., & Karabelas, A. J. (2016). Natural origin lycopene and its “green” downstream processing. *Critical reviews in food science and nutrition, 56*(4), 686-709.

Periago, M. J., Rincon, F., Agüera, M. D., & Ros, G. (2004). Mixture approach for optimizing lycopene extraction from tomato and tomato products. *Journal of Agricultural and Food Chemistry, 52*(19), 5796-5802.

Perretti, G., Troilo, A., Bravi, E., Marconi, O., Galgano, F., & Fantozzi, P. (2013). Production of a lycopene-enriched fraction from tomato pomace using supercritical carbon

มหาวิทยาลัยศิลปากร

dioxide. *The Journal of Supercritical Fluids, 82*, 177-182.

Phinney, D. M., Frelka, J. C., Cooperstone, J. L., Schwartz, S. J., & Heldman, D. R. (2017). Effect of solvent addition sequence on lycopene extraction efficiency from membrane neutralized caustic peeled tomato waste. *Food Chemistry, 215*, 354-361.

Pinelo, M., Rubilar, M., Jerez, M., Sineiro, J., & Núñez, M. J. (2005). Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. *Journal of Agricultural and Food Chemistry, 53*(6), 2111-2117.

Poojary, M. M., & Passamonti, P. (2015a). Extraction of lycopene from tomato processing waste: kinetics and modelling. *Food Chemistry, 173*, 943-950.

Poojary, M. M., & Passamonti, P. (2015b). Optimization of extraction of high purity all-trans-lycopene from tomato pulp waste. *Food Chemistry, 188*, 84-91.

Prado, J. M., Veggi, P. C., & Meireles, M. A. A. (2014). Extraction methods for obtaining carotenoids from vegetables-review. *Current Analytical Chemistry, 10*(1), 29.

Preedy, V. R., & Watson, R. R. (2009). Lycopene–Nutritional, Medicinal and Therapeutic Properties. *Nutrition & Food Science, 39*(6), 702-703.

Pu, J., Bechtel, P. J., & Sathivel, S. (2010). Extraction of shrimp astaxanthin with flaxseed oil: effects on lipid oxidation and astaxanthin degradation rates. *Biosystems engineering, 107*(4), 364-371.

Puri, M., Sharma, D., & Barrow, C. J. (2012). Enzyme-assisted extraction of bioactives from plants. *Trends in biotechnology, 30*(1), 37-44.

Pyke, K. A., & Howells, C. A. (2002). Plastid and stromule morphogenesis in tomato. *Annals of Botany, 90*(5), 559-566.

Qu, W., Pan, Z., & Ma, H. (2010). Extraction modeling and activities of antioxidants from pomegranate marc. *Journal of Food Engineering, 99*(1), 16-23.

Ranveer, R. C., Patil, S. N., & Sahoo, A. K. (2013). Effect of different parameters on enzyme-assisted extraction of lycopene from tomato processing waste. *Food and Bioproducts Processing, 91*(4), 370-375.

Rao, A. (2002). Lycopene, tomatoes, and the prevention of coronary heart disease. *Experimental Biology and Medicine, 227*(10), 908-913.

Rao, A., Ray, M., & Rao, L. (2006). Lycopene. *Advances in food and nutrition research, 51*, 99-164.

Rao, A. V., & Agarwal, S. (2000). Role of antioxidant lycopene in cancer and heart disease. *Journal of the American College of Nutrition, 19*(5), 563-569.

Rao, L., & Rao, A. (2013). Oxidative stress and antioxidants in the risk of osteoporosis—role of the antioxidants lycopene and polyphenols *Topics in osteoporosis*: InTech.

Rastogi, N. K. (2011). Opportunities and challenges in application of ultrasound in food processing. *Critical reviews in food science and nutrition, 51*(8), 705-722.

Rath, S., Olempska-Beer, Z., & Kuznesof, P. (2013). Lycopene extract from tomato Chemical and Technical Assessment (CTA): Retrieved.

Ried, K., & Fakler, P. (2011). Protective effect of lycopene on serum cholesterol and blood pressure: Meta-analyses of intervention trials. *Maturitas, 68*(4), 299-310.

Rizk, E. M., El-Kady, A. T., & El-Bialy, A. R. (2014). Charactrization of carotenoids (lyco-red) extracted from tomato peels and its uses as natural colorants and antioxidants of ice cream. *Annals of Agricultural Sciences, 59*(1), 53-61.

Routray, W., & Orsat, V. (2012). Microwave-assisted extraction of flavonoids: a review. *Food and Bioprocess Technology, 5*(2), 409-424.

Sabio, E., Lozano, M., Montero de Espinosa, V., Mendes, R., Pereira, A., Palavra, A., & Coelho, J. (2003). Lycopene and β-carotene extraction from tomato processing waste using supercritical CO2. *Industrial & engineering chemistry research, 42*(25), 6641-6646.

Sachindra, N., & Mahendrakar, N. (2005). Process optimization for extraction of carotenoids from shrimp waste with vegetable oils. *Bioresource Technology, 96*(10), 1195-1200.

Saeid, A. (2015). *EFFECTS OF EXTRACTION AND PURIFICATION METHODS ON DEGRADATION KINETICS AND STABILITY OF LYCOPENE FROM WATERMELON UNDER STORAGE CONDITIONS.* NPI University of Bangladesh, Manikgon, Researchgate.

Saeid, A., Eun, J. B., Sagor, M. S. A., Rahman, A., Akter, M. S., & Ahmed, M. (2016). Effects

of Extraction and Purification Methods on Degradation Kinetics and Stability of Lycopene from Watermelon under Storage Conditions. *Journal of food science, 81*(11), C2630-C2638.

Sagiroglu, A., Ozcan, H. M., Isbilir, S. S., Paluzar, H., & Toprakkiran, N. M. (2013). Alkali Catalysis of Different Vegetable Oils for Comparisons of Their Biodiesel Productivity. *Journal of Sustainable Bioenergy Systems, 3*(01), 79.

Saini, R. K., & Keum, Y.-S. (2018). Carotenoid extraction methods: A review of recent developments. *Food Chemistry, 240*, 90-103.

Shen, R., Jiang, X., Ye, W., Song, X., Liu, L., Lao, X., & Wu, C. (2011). A novel and practical synthetic route for the total synthesis of lycopene. *Tetrahedron, 67*(31), 5610-5614.

Shi, J., Dai, Y., Kakuda, Y., Mittal, G., & Xue, S. J. (2008). Effect of heating and exposure to light on the stability of lycopene in tomato purée. *Food control, 19*(5), 514-520.

Shi, J., Khatri, M., Xue, S. J., Mittal, G. S., Ma, Y., & Li, D. (2009). Solubility of lycopene in supercritical CO2 fluid as affected by temperature and pressure. *Separation and Purification Technology, 66*(2), 322-328.

Shi, J., Le Maguer, M., Kakuda, Y., Liptay, A., & Niekamp, F. (1999). Lycopene degradation and isomerization in tomato dehydration. *Food research international, 32*(1), 15-21.

Shi, J., & Maguer, M. L. (2000). Lycopene in tomatoes: chemical and physical properties affected by food processing. *Critical reviews in food science and nutrition, 40*(1), 1-42.

Shi, J., Maguiere, M., & Bryan, M. (2002). Chapter 4: lycopene from tomatoes. *Functional Foods, Biochemical and Processing Aspects, 2*.

Shi, X. F., Xu, Y., Li, Y. H., Zeng, H. X., & Sun, Y. H. (2012). *Optimization of extraction process of lycopene from Watermelon (Citrullus lanatus) by response surface methodology.* Paper presented at the Applied Mechanics and Materials.

Shrestha, S. L., & Sah, R. L. (2014). Evaluation of Tomato Cultivars for Central Tarai of Nepal. *Nepal Journal of Science and Technology, 15*(2), 11-16.

Singh, P., & Goyal, G. (2008). Dietary lycopene: Its properties and anticarcinogenic

effects. *Comprehensive Reviews in Food Science and Food Safety, 7*(3), 255-270.

Stacewicz-Sapuntzakis, M., & Bowen, P. E. (2005). Role of lycopene and tomato products in prostate health. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1740*(2), 202-205.

Stahl, W., Schwarz, W., Sundquist, A. R., & Sies, H. (1992). Cis-trans isomers of lycopene and β-carotene in human serum and tissues. *Archives of biochemistry and biophysics, 294*(1), 173-177.

Strati, I., & Oreopoulou, V. (2014). Recovery of carotenoids from tomato processing by-products–a review. *Food research international, 65*, 311-321.

Strati, I. F., & Oreopoulou, V. (2011a). Effect of extraction parameters on the carotenoid recovery from tomato waste. *International journal of food science & technology, 46*(1), 23-29.

Strati, I. F., & Oreopoulou, V. (2011b). Process optimisation for recovery of carotenoids from tomato waste. *Food Chemistry, 129*(3), 747-752.

Tan, P., Tan, C., & Ho, C. (2011). Antioxidant properties: Effects of solid-to-solvent ratio on antioxidant compounds and capacities of Pegaga (Centella asiatica). *International Food Research Journal, 18*(2).

Tan, Q. L. P., Kieu, X. N. T., Kim, N. H. T., & Hong, X. N. T. (2012). Application of response surface methodology (RSM) in conditionoptimization for essential oil production from Citrus latifolia. *Emirates Journal of Food and Agriculture*, 25-30.

Tarazona‐Díaz, M. P., Viegas, J., Moldao‐Martins, M., & Aguayo, E. (2011). Bioactive compounds from flesh and by‐product of fresh‐cut watermelon cultivars. *Journal of the Science of Food and Agriculture, 91*(5), 805-812.

Taungbodhitham, A. K., Jones, G. P., Wahlqvist, M. L., & Briggs, D. R. (1998). Evaluation of extraction method for the analysis of carotenoids in fruits and vegetables. *Food Chemistry, 63*(4), 577-584.

Tello, J., Viguera, M., & Calvo, L. (2011). Extraction of caffeine from Robusta coffee (Coffea canephora var. Robusta) husks using supercritical carbon dioxide. *The Journal of Supercritical Fluids, 59*, 53-60.

Topal, U., Sasaki, M., Goto, M., & Hayakawa, K. (2006). Extraction of lycopene from tomato skin with supercritical carbon dioxide: effect of operating conditions and solubility analysis. *Journal of Agricultural and Food Chemistry, 54*(15), 5604-5610.

Vági, E., Simándi, B., Vásárhelyiné, K., Daood, H., Kéry, Á., Doleschall, F., & Nagy, B. (2007). Supercritical carbon dioxide extraction of carotenoids, tocopherols and sitosterols from industrial tomato by-products. *The Journal of Supercritical Fluids, 40*(2), 218-226.

Voehl, F., Mignosa, C., Harrington, H. J., & Charron, R. (2016). *The lean six sigma black belt handbook: tools and methods for process acceleration*: Productivity Press.

W¹sowicz, E., Gramza, A., Hêœ, M., Jeleñ, H. H., Korczak, J., Ma³ecka, M., . . . Zawirska-Wojtasiak, R. (2004). Oxidation of lipids in food. *Pol J Food Nutr Sci, 13*, 87-100.

Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science & Technology, 17*(6), 300-312.

Wang, Q., Pagan, J., & Shi, J. (2002). Pectin from fruits. *Functional foods: Biochemical and processing aspects, 2*, 264-295.

Wong, B., Tan, C., & Ho, C. (2013). Effect of solid-to-solvent ratio on phenolic content and antioxidant capacities of" Dukung Anak"(Phyllanthus niruri). *International Food Research Journal, 20*(1).

Xu, Y., & Pan, S. (2013). Effects of various factors of ultrasonic treatment on the extraction yield of all-trans-lycopene from red grapefruit (Citrus paradise Macf.). *Ultrasonics Sonochemistry, 20*(4), 1026-1032.

Zhang, Z.-f., Lv, G.-y., Jiang, X., Cheng, J.-h., & Fan, L.-f. (2015). Extraction optimization and biological properties of a polysaccharide isolated from Gleoestereum incarnatum. *Carbohydrate polymers, 117*, 185-191.

Zuorro, A., Fidaleo, M., & Lavecchia, R. (2011). Enzyme-assisted extraction of lycopene from tomato processing waste. *Enzyme and microbial technology, 49*(6-7), 567-573.

Zuorro, A., Lavecchia, R., Medici, F., & Piga, L. (2014). Use of cell wall degrading enzymes for the production of high-quality functional products from tomato

processing waste. *Chemical Engineering, 38*.

Appendices

Appendix A

Calibration curve

A.1 Calibration curve for standard lycopene using UV-VIS spectrometer

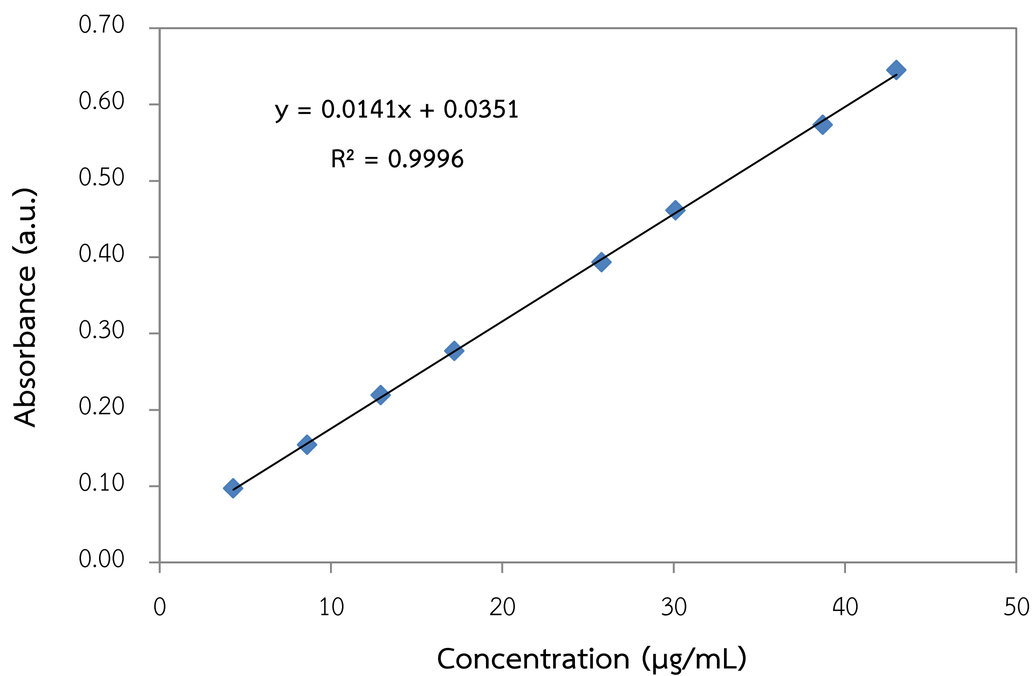
The calibration curve for standard lycopene using UV-VIS spectrometer was measured at 503 nm. Stock standard solution was prepared by dissolving standard lycopene in n-hexane (43.0 μg/ mL). The stock solution was diluted with n-hexane to obtain concentration between 4.3-43.0 μg/mL as follows:

Concentration (μg/ mL)	Stock standard solution (μL)	n-Hexane (μL)
4.3	500	4500
8.6	1000	4000
12.9	1500	3500
17.2	2000	3000
25.8	3000	2000
30.1	3500	1500
38.7	4500	500
43.0	5000	0

มหาวิทยาลัยศิลปากร

The calibration curve is plotted between the absorbance and concentration according to the data as follows:

Concentration (μg/ mL)	Absorbance (a.u.)		Average Absorbance	%RSD
	no. 1	no. 2		
4.3	0.096	0.098	0.097	1.46
8.6	0.154	0.153	0.154	0.46
12.9	0.219	0.217	0.219	0.65
17.2	0.277	0.276	0.277	0.26
25.8	0.394	0.391	0.393	0.54
30.1	0.463	0.463	0.461	0.00
38.7	0.573	0.572	0.573	0.12
43.0	0.642	0.645	0.645	0.33



A.2 Calibration curve for standard lycopene using HPLC

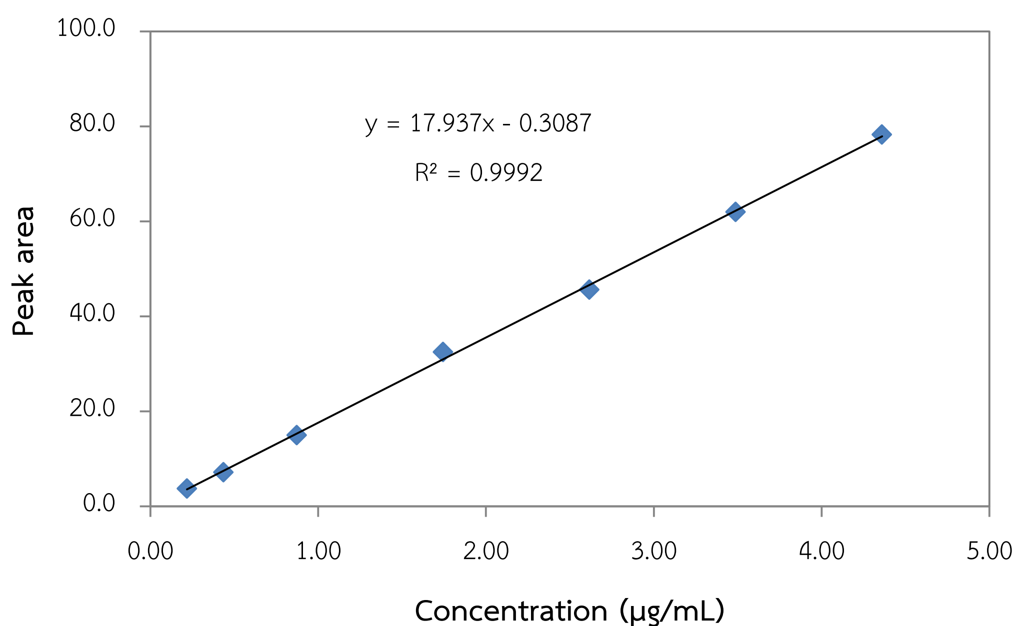
Stock standard solution was prepared by dissolving standard lycopene in THF (43.6 μg/ mL). Oil was spiked in the stock solution and the spiked oil sample was diluted with 2-propanol to obtain concentration between 0.218-4.360 μg/mL as follows:

Concentration (μg/ mL)	Stock standard solution (μL)	Oil (μL)	2-Propanol (μL)
0.218	25	200	4775
0.436	50	200	4750
0.872	100	200	4700
1.744	200	200	4600
2.616	300	200	4500
3.488	400	200	4400
4.360	500	200	4300

มหาวิทยาลัยศิลปากร

The calibration curve is plotted between the peak area and concentration according to the data as follows:

Concentration (μg/ mL)	Peak area		Average Peak area	%RSD
	no. 1	no.2		
0.218	3.639	3.859	3.749	4.15
0.436	7.151	7.240	7.196	0.87
0.872	15.036	14.917	14.977	0.56
1.744	32.665	32.273	32.469	0.85
2.616	46.363	44.829	45.596	2.38
3.488	62.474	61.438	61.956	1.18
4.360	79.492	77.001	78.247	2.25



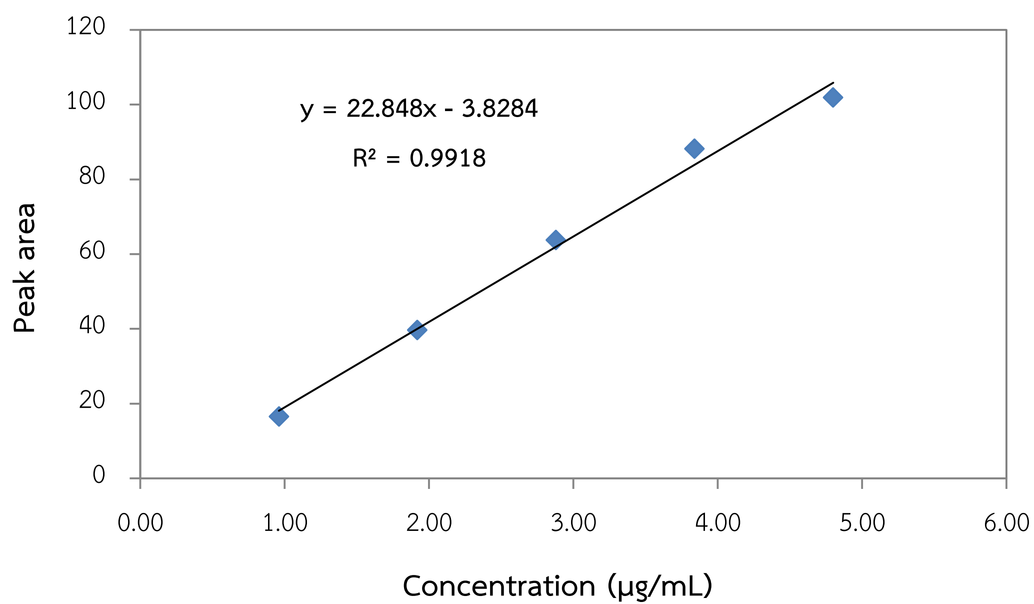
A.3 Calibration curve for standard β-carotene using HPLC

Stock standard solution was prepared by dissolving standard β-carotene in THF (43.6 µg/ mL). Oil was spiked in the stock solution and the spiked oil sample was diluted with 2-propanol to obtain concentration between 0.960-4.800 µg/mL as follows:

Concentration (µg/ mL)	Stock standard solution (µL)	Oil (µL)	2-Propanol (µL)
0.960	100	200	4700
1.920	200	200	4600
2.880	300	200	4500
3.840	400	200	4400
4.800	500	200	4300

The calibration curve is plotted between the peak area and concentration according to the data as follows:

Concentration (µg/ mL)	Peak area		Average Peak area	%RSD
	no. 1	no.2		
0.960	16.392	16.550	16.471	0.68
1.920	40.297	38.944	39.621	2.41
2.880	63.298	64.181	63.734	0.98
3.840	90.269	86.086	88.178	3.35
4.800	99.898	103.830	101.864	2.73



มหาวิทยาลัยศิลปากร

Appendix B

Calculation for yield of lycopene extract and purity

B.1 Calculation for yield of lycopene extracted from tomato paste using UV-VIS spectroscopy.

Example: Extraction of lycopene from tomato paste using coconut oil under the conditions as follows: tomato paste 0.435 g, extraction time of 30 min and extraction temperature of 30°C was diluted with 2 ml of n-hexane. Volume of oil phase after extraction is 30 mL. The absorbance of sample is 0.0759 at 503 nm.

Base on the equation of calibration curve in Appendix A.1:

$$y = 0.0141x + 0.0351$$

where ***x*** = concentration of lycopene extract (μg/mL)

y = absorbance of sample at 503 nm

So, the concentration of lycopene in the sample is 2.84 μg/mL

The yield of lycopene can be calculated from the following equation:

$$Y = \frac{D \times x \times V}{g \times 1000}$$

where ***Y*** = yield of lycopene (mg/g paste)

D = dilution factor

V = volume of oil phase after extraction (mL)

g = amount of tomato paste (g)

Substitute the variables in the equation above,

$$Y = \frac{5 \times 2.84 \times 30}{0.435 \times 1000}$$

So, the yield of lycopene in the sample is 0.98 mg/g paste

B.2 Calculation for purity of lycopene extracted from tomato peel waste using HPLC

Example: Extraction of lycopene from tomato peel waste using sunflower oil and ethanol mixture solvents (50%v/v) under the optimum conditions as follows: particle size 0.2 mm, extraction time of 90 min, extraction temperature of 30°C and solvent to paste ratio of 40 mL/g. 200 μL of oil sample was diluted with 4800 μL of 2-propanol. Volume of oil phase after extraction is 17 mL. The peak areas of sample at 6.90 min and 10.21 min are 60.964 and 6.425, respectively.

Base on the equation of calibration curve in Appendix A.2:

$$y = 17.937x - 0.3087$$

where ***x*** = concentration of lycopene extract (μg/mL)

y = peak area

So, the concentration of lycopene in the sample is 3.42 μg/mL

The yield of lycopene can be calculated from the following equation:

$$Y_1 = \frac{D \times x \times V}{g \times 1000}$$

where ***Y*** = yield of lycopene (mg/g paste)

D = dilution factor

V = volume of oil phase after extraction (mL)

g = amount of tomato dry peel waste (g)

Substitute the variables in the equation above,

$$Y_1 = \frac{25 \times 3.42 \times 17}{0.7504 \times 1000}$$

So, the yield of lycopene in the sample is 1.93 mg/g dry peel

Base on the equation of calibration curve in Appendix A.3:

$$y = 22.848x - 3.8284$$

where x = concentration of β-carotene extract (μg/mL)

y = peak area

So, the concentration of β-carotene in the sample is 0.36 μg/mL

The yield of β-carotene can be calculated from the following equation:

$$Y_2 = \frac{D \times x \times V}{g \times 1000}$$

where Y = yield of β-carotene (mg/g paste)

D = dilution factor

V = volume of oil phase after extraction (mL)

g = amount of tomato dry peel waste (g)

Substitute the variables in the equation above,

$$Y_2 = \frac{25 \times 0.45 \times 17}{0.7504 \times 1000}$$

So, the yield of β-carotene in the sample is 0.20 mg/g dry peel

The purity of lycopene can be calculated from the following equation:

$$\%\ Purity = \frac{Y_1}{Y_1 + Y_2} \times 100$$

So, the purity of lycopene in the sample is 91 % of total carotenoid.

VITA

NAME	Naphaphan Kunthakudee
DATE OF BIRTH	17 May 1987
PLACE OF BIRTH	Chachoengsao
INSTITUTIONS ATTENDED	Department of Chemical Engineering, Faculty of Engineering and Industrial Technology, Silpakorn University
HOME ADDRESS	81 Moo 5 Mueang Mai Subdistrict, Ratchasan District, Chachoengsao Province, 24120
PUBLICATION	1. Naphaphan Kunthakudee, Ura Pancharoen, Katarína Fulajtárová, Tomáš Soták, Milan Hronec, Prakorn Ramakul, Influence of inorganic salts on the liquid–liquid equilibrium of water + furfuryl alcohol + cyclopentanone system at 298.15 K, Chemical Papers 72 (2018) 337–348 2. Naphaphan Kunthakudee, Ura Pancharoen, Katarína Fulajtárová, Tomáš Soták, Milan Hronec, Prakorn Ramakul, Salt effect on the liquid-liquid equilibrium of water-furfuryl alcohol-furfural system at 298.15 K, Korean Journal of Chemical Engineering, 34(8) (2017) 2293-2300 3. Naphaphan Kunthakudee, Niti Sunsandee, Ura Pancharoen, Prakorn Ramakul, Separation of yttrium from rare earth using hollow fiber-supported liquid membrane: factorial design analysis, Desalination and Water Treatment 57 (2016) 3985–3994 4. Thidarat Wongsawa, Tanakon Koonsang, Naphaphan Kunthakudee, Tatchanok Prapasawat, Kreangkrai Maneeintr, Ura Pancharoen, The experimental investigations on viscosity, surface tension, interfacial

มหาวิทยาลัยศิลปากร

tension and solubility of the binary and ternary systems for tributyl phosphate (TBP) extractant in various organic solvents with water: Thermodynamic NRTL model and molecular interaction approach, Journal of Molecular Liquids 251 (2018) 229-237

5. Niti Sunsandee, Naphaphan Kunthakudee, Boonta Chutvirasakul, Suphot Phatanasria, Prakorn Ramakul, Enantioseparation of (S)-amlodipine from pharmaceutical wastewater by hollow-fiber supported liquid membrane: central composite design and optimization, Desalination and Water Treatment 72 (2017) 207–215