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Development of Assays for Biomarkers of Oxidative Damage to Assess the Efficacy of Fruit-derived Antioxidants

A thesis presented to in partial fulfilment of the requirements for the degree of
Master of Science in Biochemistry at
Massey University

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2003

Acknowledgements

Lord, thank You for your never-ending Grace in all aspects of my life. Thank You for surrounding me with people who are a true blessing to me in so many ways.

Thank you to Mum and Dad for your continual support, love and encouragement. Thanks to my groovy troovy sister Claire, I have been blessed to have you as my sister. Well done with everything you have achieved and will achieve in the future. Thanks also to Sara, Andy and the rest of my family for encouraging me!!

Thanks heaps to Amanda King (Brp), for supporting me and for just being there. I know you're going to go far and I hope I can be as much of a blessing to you as you have been to me!

Thank you to Daryl Rowan, for the invaluable discussions and for proof-reading my work. Special thanks to Linda Rowan for your advice and guidance in my journey and for always being there to listen.

To Tony McGhie, my supervisor at HortResearch, thank you for supporting and guiding me, and for allowing me to work independently and figure things out for myself.

Kathryn Stowell, my Massey supervisor, thank you for encouraging me and helping me gain confidence in my scientific ability. Thanks too for helping me with all the 'admin stuff'.

Thanks to Adam Matich, for your continuing support, humour and for helping me with calculations, statistics and proof-reading my thesis.

Thanks to Martin Hunt for doing the MDA analysis and for encouraging me and making me laugh when I really needed to!

Thank you to staff of HortResearch, past and present, who have supported and encouraged me from the start. Thank you to HortResearch for the financial support (from The New Zealand Foundation for Research, Science and Technology funding).

Thanks to all involved in organising and looking after the rats at SAPU, especially Anne Broomfield from the Institute of Food, Nutrition and Human Health at Massey University.

To my friends, thank you for the encouragement, support and prayers. God Bless You!.

Abstract

The diet is a very important part of maintaining a healthy lifestyle. Increased consumption of fruits and vegetables is one practice postulated to decrease the incidence of diseases such as cancer, cardiovascular disease and other disorders. Although there are a number of possible beneficial compounds in fruit, it is believed that the antioxidant components found in these foods may decrease the oxidative damage that could lead to such diseases. Oxidative damage to cellular proteins, lipids and DNA is considered to result from an increase in the production of free radicals, which overwhelm the body's defence system.

This research investigated fruit-derived antioxidants, and developed biomarker assays to measure the potential health benefits they may offer. To determine the *in vivo* antioxidant efficacy of berry fruit anthocyanins, oxidative damage to proteins, lipids and DNA was measured in rats fed several combinations of natural and synthetic diets. Mild oxidative damage was induced by the inclusion of fish oil in these diets.

DNA oxidation was determined by measuring urinary 8-hydroxy-2'-deoxyguanosine using reversed-phase high performance liquid chromatography with electrochemical detection. ELISA and colorimetric techniques were used to measure protein carbonyl content of plasma as a reflection of protein oxidation. Oxidation to lipids was assessed by measuring malondialdehyde, which results from lipid peroxidation.

Supplementation with fish oil induced a mild form of dietary oxidative damage, as shown by an increase in lipid and protein oxidation. In most cases the berry fruit extracts had little effect on the level of fish oil-induced oxidative damage, however, boysenberry anthocyanin extract significantly reduced protein oxidation when used in combination with the natural diet. Taken together the results suggest that oxidative damage to biomacromolecules may occur by different pathways of oxidative stress, which selectively target either DNA, protein or lipids at varying levels, and the antioxidant is effective only with selected mechanisms of oxidative damage.

Abbreviations

8OHdG	8-hydroxy-2'-deoxyguanosine
AAPH	2,2'-azobis(2-amidinopropane) dihydrochloride
ABTS	2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid)
AUC	area under curve
BHT	butylated hydroxytoluene
BSA	bovine serum albumin
$CHCl_3$	carbon tetrachloride
CoA	coenzyme A
DNA	deoxyribonucleic acid
DNPH	dinitrophenylhydrazine
EDTA	ethylene diamine tetra-acetic acid
ELISA	enzyme-linked immunoabsorbent assay
FPG	formamidopyrimidine DNA N-glycosylase
FO	fish oil
FRAP	free radical antioxidant power
GC-MS	gas chromatography – mass spectrometry
HCl	hydrochloric acid
HPLC	high-performance liquid chromatography
IPA	isopropyl alcohol
LC-MS	liquid chromatography with mass spectrometry
LDL	low density lipoprotein
MDA	malondialdehyde
MeOH	methanol
NaOAc	sodium acetate
ORAC	oxygen radical absorbance capacity
$ORAC_{FL}$	oxygen radical absorbance capacity assay using fluorescein
PUFA	polyunsaturated fatty acid
RP-HPLC	reversed-phase high-performance liquid chromatography
SBO	soybean oil
SEM	standard error of the mean

SPE	solid phase extraction
TBA	thiobarbituric acid
TCA	trichloroacetic acid
TE	trolox equivalent
TEAC	trolox equivalent antioxidant capacity
Trolox	6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid

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