BERRY FRUIT ANTHOCYANINS IN HUMAN NUTRITION – BIOAVAILABILITY AND ANTIOXIDANT EFFECTS

A thesis presented in partial fulfilment
of the requirements for the degree of

Doctor of Philosophy
in
Nutritional Science

at Massey University, Palmerston North,
New Zealand.

Michaela C. Walton

July 2006

ABSTRACT

Anthocyanins (ACNs), which are responsible for the red and blue colours displayed by many vegetables and fruits (particularly berries), belong to secondary plant metabolites, and are a component of our daily diet. There is an increasing interest on their biological activities as they are claimed to enhance health by protecting against some chronic diseases. However, before ACNs can perform health-promoting effects *in vivo*, they must first be sufficiently absorbed, distributed within the human body, and reach target tissues in adequate concentrations. To date, all studies investigating ACN absorption and metabolism came to the conclusion that their bioavailability is extremely low. To benefit from the proposed health effects of ACNs, their bioavailability, including absorption, metabolism, and excretion must first be understood. The main objective of this thesis was to provide further knowledge on ACN absorption, including the absorption site and mechanism, and the influence of food and other flavonoids on ACN absorption, as well as the investigation of their antioxidant effects *in vivo*. *In vitro* experiments using Ussing chambers showed that a strong absorption of ACNs occurred from the jejunum in mice. This was supported with a further *in vivo* study, where the major absorption site for ACNs may be the jejunum in rats. The limitation of ACN absorption to mainly one part of the intestine suggested the participation of a particular transport mechanism. In a further Ussing chamber study it was shown that flavonols, another common flavonoid group present in many fruits and vegetables, strongly inhibit ACN absorption, indicating a specific transport mechanism, with preference for other flavonoid compounds. Further *in vivo* studies have shown that the simultaneous ingestion of food components, such as breakfast cereals, resulted in a delayed absorption profile in two animal species. However, the additional food did not influence the antioxidant effect of ACNs. During a human intervention study, several measures of oxidative stress improved, but this improvement occurred equally in the treatments and placebo control, and may have resulted from changes in lifestyle. The results of these studies aid to understand details of ACN absorption and help to formulate future recommendations for ACN intake with increased bioavailability in humans.

ACKNOWLEDGEMENTS

Firstly I would like to thank my supervisors – Professor Wouter H. Hendriks for his guidance, encouragement, and scientific insight, and Dr. Tony K. McGhie for providing excellent laboratorial equipment for sample analysis, expert knowledge in anthocyanin chemistry, and valuable discussions – for the practical realisation of this thesis. Furthermore, I thank Professor Marlena C. Kruger for taking over the role of supervision from Professor Wouter H. Hendriks for the last third of my thesis, and her support and helpful discussions during the last part of my thesis.

I sincerely thank Dr. Gordon W. Reynolds for his support and skills with the Ussing chamber experiments and valuable scientific discussions.

I would like to thank Sheinach P. Dunn for her excellent technical support with the Ussing chambers, Martin B. Hunt, Laura E. Barnett and Rosheila Vather for their skilled assistance with the HPLC analysis, as well as the analysis of several parameters related to oxidative stress, Dr. Phil D. Pearce for the creatinine analysis, Dr. Janine M. Cooney for the LC-MS analysis, and Dr. Alasdair D. Noble and Mrs Padmaja Ramankutty for their advice on statistical matters.

Furthermore, I would like to acknowledge several staff members and volunteers of the Institute of Food, Nutrition and Human Health at Massey University for their help and support during animal trial work, in particular Mrs Chris L. Booth, Mrs Anne M. Broomfield, Ms Claire Browne, Ms Hilary L. Mckinnon, and Mr Ian Johnston.

I wish to acknowledge the support of a Massey University Doctoral Scholarship, and a HortResearch Scholarship.

I gratefully thank Just the Berries Ltd. (Palmerston North, New Zealand) for providing the blackcurrant material for both animal trials.

I wish to acknowledge the financial support for this thesis, which was mainly funded by the New Zealand Foundation for Research Science and Technology.

Finally I would like to thank my parents and my husband for their encouragement and support during my studies.

CONTENTS

GENERAL INTRODUCTION		1
CHAPTER 1	Literature Review: Berry Fruit Anthocyanins – Bioavailability and Antioxidant Effects	3
CHAPTER 2	The Jejunum is the Main Site of Absorption for Anthocyanins in Mice	43
CHAPTER 3	The Flavonol Quercetin-3-Glucoside Inhibits Cyanidin-3-Glucoside Absorption *In Vitro*	61
CHAPTER 4	A Viscous Food Matrix Influences Absorption and Excretion but not Metabolism of Anthocyanins From Blackcurrants in Rat	85
CHAPTER 5	Anthocyanin Absorption and Antioxidant Status in Pigs	111
CHAPTER 6	Berry Fruit and Oxidative Stress – A Human Intervention Study	133
CHAPTER 7	General Discussion	159
LIST OF PUBLICATIONS		169

GENERAL INTRODUCTION

Anthocyanins (ACNs) are widely distributed in the human diet through crops, beans, fruits, vegetables and red wines, suggesting that we ingest considerable amounts from our daily diets. Berry fruits in particular are rich dietary sources, and some can contribute 100-300 mg ACNs in a single serving.

As a potential major component of our daily diet, more and more research has concentrated on their biological activities and possible health benefits in protecting against some chronic diseases, including cancer, atherosclerosis, and diabetes. Recently, some research has also shown that an increasing dietary intake of fruit and vegetables rich in antioxidants like ACNs may retard age-related declines in brain function as well as improve cognitive and motor performance in rats.

Nevertheless, to perform their multiple biological effects, the bioavailability of ACNs present in different fruits and vegetables is an important, but still not well-understood issue. So far, there is only a small number of data available on their ability, in intact or metabolised form, to reach the systemic circulation in humans. Despite the relatively high amounts in food and potential intake in humans, the physiological impact of the ACNs is not well studied and investigations regarding their bioavailability in humans have been conducted only within the last few years. To act as systemic antioxidants and perform health effects for humans, ACNs first need to be ingested and distributed within the body successfully. Therefore, the bioavailability including absorption, metabolism, and excretion must be known.

The main objectives of the present thesis were to provide further information on the absorption site, and mechanisms involved in ACN absorption, with the aim to generate future recommendations on ACN intake with an increased bioavailability. As ACNs are mainly ingested in combination with other food sources, the effect of food matrixes on ACN absorption was also taken into account. Furthermore, the antioxidant capacity of ACNs was investigated, as well as the effect of other food intake on this capacity.