Dietary Isoflavones: Aglycones and Glycosides

Jingjun Tan

Submitted in accordance with the requirements for the degree of

Doctor of Philosophy

The University of Leeds



School of Food Science and Nutrition

June 2011

The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others.

This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

The right of Jingjun Tan to be identified as Author of this work has been asserted by her in accordance with the Copyright, Designs and Patents Act 1988.

© 2011 The University of Leeds and Jingjun Tan

Acknowledgements

I would firstly like to thank my supervisors, Professor Mike Morgan and Dr. Andrea Day, for their continual support, guidance, patience and encouragement throughout my doctoral studies. I thank you for your time, helpful discussions, valuable advice and for giving me opportunities to attend conferences. Special thanks to Mike's help in revising the thesis.

Many thanks to all members of the Food Biochemistry research group, and to all those who have helped me and give me advice in some way or other, both past and present, Joe, Tristan, Yichao, Tariq, Sharareh, Esti, Mohammad, Michael, Susana, Dennis, James, Juan, Nassan, Yoko, Segun, and others, for their kind assistance and interesting discussions, as well as providing me with a friendly environment in which to work. I would also like to thank Ian Hardy for his technical support during the preparation of biscuits in the food technology lab, and to thank all people of the common room for donating apple cores for the enzyme materials collection.

I also wish to thank all members of School of Food Science and Nutrition, for their friendly help. Special thanks to Katie from the international office and to Katie from the student advice centre, for their kind help during my hardest time of being an innocent victim of a criminal.

I would like to acknowledge the BBSRC and Hutchison Whampoa Ltd, for supplying me with the Dorothy Hodgkin Postgraduate Award (DHPA) that made this work possible.

I would like to take this opportunity to express my most sincere gratitude and deepest thanks to my parents, Yong Tan and Yongzhen Wei, for their never-ending care and unconditional support throughout my life, also to my brother and sister for their continuous support and encouragement. I dedicate this thesis to my father who passed away during the study.

Finally, and most importantly, I would like to thank my husband, Li Chen, for his love, emotional support and endless patience. I could not finish this study without his support.

Publications and Presentations

Tan, J., Day, A. J. and Morgan, M. R. A. Poster presentation: *The* β -*D-Glucosidase on De-glycosylation of Flavonoids*. Sixth Royal Society of Chemistry Food Group Postgraduate Research Conference, Leeds, UK, July, 2006

Tan, J., Day, A. J. and Morgan, M. R. A. Poster presentation: *Preliminary Characterisation Using Flavonoid Glycosides of \beta-Glucosidases from Different Plant Sources*. Third International Conference on Polyphenols and Health, Kyoto, Japan, December, 2007

Tan, J., Day, A. J. and Morgan, M. R. A. *Identification and Characterisation of* β -*D-Glucosidases from Diverse Plant Sources Using Flavonoid Glycosides: Apple Seeds Are a Rich Source of Stable Enzyme*. (Manuscript in preparation)

Abstract

Flavonoids are non-nutrient secondary metabolites ubiquitous in plants, associated with protection against various diseases, such as cancer and cardiovascular diseases. Dietary flavonoids are normally found as conjugated glycosides except, notably, in fermented foods where although there may be losses in total flavonoid content, levels of liberated aglycones can be relatively high. There has been considerable interest in the relationship between the form and structure of the ingested flavonoids and the consequences for efficiency of absorption.

The research focused firstly on β -D-glucosidases (β -D-glycoside glucohydrolase, EC 3.2.1.21) extracted from different plant sources and characterised. The enzyme was found at the highest levels in almond and apple seeds. The optimum reaction conditions of the enzyme from apple seeds were determined to be pH 5.5 at 65°C, and the enzyme extract was stable at 4°C for at least 12 weeks. Kinetic characterisation of the enzyme from selected materials was carried out by using para-nitrophenyl- β -D-glucopyranoside (p-NP-Glc) as substrate. The Km and Vmax of the enzyme from apple seed extract were determined, for the first time, to be 5.48 \pm 0.34 mM and 15.60 \pm 0.95 U/mg protein (n = 8), respectively, with the protein content of the extract being 0.728 \pm 0.019 mg/ml.

Secondly, isoflavone contents from different sources were investigated. Soy bean and its products are were found to be good sources of daidzin and genistin; kudzu was the best source of puerarin; red clover and chickpea were good sources of formononetin and biochanin A. Passion fruit was found to be an interesting source of isoflavones outside the legume family.

By using selected enzyme sources and isoflavone sources, a novel natural style soybased food was developed in which isoflavones existed predominately as aglycones. The food, derived using soya and enzymes from waste sources, may have further potential.

Contents

Acknowledgements	iii
Publications and Presentations	iv
Abstract	v
Contents	vi
List of Figures	xii
List of Tables	xvii
Chapter 1 General Introduction	1
1.1. Basic Structure and Classification	3
1.1.1. Basic Structure	3
1.1.2. Biosynthesis	4
1.2. Major Sub-Classes of Flavonoids	7
1.2.1. Flavones	8
1.2.2. Flavonols	8
1.2.3. Flavanones and Dihydroflavonols	10
1.2.4. Isoflavones	12
1.2.5. Flavanols	14
1.2.6. Anthocyanins	18
1.2.7. Minor Sub-Classes	20
1.3. Functions in the Plants	25
1.3.1. Functions in Plant Growth	25
1.3.2. Protective Functions	27
1.4. Flavonoids and Human Health	30
1.4.1. Epidemiology Studies	30
1.4.2. Antioxidative Activities	34
1.4.3. Enzyme Inhibition	38
1.4.4. Health Benefits	39
1.5. Bioavailability	42
1.5.1. Dietary Intake	42
1.5.2. Absorption and Metabolism	44
1.5.3. Determination of Isoflavone Metabolites in Biofluids	48
1.5.4. Factors Affecting Isoflavone Metabolism	51

1.5.5. Intra-individual Differences	53
1.6. Enzymatic Hydrolysis of Flavonoid Glycosides	56
1.7. Aims of the Project	58
Chapter 2 Materials and Methods	59
2.1. Chemicals	60
2.1.1. Flavonoids	60
2.1.2. Solvents	61
2.1.3. Buffer Salts, Acids and Bases	62
2.1.4. Water	62
2.1.5. Preparation of Buffers	63
2.2. Foods	64
2.2.1. Food Materials for Enzyme Studies	64
2.2.2. Food Materials for Isoflavone Sources	64
2.3. Equipment	65
2.3.1. Spectrophotometer	66
2.3.2. Other Consumables	66
2.4. High Performance Liquid Chromatography (HPLC)	67
2.4.1. Merck-Hitachi HPLC System	67
2.4.2. Agilent 1200 Series HPLC System	68
2.4.3. Consumables and Solvents	69
2.5. HPLC Methods	69
2.5.1. Merck-Hitachi HPLC Method	69
2.5.2. Agilent 1200 HPLC Method	71
2.5.3. Equol Separating Method	73
2.6. Flavonoid Determination	74
2.6.1. Verifying Flavonoid Peaks	74
2.6.2. UV-VIS Spectra of Flavonoids	74
2.6.3. Standard Curves for Flavonoids	77
Chapter 3 Identification of Enzyme Sources	81
3.1. Introduction	
3.2. Aims of Chapter	
3.3. Materials and Methods	

	84
3.3.2. Stock Solutions	84
3.3.3. p-NP Standard Curves	85
3.3.4. Crude Enzyme Extracts	86
3.3.5. Hydrolysis Reactions	86
3.3.6. Optimization	87
3.3.7. Stability of Crude Enzyme Extracts	88
3.4. Results and Discussion	88
3.4.1. p-NP Standard Curves	88
3.4.2. Effects of pH on the Enzyme Activities of Whole Almond	Extracts 91
3.4.3. Effects of Temperature on the Enzyme from Whole Almon	d Extracts 92
3.4.4. Stability of Whole Almond Extracts	93
3.4.5. Optimization	94
3.4.6. Identification of Alternative Enzyme Sources	97
3.4.7. Substrate Analysis Using Additional Substrates	100
3.5. Conclusions	103
Chapter 4 Further Enzyme Studies	104
Chapter 4 Further Enzyme Studies4.1. Introduction	
	105
4.1. Introduction	
4.1. Introduction 4.1.1. General Principles 4.1.2. Enzyme Assay 4.1.3. Michaelis-Menton Kinetics 4.1.4. Other Enzyme Studies 4.1.5. Factors to Control in Enzyme Assays	
4.1. Introduction 4.1.1. General Principles 4.1.2. Enzyme Assay 4.1.3. Michaelis-Menton Kinetics 4.1.4. Other Enzyme Studies 4.1.5. Factors to Control in Enzyme Assays 4.1.6. Expression of Enzyme Activity	
4.1. Introduction 4.1.1. General Principles 4.1.2. Enzyme Assay 4.1.3. Michaelis-Menton Kinetics 4.1.4. Other Enzyme Studies 4.1.5. Factors to Control in Enzyme Assays 4.1.6. Expression of Enzyme Activity. 4.2. Aims of Chapter	
4.1. Introduction 4.1.1. General Principles 4.1.2. Enzyme Assay 4.1.3. Michaelis-Menton Kinetics 4.1.4. Other Enzyme Studies 4.1.5. Factors to Control in Enzyme Assays 4.1.6. Expression of Enzyme Activity 4.2. Aims of Chapter 4.3. Materials and Methods	
4.1. Introduction 4.1.1. General Principles 4.1.2. Enzyme Assay 4.1.3. Michaelis-Menton Kinetics 4.1.4. Other Enzyme Studies 4.1.5. Factors to Control in Enzyme Assays 4.1.6. Expression of Enzyme Activity 4.2. Aims of Chapter 4.3. Materials and Methods 4.3.1. Chemicals	
4.1. Introduction	
4.1. Introduction 4.1.1. General Principles 4.1.2. Enzyme Assay 4.1.3. Michaelis-Menton Kinetics 4.1.4. Other Enzyme Studies 4.1.5. Factors to Control in Enzyme Assays 4.1.6. Expression of Enzyme Activity 4.2. Aims of Chapter 4.3. Materials and Methods 4.3.1. Chemicals 4.3.2. Preparation of Protein Reagents 4.3.3. Optimization	
4.1. Introduction	

	4.4.1. p-NP Standard Curves	. 122
	4.4.2. Stability of Apple Seed Extracts	. 123
	4.4.3. Optimum Reaction Conditions	. 125
	4.4.4. Content of Protein	. 130
	4.4.5. Progress Curves	. 133
	4.4.6. The Linearity of Velocity against Enzyme Concentration	. 134
	4.4.7. Km and Vmax	. 135
	4.5. Discussion	. 139
	4.5.1. The Optimum Conditions for Enzyme Activity	. 139
	4.5.2. Enzyme Stability	. 139
	4.5.3. Protein Determination	. 140
	4.5.4. Enzyme Kinetic Parameters	. 141
	4.6. Conclusions	. 151
CI	hapter 5 Identification of Potential Isoflavone Glycoside Sources	.152
	5.1. Introduction	. 153
	5.1.1. Distribution	. 154
	5.1.2. Isoflavone Pattern	. 156
	5.1.3. The Factors Affecting Isoflavone Contents	. 156
	5.1.4. Determining Isoflavone Contents	. 160
	5.2. Aims of Chapter	. 163
	5.3. Materials and Methods	. 164
	5.3.1. Sources of Plant Materials	. 164
	5.3.2. Acid and Base	. 164
	5.3.3. Sample Preparation	. 164
	5.3.4. Isoflavone Extraction	. 165
	5.3.5. Enzymatic Hydrolysis	. 165
	5.3.6. Acidic Hydrolysis	. 165
	5.3.7. Recovery	. 166
	5.4. Results and Discussion	. 166
	5.4.1. Soy Flour	. 167
	5.4.2. Broad Bean	. 169
	5.4.3. Chickpea	. 170
	5.4.4. Clover	. 171

	173
5.4.6. Mung Bean	175
5.4.7. Passion Fruits	178
5.4.8. Soy Nuts-Dried	179
5.4.9. Soy Nuts-Fresh	181
5.5. General Discussion	184
5.5.1. The Method of Determination	184
5.5.2. Soy Isoflavones	186
5.5.3. Broad Beans	188
5.5.4. Chickpea Isoflavones	188
5.5.5. Clover	189
5.5.6. Kudzu Isoflavones	191
5.5.7. Mung Bean	192
5.5.8. Passion Fruits	192
5.5.9. Other Food Materials	193
5.5.10. Summary of Isoflavone Contents	195
· · · · · · · · · · · · · · · · · · ·	
5.6. Conclusions	
5.6. Conclusions Chapter 6 Preparation of a Novel Food Source of Isoflavone Glycoand Aglycones for Possible Use in Human Studies of Isofl	osides
Chapter 6 Preparation of a Novel Food Source of Isoflavone Glyco	osides avone
Chapter 6 Preparation of a Novel Food Source of Isoflavone Glycoand Aglycones for Possible Use in Human Studies of Isofl	osides avone 198
Chapter 6 Preparation of a Novel Food Source of Isoflavone Glyco and Aglycones for Possible Use in Human Studies of Isofl Absorption and Metabolism	osides avone 198 199
Chapter 6 Preparation of a Novel Food Source of Isoflavone Glycoand Aglycones for Possible Use in Human Studies of Isofl Absorption and Metabolism	osides avone 198 199
Chapter 6 Preparation of a Novel Food Source of Isoflavone Glyco and Aglycones for Possible Use in Human Studies of Isofl Absorption and Metabolism	osides avone 198 199 201
Chapter 6 Preparation of a Novel Food Source of Isoflavone Glyco and Aglycones for Possible Use in Human Studies of Isofl Absorption and Metabolism	avone198199199201202
Chapter 6 Preparation of a Novel Food Source of Isoflavone Glyco and Aglycones for Possible Use in Human Studies of Isofl Absorption and Metabolism	avone198199199201202
Chapter 6 Preparation of a Novel Food Source of Isoflavone Glyco and Aglycones for Possible Use in Human Studies of Isofl Absorption and Metabolism	avone198199201206206
Chapter 6 Preparation of a Novel Food Source of Isoflavone Glyco and Aglycones for Possible Use in Human Studies of Isofl Absorption and Metabolism	osides avone 198 199 201 206 206 206
Chapter 6 Preparation of a Novel Food Source of Isoflavone Glyco and Aglycones for Possible Use in Human Studies of Isofl Absorption and Metabolism	osides avone 198 199 201 206 206 206 207
Chapter 6 Preparation of a Novel Food Source of Isoflavone Glyco and Aglycones for Possible Use in Human Studies of Isofl Absorption and Metabolism	avone198199201206206206206
Chapter 6 Preparation of a Novel Food Source of Isoflavone Glyco and Aglycones for Possible Use in Human Studies of Isofl Absorption and Metabolism	avone198199201206206206206
Chapter 6 Preparation of a Novel Food Source of Isoflavone Glyco and Aglycones for Possible Use in Human Studies of Isofl Absorption and Metabolism	osides avone 198 199 201 206 206 206 207 208 209

6.5. Discussion	212
6.5.1. Contradictory Results of Bioavailability of Isoflavones	212
6.5.2. Isoflavone Contents in the Biscuits	215
6.5.3. Soy Food Samples Used in Human Studies	216
6.6. Conclusions	218
Chapter 7 General Discussion and Future Work	219
7.1. Comparison of the Properties of the Glycosidase Isolated from A	pple with
Glycosidases from other Sources	220
7.1.1. Physiochemical Characteristics of β-D-Glycosidases from Diffe	erent
Sources	220
7.1.2. Difference in Kinetic Properties of β-D-Glycosidases from Diff	ferent
Sources	222
7.1.3. Sources of β-D-Glucosidases	224
7.2. Possible Human Intervention Study	225
7.2.1. Subjects and Diet	225
7.2.2. Study Design	225
7.2.3. Urine Sample Collection	226
7.2.4. Conditions for Urine Sample Analysis	228
7.2.5. Soy Isoflavone Metabolites in Human Urine	229
7.2.6. Inter-individual Differences	233
7.3. Concluding Statements	235
Deferences	226

List of Figures

Figure 1-1 Basic monomeric structure of flavonoids	3
Figure 1-2 Structure of chalcone	4
Figure 1-3 Structure of aurone	4
Figure 1-4 General phenylpropanoid and flavonoid biosynthetic pathways (from	
Harborne & Baxter, 1999a, Davies & Schwinn, 2006)	6
Figure 1-5 Structures of flavonoids sub-classes. Classification is based on variation in	1
the heterocyclic C ring (from Hollman & Arts, 2000).	7
Figure 1-6 Structures of common flavones	8
Figure 1-7 Structures of common flavonols	0
Figure 1-8 Structures of (2S)-flavanones and (2S, 3R)-dihydroflavonols 1	1
Figure 1-9 Structures of common flavanones	2
Figure 1-10 Structures of common isoflavones	3
Figure 1-11 Structures of different flavanols	5
Figure 1-12 Stereoisomeric structures of flavanols	6
Figure 1-13 Flavanol polymers1	7
Figure 1-14 Structures of common anthocyanidins	8
Figure 1-15 Colour reactions of anthocyanidins	9
Figure 1-16 Structure of typical neoflavonoid	0
Figure 1-17 Chalcone and dihydrochalcone structures	2
Figure 1-18 Typical aurone and auronol structures	4
Figure 1-19 Hypotheses of the links between the working mechanisms of flavonoids	
and their effects on disease (Nijveldt et al., 2001)	9
Figure 1-20 Routes for dietary flavonoids and their metabolites in humans (Scalbert e	?t
al., 2002)	8
Figure 1-21 Comparison of the structure of isoflavone metabolite equal with that of	of
estradiol showing the striking similarity in planar spatial arrangement of the tw	O
molecules (Setchell & Cassidy, 1999)54	4
Figure 2-1 Solvent gradient over the course of a run for the Merck-Hitachi Lachrom	
L-series model	1

Figure 2-2 Solvent gradient over the course of a run for the Agilent 1200 series model
Figure 2-3 Solvent gradient for the equol separating method
Figure 2-4 UV-VIS absorption spectra of tested isoflavone glucosides in 50%
methanol
Figure 2-5 UV-VIS absorption spectra of tested isoflavone aglycones and equol in
50% methanol
Figure 2-6 UV-VIS absorption spectra of tested flavones in 50% methanol
Figure 2-7 UV-VIS absorption spectra of tested flavonols in 50% methanol
(abbreviations: Q: quercetin; Q3: quercetin-3-glucoside; Q4': quercetin-4'-glucoside;
Q34': quercetin-3, 4'-diglucoside; R: rutin)
Figure 2-8 UV-VIS absorption spectra of tested flavonones in 50% methanol 77
Figure 2-9 Standard curve for daidzein by the Agilent 1200 method
Figure 2-10 Standard curve for genistein by the Agilent 1200 method
Figure 2-11 Standard curve for daidzin by the Agilent 1200 method
Figure 2-12 Standard curve for genistin by the Agilent 1200 method
Figure 2-13 Standard curve for equol obtained by the equol separating method 80
Figure 2-14 Standard curve for genistein obtained by the equol separating method 80
Figure 3-1 Enzyme catalysed reaction of p-NP-G producing p-NP 83
Figure 3-2 Standard curve for p-NP at pH 3.5
Figure 3-3 Standard curve for p-NP at pH 4.5
Figure 3-4 Standard curve for p-NP at pH 5.5
Figure 3-5 Standard curve for p-NP at pH 6.5
Figure 3-6 Standard curve for p-NP at pH 7.5
Figure 3-7 Standard curve for p-NP at pH 10.5
Figure 3-8 Effects of pH on the enzyme from almond extracts
Figure 3-9 Effects of temperature on the enzyme from almond extracts
Figure 3-10 Stability of $\beta\text{-glucosidase}$ from almond (for conditions of storage, see text)
94
Figure 3-11 Additional experiment on the effects on temperature on activity of β -
glucosidase from almond extracts
Figure 3-12 Percentage hydrolysis of flavonoid glycosides catalysed by different plant
ticques

Figure 3-13 Chromatogram of quercetin and its glucosides. Peaks identified: P1:	
quercetin-3, 4'-diglucoside; P2: rutin; P3: quercetin-3-glucoside; P4: quercetin-4'-	
glucoside; P5: quercetin	. 102
Figure 3-14 Chromatogram of quercetin-3, 4'-diglucoside after hydrolysis. Peaks	
identified: P1: quercetin-3, 4'-diglucoside; P3: quercetin-3-glucoside. For further	
details, see text.	. 102
Figure 4-1 Single-substrate mechanism for an enzyme reaction (Michaelis-Menter	n
kinetic model)	. 105
Figure 4-2 Saturation curve for an enzyme showing the relationship between subs	trate
concentration and reaction rate	. 106
Figure 4-3 Progress curve for an enzyme reaction	. 107
Figure 4-4 Displaying enzyme kinetic data on a Lineweaver-Burk plot	. 110
Figure 4-5 Kinetics scheme for reversible enzyme inhibitors	. 112
Figure 4-6 Lineweaver-Burk plots of different types of reversible enzyme inhibitor	ors.
The arrow shows the effect of increasing concentrations of inhibitor.	. 114
Figure 4-7 Standard curve for p-NP at pH 5.5	. 123
Figure 4-8 Standard curve for p-NP (high concentration) at pH 5.5	. 123
Figure 4-9 Stability of enzyme extracted from apple seeds	. 125
Figure 4-10 Effect of temperature on activity of apple seed extracts	. 128
Figure 4-11 Optimum reaction conditions for almond and apple seed extracts	. 129
Figure 4-12 Standard curve for protein determination by the Bradford method (for	r
further details, see text)	. 132
Figure 4-13 Product concentration against time (apple seed extracts)	. 133
Figure 4-14 Product concentration against time (almond extracts)	. 133
Figure 4-15 Velocity against enzyme concentration (apple seed extracts)	. 134
Figure 4-16 Velocity against enzyme concentration (whole almond extracts)	. 135
Figure 4-17 Protein-dye complex formation rate and colour stability (Bradford, 19	976)
	. 140
Figure 4-18 Real progress curve for an enzyme reaction	. 141
Figure 4-19 Graph of V vs. [S] to test co-operativity (apple seed extracts, data	
converted from table 4-12)	. 146
Figure 4-20 Graph of V vs. [S] to test co-operativity (almond extracts, data convergence)	rted
from table 4-13)	. 147
Figure 5-1 Structures of common isoflavones	153

Figure 5-2 Plants reported rich in isoflavones. (For key, see table 5-1)
Figure 5-3 Chromatograms of toasted soy flour. Top: unhydrolysed; Bottom:
hydrolysed by apple seed extracts. (For discussion of peaks, see text)
Figure 5-4 Chromatograms of broad bean (whole bean). Top: unhydrolysed; Bottom:
hydrolysed by apple seed extracts. No peak can be identified as isoflavone 169
Figure 5-5 Chromatograms of chickpea. Top: unhydrolysed; Bottom: hydrolysed by
apple seed extracts. (Peaks identified: P1: daidzein; P2 maybe ononin (biochanin-A-7-
glucoside); P3: genistein; P4: biochanin-A)
Figure 5-6 Chromatograms of clover. Top: original extract; Bottom: original extract
but changing Y axis scale in order to show other peaks. Both chromatograms were of
an unhydrolysed sample. (Peaks identified: P1: rutin; P2: quercetin-3-glucoside; P3:
maybe sissotrin (formononetin-7-glucoside); P4: daidzein; P5: maybe ononin
(biochanin-A-7-glucoside); P6: genistein; P7: formononetin; P8: biochanin-A) 171
Figure 5-7 UV Spectra of formononetin and biochanin-A. Absorption maxima have
been reported to be 248nm for formononetin, 300nm and 260nm for biochanin-A
respectively (adapted from Mabry et al., 1970).
Figure 5-8 Chromatograms of kudzu roots. Top: unhydrolysed; Bottom: hydrolysed
by apple seed extracts. (Peaks identified: P1: puerarin; P2: daidzin; P3: genistin; P4:
daidzein; P5; genistein)
Figure 5-9 Spectrum of puerarin, highest absorbance appeared at 250nm and 306nm.
Figure 5-10 Chromatograms of dried mung bean extracts. Top: unhydrolysed; Bottom:
hydrolysed by apple seed extracts. No peak can be identified as isoflavone. For
discussion of P1 and P2, see text
Figure 5-11 Spectra of the peaks in mung bean extracts. Absorbance maxima
appeared at 280nm for peak 1 (eluting at 6.704min), 270nm and 336nm for peak 2
(eluting at 10.189min)
Figure 5-12 Chromatogram of mung bean sprout extracts. (Peaks identified: P1:
daidzin; P2: genistin; P3: genistein)
Figure 5-13 Chromatograms of passion fruit extracts. Top: passion fruit flesh; Bottom:
passion fruit shell. (Peaks identified: P1: daidzin; P2 may be biochanin-A but was not
further identified)
Figure 5-14 Chromatograms of dried soy nuts. Top: unhydrolysed; Middle:
hydrolysed by apple seed extracts; Bottom: hydrolysed by 1M hydrochloric acid.

Peaks identified: P1: daidzin; P2; glycitin: P3: genistin; P4: ?; P5: ?; P6: daidzein; P7:
glycitein; P8: genistein
Figure 5-15 Spectra of P4 and P5 from HPLC of dried soy nuts. The highest
absorbance appeared at 256nm and 260nm respectively
Figure 5-16 Chromatograms of the different fresh soy nut tissues compared. Top: soy
nuts seed coat; Bottom: soy nuts hypocotyl. Both extracts are unhydrolysed. Note the
difference of the Y axis. Peaks identified: P1: daidzin; P2; glycitin; P3 was the peak
P4 in dried soy nuts; P4 was the peak 5 in dried soy nuts. The peaks after 25mins
were so-called "ghost peaks".
Figure 5-17 Diagram of pea (Pisum sativum) seed germination shows an idea of the
position of cotyledon and hypocotyls (adapted from
http://www.seedbiology.de/hormones.asp, accessed 09/2010)
Figure 5-18 Conjugate forms of soy isoflavones
Figure 5-19 White and green chickpeas (adapted from
www.wikipedia.org/wiki/chickpea, accessed 02/2008)
Figure 5-20 Similarity of 3 species of clovers before flowering (adapted from
www.uwyo.edu/Plants/Forages/3clovers.jpg, accessed 02/2008)
Figure 5-21 Structure of puerarin
Figure 5-22 Pictures of passion fruits (passion fruit; flower of passion fruit; passion
fruit on the tree). (Adapted from www.wikipedia.org/wiki/passionfruit, accessed
03/2008)
Figure 6-1 Glucuronidation of flavonoids by UDP-GT
Figure 6-2 Sulfation of flavonoids by sulfotransferase
Figure 6-3 Chromatograms of isoflavones in biscuits (Top: Using unhydrolysed soy
flour; Bottom: Using soy flour pre-heated with apple seed extracts). Peak
identification: P1: daidzin; P2: glycitin; P3: genistin; P4: daidzein; P5: glycitein; P6:
genistein. The isoflavone contents of the biscuits are shown in table 6-2
Figure 7-1 Flow diagram of a possible human study
Figure 7-2 Degradation of daidzein in the colon (adapted from (Day et al., 2004)). 230
Figure 7-3 Postulated metabolic breakdown of genistein (Joannou et al., 1995) 231
Figure 7-4 Percentages of glucuronide and sulfate conjugates and isoflavone
aglycones in urine and plasma from women fed soymilk isoflavones (Hendrich, 2002)

List of Tables

Table 1-1 Summary of cohort studies on flavonoids and risk of CHD or stroke	32
Table 1-2 Pharmacokinetic parameters of flavonoids	44
Table 3-1 Factor-level table for almond extract optimizations	94
Table 3-2 Design of orthogonal experiment, results and statistics (L $_9(3^4)$) (whole	
almond extracts)	95
Table 3-3 Results of variance analysis (whole almond extracts)	95
Table 3-4 Percentage hydrolysis of flavonoid glycosides catalysed by different pla	ant
tissues	98
Table 3-5 Effect of crude enzyme extracts on hydrolysis of other flavonoid glycos	ides
	. 100
Table 4-1 Inhibitors' effects on kinetic parameters	. 113
Table 4-2 Stability tests of the enzyme extracted from apple seeds	. 124
Table 4-3 Factor-level table for apple seed extracts optimization	. 125
Table 4-4 Design of orthogonal experiment, results and statistics (L $_9$ (3 4)) (apple s	seed
extracts)	. 126
Table 4-5 Result of variance analysis (apple seed extracts)	. 127
Table 4-6 Effect of temperature on apple seed extracts	. 127
Table 4-7 Confirmation of optimum conditions	. 129
Table 4-8 Summary of protein determination of apple seed extracts	. 131
Table 4-9 Results of protein determination	. 132
Table 4-10 Relationship between velocity and enzyme concentration (apple seed	
extracts)	. 134
Table 4-11 Relationship between velocity and enzyme concentration (whole almo	
extracts)	. 135
Table 4-12 Data for Vmax and Km determination (apple seed extracts). Columns	
contain data from replicate determinations at the conditions described	. 136
Table 4-13 Data for Vmax and Km determination (whole almond extracts). Colum	nns
contain data from triplicate determinations at the conditions described	. 137
Table 4-14 Summary of Vmax and Km determinations	. 138
Table 5-1 Isoflavone contents of selected plants (Cui 2005: Marin et al. 2005)	155

Table 5-2 Isoflavone distribution pattern in different food plants	156
Table 5-3 Isoflavone contents in four samples of toasted soy flour (mg/100g)	168
Table 5-4 Isoflavone contents in three samples of kudzu (mg/100g)	174
Table 5-5 Isoflavone contents of three samples of dried soy nuts (mg/100g)	181
Table 5-6 Isoflavone contents in fresh soy nuts (mg/100g wet sample, n=3)	183
Table 5-7 Summary of isoflavone contents in tested materials	195
Table 6-1 Results for hydrolysis efficiency of apple seed extracts towards soy flo	ur
isoflavones	209
Table 6-2 Isoflavone contents of biscuits (mg/biscuit meal)	211
Table 7-1 Physiochemical characteristics of β -D-glycosidase from different sources	
	221
Table 7-2 Kinetic parameters of β-D-glycosidase from different sources	223