

PHENOLIC ANTIOXIDANTS EXTRACTION FROM HAZELNUT SHELLS

by

Bo Yuan

A DISSERTATION

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Major: Food Science and Technology

Under the Supervision of Professor Milford A. Hanna

Lincoln, Nebraska

May, 2016

ProQuest Number: 10097962

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest 10097962

Published by ProQuest LLC (2016). Copyright of the Dissertation is held by the Author.

All rights reserved.

This work is protected against unauthorized copying under Title 17, United States Code
Microform Edition © ProQuest LLC.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

PHENOLIC ANTIOXIDANTS EXTRACTION FROM HAZELNUT SHELLS

Bo Yuan, Ph.D.

University of Nebraska, 2016

Advisor: Milford A. Hanna

Hazelnut shells are rich in potentially health-promoting phenolic antioxidants. Various novel extraction techniques, which are considered as efficient alternatives to conventional extraction methods, have been developed for the extraction of nutraceuticals from different feedstocks. However, limited research has been conducted on novel techniques for the highly effective extraction of phenolic antioxidants from hazelnut shells. From a commercial point of view, conducting research to extract phenolic compounds from hazelnut shells efficiently in terms of high extraction rate and yield, using newly developed techniques, is important and timely. Therefore, the goal of this project was to develop and optimize novel extraction methods for the production of phenolic antioxidants from hazelnut shells.

In this dissertation, effects of different extraction techniques, including conventional shaking bath extraction (SBE), and novel ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and high pressure/temperature extraction (HPTE), and their extraction conditions on total phenolic content and antioxidant capacities in the extracts were investigated and compared. Phenolic composition of the extracts under different extraction processes were analyzed. The total phenolic content and antioxidant capacities of the phenolic extracts under the optimal conditions from Nebraska hybrid hazelnut shells also were evaluated and compared.

Results showed that hazelnut shells were rich in phenolic compounds with high antioxidant capacity. MAE could recover almost double the amount of total phenolics compared with SBE and UAE under their individual optimal extraction conditions, meanwhile, spending much shorter extraction time. Of these extraction methods, HPTE could recover the highest amount of phenolic compounds from hazelnut shells. Under the optimal HPTE conditions, the total phenolic content in the extracts was about 1.5 times higher than that of MAE, while the extraction time of HPTE was much longer than that of MAE and UAE using probe-type sonication, and the extraction temperature of HPTE was much higher than any other method. Among the tested Nebraska hybrid hazelnuts, cultivar 6-2 had the highest total phenolic content and antioxidant capacity.

Acknowledgements

I would like to acknowledge all the people who have been on my journey in pursuing my Doctoral degree. First and foremost, I would like to express heartfelt gratitude to my advisor Dr. Milford Hanna. Without the opportunity from him, I could not have studied at the University of Nebraska-Lincoln, and my doctoral work would not have been possible. I would like to thank him for his valuable advices and encouragement, and for the freedom he allowed me in my research. I learned a lot from Dr. Hanna, not only in the research, but also in the daily life.

I would like to thank my committee members Dr. Kent Eskridge, Dr. Deepak Keshwani, Dr. Randy Wehling, and Dr. Curtis Weller. I really appreciate their help on my research and my coursework. Also, several of my fellowship applications were made possible by recommendation letters from Dr. Hanna, Dr. Wehling, and Dr. Weller.

Special thanks to Mr. Loren Isom for assistance with hazelnuts harvest, chemicals ordering, and many more things for my research. I also would like to acknowledge Mr. Terry Bartels and Ms. Amber Patterson for their assistance during my research.

I would like to thank Dr. Daniel Snow, Mr. David Cassada, and Dr. Sathaporn Onanong from the Water Science Laboratory for the LC-MS/MS analysis, and Dr. Vicki Schlegel, Dr. Devin Rose, and Dr. Junyi Yang from the Food Science and Technology Department, and Mr. Marc Walter from Wheat Quality Laboratory for the HPLC analysis. I also would like to acknowledge Dr. Yiqi Yang, Dr. Jeyamkondan Subbiah, and Dr. Sibel Irmak, for their generous permission to use their instruments. Thanks to Dr. Chao Tai, Dr. Jiajia Chen, Dr. Helan Xu, Mr. Gangwei Pan, Dr. Zhen Shi, Ms. Autumn Longo, and Dr. Yun Zhang for their assistance on the operation of the instruments.

I would like to thank all my friends in the Food Science and Technology Department and Biological Systems Engineering Department for their support of my research. My sincere thanks also go to all my friends in Lincoln, because my life would not have been so happy and colorful without you.

I also would like to thank the Chinese Scholarship Council for providing me a partial scholarship for my Ph.D. studies at the University of Nebraska-Lincoln.

Last but not least, I am eternally grateful to my family. My parents, Sheshi Yuan and June Wang, who always encourage and support me. My wife, Mei Lu, who always takes care of me without any complaint. And my little princess Miranda Yuan, who makes me become a father, and makes me understand the meaning of life.

Overall, these five years at the University of Nebraska-Lincoln have been a great and invaluable experience, with ups and downs, I could never have done it without all of you in my journey.

Thank you!

Table of Contents

CHAPTER I.....	1
INTRODUCTION	1
1.1. Background	1
1.2. Objectives.....	5
References	5
CHAPTER II.....	11
PHENOLIC COMPOUNDS EXTRACTION FROM HAZELNUT SHELLS BY CONVENTIONAL EXTRACTION TECHNIQUE.....	11
Abstract	11
2.1. Introduction	13
2.2. Materials and methods	15
2.2.1. Raw materials	15
2.2.2. Chemicals and reagents	15
2.2.3. Grinding and sifting.....	16
2.2.4. Shaking bath extraction (SBE)	16
2.2.5. Experimental design	16
2.2.6. Total phenolic content (TPC)	17
2.2.7. Antioxidant capacity.....	17
2.2.8. Phenolic composition analysis.....	19
2.2.9. Statistical analyses	21
2.3. Results and discussions	22
2.3.1. Effect of particle size.....	22
2.3.2. Effect of different solvents	23
2.3.3. Effect of temperature	24
2.3.4. Effect of solid to liquid ratio, acetone concentration, and extraction time.....	25
2.3.5. TPC and antioxidant capacity of the extracts in Nebraska hybrid hazelnut shells under SBE	28
2.3.6. Identification of phenolic compounds	29
2.3.7. Quantitative analysis of the identified individual phenolic compounds.....	30
2.4. Conclusions	32
Acknowledgement.....	32
References	33

CHAPTER III	54
ULTRASOUND-ASSISTED EXTRACTION OF PHENOLIC ANTIOXIDANTS FROM HAZELNUT SHELLS USING ULTRASONIC BATH	54
Abstract	54
3.1. Introduction	56
3.2. Materials and methods	58
3.2.1. Plant materials	58
3.2.2. Chemicals	59
3.2.3. Phenolic compounds extraction.....	59
3.2.4. Experimental design	60
3.2.5. Total phenolic content (TPC)	61
3.2.6. Ferric reducing antioxidant power (FRAP) assay	61
3.2.7. DPPH radical scavenging capacity (DRSC).....	62
3.2.8. Phenolic composition analysis.....	62
3.2.9. Statistical analyses	63
3.3. Results and discussions	64
3.3.1. Single factor tests.....	64
3.3.2. Optimization of UAE parameters	68
3.3.3. TPC and antioxidant capacity of the extracts of Nebraska hybrid hazelnut shells.....	72
3.3.4. Phenolic composition of the extracts of hazelnut shells under UAE	73
3.4. Conclusions	74
Acknowledgement.....	74
References	75
CHAPTER IV	96
ULTRASOUND-ASSISTED EXTRACTION OF PHENOLIC ANTIOXIDANTS FROM HAZELNUT SHELLS USING PROBE-TYPE SONICATION.....	96
Abstract	96
4.1. Introduction	98
4.2. Materials and methods	101
4.2.1. Plant materials	101
4.2.2. Chemicals	101
4.2.3. Extraction process.....	102
4.2.4. Experimental design	103

4.2.5. Total phenolic content (TPC)	103
4.2.6. Ferric reducing antioxidant power (FRAP) assay	104
4.2.7. DPPH radical scavenging capacity	104
4.2.8. Phenolic composition analysis	105
4.2.9. Statistical analyses	106
4.3. Results and discussions	106
4.3.1 Effect of tip depth dipped into the solvent	106
4.3.2. Effect of ultrasound power	107
4.3.3. Effect of duty cycle	108
4.3.4. Effect of solvent type	109
4.3.5. Effect of aqueous acetone concentration	110
4.3.6. Effect of solid to liquid ratio	111
4.3.7. Effect of extraction temperature	112
4.3.8. Effect of extraction time	112
4.3.9. Comparison of effects of probe-type sonication with other extraction methods on phenolic extraction	113
4.3.10. Phenolic composition in the extracts of hazelnut shells under UAE	116
4.3.11. TPC and antioxidant capacity of the extracts in Nebraska hybrid hazelnut shells under	117
4.4. Conclusions	118
Acknowledgement	119
References	119
CHAPTER V	141
MICROWAVE-ASSISTED EXTRACTION OF PHENOLIC ANTIOXIDANTS FROM HAZELNUT SHELLS	141
Abstract	141
5.1. Introduction	143
5.2. Materials and methods	145
5.2.1. Plant materials	145
5.2.2. Chemicals	146
5.2.3. Microwave-assisted extraction (MAE)	146
5.2.4. Experimental design	147
5.2.5. Total phenolic content (TPC)	148

5.2.6. Ferric reducing antioxidant power (FRAP) assay	149
5.2.7. DPPH radical scavenging capacity (DRSC).....	149
5.2.8. Phenolic composition analysis.....	150
5.2.9. Statistical analyses.....	151
5.3. Results and discussion.....	151
5.3.1. Effect of solvent type at different temperatures	151
5.3.2. Single factor tests.....	154
5.3.3. Optimization of MAE parameters	158
5.3.4. TPC and antioxidant capacity of the extracts of Nebraska hybrid hazelnut shells using MAE	162
5.3.5. Phenolic composition of the extracts of hazelnut shells.....	163
5.4. Conclusions	165
Acknowledgement.....	166
References	166
CHAPTER VI.....	189
HIGH PRESSURE/TEMPERATURE EXTRACTION OF PHENOLIC ANTIOXIDANTS FROM HAZELNUT SHELLS	189
Abstract	189
6.1. Introduction	190
6.2. Materials and methods	192
6.2.1. Plant materials	192
6.2.2. Chemicals	192
6.2.3. High pressure/temperature extraction (HPTE).....	193
6.2.4. Phenolic compounds.....	193
6.2.5. Antioxidant capacity.....	195
6.2.6. Statistical analyses.....	196
6.3. Results and discussion.....	196
6.3.1. Effects of different atmospheres and temperatures on TPC	196
6.3.2. Effects of extraction solvents at different temperatures	198
6.3.3. Effect of extraction time on TPC.....	203
6.3.4. Comparison between HPTE and other extraction methods.....	205
6.4. Conclusions	205
Acknowledgement.....	206

References	206
CHAPTER VII.....	223
CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH.....	223
7.1. Conclusions	223
7.2. Recommendations for future study	225
References	226

PREVIEW

List of Tables

Table 2.1. Experimental design for the evaluation of the effect of particle size at different time on phenolics recovery using shaking bath extraction.	38
Table 2.2. MRM channels, cone voltages and collision energies used for each phenolic compound in HPLC-MS/MS analysis.	39
Table 2.3. Analysis of variance (ANOVA) for the experiment with the experiment with the design of 4 particle sizes \times 4 extraction times.	40
Table 2.4. Analysis of variance (ANOVA) for the experiment with the design of 3 solvents \times 3 solvent concentrations.	41
Table 2.5. Three-way analysis of variance (ANOVA) for the experiment with the design of 3 ratios \times 3 solvent concentrations \times 5 extraction times.	42
Table 2.6. Parameter estimates and fitness of the models with transformation of time for the response variable of TPC.	43
Table 2.7. Optimal extraction conditions and maximal values of TPC for SBE.	44
Table 2.8. TPC and antioxidant capacity of the antioxidants in hazelnut shells from Nebraska and Oregon under the optimal SBE conditions for Lewis cultivar hazelnut shells.	45
Table 2.9. Tentative identification of phenolic compounds in Lewis and Nebraska hybrid hazelnut shells by HPLC-MS/MS in MRM mode.	46
Table 2.10. Quantification of phenolic compounds ($\mu\text{g/g}$ shell) in hazelnut shell extracts.	47
Table 3.1. Coded and original values of the independent variables of the ultrasound bath extraction process.	81
Table 3.2. Central composite design matrix along with the experimental responses of the extracts obtained from hazelnut using ultrasound-assisted extraction.	82
Table 3.3. Analysis of variance (ANOVA) for the experimental results obtained by using ultrasound-assisted extraction.	83
Table 3.4. Second order polynomial equations, regression coefficients of the response variables.	84
Table 3.5. Optimal UAE conditions and maximal values of response variables.	85
Table 3.6. Total phenolic content and antioxidant capacity of the extracts in hazelnut shells from Nebraska and Oregon.	86
Table 3.7. Quantification of phenolic compounds ($\mu\text{g/g}$ shell) in hazelnut shell extracts.	87
Table 4.1. Quantification of phenolic compounds in hazelnut shell extracts.	126
Table 4.2. Total phenolic content and antioxidant capacity in the extracts of hazelnut shells from Nebraska and Oregon after probe sonication.	127
Table 4.3. Comparison of optimal extraction conditions and total phenolic content under these conditions for each extraction method.	128
Table 5.1. Coded and original values of the independent variables of the MAE process.	173

Table 5.2. Central composite design matrix along with the experimental responses for MAE.	174
Table 5.3. Analysis of variance (ANOVA) for the experiment results of the effects of solvent type and temperature on responses using MAE.....	175
Table 5.4. Analysis of variance (ANOVA) for the experimental results of CCD obtained by using microwave-assisted extraction.	176
Table 5.5. Predictive models and regression coefficients of the response variables for MAE...	177
Table 5.6. Comparison of optimal extraction conditions and total phenolic content under these conditions for each extraction method.	178
Table 5.7. Total phenolic content and antioxidant capacity in the extracts of hazelnut shells from Nebraska and Oregon under the optimal MAE conditions for TPC of Lewis cultivar shells.	179
Table 5.8. Main phenolic compounds in hazelnut shell extracts using MAE under optimal conditions for TPC of Lewis cultivar.....	180
Table 6.1. Percentage of total tannin content in total phenolic content.	212
Table 6.2. Pearson correlation coefficient matrix of phenolic compounds content and antioxidant capacity of hazelnut shells.	213
Table 6.3. Parameters of second-order kinetic model for phenolics extraction from hazelnut shells.	214
Table 6.4. Comparison of optimal extraction conditions and total phenolic content under these conditions of extracts from Lewis cultivar hazelnut shells using each extraction method.	215

List of Figures

Figure 2.1. Lewis cultivar hazelnut shells.	48
Figure 2.2. Effect of particle size on TPC, FRAP, and DPPH radical scavenging capacity.	49
Figure 2.3. Particle size distribution of hazelnut shells ground by Wiley mill.....	50
Figure 2.4. Effect of solvent concentration on total phenolic content.	51
Figure 2.5. Effect of extraction temperature on total phenolic content.	52
Figure 2.6. Surface plot of the TPC of hazelnut shell extract as affected by solid to liquid ratio, acetone concentration, and extraction time or square root of time at 50 °C.....	53
Figure 3.1. Effect of different solvents on TPC, FRAP, and DRSC.....	88
Figure 3.2. Effect of extraction temperature on total phenolic content and antioxidant capacity.	89
Figure 3.3. Effect of solid to liquid ratio on total phenolic content and antioxidant capacity.....	90
Figure 3.4. Effect of acetone concentration on total phenolic content and antioxidant capacity.	91
Figure 3.5. Effect of extraction time on total phenolic content and antioxidant capacity.	92
Figure 3.6. Response surface plots (left) and contour plots (right) of TPC.....	93

Figure 3.7. Response surface plots (left) and contour plots (right) of FRAP.	94
Figure 3.8. Response surface plots (left) and contour plots (right) of DPPH radical scavenging capacity.	95
Figure 4.1. Schematic representation of the experimental setup.	129
Figure 4.2. Effect of tip depth under the aqueous acetone solution surface on extraction of phenolics.	130
Figure 4.3. Effect of ultrasound power on extraction of phenolics.	131
Figure 4.4. Effect of duty cycle on extraction of phenolics.	132
Figure 4.5. Effect of solvent type on extraction of phenolics.	133
Figure 4.6. Effect of acetone concentration on extraction of phenolics.	134
Figure 4.7. Effect of solid to liquid ratio on extraction of phenolics.	135
Figure 4.8. Effect of extraction temperature on extraction of phenolics.	136
Figure 4.9. Effect of extraction time on extraction of phenolics.	137
Figure 4.10. Influence of extraction method on the total phenolic content under the optimal UAE-p conditions except extraction time.	138
Figure 4.11. The residues above the filter papers after separation of extracts obtained by (A) conventional shaking bath extraction and (B) ultrasound-assisted extraction.	139
Figure 4.12. The effect of conjugation of ultrasound-assisted extraction and shaking bath extraction on phenolics recovery.	140
Figure 5.1. Effect of different solvents on TPC, FRAP, and DRSC at different temperatures. .	181
Figure 5.2. Effect of solid to liquid ratio on total phenolic content and antioxidant capacity....	182
Figure 5.3. Effect of ethanol concentration on total phenolic content and antioxidant capacity.	183
Figure 5.4. Effect of extraction temperature on total phenolic content and antioxidant capacity.	184
Figure 5.5. Effect of extraction time on total phenolic content and antioxidant capacity.	185
Figure 5.6. 3D Response surface plots and 2D contour plots of total phenolic content (TPC) for MAE.	186
Figure 5.7. 3D Response surface plots and 2D contour plots of ferric reducing antioxidant power (FRAP) for MAE.	187
Figure 5.8. 3D Response surface plots and 2D contour plots of DRSC for MAE.	188
Figure 6.1. Effects of atmosphere and temperature on TPC after HPTE with 37.5% ethanol for 30 min.	216
Figure 6.2. Effects of solvent and temperature on total phenolic content (TPC) after HPTE for 30 min under nitrogen atmosphere..	217
Figure 6.3. Effects of solvent and temperature on total tannins content (TTC) after extraction for 30 min under nitrogen atmosphere..	218

Figure 6.4. Effects of solvent and temperature on condensed tannins content (CTC) after extraction for 30 min under nitrogen atmosphere.	219
Figure 6.5. Effects of solvent and temperature on ferric reducing antioxidant capacity (FRAP) after extraction for 30 min under nitrogen atmosphere.	220
Figure 6.6. Effects of solvent and temperature on DPPH radical scavenging capacity (DRSC) after extraction for 30 min under nitrogen atmosphere.	221
Figure 6.7. Effect of extraction time on TPC (A) and the kinetic model (B).	222

PREVIEW

CHAPTER I

INTRODUCTION

1.1. Background

Hazelnuts, originated in the Mediterranean region, are an important commercial crop in many countries. The United States is the third largest hazelnut producer in the world, producing about 7% of the world's total hazelnuts, behind Turkey (~80%) and the European Union (~13%) (Huntrods, 2013). In the United States, commercial hazelnut production is mostly in the Willamette Valley in Oregon (Xu and Hanna, 2010b), and an average 37,625 tons were produced each year from 2008 to 2015 according to USDA (2015).

Hazelnuts are considered very healthy, and hold an important place among the types of dried nuts in terms of many of the important health categories because they are a rich source of unsaturated fatty acids, essential amino acids (mostly as arginine and leucine), dietary fiber, B vitamins, vitamin E, and minerals (Köksal et al., 2006). In the food industry, hazelnuts are currently eaten raw, roasted, blanched, minced, sliced, powdered, and pureed. Due to the distinctive flavor and nutritional value of hazelnuts, they are used as a premium ingredient in chocolates, caked foods, confectionary products, nut butter products, ice cream, and in meals and salads (Huntrods, 2013).

Based on different ways of consumption, the hazelnut market is segmented into two major categories: the in-shell and the shelled hazelnuts. In-shell hazelnuts are produced and marketed with the shell intact. However, the most common form of nuts used in the food-processing sector is the shelled nut (Ozdemir and Akinci, 2004).

Hazelnut shells represent more than 50% of the total nut weight (Xu and Hanna, 2009), and they are the major byproduct of hazelnut industry production. Hazelnut shells are composed of about

30% hemicellulose, 27% cellulose, and 43% lignin (Demirbas, 2006), so they are mainly utilized as a low-value heat source (Arslan et al., 2012). Other direct applications of the hazelnut shells in the production of activated carbon (Demirbaş et al., 2002; Kobya, 2004; Şayan, 2006) and bioplastic (Balart et al., 2016), and in pyrolysis (Di Blasi et al., 2015) have been carried out. Moreover, the conversion of hazelnut shells into useful chemicals such as methanol (Güllü and Demirbaş, 2001), hemicellulosic sugar (Arslan et al., 2012), reducing sugar (Uzuner and Cekmecelioglu, 2014), and furfural (Demirbas, 2006) have been reported. Recently, some effort has been made to utilize hazelnut shells as a low cost raw material for phenolic compounds extraction (Ciemniewska-Żytkiewicz et al., 2015; Contini et al., 2008; Shahidi et al., 2007; Xu et al., 2012).

Currently, commercial hazelnut cultivars in the USA are derived from the European hazelnuts and cultivated primarily in Oregon because European cultivars produce larger, higher quality nuts with thinner shells. However, the European cultivars cannot tolerate the harsh winters of the Upper Midwest, nor are they resistant to Eastern Filbert Blight (EFB). On the other hand, the native American species are cold-tolerant and EFB resistant, but the nuts are smaller and of less commercial value (Xu et al., 2012). Hybrid hazelnut shrub cultivars, which combine the superior qualities of the European hazelnuts and EFB resistance and cold hardiness of the native American hazelnuts, are emerging as a promising oil seed crop in the Upper Great Plains region of the USA. They require relatively low inputs and can be planted on marginal lands (Hammond, 2006). The results of a series of evaluations already completed by the Industrial Agricultural Products Center (IAPC) at the University of Nebraska - Lincoln (UNL) indicate that hybrid hazelnut kernels are potential feedstocks for food and value-added industrial applications (Xu and Hanna, 2010a; Xu and Hanna, 2010b; Xu and Hanna, 2011). Meanwhile, hazelnut shells, as the major byproduct of

processing, are rich in phenolic compounds that may serve as a potential source of natural antioxidants (Xu et al., 2012).

Plant-derived products contain a wide range of phytochemicals and phenolic compounds that possess substantial antioxidant and antiradical activities, anticarcinogenic (Kaliyara et al., 2014), anti-inflammatory (Raso et al., 2001) and antimutagenic effects (Surh, 2003), and antiproliferative potential (de la Rosa et al., 2014). These phenolics provide protection against harmful effects of free radicals and are known to reduce the risk of certain types of cancer, coronary heart disease, cardiovascular disease, stroke, atherosclerosis, osteoporosis, inflammation, and other neurodegenerative diseases associated with oxidative stress (Shahidi et al., 2007). Thus, they are now used widely in the fields of biology, medicine, food, and cosmetics. Currently, many synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and *t*-butylhydroxyquinone (TBHQ) are being used to retard the oxidation process, particularly in food systems. However, the application of synthetic antioxidants in food products is of high concern, and they are strictly regulated due to potential health hazards (Park et al., 2001). Consequently, the utilization of natural phenolic antioxidants, as alternatives, and the development of phenolic extraction methods have attracted global interest.

Extraction is a very important stage in the isolation, identification, and use of phenolic compounds (Lapornik et al., 2005). Phenolic compounds traditionally have been extracted by long maceration (Contini et al., 2008; Lu et al., 2011), heat reflux (Zhang et al., 2008), or Soxhlet extraction (Locatelli et al., 2010; Tyman et al., 1989). These extraction techniques are laborious and time-consuming. Moreover, these procedures require large amounts of toxic organic solvents, and thermo-labile compounds may suffer thermal decomposition (Luque de Castro and García-Ayuso, 1998). Due to the prescribed limitations of conventional techniques and the demands for more

efficient and economical processes of extraction in the food and chemical industries, alternative extraction techniques are being given more and more attention by researchers. Recently, various novel extraction techniques have been developed for the extraction of nutraceuticals from plants, including ultrasound-assisted extraction (UAE) (Roselló-Soto et al., 2015; Wang et al., 2013; Zhang et al., 2014), microwave-assisted extraction (MAE) (Simsek et al., 2012; Sun et al., 2016; Upadhyay et al., 2012), supercritical fluid extraction (SFE) (Roseiro et al., 2013; Solana et al., 2016), subcritical water extraction (SWE) (Etoh et al., 2010; Rangsrivong et al., 2009; Singh and Saldaña, 2011), high hydrostatic pressure (HHP) extraction (Corrales et al., 2008), high pressure/temperature extraction (HPTE) (Casazza et al., 2012; Yang et al., 2013), pyrolytic distillation (Ma et al., 2011; Wei et al., 2010a; Wei et al., 2010b), pulsed electric field (PEF) extraction (Boussetta et al., 2014; Boussetta et al., 2012), and high voltage electrical discharge (HVED) extraction (Boussetta et al., 2009a; Boussetta et al., 2009b; Boussetta et al., 2012). Compared with traditional extraction methods, the newly developed techniques are more efficient in terms of higher yields, shorter extraction times, lower amounts of extraction solvents, and sometimes lower temperatures.

Although hazelnut shells are rich in phenolic compounds, limited research has been conducted to extract phenolic compounds from hazelnut shells using those novel extraction techniques for the high efficiency production of phenolic compounds. The goal of this project was to develop and optimize novel extraction methods (UAE, MAE, and HPTE) for the production of phenolic compounds from hazelnut shells. The long-term goal is the application of the optimized process to increase the value of hazelnuts.

1.2. Objectives

Overall, this dissertation research was carried out to explore the extraction techniques for phenolic antioxidants production from hazelnut shells, with the specific objectives to:

- 1) evaluate the phenolic content and composition, and antioxidant activities of extracts from hazelnut shells by conventional shaking bath extraction;
- 2) develop an ultrasound-assisted extraction method with an ultrasound bath for the phenolics extraction from hazelnut shells;
- 3) develop an ultrasound-assisted extraction method with a probe sonicator for the phenolics extraction from hazelnut shells;
- 4) develop a microwave-assisted extraction method for the phenolics extraction from hazelnut shells; and
- 5) develop a high pressure/temperature extraction method for the phenolics extraction from hazelnut shells.

References

- Arslan, Y., Takaç, S., Eken-Saraçoğlu, N., (2012). Kinetic study of hemicellulosic sugar production from hazelnut shells. *Chemical Engineering Journal* 185, 23-28.
- Balart, J.F., Fombuena, V., Fenollar, O., Boronat, T., Sánchez-Nacher, L., (2016). Processing and characterization of high environmental efficiency composites based on PLA and hazelnut shell flour (HSF) with biobased plasticizers derived from epoxidized linseed oil (ELO). *Composites Part B: Engineering* 86, 168-177.
- Boussetta, N., Lanoisellé, J.L., Bedel-Cloutour, C., Vorobiev, E., (2009a). Extraction of soluble matter from grape pomace by high voltage electrical discharges for polyphenol recovery: Effect of sulphur dioxide and thermal treatments. *Journal of Food Engineering* 95(1), 192-198.

- Boussetta, N., Lebovka, N., Vorobiev, E., Adenier, H., Bedel-Cloutour, C., Lanoisellé, J.L., (2009b). Electrically assisted extraction of soluble matter from Chardonnay grape skins for polyphenol recovery. *Journal of Agricultural and Food Chemistry* 57(4), 1491-1497.
- Boussetta, N., Soichi, E., Lanoisellé, J.L., Vorobiev, E., (2014). Valorization of oilseed residues: Extraction of polyphenols from flaxseed hulls by pulsed electric fields. *Industrial Crops and Products* 52(0), 347-353.
- Boussetta, N., Vorobiev, E., Le, L., Cordin-Falcimaigne, A., Lanoisellé, J.L., (2012). Application of electrical treatments in alcoholic solvent for polyphenols extraction from grape seeds. *LWT-Food Science and Technology*.
- Casazza, A.A., Aliakbarian, B., Sannita, E., Perego, P., (2012). High-pressure high-temperature extraction of phenolic compounds from grape skins. *International Journal of Food Science & Technology* 47(2), 399-405.
- Ciemniewska-Żytikiewicz, H., Verardo, V., Pasini, F., Bryś, J., Koczoń, P., Caboni, M.F., (2015). Determination of lipid and phenolic fraction in two hazelnut (*Corylus avellana* L.) cultivars grown in Poland. *Food Chemistry* 168, 615-622.
- Contini, M., Baccelloni, S., Massantini, R., Anelli, G., (2008). Extraction of natural antioxidants from hazelnut (*Corylus avellana* L.) shell and skin wastes by long maceration at room temperature. *Food Chemistry* 110(3), 659-669.
- Corrales, M., Toepfl, S., Butz, P., Knorr, D., Tauscher, B., (2008). Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: a comparison. *Innovative Food Science & Emerging Technologies* 9(1), 85-91.
- de la Rosa, L.A., Vazquez-Flores, A.A., Alvarez-Parrilla, E., Rodrigo-García, J., Medina-Campos, O.N., Ávila-Nava, A., González-Reyes, S., Pedraza-Chaverri, J., (2014). Content of major classes of polyphenolic compounds, antioxidant, antiproliferative, and cell protective activity of pecan crude extracts and their fractions. *Journal of Functional Foods* 7, 219-228.
- Demirbas, A., (2006). Furfural production from fruit shells by acid-catalyzed hydrolysis. *Energy Sources, Part A* 28(2), 157-165.
- Demirbaş, E., Kobya, M., Öncel, S., Şencan, S., (2002). Removal of Ni(II) from aqueous solution by adsorption onto hazelnut shell activated carbon: equilibrium studies. *Bioresource Technology* 84(3), 291-293.
- Di Blasi, C., Branca, C., Galgano, A., Gallo, B., (2015). Role of pretreatments in the thermal runaway of hazelnut shell pyrolysis. *Energy & Fuels* 29(4), 2514-2526.
- Etoh, H., Ohtaki, N., Kato, H., Kulkarni, A., Morita, A., (2010). Sub-critical water extraction of residual green tea to produce a roasted green tea-like extract. *Bioscience, Biotechnology, and Biochemistry* 74(4), 858-860.

- Güllü, D., Demirbaş, A., (2001). Biomass to methanol via pyrolysis process. *Energy Conversion and Management* 42(11), 1349-1356.
- Hammond, E.A., (2006). Identifying superior hybrid hazelnut plants in southeast Nebraska. University of Nebraska--Lincoln.
- Huntrods, D., (2013). Hazelnut profile, http://www.agmrc.org/commodities_products/nuts/hazelnut-profile/.
- Kaliora, A.C., Kogiannou, D.A., Kefalas, P., Papassideri, I.S., Kalogeropoulos, N., (2014). Phenolic profiles and antioxidant and anticarcinogenic activities of Greek herbal infusions; balancing delight and chemoprevention? *Food Chemistry* 142, 233-241.
- Kobyas, M., (2004). Removal of Cr (VI) from aqueous solutions by adsorption onto hazelnut shell activated carbon: kinetic and equilibrium studies. *Bioresource Technology* 91(3), 317-321.
- Köksal, A.İ., Artik, N., Şimşek, A., Güneş, N., (2006). Nutrient composition of hazelnut (*Corylus avellana* L.) varieties cultivated in Turkey. *Food Chemistry* 99(3), 509-515.
- Lapornik, B., Prošek, M., Golc Wondra, A., (2005). Comparison of extracts prepared from plant by-products using different solvents and extraction time. *Journal of Food Engineering* 71(2), 214-222.
- Locatelli, M., Travaglia, F., Coisson, J.D., Martelli, A., Stévigny, C., Arlorio, M., (2010). Total antioxidant activity of hazelnut skin (Nocciola Piemonte PGI): Impact of different roasting conditions. *Food Chemistry* 119(4), 1647-1655.
- Lu, M., Yuan, B., Zeng, M., Chen, J., (2011). Antioxidant capacity and major phenolic compounds of spices commonly consumed in China. *Food Research International* 44(2), 530-536.
- Luque de Castro, M., Garcia-Ayuso, L., (1998). Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. *Analytica Chimica Acta* 369(1), 1-10.
- Ma, X., Wei, Q., Zhang, S., Shi, L., Zhao, Z., (2011). Isolation and bioactivities of organic acids and phenols from walnut shell pyrolytic acid. *Journal of Analytical and Applied Pyrolysis* 91(2), 338-343.
- Ozdemir, F., Akinci, I., (2004). Physical and nutritional properties of four major commercial Turkish hazelnut varieties. *Journal of Food Engineering* 63(3), 341-347.
- Park, P.-J., Jung, W.-K., Nam, K.-S., Shahidi, F., Kim, S.-K., (2001). Purification and characterization of antioxidative peptides from protein hydrolysate of lecithin-free egg yolk. *Journal of the American Oil Chemists' Society* 78(6), 651-656.

- Rangsriwong, P., Rangkadilok, N., Satayavivad, J., Goto, M., Shotipruk, A., (2009). Subcritical water extraction of polyphenolic compounds from *Terminalia chebula* Retz. fruits. *Separation and Purification Technology* 66(1), 51-56.
- Raso, G.M., Meli, R., Di Carlo, G., Pacilio, M., Di Carlo, R., (2001). Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by flavonoids in macrophage J774A. 1. *Life sciences* 68(8), 921-931.
- Roseiro, L.B., Duarte, L.C., Oliveira, D.L., Roque, R., Bernardo-Gil, M.G., Martins, A.I., Sepúlveda, C., Almeida, J., Meireles, M., Gírio, F.M., (2013). Supercritical, ultrasound and conventional extracts from carob (*Ceratonia siliqua* L.) biomass: Effect on the phenolic profile and antiproliferative activity. *Industrial Crops and Products* 47, 132-138.
- Roselló-Soto, E., Galanakis, C.M., Brnčić, M., Orlien, V., Trujillo, F.J., Mawson, R., Knoerzer, K., Tiwari, B.K., Barba, F.J., (2015). Clean recovery of antioxidant compounds from plant foods, by-products and algae assisted by ultrasounds processing. Modeling approaches to optimize processing conditions. *Trends in Food Science & Technology* 42(2), 134-149.
- Şayan, E., (2006). Ultrasound-assisted preparation of activated carbon from alkaline impregnated hazelnut shell: An optimization study on removal of Cu^{2+} from aqueous solution. *Chemical Engineering Journal* 115(3), 213-218.
- Service, N.A.S., (2015). Hazelnut forecast 2015. United States Department of Agriculture, Washington, DC.
- Shahidi, F., Alasalvar, C., Liyana-Pathirana, C.M., (2007). Antioxidant phytochemicals in hazelnut kernel (*Corylus avellana* L.) and hazelnut byproducts. *Journal of Agricultural and Food Chemistry* 55(4), 1212-1220.
- Simsek, M., Sumnu, G., Sahin, S., (2012). Microwave assisted extraction of phenolic compounds from sour cherry pomace. *Separation Science and Technology* 47(8), 1248-1254.
- Singh, P.P., Saldaña, M.D.A., (2011). Subcritical water extraction of phenolic compounds from potato peel. *Food Research International* 44(8), 2452-2458.
- Solana, M., Mirofci, S., Bertucco, A., (2016). Production of phenolic and glucosinolate extracts from rocket salad by supercritical fluid extraction: Process design and cost benefits analysis. *Journal of Food Engineering* 168, 35-41.
- Sun, Z., Kong, X., Zuo, L., Kang, J., Hou, L., Zhang, X., (2016). Rapid extraction and determination of 25 bioactive constituents in *Alpinia oxyphylla* using microwave extraction with ultra high performance liquid chromatography with tandem mass spectrometry. *Journal of Separation Science*.
- Surh, Y.-J., (2003). Cancer chemoprevention with dietary phytochemicals. *Nature Reviews Cancer* 3(10), 768-780.

- Tyman, J., Johnson, R., Muir, M., Rokhgar, R., (1989). The extraction of natural cashew nut-shell liquid from the cashew nut (*Anacardium occidentale*). *Journal of the American Oil Chemists' Society* 66(4), 553-557.
- Upadhyay, R., Ramalakshmi, K., Jagan Mohan Rao, L., (2012). Microwave-assisted extraction of chlorogenic acids from green coffee beans. *Food Chemistry* 130(1), 184-188.
- Uzuner, S., Cekmecelioglu, D., (2014). Hydrolysis of Hazelnut Shells as a Carbon Source for Bioprocessing Applications and Fermentation. *International Journal of Food Engineering* 10(4), 799-808.
- Wang, X., Wu, Y., Chen, G., Yue, W., Liang, Q., Wu, Q., (2013). Optimisation of ultrasound assisted extraction of phenolic compounds from *Sparganii rhizoma* with response surface methodology. *Ultrasonics Sonochemistry* 20(3), 846-854.
- Wei, Q., Ma, X., Dong, J., (2010a). Preparation, chemical constituents and antimicrobial activity of pyroligneous acids from walnut tree branches. *Journal of Analytical and Applied Pyrolysis* 87(1), 24-28.
- Wei, Q., Ma, X., Zhao, Z., Zhang, S., Liu, S., (2010b). Antioxidant activities and chemical profiles of pyroligneous acids from walnut shell. *Journal of Analytical and Applied Pyrolysis* 88(2), 149-154.
- Xu, Y., Hanna, M.A., (2010a). Composition and oxidative stabilities of oils extracted from hybrid hazelnuts grown in Nebraska, USA. *International Journal of Food Science & Technology* 45(11), 2329–2336.
- Xu, Y., Hanna, M.A., (2010b). Evaluation of Nebraska hybrid hazelnuts: Nut/kernel characteristics, kernel proximate composition, and oil and protein properties. *Industrial Crops and Products* 31(1), 84-91.
- Xu, Y., Hanna, M.A., (2011). Nutritional and anti-nutritional compositions of defatted Nebraska hybrid hazelnut meal. *International Journal of Food Science & Technology* 46(10), 2022-2029.
- Xu, Y., Sismour, E.N., Parry, J., Hanna, M.A., Li, H., (2012). Nutritional composition and antioxidant activity in hazelnut shells from US-grown cultivars. *International Journal of Food Science & Technology*.
- Xu, Y.X., Hanna, M.A., (2009). Synthesis and characterization of hazelnut oil-based biodiesel (journal version, without notes). *Industrial Crops and Products* 29(2–3), 473-479.
- Yang, J., Maldonado-Gómez, M.a.X., Hutkins, R.W., Rose, D.J., (2013). Production and in vitro fermentation of soluble, non-digestible, feruloylated oligo-and polysaccharides from maize and wheat brans. *Journal of Agricultural and Food Chemistry* 62(1), 159-166.