OXALATE AND ANTIOXIDANT CONCENTRATIONS OF LOCALLY GROWN AND IMPORTED FRUIT IN NEW ZEALAND

A thesis

submitted in partial fulfilment

of the requirements for the Degree of

Doctor of Philosophy

at

Lincoln University

by

Nguyen Vu Hong Ha

Lincoln University

2012

Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

OXALATE AND ANTIOXIDANT CONCENTRATIONS OF LOCALLY GROWN AND IMPORTED FRUIT IN NEW ZEALAND

by

Ha Vu Hong Nguyen

Locally grown and some imported fruits were analysed for their antioxidant and oxalate concentrations. Total phenolic and ascorbic acid concentrations and the antioxidant capacity using ABTS and ORAC methods showed that the fruits were a source of beneficial nutrients. In contrast, the fruits contained variable amounts of soluble and insoluble oxalates as anti-nutritive compounds. Fruit available in New Zealand contained a wide range of total phenolic compounds (27.4 – 2731.9 mg gallic acid equivalents (GAE)/100g fresh weight (FW)) and vitamin C (6.2-201.3 mg ascorbic acid/100 g FW). The ABTS capacity of these fruits ranged from 19.5 - 6045.9 μmol Trolox equivalents (TE)/100 g FW and the ORAC values ranged from 1121.2-13631.6 μmol TE/100 g FW.

Extraction using 2 M HCL at 21°C was the optimum condition to extract total oxalate from fruit. This resulted in the highest mean value for total oxalate concentration (123.1 ± 2.5 mg/100 g DW) as compared to extraction using 0.2 M HCL at the same temperature (102.2 ± 2.1 mg/100 g DW) or extracting with 0.2 M HCL and 2 M HCL at 80^0C (85.2 ± 3.0 and 114.5 ± 2.2 mg/100 g DW, respectively). Similarly, at 21^0C, the mean soluble oxalate concentrations for the four analysed fruits were significantly higher than those extracted at 80^0C (54.7 vs. 39.8 mg/100 g DM).

The total oxalate concentration of locally grown fruit determined by extracting with 2M HCL at 21^0C ranged from 2.9 – 7566.5 mg/100 g FW while the soluble oxalate extracted with deionised water ranged from 5 to 56% of the total oxalate concentration. Very high

concentrations of total oxalate were measured in Indian gooseberry, rhubarb and carambola, 7566.5, 640.2 and 436.1 mg/100 g FW, respectively.

Storage of kiwifruit for 15 days at 20 ± 1^0C showed reductions in soluble oxalate concentration and increases in insoluble oxalate concentration in the three kiwifruit fractions measured (whole fruit, skin and pulp), but not the seeds. Green kiwifruit (*Actinidia deliciosa*) contained significantly higher concentrations of oxalates ($p < 0.001$) than golden kiwifruit (*A. chinensis*).

Processing kiwifruit to produce juice considerably reduced the oxalate concentration because most of the insoluble oxalate was retained in the pomace. Concentrations of total and soluble oxalates found in green kiwifruit juice obtained by enzymatic extraction were significantly higher ($p < 0.01$) than the values obtained by pressing. In the juice, soluble oxalate concentration contributed to 60.3 - 70.3% of total oxalate concentration compared with 31.7% found in the fresh tissue. Pasteurization temperatures significantly affected the soluble oxalate concentration but did not impact on the concentrations of total oxalate in the juice.

Boiling rhubarb petioles (*Rheum rhabarbarum* L.) in water or cooking with trim (low fat) or standard milk significantly ($p < 0.05$) reduced the total and soluble oxalate concentrations of the mixtures by dilution. Total oxalate in the raw rhubarb was 902.7 mg/100 g FW and in cooked rhubarb it was 454.3 mg/100 g FW. Cooking rhubarb petioles with standard and trim milk resulted in further reductions in soluble oxalate concentrations of 65.9% and 74.5%, respectively, when compared to the soluble oxalate concentration of the raw petioles. When using an *in vitro* method (to simulate the gastric and intestinal environments) to extract oxalates from the raw and cooked rhubarb, the amounts of soluble oxalates binding with fibre at pH 2.0 were significantly higher ($p < 0.01$) than at pH 7.0 (an average of 74.5 vs. 39.4 mg/100 g FW, respectively), while, the insoluble oxalate concentrations in the fibre increased when the extractions were carried out in pH conditions similar to the intestinal environment. This trapping of oxalate in the fibre fraction suggests that dietary fibre can partly reduce oxalate absorption.

Keywords: Fruit, antioxidant, antioxidant capacity, total phenolic compounds, ascorbic acid, oxalates, kiwifruit, *Actinidia deliciosa*, *Actinidia chinensis*, rhubarb, *Rheum rhabarbarum* L., *in vitro* digestion assay, cooking medium, mineral bioavailability, dietary fibre, oxalate availability, oxalate absorption.

Acknowledgements

I would like to express my sincere thanks to to all those who helped me in various ways during the work represented by this thesis.

I wish to express my sincerest gratitude to my supervisor, Professor Geoffrey Savage, for his support, guidance, wisdom and inexhaustible patience in the correction of this thesis for an excellent work. A great gratefulness too, to his encouraging me to do a Doctor of Philosophy from the very beginning of my study.

A special thanks to my co-supervisor, Dr Sue Mason, for her advice, encouragement and meticulous suggestions. Thanks for standing beside me when I felt frustrated and depressed.

Thanks Leo Vanhanen for your assistance with technical issues. I really appreciated all your patience in working with problems of the HPLC, especially at the time laboratory work needed to be finished. Thank you to Allison Lister for your guidance with the statistical analyses. A grateful acknowledgment to the research funding from Lincoln University, Canterbury, New Zealand for financial support for this study.

Thanks to my beloved husband for his encouragement and sharing of knowledge. A huge thanks to my loving son as his smile was the strongest force pushing me to finish my PhD. Most importantly, special thanks my dear parents for supporting me to pursue my PhD.

Contents

Appendix

List of Tables

List of Figures

Chapter 1
Introduction

1.1. Research background

Fruit is an important component of a healthy diet. Fruit supplies many health promoting nutrients including phenolic compounds, vitamin C, vitamin E, β-carotene, phyto-chemicals, dietary fibre, folate, potassium and plant proteins (Lyn, 2006), in which phenolic compounds have been identified as the most effective and make up a major portion of antioxidants found in plant foods (Dai and Mumper, 2010). If consumed daily in sufficient amounts, the health benefits of fruit can contribute to the prevention of major diseases such as cardiovascular diseases and certain cancers (Van Duyn and Pivonka, 2000) and could potentially save almost three million lives each year (WHO, 2002). Recently, Holoch and Tracy (2011) demonstrated the role of some natural antioxidants in preventing kidney stone formation. The New Zealand Ministry of Health (2011) has updated a message in *Food and Nutrition Guidelines for Healthy Adults: A Background Paper* to recommend that a minimum of five portions of fruit and vegetables per day contributes towards a healthy diet. The report of a Joint FAO/WHO Expert Consultation on diet, nutrition and the prevention of chronic diseases recommended a minimum intake of 400 g fruit and vegetables per day to protect people from chronic diseases as well as help to prevent the occurrence of a number of micronutrient deficiencies (FAO, 2002). Consumer demand for fresh fruit has increased, for example, in the USA demand increased from 40.2 kg/capita/year between 1980-1983 to 45.9 kg/capita/year between 2003-2005 (Huang and Huang, 2007).

Natural antioxidants present in fruit have received increased attention because of their potential to reduce the level of oxidative stress, which has been identified as a major factor in the development many human diseases (Wu *et al*., 2004a). Quantitatively, polyphenols are the main dietary antioxidants, followed by vitamins and carotenoids (O'Neill *et al*., 2001; Saura-Calixto and Goñi, 2006). Since antioxidants in plant foods vary according to cultivar and environmental growing conditions (Connor *et al*., 2002), establishing a database of antioxidants of fruits from different geographic areas is essential. Although several databases of antioxidants in fruit have been published for specific areas such as the USA (Wu *et al*., 2004b; Mahattanatawee, *et al*., 2006), Singapore (Leong and Shui, 2002), Ecuador (Vasco, *et al*., 2008), Brazil (Rufino *et al*., 2010) and Colombo (Contreras-Calderón *et al*., 2011), there is no database for New Zealand fruit.

Depending on the reactions involved, these assays can be classified into two main types: hydrogen atom transfer (HAT) reactions and electron transfer (ET) - based assays (Huang *et al*., 2005). Using one-dimensional methods to evaluate multifunctional foods and biological antioxidants cannot sufficiently evaluate all relevant parameters (Frankel and Meyer, 2000). Therefore, it has been recommended to use at least two different types of antioxidant assays to comprehensively assess the antioxidant capacity of each food (Pérez-Jiménez *et al*., 2008).

The health benefits from consuming fruit are due to their rich vitamin and non-vitamin antioxidant concentrations and these have been studied extensively (Liu *et al*., 2003); however, other components also found in fruit, may cause adverse health effects, and these have been largely overlooked. One undesirable component is oxalates. Oxalates are widely found in plant foods and can range from 3-80% of a plant's dry weight (Libert and Franceschi, 1987). Oxalates are present as water-soluble and insoluble salts of oxalic acid, a toxic dicarboxylic acid that if ingested frequently in very high amounts can cause renal failure and mineral deficiency-induced bone diseases (Hodgkinson, 1977; Sakhaee *et al.,* 2011). Once ingested, soluble oxalates are absorbed into the blood and then rapidly cleared by the kidneys and excreted in the urine (Holmes and Assimos, 2004). Oxalates can combine with calcium in the urinary tract to form insoluble calcium oxalates that are responsible for approximately 80% of all kidney stones (Noonan and Savage, 1999).

Renal stone disease places a considerable burden on the community. They cause intense pain and, if untreated, can lead to kidney damage. The costs of diagnosing kidney stones are $NZ 450,000 per 100,000 people/year (Davidson *et al.,* 2009). In the USA, the cost is estimated to be $U.S 2.1 billion/year (Pearle *et al*., 2005). According to Southern Cross Medical Library (2007), kidney stones occur in 5 to 10% of the population in New Zealand. In contrast, kidney stones occur in 2–5% of Asian populations, 8–15% of people in Europe and North America and about 20% of people in Saudi Arabia (Charles, 1998). Overall, the incidence of stone formation appears to be increasing in many countries around the world (Romero *et al*., 2010). It is possible that this is a result of the increased consumption of vegetables and fruit as people strive to consume a more healthy diet.

In mammals, urinary oxalate concentration, which is considered as a risk factor for oxalate containing kidney stones, primarily originates from dietary sources or as an end-product of endogenous metabolism of ascorbate, glyoxylate and glycine (Noonan and Savage, 1999). The contribution of dietary oxalate to total urinary oxalate excretion has been estimated to be around 50% under normal conditions (Leibman and Chai, 1997; Holmes *et al.,* 2001),

however, this may reach 67% in higher oxalate absorbers (Holmes *et al.,* 1995). As a large proportion of renal oxalates come from the diet, the main way to control the formation of kidney stones is by reducing the dietary intake of oxalates. This can only be achieved when it is known which foods contain high concentrations of oxalates. Depending on food choice and serving sizes, dietary oxalate ranges from 70-930 mg/day in a typical western diet (Zarembski and Hodgkinson, 1962) but can reach 2,000 mg/day in the Indian diet where a large percentage of plant foods are consumed (Singh *et al.,* 1972). Some Indian spices contain surprising amounts of oxalates that can reach 4014 mg total oxalate and 3977 mg soluble oxalate/100 g dry matter (Ghosh Das and Savage, 2012).

Concentrations of oxalates in leafy vegetables and the proportion absorbed in the digestive tract have been well studied (Noonan and Savage, 1999; Radek and Savage, 2008). A small amount of data giving values for some fruits using older methods of analysis has been published; however, information on locally grown and imported fruits in New Zealand is very limited. As fruit are usually consumed fresh, antioxidants are not lost by heating or from oxidative effects during processing, hence, fruit can be considered as a good source of antioxidant compounds for daily intake. However, with the recommendation that 400 g of fruit and vegetables should be consumed per day (FAO, 2002), it is not clear how much oxalates will be taken into the body. It is also not clear what percentage of oxalate will be consumed when fruits are processed into juice, which is the most popular fruit product used as an alternative source for fresh fruit.

It is thought that only the soluble oxalate fraction is absorbed into the body of normal individuals (Holmes *et al.,* 1995) and that the absorption of oxalate occurs predominately in the small intestine (Holmes *et al.,* 1995; Hatch and Freel, 2005*).* However, Hautmann (1993) observed that a relatively high percentage of oxalates was absorbed in the stomach, suggested that the pH changes along the gastrointestinal tract may have a large effect on the form and absorption of oxalates. Moreover, the presence of other food components such as dietary fibre and minerals that are ingested at the same time as oxalates may also affect oxalate absorption (Jaeger and Robertson, 2004). To date, several feeding studies suggest that there are many digestive factors interfering with oxalate absorption (Liebman and Chai, 1997; Liebman and Chai, 2004; Holmes *et al.*, 2004). However, these studies have not been able to clearly identify which factor affects oxalate availability during digestion. An *in vitro* digestive technique (Savage and Catherwood, 2007) that closely simulates the environment and the processes involved in the human gastric and intestinal digestion of ingested food allows a

direct measure of the solubility of oxalate in the presence of other digestive factors, such as pH, in the stomach and small intestine.

Advice to reduce dietary oxalates requires knowledge of food oxalate values including fruit oxalate concentrations; however, oxalate concentrations in food plants vary depending on genotype, maturity stage, growing conditions, plant parts eaten and analytical methods used (Libert and Franceschi, 1987; Massey, 2007; Nguyen and Savage, 2012). Therefore, it is critical to determine the oxalate concentrations in plant foods from specific geographic areas, as well as to identify dietary factors which affect food oxalate absorption. This would give a clear understanding to help prevent oxalate-containing kidney stones and their complications and, hence, help reduce morbidity and adverse financial effects for the community.

References

Chai, W., & Liebman, M. (2004). Assessment of oxalate absorption from almonds and black beans with and without the use of an extrinsic label. *The Journal of Urology, 172*(3), 953-957.

Charles, Y.C.P. (1998). Kidney stones. *The Lancet, 351*(9118), 1797-1801.

Connor, A. M., Luby, J. J., Tong, C. B. S., Finn, C. E., & Hancock, J. F. (2002). Genotypic and environmental variation in antioxidant activity, total phenolic content, and anthocyanin content among blueberry cultivars. *Journal of the American Society for Horticultural Science, 127*(1), 89-97.

Contreras-Calderón, J., Calderón-Jaimes, L., Guerra-Hernández, E., & García-Villanova, B. (2011). Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia. *Food Research International, 44*(7), 2047-2053.

Dai, J., & Mumper, R.J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules,15*, 7313-7352.

Davidson, P.J., Sheerin, I.G., & Frampton, C. (2009). Renal stone disease in Christchurch, New Zealand. Part 2: a community study on the burden of renal stone disease. *Journal of the New Zealand Medical Association, 122*(1297), 57-67

FAO . (2002). Diet, nutrition and the prevention of chronic diseases. Retrieved February 18, 2012, from http://www.fao.org/docrep/005/ac911e/ac911e00.htm

Frankel, E. N., & Meyer, A. S. (2000). The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture, 80*(13), 1925-1941.

Hatch, M., & Freel, R. W. (2005). Intestinal transport of an obdurate anion: oxalate. *Urological Research, 33*(1), 1-16.

Hautmann, R.E. (1993). The stomach: a new and powerful oxalate absorption site in man. *Journal of Urology, 149*, 1401-1404.

Hodgkinson, A. (1997). Oxalate metabolism in animals and man. In J. W. Arrowsmith (Ed.), *Oxalic acid in biology and medicine* (pp. 159–200). New York: Academic Press.

Holmes, P. R., Goodman, H. O., & Assimos, D. G. (1995). Dietary oxalate and its intestinal absorption. *Scanning Microscopy, 9*, 1109–1120.

Holmes, R. P., & Assimos, D. G. (2004). The impact of dietary oxalate on kidney stone formation. *Urological Research, 32*(5), 311-316.

Holmes, R. P., Goodman, H. O., & Assimos, D. G. (2001). Contribution of dietary oxalate to urinary oxalate excretion. *Kidney International, 59*(1), 270-276

Holoch, P.A., & Tracy, C.R. (2011). Antioxidants and self-reported history of kidney stones: the national health and nutrition examination survey. *Journal of Endourology, 25*(12), 1903-1908

Huang, S., & Huang, K. (2007). Increased U.S. imports of fresh fruit and vegetables. Retrieved March 5, 2012, from http://www.ers.usda.gov/Publications/fts/2007/08Aug/fts32801/fts32801.pdf

Jaeger, Ph., & Robertson, W.G. (2004). Role of dietary intake and intestinal absorption of oxalate in calcium stone formation. *Nephron Physiology, 98*, 64-71

Leong, L. P., & Shui, G. (2002). An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chemistry, 76*(1), 69-75.

Libert, B. & Franceschi, V.R. (1987). Oxalate in crop plants. *Journal of Agricultural and Food Chemistry, 35,* 926-938.

Liebman, M., & Chai, W. (1997). Effect of dietary calcium on urinary oxalate excretion after oxalate loads. *American Journal of Clinical Nutrition, 65,* 1453–1459.

Liebman, M., & Chai, W. (2004). Assessment of oxalate absorption from almonds and black beans with and without the use of an extrinsic label. Journal of Urology, 172(3), 953-957

Liu, R. H. (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *The American Journal of Clinical Nutrition, 78*(3), 517S-520S.

Lyn M, S. (2006). Eat your fruit and vegetables. *The Lancet, 367*(9507), 278-279.

Mahattanatawee, K., Manthey, J. A., Luzio, G., Talcott, S. T., Goodner, K., & Baldwin, E. A. (2006). Total antioxidant activity and fiber content of select florida-grown tropical fruits. *Journal of Agricultural and Food Chemistry, 54*(19), 7355-7363.

Massey, L.K. (2007). Food oxalate: factors affecting measurement, biological variation, and bioavailability. *Journal of the American Dietetic Association, 107*(7), 1191-1194

Ministry of Health. (2011). Food and Nutrition Guidelines for Healthy Adults: A Background Paper. Retrieved March 12, 2012, from http://www.health.govt.nz/publication/food-and-nutrition-guidelines-healthy-adults-background-paper

Nishiura, J. L., Martini, L. A., Mendonça, C. O. G., Schor, N., & Heilberg, I. P. (2002). Effect of calcium intake on urinary oxalate excretion in calcium stone-forming patients. *Brazilian Journal of Medical and Biological Research, 35,* 669-675.

Noonan, S.C. & Savage, G.P. (1999). Oxalate content of foods and its effect on humans. *Asia Pacific Journal of Clinical Nutrition, 8*(1), 64-74.

O'Neill, M.E., Carroll, Y., Corridan, B., Olmedilla, B., Granado, F., Blanco, I., Van den Berg, H., Hininger, I., Rousell, A-M., Chopra, M., Southon S., & Thurnham, D.I. (2001). A European carotenoid database to assess carotenoid intakes and its use in a five-country comparative study. *British Journal of Nutrition, 85,* 499-507

Pearle. M.S., Calhoun, E.A., & Curhan, G.C. (2005). Urologic diseases in America project: urolithiasis. *The Journal of Urology, 173*(3), 848-57

Pérez-Jiménez, J., Arranz, S., Tabernero, M., Díaz- Rubio, M. E., Serrano, J., Goñi, I., & Saura-Calixto, F. (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. *Food Research International, 41*(3), 274-285.

Radek, M., & Savage, G. P. (2008). Oxalates in some Indian green leafy vegetables. *International Journal of Food Sciences and Nutrition, 59*(3), 246-260.

Romero, V., Akpinar, H., & Assimos, D.G. (2010). Kidney stones: a global picture of prevalence, incidence, and associated risk factors. *Reviews in Urology, 12*(2/3), e86-e98.

Rufino, M. d. S. M., Alves, R. E., de Brito, E. S., Pérez-Jiménez, J., Saura-Calixto, F., & Mancini-Filho, J. (2010). Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chemistry, 121*(4), 996-1002.

Sakhaee, K., Maalouf, N. M., Kumar, R., Pasch, A., & Moe, O. W. (2011). Nephrolithiasis-associated bone disease: pathogenesis and treatment options. *Kidney International, 79*(4), 393-403.

Saura-Calixto, F., & Goñi, I. (2006). Antioxidant capacity of the Spanish mediterranean diet. *Food Chemistry, 94*(3), 442-447.

Savage, G. P., & Catherwood, D. J. (2007). Determination of oxalates in Japanese taro corms using an *in vitro* digestion assay. *Food Chemistry, 105*(1), 383-388.

Singh, P. P., Kothari, L. K., Sharma, D. C., & Saxena, S. N. (1972). Nutritional value of foods in relation to their oxalic acid content. *The American Journal of Clinical Nutrition, 25*(11), 1147-1152.

Southern Cross Medical Library. (2007). Kidney stones. Retrieved March 7, 2012 from http://www.southerncross.co.nz/AboutTheGroup/HealthResources/MedicalLibrary/tabid/178/vw/1/ItemID/148/Kidney-Stones.aspx

Van Duyn, M. A. S., & Pivonka, E. (2000). Overview of the health benefits of fruit and vegetable consumption for the dietetics professional: selected literature. *Journal of the American Dietetic Association, 100*(12), 1511-1521.

Vasco, C., Ruales, J., & Kamal-Eldin, A. (2008). Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. *Food Chemistry, 111*(4), 816-823.

WHO. (2002). The world health report 2002: reducing risks, promoting health. Retrieved from http://www.who.int/whr/2002/Overview_E.pdf

Williams, A.W., & Wilson, D.M. (1990). Dietary intake, absorption, metabolism, and excretion of oxalate. *Seminars in Nephrology, 10*(1), 2-8

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2004a). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agricultural and Food Chemistry, 52*(12), 4026-4037.

Wu, X., Gu, L., Holden, J., Haytowitz, D. B., Gebhardt, S. E., Beecher, G., & Prior, R. L. (2004b). Development of a database for total antioxidant capacity in foods: a preliminary study. *Journal of Food Composition and Analysis, 17*(3-4), 407-422.

Zarembski, P. M. & Hodgkinson, A. (1962). The oxalic acid content of English diets. *British Journal of Nutrition, 16*, 627-634.

1.2 Thesis approach

1.2.1. Thesis aim

The study aimed to investigate potential factors that could affect oxalate concentrations in post-harvest fruits and, consequently, impact on oxalate availability for absorption. This study also aimed to establish a general vision about nutritive and anti-nutritive values of New Zealand fruit in which antioxidants and oxalates are analysed. These data would be useful for clinical practice as well as diet and nutrition advisers designing balanced diets for people who are at high risk of developing kidney stones.

1.2.2. Thesis objectives

Accordingly, in terms of fruit, this study was conducted with several principal objectives:

- To measure antioxidant concentrations and antioxidant capacities of fruit available in New Zealand.
- To determine the effects of extraction factors on the measurement of oxalates.
- To measure oxalate concentrations of fruit grown and imported in New Zealand using the optimum extraction conditions.
- To investigate changes of oxalates which may occur during fruit storage and processing.
- To study the effect of digestive pH changes on oxalate availability for absorption.
- To investigate the effects of dietary fibre and other food digestive components on oxalate available for absorption using an *in vitro* digestion method.

Chapter 2

Literature Review

2.1. Introduction

Antioxidants are substances that neutralize free radicals or their actions. Antioxidants are involved in all processes that slow down or stop free radical oxidation (Michael, 2000). Natural antioxidants present in fruit have been demonstrated to be beneficial in chronic disease and certain cancers (Liu, 2003), and the consumption of fruit has been encouraged worldwide (WHO, 2003). However, undesirable substances, which can have negative effects on human health can be found in fruit and these, are often overlooked. Oxalic acid is a strong organic acid widely found in the plant world (Leenheer *et al.*, 2003). It occurs either as a free acid, water-soluble salt, formed with K^+, Na^+ and NH_4^+ (soluble oxalates), or as a water-insoluble salt of Ca^{2+}, Fe^{2+}, Mg^{2+} (insoluble oxalates), or accompanied by other acids (Ranson, 1965). Insoluble oxalates are predominantly found as calcium oxalate, which is usually the largest amount in plant foods (Noonan and Savage, 1999). A very important feature of oxalates is that they are an end point of metabolism in plants and animals and once absorbed by an animal oxalates have to be excreted in the urine (Noonan and Savage, 1999). Once oxalate-rich foods are eaten they cause adverse effects on mineral bioavailability because of the combination of oxalate ions with metal cations to create soluble or insoluble forms. Consumption of large amounts of oxalates may result in kidney disease or even death due to oxalate poisoning (James, 1972); the main effect is the removal of calcium ions from plasma (Morrison and Savage, 2003). Oxalate, therefore, is considered as an end-product and an undesired compound in mammalian metabolism. This literature review, thus, contains information concerning antioxidants and oxalates in plant foods as well as factors affecting the form and availability of oxalates, the main subject of this study.

2.2. Antioxidants – components of a healthy diet

2.2.1. What is antioxidant?

An antioxidant is broadly defined as any substance capable of inhibiting or deactivating free radical generation from an oxidisable substrate, even when the antioxidant substance is present in a significantly lower concentration than the oxidisable substrate (Halliwell, 1995). After participating in oxidation processes, the antioxidant compounds can be damaged or can

be recycled in the cell; however, their oxidation products are less harmful or can be further converted to nontoxic substances (Halliwell and Gutteridge, 2007). There are four major antioxidant processes; (1) preventing free radical generation through scavenging radical capacity, (2) enzymatic hydrolysis of ester bonds to remove peroxidised fatty acids from lipids, (3) segregation of transition metal ions and (4) enzyme-catalysed reduction of peroxides. Process (1) describes a direct way that a dietary antioxidant usually works. Meanwhile, the other three processes do not stop the reactions of radicals; instead, they prevent the accumulation of molecules that can promote free radical reactions. To protect the cells and organs in the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system. This involves both endogenous and dietary antioxidant sources that function interactively and synergistically to neutralize free radicals (Robert, 1995).

2.2.2. Free radicals and oxidative stress

Free radicals are any chemical species capable of independent existence that possess one or more unpaired electrons. Free radicals are highly reactive species. Once formed, free radicals are less stable than non-radicals. They can react either with another radical or with another molecule by capturing electrons from these substances through various interactions in order to neutralize themselves. Although the initial attack causes the free radical to become neutralized, the molecule from which the free radical 'steals' the electron becomes another free radical, causing a self-perpetuating chain reaction (Halliwell and Gutteridge, 1989). Consequently, until subsequent free radicals are deactivated, thousands of free radical reactions can occur within a cell. Free radicals in living organisms include hydroxyl ($OH\cdot$), superoxide ($O_2\cdot^-$) nitric oxide ($NO\cdot$), and peroxyl ($RO_2\cdot$). Peroxynitrite ($ONOO^-$), hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and ozone (O_3) are not free radicals but can easily lead to free-radical reactions in living organisms (Aruoma, 1998). They are capable of biologically damaging relevant molecules in the nucleus and membranes of cells such as DNA, proteins, carbohydrates and lipids and, therefore, can damage the structure and function of cells (Young and Woodside, 2001). The damaged membranes lose their ability to transport nutrients, lipoproteins are changed into oxidized forms and damaged DNA has the potential to accumulate consecutive mutations, which can lead to carcinogenesis (Ames *et al*., 1993).

Free radicals, particularly reactive oxygen species (ROS), are derived either from normal essential metabolic processes in the human body involving with the mitochondria, xanthine

oxidase, peroxisomes, inflammation, phagocytosis, arachidonate pathways, exercise, ischemia/reperfusion injury or from external sources such as exposure to UV light, X-rays, ozone, cigarette smoking, air pollutants or industrial chemicals (Bagchi and Puri, 1998). When concentrations of free radicals overcome the natural defences of the organism, a situation of oxidative stress will occur (McCord, 2000). Oxidative stress, which occurs in plants as well as animals (Jomova *et al*., 2010; Grill and Tuteja, 2010), causes oxidative damage resulting in tissue injuries to cellular and extracellular macromolecules (proteins, lipids and nucleic acids); however antioxidant operation can reduce this (Ames *et al*., 1993). To maintain proper physiological function, a balance between free radicals and antioxidants is required.

2.2.3. The antioxidant functions against oxidative stress

To defend the body from a variety of sources of free radicals, a series of defence mechanisms have been developed in the aerobic organisms (Cadenas, 1997). The antioxidant defence system includes both enzymatic and non-enzymatic systems. Enzymatic antioxidant defences include superoxide dismutase, glutathione peroxidase and catalase. Non-enzymatic antioxidants are represented by polyphenols, ascorbic acid, α-tocopherol, glutathione, carotenoids and other antioxidants (Valko *et al*., 2007). Under normal conditions, there is a balance, which is essential for the survival of organisms and their health, between the antioxidant activities and the intracellular concentrations of these antioxidants. Pathways for the management of oxidative stress by glutathione and other antioxidants have been established (Valko *et al*, 2007). Defence mechanisms against free radical-induced oxidative stress involve: (i) preventative mechanisms, (ii) repair mechanisms, (iii) physical defences, (iv) antioxidant defences.

Based on these mechanistic functions, antioxidants could be classified into preventing antioxidants, scavenging antioxidants, repair antioxidants and *de novo* antioxidants (Niki *et al*., 1995). The preventing antioxidants function as the first line of defence by inhibiting the formation of reactive oxygen and nitrogen species (ROS/RNS) by, for example, reducing hydrogen peroxide and lipid hydroperoxides to water and lipid hydroxides, respectively, or by sequestering metal ions such as iron and copper. The scavenging antioxidants, which act as the second line defence in aerobic organism, remove active species rapidly before these species attack biologically relevant molecules. Phenolic compounds and aromatic amines act as free radical-scavenging antioxidants (Dai and Mump, 2010). Repairing function of antioxidants is the third line of defence involves various enzymes that can clear the wastes

and reconstitute the lost function of the damaged cells. The adaptation mechanism functions as the fourth line of defence, where appropriate antioxidants are generated at the right time and transferred to the right position at the right concentration (Niki, 2010).

2.2.4. Dietary antioxidants

The cooperative nature of various antioxidant molecules and antioxidant enzymes is essential for inhibiting free radical reactions in an organism's cells. Other metabolism-derived molecules such as urate, bilirubin, ceruloplasmin, transferrin and hormones also contribute to the antioxidant defence (Halliwell and Gutteridge, 1989). However, the concentrations of these antioxidants are stable in human plasma and depend on different metabolic pathways. Their contributions to antioxidant processes are, thus, limited. Therefore, supplying dietary antioxidant sources is required to maintain a healthy biological balance in the body.

Much attention has focused on fruit and vegetable use as natural antioxidant sources (Cai *et al.,* 2004; Katalinic *et al.,* 2006) as alternatives for synthetic antioxidant supplements that have been restricted because the synthetic chemicals may cause potential hazardous toxicity and health problems such as asthma, allergies, liver swelling and carcinogenicity when being used long term (Halladay *et al*., 1980; Ito *et al*., 1983). Recently, in a systematic review and meta-analysis, Bjelakovic *et al*., (2007) demonstrated that single antioxidant supplements have not shown any beneficial effects on human health, in fact, single vitamins supplements increased the risk of mortality. The antioxidants present in fruits and vegetables exhibit various beneficial properties in humans. Diets rich in these foods are associated with a lower risk from the chronic diseases, certain cancers and heart disease (Liu *et al.,* 2003). Dietary antioxidant requirements can be obtained by consumption of a variety of fruit and vegetables and the message at least five serving of these foods a day has been promoted in New Zealand (New Zealand Ministry of Health, 2011).

Increased plasma antioxidant capacity following increased fruit intake has been reported in numerous studies (Marniemi *et al*., 2000; Mazza *et al*., 2002; Prior *et al*., 2007; Henning *et al*., 2010). Additionally, the total antioxidant capacity of a given fruit is the incorporated actions from hundreds of antioxidant compounds rather than from any single compound (Halvorsen *et al.*, 2006), thus, the activities of individual antioxidants cannot sufficiently reflect the total antioxidant capacity. Due to the chemical diversity of the antioxidant compounds present in plant foods, it was suggested that regular consumption of a mixture of fruit and vegetable provided better protection than a single supplement (Liu, 2004). As fruit are usually consumed fresh, the antioxidants in fruit are not lost by heating and oxidation

effects during cooking or processing, hence, fruit can be considered as a good source of antioxidant compounds.

Natural dietary antioxidants occurring widely in fruit mainly comprise vitamins and phytochemicals including polyphenols, glucosynolates and carotenoids (Blasa *et al*., 2010) of which polyphenols are present in great quantity (Hamid *et al*., 2010). Plant-derived antioxidants are capable of free radical scavenging, singlet and triplet oxygen quenchers, peroxide decomposers, enzyme inhibitors and synergists (Larson, 1988). The disease preventability of fruit antioxidant compounds are considered to reduce oxidative stress, lower blood pressure, increase insulin sensitivity, be antiviral, antitumor, antiallergic, anti-inflammatory, improve lipoprotein profiles and improve a hemostat regulation (Nakagami *et al.*, 1995; Harborne and Williams, 2000; Van Duyn *et al.*, 2000). The antioxidant capacity of phytochemicals present in fruits can largely be attributed to polyphenols, particularly flavonoid compounds (Lotito and Frei, 2006). In the animal and human *in vitro* studies of Bravo (1998), Ader *et al*., (2000) and Mazza *et al.*, (2002), some phenolic compounds showed antioxidant capacities more powerful than vitamin C and vitamin E and demonstrated high bioavailability.

It is important to note that concentrations of antioxidants and their antioxidant capacities measured in foods may not correspond to their activity after digestion and absorption (Burton and Ingold, 1984; Halliwell, 1996). The actual health benefits from consuming food containing antioxidants are strongly dependant on physiological responses of the body to the dietary antioxidants (Halliwell, 1996). Antioxidants could be changed into biologically inactive or more activated forms, resulting in reducing or increasing their scavenging free radical ability or may act in other biological roles within the body for example stimulation of defense mechanisms.

2.2.4.1. Phenolic compounds

Phenolic compounds are the most abundant secondary metabolites of both edible and non-edible plants and comprise more than 8000 phenolic structures possessing aromatic rings with hydroxyl groups (Ross and Kasum, 2002; Manach *et al*., 2004), increasing the number of hydroxyl groups increase the antioxidant capacity. The structures of natural phenolic compounds vary from single molecules, such as phenolic acid, to highly polymerized compounds, such as tannin (Harborn, 1980). There is a growing interest in research focusing on the antioxidant role of phenolic compounds in preventing degenerative disease in humans (Steinmetz and Potter, 1996; Rimm *et al.*, 1996). The antioxidant activity of polyphenols is

through scavenging free radicals and diminishing oxidative stress (Heim *et al.*, 2002). According to their redox properties, polyphenols act as reducing agents, hydrogen donators, singlet oxygen quenchers and metal chelating agents in free radical production (Rice-Evan *et al.*, 1995; Yao *et al.*, 2004). The antioxidant activity of phenolic compounds are beneficial in both food preservation and the human body where they are preferentially oxidised, therefore maintaining nutritive factors and protecting body cell components from damages by free radicals.

Phenolic compounds are involved with the colour, aroma and flavour of fruits (Arts and Hollman, 2005). They also have roles in plant development and plant protection from infection and injury (Heldt, 1997). Phenolic compounds vary with maturity stage, light exposes, cultivar and different parts of the fruit (Ribera *et al.*, 2010). The main phenolic compounds in fruit are flavonoids, tannins and phenolic acids and, less commonly, stilbenes, lignans and lignin (Macheix *et al*., 1990).

Flavonoids are divided into six subgroups whose structure depends on the level and pattern of hydroxylation, methoxylation, prenylation, or glycosylation. These subgroups comprise flavones, flavonols, flavanols, flavanones, isoflavones and anthocyanins. Anthocyanins are found mostly in berries and dark coloured fruits and vegetables (Macheix *et al*., 1990; Qian *et al*., 2004). Fruit rich in flavonoid compounds are red, yellow, dark blue and purple, such as orange, berries, dark grapes and cherries (Cieslik *et al.*, 2006). Constatino *et al.*, (1992) found that phenolic berry extracts, including blackberries, black and redcurrant, blueberries, black and red raspberries, have a marked scavenging activity and are capable of preventing oxidation of low-density lipoprotein (LDL) (Heinonen *et al.*, 1998).

Phenolic acids are divided into two classes, one derived from benzoic acid such as gallic acid and the other derived from cinnamic acids such as coumaric, caffeic and ferulic acids. While caffeic acid is found abundantly in fruits and vegetables and is the majority of hydroxycinnamates in citrus fruit, ferulic acid is found mainly in cereals and as a small percentage of total hydroxycinnamates in fruit. Although less abundant than caffeic acid in most fruit, coumaric acid predominates in the skin of red cultivars of *Vitis vinifera* (Rice-Evans *et al.*, 1997).

Tannins are also major phenolic compounds found in fruit. Based on the structure diversity of tannins, they have been subdivided into three groups: hydrolysable tannins, condensed tannins and complex tannins. Hydrolysable tannins that can be fractioned hydrolytically into

components contain a central core of glucose or another polyol esterified with gallic acid (gallotannins) or hexahydroxydiphenic acid (ellagitannins). Condensed tannins are oligomers or polymers of flavonoids consisting of a flavan-3-ol linked through an interflavan carbon bond. Once condensed tannins are broken down to anthocyanidins through an acid-catalyzed oxidation reaction by heating in acidic alcohol solutions, they are referred to as proanthocyanidins (Dai and Mumper, 2010).

2.2.4.2. Vitamin C

Vitamin C (ascorbic acid) is synthesized from glucose in the liver of most mammalian species, but not by humans, non-human primates and guinea pigs because these species lack the enzyme l-gulono-γ-lactone oxidase, which is essential for synthesis of the 2-keto-l-gulonolactone, the immediate precursor of ascorbic acid (Nishikimi and Yagi, 1991). Therefore, humans can only obtain vitamin C from their diets. Vitamin C plays an important role in humans through the evolution of an efficient carrier protein to take it up from the digestive tract and through preventing scurvy symptoms that result from prolonged deficiency of vitamin C in humans, characterized by spongy and bleeding gums, bleeding under the skin and extreme weakness (Halliwell, 2001).

Vitamin C is an electron donor and, therefore, acts as an antioxidant or reducing agent. It can prevent being oxidized by other compounds by donating two electrons from the double bond between the second and third carbons of the 6-carbon molecule (Padayatty *et al.*, 2003). Vitamin C can be oxidized by many radical species that are involved in human diseases (Halliwell, 1999). When vitamin C donates electrons, it becomes a free radical named semi-dehydroascorbic acid or ascorbyl radical that is relatively stable compared to other free radicals and fairly unreactive. Due to this property, vitamin C is considered to be a preferred antioxidant or good free radical scavenger (Bielski *et al.*, 1975).

A number of earlier studies reported the ascorbic acid's capacity to prevent oxidation of low-density lipoprotein by scavenging free radicals and other reactive oxygen species (Frei *et al*., 1989; Lehr *et al*., 1995; Martin and Frei, 1997). However, vitamin C may show pro-oxidant effects in the presence of redox-cycling oxidizing agents, such as Fe, Cu or Cr, to form hydroxyl radicals in biological fluid according to Fenton-like chemistry (Rietjens *et al.*, 2002; Poljsak *et al*., 2005)

Vitamin C can be found widely in fruits and vegetables (Kader *et al*., 2003). The New Zealand Nutrition Foundation (2012) recommends that vitamin C daily intake is about 45 mg

for adults and 60 mg for pregnant and breastfeeding women. If five servings of fruit and vegetable are consumed, it is estimated to supply about 200-300 mg vitamin C (Padayatty *et al.*, 2003).

2.2.5. Antioxidant assays

Antioxidant and oxidative issues have received increased attention over recent decades (Huang *et al.*, 2005). An inverse correlation between the consumption of dietary antioxidants from fruits and vegetables and the occurrence of inflammation, cardiovascular disease, cancer and aging-related disorders has been well reported (Liu, 2003). Due to the complexity of food composition, separating and studying individual antioxidant compounds is difficult, costly and inefficient. Moreover, the antioxidant capacities from foods derive from the synergistically cooperative interactions among the antioxidant compounds in a food mixture. Therefore, establishing standardized methods that can measure the total antioxidant power directly from the food extracts is desirable.

Depending on the reaction mechanism, the assays for measuring antioxidant capacity are classified into two groups: assays based on hydrogen atom transfer (HAT) and assays based on electron transfer (ET) (Huang *et al*., 2005). The HAT-based assays involve a competitive reaction scheme, in which antioxidant and substrate compete for thermally generated peroxyl radicals through the decomposition of azo-compounds. Meanwhile, ET-based assays measure the capacity of an antioxidant in the reduction of an oxidant, which changes colour when reduced. The degree of colour change is correlated with the sample's antioxidant concentrations (Huang *et al.*, 2005). Using one-dimensional assays, therefore, cannot sufficiently evaluate all multifunctional food and biological antioxidants (Frankel and Meyer, 2000). It has been recommended that at least two different types of antioxidant assays should be used for evaluating the total antioxidant capacity of foods (Pérez-Jiménez *et al*., 2008). Prior *et al*., (2005) suggested for three assays: Folin-Ciocalteu, Trolox Equivalent Antioxidant Capacity (TEAC) and Oxygen Radical Antioxidant Capacity (ORAC) assay, which is most relevant to human biology, should be used as standardized ET and HAT-based assays.

Folin-Ciocalteu (F-C) or total phenolics assay, which is an ET-based assay, is simple, sensitive and precise (Prior *et al*., 2005). The F-C assay has been used to measure total phenolic concentrations in natural products with the basic mechanism of an oxidation/reduction reaction, therefore it can be considered as an antioxidant method (Prior *et al*., 2005). The assay, which developed by Folin (1927) and later improved by Singleton and Rossi (1965), produces predictable results for a wide range of phenolics (Singleton and Rosi,

1965). The improved protocol, using a molybdotungstophosphoric heteropolyanion as a reagent, contains defined steps and conditions to ensure obtaining reliable and predictable results. These include: (i) proper volume ratio of alkali and F-C reagent, (ii) optimal reaction time and temperature for colour development, (iii) monitoring of optical density at 765 nm, (iv) gallic acid used as the standard reference phenol. There are few published studies which follow the exact steps of the improved F-C method. However, a number of researchers have applied different reagent concentrations, timing of additions and incubation. Additionally, using gallic acid equivalent as a standard has been replaced by other chemicals such as catechin equivalents (Vinson *et al.*, 2001), tannic acid equivalents (Nakamura *et al*., 2003), chlorogenic acid equivalents (Mingfu *et al*., 2003), caffeic acid equivalents (Maranz *et al*., 2003), protocatechuic acid equivalents (Cai *et al*., 2003), vanillic acid equivalents (Jayasinghe *et al*., 2003) and ferrulic acid equivalents (Velioglu *et al*., 1998). Although there are several interfering substances in the F-C assay, a uniformly acceptable method of total phenolics analysis could be established if the analytical steps are carried out exactly as laid out in the original improved assay, appropriate correction is conducted and gallic acid is used as the reference standard, results can then be compared. Lack of standardisation of analytical methods can result in variations of data obtained from the same samples (Prior *et al*., 2005).

The Trolox equivalent antioxidant capacity (TEAC) assay, which is also called the ABTS assay was first reported by Miller *et al*., (1993) and then modified further by Re *et al*., (1999). The ABTS assay is based on the scavenging ability of antioxidants to the long-life radical cation of 2,2-azinobis (3-ethylbenzothiazoline 6-sulfonate) (ABTS). In the modified protocol (Re *et al*., 1999), ABTS radicals are produced by oxidation of ABTS with potassium persulfate. This ABTS radical is stable for at least two days when stored in the dark at room temperature (Miller *et al*., 1996): $ABTS^{\bullet-}$, the oxidant, is generated by potassium persulfate oxidation of $ABTS^{2-}$ and a stable form of $ABTS^{\bullet+}$ radical is then generated directly prior to the reaction with antioxidants, to create a intense blue–green $ABTS^{\bullet+}$ chromophore with characteristic absorption at 734 nm. The antioxidant capacity of antioxidants to quench the $ABTS^{\bullet+}$ radical are compared with that of Trolox, a water-soluble vitamin E, in a Trolox standard analogue and expressed as trolox equivalents (TE)/gram sample.

Due to its applicability to aqueous and lipid phases (MacDonald-Wicks *et al*., 2006), the ABTS method has been broadly applied to measure the antioxidant capacity of many natural products such as fruits (Leong and Shui, 2002; Ozgen *et al*., 2006), vegetables (Sun *et al*., 2007), medicinal plants (Surveswaran *et al*., 2007), cereals (Hu *et al*., 2007), wine and grapes

(Rivero-Pérez et al., 2008), beverages (Gómez-Ruiz *et al*., 2007) and essential oils (Ekran *et al.,* 2008).

For assays that measure hydrogen atom transfer (HAT), Huang *et al*., (2005) recommend the oxygen radical absorbance capacity (ORAC) assay. The ORAC assay measures inhibition of peroxyl radical induced oxidations by antioxidants from food components and thus reflects classical radical chain-breaking antioxidant activity by H-atom transfer. This assay has been used widely to measure the net outcome antioxidant capacity, particularly peroxyl radical absorbance capacity, of plants and biological samples (MacDonald-Wicks *et al*., 2006). Fluorescein (FL) (3',6'- dihydroxyspiro[isobenzofuran- 1[3H],9'[9H]-xanthen]-3-one) is currently used as the probe replacing for B - phycoerthrin (B-PE) because of its stability and lower activity compared to B-PE (Prior *et al*., 2005). However, FL is pH sensitive and this must be carefully monitored. As the reaction progresses, FL is oxidised and the fluorescence diminishes. In the presence of an antioxidant, the decay of FL is prevented. The antioxidant capacity of samples are determined as the difference between the area under the fluorescence decay curves (fluorescence intensity vs. time) when FL is incubated with the sample extract or with a blank. The data from the assay are calculated by comparison to a Trolox standard analogue and expressed as trolox equivalents (TE)/gram sample.

Applying the area under-curve technique allows the combination of both inhibition percentages and length of inhibition time of free radical action by an antioxidant into a single quantity. This makes the ORAC assay superior to similar methods that use either an inhibition percentage at a fixed time or a length of inhibition time at a fixed inhibition percentage (Cao and Prior, 1998). Moreover, other advantages of the ORAC assay are also available through the use of peroxyl radical as reactants with redox potential and mechanism of reaction (i.e. hydrogen atom vs. electron transfer) similar to those of physiological oxidants and the use of a physiological pH so that the antioxidants react with an overall charge and protonation state similar to that in the body (Bisby *et al.,* 2008). The adaptation to detect both hydrophilic and lipophilic antioxidants of samples by altering the radical source and solvent used for solubilisation of the extract is also an advantage of the assay (Zulueta *et al.,* 2009). Due to these main advantages, the ORAC assay has been used in a broad range of sample types such as fruit and vegetable, plasma and pure phytochemicals (Cao *et al.,* 1993).

2.2.6. Contribution of antioxidants from fruit

Antioxidants vary according to genotype variation, concentrations of maturity, environmental growth conditions and the part of fruit (Vieira *et al*., 2009; Wang *et al.,* 2011). Environmental

factors including temperature, climate conditions, illumination and soil history impact directly on the phenolic composition and concentration of fruit. Fruit grown under the coolest temperature conditions contained lowest phenolic acid, anthocyanins, flavonols and, therefore, showed the lowest radical absorbance capacity compared with fruit grown under higher temperatures (Wang and Zheng, 2001). Howard *et al.,* (2003) also reported the effect of growing season on the correlation between antioxidant capacity and phenolic compounds. In addition, Connor *et al.,* (2002) found significant differences in antioxidant capacity, total phenolic and anthocyanins concentration between nine blueberry genotypes harvested at different locations.

Phenolic compounds are reportedly synthesized in parallel with the development and maturation of fruit resulting in antioxidant capacity varing considerably with concentrations of maturity. Prior *et al.,* (1998) carried out a small comparative study measuring ORAC, anthocyanin and total phenolic concentration of two different cultivars of blueberry. The results showed that there were significantly higher ORAC values, anthocyanins and total phenolic concentration in mature blueberries harvested 49 days later, in comparison with younger fruits harvested immediately after turning blue. Likewise, blackberry, raspberry and strawberry fruits harvested at ripe stage consistently yielded higher antioxidant values than those harvested at the early stage (Wang and Lin, 2000).

The level of antioxidant compounds also varies in the different parts of fruit. Indeed, peels of apples, peaches, pears and yellow- and white-flesh nectarines were found to contain twice the level of total phenolics as found in the flesh (Gorinstien *et al.,* 2002, Gil *et al.,* 2002). In addition, there are high concentrations of phenolic compounds in seeds, considered a by-product of the fruit processing industry. Numerous studies record an abundant concentration of polyphenols found in seeds of several fruits, such as grapes, mango, avocado, longan and jackfruit and these tend to be higher than the edible parts (Torres and Bobet, 2001; Soong and Barlow, 2004) . Similar to these observations, Toor and Savage (2005) found the total phenolics concentration of skin and seeds of tomatoes were 29.1 and 22.0 mg gallic equivalent/100 g fresh weight, respectively, as compared to 12.7 mg gallic equivalent/100 g fresh weight found in the pulp.

Antioxidants can be found in all parts of fruits, so increased consumption of fruit is believed to bring benefits to human health. However, designing a healthy diet for increasing fruit intake should carefully consider the contribution of existing anti-nutritional constituents of

which oxalates are regarded as one of undesirable components that are found widely in the plant world.

2.3. Oxalates – anti-nutritional components in plant tissues

2.3.1. Biosynthesis of oxalate

Oxalates are found widely in 215 plant families (McNair, 1932) including many crop plants; oxalate range from 3-80% of a plant's dry weight (Libert and Franceschi, 1987). The compounds accumulate in vacuoles of specialised idioblast cells and can incorporate up to 90% of the total plant calcium in tissues of oxalate-producing plants (Gallaher, 1975; Franceschi and Horner, 1980). Although several models and numerous proposed pathways for oxalate biosynthesis in plants (Libert and Franceschi, 1987; Keastes *et al*., 2000; Kostman *et al*., 2001; Xu *et al.*, 2006; Nataka and McCorn, 2007), the biosynthesis pathways for oxalate are based around glycolate metabolism and the cleavage of ascorbic acid (Franceschi and Horner, 1980).

Early studies on rhubarb, buckwheat and other plants indicate that oxalate formation is related to photosynthetically-derived carbon compounds (Pucher *et al*., 1939; Myers, 1947; Stutz and Burris, 1951). Noll and Burris (1954) observed that glyoxylate oxidase, peroxisomal enzyme, was present in all plants analyzed and illumination greatly enhanced their activity. Glycolate^{-} ^{14}C was oxidized by glycolate oxidase (EC 1.1.3.15) to glyoxylate that was oxidized further by the same enzyme to oxalate (Seal and Sen, 1970). However, the characteristic of the enzyme varies between different plant species.

The glycolate/glyoxylate pathway for oxalate synthesis has been argued about and has several controversial themes. Raven *et al.,* (1982) showed that the photosynthetic incorporation of $^{18}O_2$ into glycolate and glyoxylate did not appear in oxalate in spinach. In several C_4 plants such as amaranth (*Amaranthus* family), sorghum (*Poaceae* family) that utilize C_4 photosynthesis where there is little or no photorespiration, surprising amounts of oxalate were found in the tissue. Oxalate can be deposited in the dark or even in low-light stage where there is apparently no photorespiration (Libert and Franceschi, 1987), indicating that photosynthesis is not an essential requirement for oxalate synthesis. Although Xu *et al.*, (2006) did not deny the role of glycolate/glyoxylate as the major precursor for oxalate synthesis they suggested that glycolate oxidase was not completely involved with accumulation and regulation of oxalate synthesis in rice leaves and that there is a undetected enzyme catalyzing oxalate synthesis from the glycolate/glyoxylate pathway. Overall, it is

accepted that glycolate and glyoxylate are the immediate precursors of oxalate and the photorespiratory glycolate/glyoxylate oxidation is the primary oxalate biosynthesis pathways in certain plants in which glycolate oxidase catalyzed both oxidations (Vickery and Palmer, 1956; Chang and Beevers, 1968; Nataka, 2003; Yu *et al*., 2010).

Not only was glyoxylic acid found as the oxalate precursor in plants but it was also shown that L-ascorbic acid can be partly converted to oxalate in several plants (Wagner and Loewus, 1973; Loewus, 1999; Keast *et al.*, 2000; Kostman et al., 2001). The synthesis of ascorbic acid (vitamin C) from D-glucose-6-phosphate via the D-mannose, cell-wall pectin breakdown and myo-inositol-hexuronic acid pathways in higher plants (Valpuesta and Botella, 2004) is well understood. The cleavage of ascorbic acid to oxalic acid and L-threonine has recently been suggested by Keates *et al.*, (2000) but no clear pathway has been identified. Several studies showed convincing evidence about oxalate formation from C^1 and C^2 cleavage of L-ascorbic acid. There was a significant increase in amount of labelled oxalate when ascorbic acid labelled at C^1 and C^2 was fed to an oxalate accumulator such as spinach, begonia and oxalis in which the conversion took place equally in the dark or light phase was observed in *Oxalis oregano*. In contrast, there was no conversion seen in barley a non-oxalate accumulator (Yang and Loewus, 1975). Likewise, Nuss and Loewus (1978) recorded that in oxalate accumulators, 22-50% of labelled [1-^{14}C] ascorbic acid was converted to oxalate while 2-19% was converted in low-oxalate formers. By using radioactive isotopic labelling, Keates *et al*., (2000) discovered that labelled carbons from L-galactose and L-ascorbic acid were carried through to oxalate and were incorporated into calcium oxalate in idioblast cells. Supplementation with glycolate did not lead to a high specific radioactivity in the idiobalst, therefore, it was suggested that glycolate and glyoxylate were of secondary importance to ascorbic acid. Furthermore, Keates *et al.*, (2000) stated that idioblast cells must contain the biochemical pathway for oxalate synthesis from L-ascorbic acid when oxalate could not be transported from other cells to idioblasts to form calcium oxalate idioblasts. Kostman and Koscher (2003) supported this suggested pathway by discovering the key enzyme for ascorbic acid synthesis, L-gulono-1,4-lactone dehydrogenase, in calcium oxalate idioblast cells of *Pistia stratiotes* L. and *Medicago truncatula*.

However, Xu *et al*., (2006) demonstrated conflicting data concerning the involvement of oxalate synthesis with the metabolism of ascorbic acid in rice seedlings. By giving ascorbate to low oxalate containing rice seedlings, there was the resulting increase of oxalate synthesis that was significantly lower than when glyoxylate and oxaloacetate were fed to the growth media. Moreover, it also was observed that ascorbate accumulated much later than oxalate

during nitrate feeding in rice leaves, suggesting that ascorbic acid was not a precursor for oxalate production in this plant.

Recently, genetic identification of calcium oxalate deficient mutants (Cod) and crystal structure in *Medicago truncatula* (Nataka and McConn, 2000; Nataka and McConn, 2003; Nataka and McConn, 2007) through the isolation of specific oxalate encoding genes led to an understanding of the role of these genes in calcium oxalate crystal development as well as the viability of different oxalate biosynthesis pathways operating synergistically in the plants. Ascorbate induction studies with wild type, Cod4, Cod4/5 and Cod5 in *Medicago truncatula*, Nataka and McConn (2007b) suggested that there were different pathways for druse and prismatic calcium crystal formation in which they used different precursors (ascorbate, glycolate or oxaloacetate) and different enzymes. The pathways may operate in parallel if they use the same precursor (e.g. ascorbic acid); however, there are cell-specific transcription factors and/or isozymes (encoded by different nuclear genes) responsible for the conversion of ascorbic acid into oxalate.

Oxalates are also produced by the activity of the enzyme isocitrate lyase on isocitrate (Giachetti *et al.*, 1987) and by oxidation of oxaloacetate (Hayaishi *et al.*, 1958), however, these are minor pathways involved in oxalate synthesis and they are employed in the formation of calcium oxalate crystals in certain plants (Franceschi and Nakata, 2005). The cleavage of isociatrate is catalyzed by isocitrate lysase to produce glyoxylate and succinate through the glyoxylate cycle was found to occur only in the early stage of seed germination in several oil-rich seeds (Tolbert, 1981; Tolbert and Essner, 1981). This removed this biosynthesis cycle from being a general source of oxalate synthesis in plants. Oxaloacetate has been shown to be cleaved from oxaloacetase to oxalate and acetate through the primary pathway for oxalate synthesis in fungi (Kubicek *et al*., 1988); however, oxaloacetase has only been discovered in beetroot and spinach (Chang and Beevers, 1968).

The question is which pathway is involved with the biosynthesis oxalate in plants? Regarding the glycolate/glyoxylate pathway, there have been some discussions. First, oxalate and calcium oxalate have been found abundantly in many C_4 plants where little or no photorespiration occurs (Libert and Franceschi, 1987) and oxalate produced from dark-grown plants was four times higher than from light-grown plants (Franceschi, 1987). Further, glycolate oxidase was absent in two raphide-producing systems of crystal idioblast cells (Li and Franceschi, 1990). Therefore, it can be seen that the oxalate biosynthesis in plants is generally unclear. It is possible that there is more than one viable pathway operating for the

biosynthesis of oxalate in plants. Glycolate/glyoxylate may be intermediates in the ascorbate cleavage pathway or both pathways may operate synergistically in oxalate synthesis in plants (Franceschi, 1987; Franceschi and Nataka, 2005; Nataka and Mccorn, 2007). Further biochemical, molecular and genetic studies needs to be accomplished to indentify novel enzymes and elucidate the primary pathways for oxalate synthesis in certain plant types.

2.3.2. Metabolism in plants

Although oxalates are considered as an end product of plant metabolism, some of plants can metabolise oxalate under certain conditions and this is observed in some micro-organisms (Khambata and Bhat, 1954, Khambata and Bhat, 1955; Jakoby *et al*., 1956; Jin *et al*., 2007), in mosses (Data and Meeuse, 1955) and in some plants (Bake and Eden, 1954; Seal and Sen, 1970; Nuss and Loewus, 1978). Oxalate metabolism in higher plants has been implicated with activation and decarboxylation by oxalate decarboxylase (Giovanelli and Tobin, 1964) and oxidation by oxalate oxidase in which the oxalate oxidase has attracted considerable interest (Arnon and Whatley, 1954; Meeuse and Campbell, 1959; Srivastava and Krishnan, 1962).

In sugar beet leaves, Bake and Eden (1954) found that oxalate concentration decreased as the season advanced while Seal and Sen (1970) recorded periodic fluctuations of oxalate level in *Oxalis corniculata*. Srivastava and Krishnan (1962) found an enzyme on the leaves of *Bougainvillea spectabilis* that can decompose oxalate in the presence of oxygen and released CO_2. Minimum enzyme activity occurs at 4 p.m. and a maximum occurs at 12 p.m., showing a significant diurnal variation. Related to enzyme activity, total oxalate concentrations of the tissue also fluctuated periodically but in the opposite direction to the enzyme. Pundir and Nath, (1984) discovered the presence of oxalate oxidase in *Sorghum* leaves, which had an affinity for oxalate twenty times lower than that in barley roots (K_m = 2.4 x 10^{-5}M and 2.1 x 10^{-4}M , respectively). It was also observed that there was a hyperbolic relationship between oxalate concentration and enzyme activity in oxalate concentration of 10^{-5} up to 2.5 x 10^{-4}M, above which the enzyme showed substrate inhibition. Regarding of localization of the enzyme, it was shown that oxalate oxidase in certain plants such as sugar beet leaves, *Begonia* and *Chenopodiu,* was associated with cytoplasmic particles from the chloroplast fragment in their isolation procedure (Arnon and Whatley, 1954; Finkle and Arnon, 1954; Nagahisa and Hattori, 1964) or mitochondrial-type particles in beet root (Meeuse and Campbell, 1959).

Oxalate decarboxylase in pea seeds and cotyledons that catalyzed oxalates in a different manner with oxalate oxidase was found by Giovanelli and Tobin (1964). It was proposed that ATP and coenzyme A-dependent decarboxylation of oxalate was catalysed by this enzyme to

give oxalyl CoA via a thiokinase reaction. A further decarboxylation of oxalyl CoA produced CO_2 and formyl CoA that may be used as source of 1-carbon units in the plant. Rapid decarboxylation of oxalic acid during the climacteric in bananas (Shimokawa *et al*., 1972) also accounted for the activity of this enzyme. Oxalate decarboxylase also was found in the wood-destroying fungus *Collybia velutipes* (Shimozono and Hayaishi, 1957). Reutilisation of oxalate was recognised as converting ^{14}C from labelled oxalate into glycolate, succinate and malate in buckwheat leaves (Zbinovsky and Burris, 1952) and for amino acid and sugar formation in sugar beet (Joy, 1964). In sterile cultures, oxalates supplied to *Lemna gibba* L, acted as carrier molecules that, subsequently, could be reduced and condensed with acetyl CoA to form malic acid (DeKock *et al*., 1973).

2.3.3. The role of oxalate in plants

Although there are numerous speculations, the biological function of oxalate in plants is not well understood. Among the proposed functions, calcium regulation (Borchert, 1986; Franceschi , 1989; Monje and Baran, 2002; Volk *et al*., 2002), plant protection (Ward *et al*., 1997; Bruynzeel, 1997; Finley, 1999; Saltz and Ward, 2000; Ruiz *et al*., 2002; Molano-Flores, 2001; Salinas *et al*., 2001) and detoxification (Foy *et* al., 1978; Franceschi and Schueren, 1986; Ma *et al*., 1997; Choi *et al.*, 2001; Ma *et al*., 2001) have attracted most studies. Others functions include tissue support/rigidity, pH regulation, osmoregulation and ion balance in the cell.

2.3.3.1. Calcium regulation

Calcium has an important biological role in plant growth and is also a major component of plant oxalate crystals (Franceschi and Horner, 1980). It is necessary for respiration efficiency, maintenance of chlorophyll concentration, fluidity of the cell membrane, cell wall formation, membrane stabilization, fruit ripening and senescence rate of leaves (Suutarinen *et al.*, 2000). The uptake of calcium into many plants is not regulated by metabolic requirements but rather by the mineral abundance and calcium permeability of the apoplastic pathway to the root xylem (White, 2000). Calcium ions may be concentrated during periods of high water evaporation from plant surfaces and build up over time, becoming a potential toxicant for plants. Since calcium is involved in signal transduction pathways and in the regulation of other biochemical and cellular processes, it is considered that deliberate control of calcium concentration and availability within the plant cell is critical to the maintenance of normal cellular metabolism (Hepler, 2005). In a high calcium environment, free oxalates are primarily synthesised from the cleavage of ascorbate and used for crystal formation, and this

would bind with a large excess of calcium ions to create calcium oxalate (CaOx), which is physiologically and osmotically inactive (Kosmant *et al.*, 2001; Nataka and McCorn, 2003). As excess calcium can interfere with cell processes such as calcium-dependent signalling, microskeletal dynamics (Webb, 1999), CaOx production demonstrates a high-capacity mechanism for regulating calcium concentrations in plant tissue (Mazen, 2004a). This is necessary for plants growing in an abundant soluble calcium environment and where the control of Ca entering the root zone is not controlled adequately (Franceschi, 1989). The disappearance of the crystals under conditions of Ca deficiency such as during maturation (Ilarslan *et al*., 1997; Ilarslan *et al.*, 2001; Storey *et al*., 2003) or the spring flush where rapid growth is present (Franceschi and Nataka, 2005) is further evidence of the function of oxalate in Ca regulation. The CaOx crystals are localized in special cells called crystal idioblasts, the size, shape; intracellular structures are entirely different from non-crystal forming cells in the same tissue (Foster, 1956).

A number of studies (Zindler-Frank *et al*., 2001; Volk *et al*., 2002; Mazen *et al*., 2004) which show the relationship between concentrations of calcium absorbed with the presence of oxalate in plants and support the function of CaOx in calcium regulation. They found that the number and size of CaOx crystals are correlated to change of calcium concentration in the nutrient medium.

Although the plant cell wall is fully capable of substituting the binding activity to ionic calcium, calcium accumulation in the apoplast of the developing cells may interfere with normal cell expansion process in plants by cross-linking with the acidic residues of the cell wall polymers (Franceschi and Nataka, 2005). Therefore, it is possible that a primary function of crystal idioblast formation in developing tissues may be to serve as a localized calcium sink to reduce the apoplastic calcium concentration around adjacent cells, allowing them to develop normally (Borchert, 1986; Ilarslan *et al*., 2001; Nataka, 2003). A study of the crystal development (Kostman and Franceschi, 2000) indicated that Ca oxalate formation is a highly controlled cellular process rather than a simple precipitation phenomenon and that specialized mechanisms must be present in crystal idioblasts to deal with the large fluxes of calcium.

2.3.3.2. Plant protection

CaOx crystal formation is not only thought as calcium regulation process but also as an herbivore defence mechanism in plants (Sunell and Healey, 1985; Perera *et al*., 1990; Ward *et al.*, 1997). The plant protection role of CaOx crystals is primarily based on their morphologies in plants and appears in five major forms: prisms, styloids, raphides, druses and crystal sand.

Raphides are commonly found in most oxalate accumulating plants and are needle-like complexes (Franceschi and Horner, 1980) that can penetrate into tissues of animals through either ingestion or skin contact and cause pain and irritation (Sakai *et al*., 1972; Finley, 1999; Bradbury and Nixon, 1998; Saltz and Ward, 2000). A dermal irritation and painful stinging sensation appears at the wound by channelling a toxin in the crystal to the wound site (Thurston, 1976). Bradbury and Nixon (1998) observed severe swelling and irritation of human forearms from rubbing taro that was caused by two kinds of raphides, thick and thin, existing in taro corm and leaves, one having one end shaper than the other end, were observed. Furthermore, in some plant species, an increase of CaOx crystal accumulation occurs following mechanical wounding of plant tissues and this response contributes to the proposal of induced defence mechanism (Tillman-Sutela and Kauppi, 1999; Molano-Flores, 2001).

Regardless of CaOx crystals, soluble oxalates can function as a toxic compound to grazing animals and sucking insects (Yoshihara *et al*., 1980; Frutos *et al*., 1998) by interfering with mineral absorption, especially calcium, leading to crystal precipitation in tissues and organs of feeding animals and this consequently contributes to renal disorders (James, 1972; Jonassen *et al*., 2005). Halogeton, an herb which contains high concentrations of oxalates, was considered to be one of the most economically destructive poisonous plants, from the 1940s to the 1970s, causing the death of many sheep and cattle following CaOx crystal formation in the rumen walls, arteries and kidneys (Erickson *et al*., 1952; Jame and Butcher, 1972; James and Cronin, 1974). Similar pathologic conditions can be observed in humans following ingestion of considerable amounts of oxalate containing foods (Holmes *et al*., 2001; Massey, 2003; Siener *et al*., 2003).

2.3.3.3. Metal detoxification

Heavy metals are essential for plant normal growth, but elevated concentrations of essential and non-essential metals, may lead to growth inhibition and toxicity symptoms (Reichman, 2002). Organic acids, e.g. malate, citrate and oxalate are responsible for metal detoxifying mechanism in plants by forming metal crystals and, thus, making heavy metals unavailable for absorption into plants (Zheng and Matsumoto, 1998; Ma, 2000). There are two mechanisms of oxalate utilization for metal detoxification in plants, known as exclusion and internal mechanisms (Hall, 2002). Some plants display two mechanisms while other plants have just one mechanism to detoxify metals (Franceschi and Nataka, 2005). Basically, the exclusion mechanism is the release of oxalates into heavy metal contaminated soil through the

plants rooting system to allow oxalates to bind with heavy metals to form oxalate crystals that cannot be absorbed by the plant (Ma *et al*., 1997), whereas, the internal tolerance mechanism involves the sequestration of heavy metals into a non-toxic form of metal-oxalate in specialised cells in the plant (Ma *et al*., 1998).

Several plant species can deposit heavy metals via metal-oxalate crystalline formation (Franceschi and Nataka, 2005). In addition, aluminium detoxification of oxalate has been reported in buckwheat *(Fagopyrum esculentum* Moench.) where the aluminium tolerance in the roots and leaves of buckwheat is accomplished by formation of a nontoxic aluminium-oxalate complex (Ma *et al*., 1997). Likewise, in taro (*Colocasia esculenta* L. Schott cv. Bun-long and Lehua maoli), increasing release of oxalates from taro roots with increasing aluminium stress was recorded by Ma and Miyasaka, (1998). Furthermore, De la Rosa and his colleges (2009) found that in *Salsola kali* or *S. tragus,* two desert plant species, citric and oxalic were identified as the main acids in plant extracts and there was less free oxalic acid in Cd-treated plants compared to control plants. There were no significant differences between concentration of citric acid in control and Cd-treated plants. Therefore, it was suggested that cadmium may have been precipitated as oxalate crystals.

Tolerance of plants to heavy metals through metal-oxalate formation involves a different process. Franceschi and Schueren (1986) discovered that in the presence of Ba, Cd, Co, Mn and Sr in the growing media of *Lemna minor*, only Sr was incorporated into the oxalate in the raphides at detectable concentrations. An increase in the incorporation of Sr with a decrease of incorporation of Ca during crystal formation was observed. In *Corchorus olitorius* plants that were supplemented at the same concentration of 5 μg/cm of Cd, Pb, Al or Cu in the hydroponic culture, however, only Al was incorporated into the CaOx crystals (Mazen, 2004a; Mazen, 2004b). In contrast, no incorporation of Zn and Pb into CaOx crystals was observed in leaves of *Phaseolus vulgaris* although a redistribution of cellular CaOx during heavy metal detoxification was recognized (Jáuregui-Zúñiga *et al*., 2005).

2.3.4. Oxalates in fruits and fruit products

Since oxalate is produced as a by-product of photosynthesis and by cleavage of ascorbic acid in many plants, it is not surprising that oxalate occurs and is stored in the leaves and may also be stored in the fruit. DeBold *et al.*, (2004) reported that in *Vitis vinifera* L, oxalic acid and tartaric acid were derived from cleavage of ascorbic acid via two different pathways in fruit that might be vital to control and to re-mobilize calcium concentration for specific stages of fruit development. Melino *et al.,* (2009) showed evidence for up-regulation of ascorbic acid

biosynthetic pathways and subsequent variation in oxalate accumulation during development of grape fruits in which oxalate was produced by C2/C3 cleavage of ascorbic or L-dehydroascorbic acid. However, several studies found that there was no relationship between vitamin C and oxalate concentration in kiwifruit (Rinallo and Mori, 2000; Rassam and Laing, 2005). Watanabe and Takahashi (1998) found that kiwifruit contain total oxalates in the range of 10.1- 29.9 mg/100 g FW. These oxalates are largely found as calcium oxalate raphides (Rassam and Laing, 2005), which can have a harsh effect in the mouth but are not absorbed in the intestinal tract.

There are numerous studies which focused on concentrations of oxalates in leafy plant foods and the proportion absorbed in the digestive tract (Brinkely *et al.*, 1981; Savage *et al*., 2000; Brogen and Savage, 2003; Chai and Liebman, 2005), however, the concentrations of oxalates occurring in fruits and fruit products have not received sufficient attention. Recently, Hönow and Hesse (2002) published values for fruits commonly available in Europe and a number of other authors (Andrews and Viser, 1951; Zarembski and Hodgkinson, 1962; Herrmann, 1972; Hodgkinson, 1977; Kasidas and Rose, 1980; Ogawa *et al*., 1984; Awadalia *et al*., 1985) have published values for some fruits using older methods of analysis. Information on fruits locally grown and imported into New Zealand is very limited, except kiwifruit and rhubarb. It is also important to measure the oxalate concentration of tropical fruits as these are becoming a more important part of New Zealand diets.

Concentrations of oxalates in food can be affected by the preparation, cooking and processing prior to human consumption. Numerous studies have been conducted to assess the effect of processing factors on oxalate concentration of plant foods, especially leafy vegetables. However, most temperate and tropical fruits are consumed raw; so it is not possible to reduce oxalate concentrations by soaking, boiling and steaming that have been commonly suggested for high oxalate containing leafy vegetables (Savage *et al*., 2000; Savage and Märtensson, 2009).

Fresh fruit that does not meet requirements of international and domestic markets can be processed into semi-finished or finished products such as frozen pulp, nectar, puree, canned sliced fruit or syrup, juices, wines, freeze-dried powder and freeze-dried slices (Venning *et al.*, 1989) in which juices are the most popular products. Fruit products that have been cooked and processed to reduce the moisture content of the final puree concentrate oxalate in the remaining dry matter (Perera *et al.*, 1990). These types of products are widely added as fruit concentrates into fruit pies, ice cream products and muesli bars. Processing fruit to juice

at an acid pH may solubilise the insoluble oxalates making more soluble oxalate available for absorption. In addition, all fruits contain vitamin C, which when boiled under acid conditions may be degraded to oxalates (Hönow and Hesse, 2002). Since such processing involves pasteurization of the juice at high temperatures, it is possible that further vitamin C breakdown may occur and lead to an increase in oxalate concentration. Analysis of these processes has not been undertaken.

2.3.5. Oxalate in human health

2.3.5.1. Kidney stones

Kidney stones are a most painful urological disorder and may be affected by genetic, nutritional and environmental factors. It affects men twice as much as women and seems to be more common in older people. The incidence of stone formation is reported to be increasing across the world (Romero *et al*., 2010). It is possible that this is a result of an increased consumption of vegetables and fruit as people strive to consume a more healthy diet.
Kidney stones that consist primarily of calcium oxalate contribute up to 80% of all kidney stone incidences (Noonan and Savage, 1999). Calcium oxalate is usually present as calcium oxalate monohydrate, which is most common in hyperoxaluria patients or calcium oxalate dehydrates, which is found in patients with hypercalciuria (Ogawa *et al*., 2000).

Oxalates, derived from dietary intake and from endogenous sources in the body, are an end product of mammalian metabolism and have no physiological function; thus, oxalates will be disposed through the renal system. Urinary oxalate concentration is considered as the most important risk factor in the formation calcium oxalate kidney stones (Tiselius, 1997; Ogawa *et al*., 2000). If oxalate concentrations in the urine exceed 40-50 mg/day hyperoxaluria can occur (Nishiura *et al*, 2002), which results from intestinal dysfunction, allowing over absorption of oxalate from food or can be related to an over production of oxalate in the body (Siener *et al.*, 2003).

The risk of stone formation increases dramatically once urinary oxalate exceeds 450 μmol/day (Massey *et al.*, 1993) and the amount of excreted oxalate in stone-forming individuals is much higher than of those non-stone forming individuals (Goldfarb 1988).

2.3.5.2. Mineral deficiency

Oxalates have attracted much attention due to their ability to bind with minerals, especially calcium, to form insoluble salts in the gut and, therefore, reduce the bioavailability of a mineral essential for human health. Groenendyk and Seawright (1974), Waltner-Toews and

Meadows (1980) found that the formation of calcium oxalate from combination of soluble oxalate and calcium available in the intestinal tract causes an acute syndrome, hypocalcaemia, soon after an intake of large amount of oxalate. Hypocalcaemia induces mobilization of bone Ca which over a long period of time results in secondary hyperparathyroidism, or osteodystrophy fibrosa (Sleiman *et al.,* 2004). Studies by Borghi *et al.,* (2002) and Von Unruh *et al.,* (2004) showed that a low-calcium diet increases the risk of calcium oxalate stone formation. Indeed, a high intake of calcium (more than 26.2 mmol/day) in the men who have no kidney stones leads to a reduction of 34% risk of stone formation than those taking a low calcium intake (i.e. less than 15.1 mmol/day) (Curhan *et al.*, 1993).

2.3.6. Dietary oxalate intake and oxalate absorption

It has been suggested that there are two primary sources of oxalates that make up urinary oxalates: endogenous synthesis and absorption from the diet. Hepatic synthesis contributes 40-50%, non-enzymatic breakdown of ascorbic acid provides 40-50% and intestinal absorption from the diet provides 10-20% (Mendonça *et al.,* 2003). However, it has been reported from *in vivo* studies (Liebman and Costa, 2000; Holmes *et al.*, 2001) that dietary oxalates play an important role in calcium oxalate stone formation as a significant part (40-50%) of the renal oxalates comes from dietary oxalate while the remainder is derived from the endogenous source. As endogenous synthesis derives primarily from ascorbic acid and glyoxylate (Hagler and Herman, 1973) this cannot be altered by any treatment, so restriction of dietary oxalate is the only way to reduce urinary oxalate (Heilberg, 2000). Dietary oxalate ranges from 44-351 mg/day depending on various food choices, seasonal consumption and serving sizes (Massey and Kynast-Gales, 1998; Holmes and Kennedy, 2000). Particularly in India, the daily intake of oxalate can reach up to 2,000 mg/day because of habitual consumption of leafy vegetables (Singh *et al*., 1972).

The absorption of soluble oxalate in the digestive tract depends not only on the amount of oxalate but also on the availability of soluble oxalate in each food digested, digestive pH, as well as the composition of the diet and the presence of oxalate-degrading bacteria in the colon (Jaeger and Robertson, 2004). Interestingly, Holmes *et al.,* (2001) discovered that oxalate absorption is higher at lower intakes of oxalates compared to higher intakes. Holmes & Assimos (2004) suggest that oxalate transporters in the gastro-intestinal wall are easily saturated at high oxalate concentrations. Oxalate absorption may increase under fasting conditions (Ogawa, 2000).

2.3.6.1. Oxalate availability

Oxalates in plant tissues are a mixture of water-soluble and water-insoluble salts; soluble oxalates are absorbed while insoluble oxalates pass through the digestive tract (Mendonca *et al*., 2003; Chai and Liebman, 2004; Massey, 2007; Tang *et al*., 2008). However, Hanes *et al.*, (1998) reported that a small fraction of calcium oxalate (2%) was absorbed intact in rats. Chai and Liebman (2004) showed that when the same amount of oxalate was given as a single dose, significantly higher oxalate absorption occurred from almonds (5.9%) than from black beans (1.8%). This appears to be proportional to the soluble oxalate concentration in almonds (31%) and black beans (5%). In a similar study, Tang *et al*., (2008), compared the absorption of soluble oxalate between cinnamon (6% absorbed) and turmeric (91% absorbed) which confirmed that the soluble oxalate in total oxalate fraction plays critical role in the oxalate absorption. However, most data reported in the literature so far gave concentrations of total oxalate (Andrews and Viser, 1951; Zarembski and Hodgkinson, 1962; Herrmann, 1972; Hodgkinson, 1977; Kasidas and Rose, 1980; Ogawa *et al*., 1984; Awadalia *et al*., 1985).

There are numerous factors which affect oxalate distribution in plants including soil nutrient status, plant part, maturity stage and climatic conditions (Libert and Franceschi, 1987). The oxalate availability in plant foods also varies widely between different species of plants and within species of the same plant. Wilson *et al*., (1982) detected oxalate concentration in 15 cultivars of carambola and these values varied 10-fold. To eliminate the influence of growing environment fertilizer level and climactic conditions on the oxalate concentration, Libert and Creed (1985) planted 78 rhubarb cultivars in the same field, in the same year and harvested the plants on the same day. They found that oxalate amounts varied 3-fold between the rhubarb cultivars, ranging from 3.35% to 9.48% of dry weight. Biological variation of oxalates was also noted in golden kiwifruit (*Actinidia chinensis* L) and oca (*Oxalis tuberosa*) where there was a 4.6-fold variation in oxalate of 134 female siblings of an *Actinidia chinensis* genotype (Rassam *et al.*, 2007) and a 2-fold difference in 14 oca cultivars.

Regardless of genetic difference within the same plant, different plant parts may also have different oxalate concentrations. In bamboo sprouts, oxalate concentrations were found to be 3-fold higher between younger and older parts of the plant (462 vs.157 mg/100 g FW) (Kozukue *et al*., 1983). In whole grains, because oxalate is concentrated in the bran fraction, refining wheat to flour reduces the oxalate concentration by one third (Chai and Liebman, 2005). Similarly, Albihn and Savage (2001), found that in three different cultivars of raw oca *(Oxalis tuberosa* Mol.) grown in New Zealand, the oxalate was concentrated 10 times higher

in the skin compared to the outer flesh. The skin of golden kiwifruit contains significantly higher oxalate concentrations as compared to outer pulp; inner pulp and core (Rassam and Laing, 2005). Meanwhile, for leafy vegetables, oxalates are found in higher concentrations in the leaves and lowest in the stems (Savage, 2002). Early reports showed that the stems of plants, such as amaranth, rhubarb, spinach, taro and beet (Fassett, 1973; Concon, 1988; Libert and Franceschi, 1987; Savage and Märtensson, 2010), contain significantly lower oxalate concentrations than the leaves.

Oxalate accumulation at different maturity stages of plants has been reported to be uneven for several plant species. Total oxalate concentrations in spinach were highest at 10.9 (% anhydrous oxalic acid on dry weight basis) after 32 days growth which reduced to 8.4 (% anhydrous oxalic acid on dry weight basis) after the 62 days while soluble oxalate reduced from 6.8 to 5.9 (% anhydrous oxalic acid on dry weight basis) (Kitchen and Burns, 1965). Similarly, Watanabe and Takahashi (1998) analysed oxalate concentrations in four different kiwifruit cultivars and discovered oxalate concentrations in the fruits were highest in the early development stage, reducing during fruit growth and maturation. In contrast, oxalate concentration in Victoria forced rhubarb was 260 mg/100 g of fresh weight, increasing to 620 mg/100 g of fresh weight at the end of season (Zarembski and Hodgkinson, 1962). This observation was supported by Libert (1987) who measured the oxalate concentration of 56 rhubarb genotypes. Total oxalate concentration of *Corchorus olitorius*, a leafy vegetable, followed the same trend, Total oxalates significantly increased from market maturity to fruiting stage, from 3.2 to 8.8 g/100 g dry weight, respectively (Musa *et al.*, 2011).

2.3.6.2. Cooking methods

Many studies have been conducted to assess how different cooking methods affect oxalate availability in food prior to human consumption. Because free oxalate is defined as water-soluble oxalate and also extracted from plant tissue using water, cooking plant food with water showed a consistent effect on the soluble oxalate concentration. Boiling leafy vegetables should be the first choice to reduce oxalate availability if the cooking water is discarded. Indeed, Chai and Liebman, (2005) showed that boiling reduced total oxalate concentration by 30-87%; most of this loss was soluble oxalate. The decreased total oxalate concentration corresponded to the amount of oxalate found in the cooking water. Similarly, boiling was shown to be the most effective method to eliminate oxalate in silver beet leaf, spinach, rhubarb and broccoli (Savage *et al.*, 2000); however, it has been shown that no loss

of oxalate from oca occurs during cooking as losses are prevented by oca tuber skin of the oca (Albihn and Savage, 2000; Sangketkit *et al.* 2001).

In comparison to boiling, baking increased the oxalate concentration in oca by76-79% due to the loss of moisture from the tubers (Albihn and Savage, 2000; Sangketkit *et al*., 2001), however, the same effect did not occur in potatoes (Chai and Leibman, 2005), roasted peanuts (Judprasong *et al*., 2006) or sesame seeds (Toma *et al*., 1979). Although there have been no reports about degradation of oxalate under high treatment temperature, Sefa-Dedeh and Agyir-Sackey (2004) found that oxalate concentration of cocoyams decreased after they were air-dried, solar-dried or drum-dried, they suggested that high temperatures collapsed the calcium oxalate crystals, leading to the breakdown of oxalate structure. Ologhobo, (1989) found that germinated soybeans contained a much lower oxalate concentration compared to soaked and cooked soybeans. Cooking germinated soybeans reduced the oxalate concentration to below that of uncooked germinated soybeans (Ologhobo, 1989). Fermented products, commonly used in Asian countries, were found to contain significantly lower concentrations of oxalate compared to the concentrations before processing. Fermenting poi, a cooked taro paste, decreased oxalate concentration by 37% after seven day at 20^0C (Huang *et al.*, 1994*).* Likewise, the oxalate concentration was reduced by 78% following fermentation of a starch containing tuber, *Icacinia manni* (Antai and Obong, 1992).

Cooking and consumption of high calcium containing foods (e.g. milk and milk products) along with known oxalate containing foods has been shown to considerably reduce oxalate absorption (Brogren and Savage, 2003; Johansson and Savage, 2011).

2.3.6.3. Digestive pH

Holmes and Assimos (2004) suggest that oxalate may be absorbed passively at all segments of the gastrointestinal tract, however, depending on the form of oxalate and food composition, which can change conditions of the digestive pH, oxalate absorption is more likely to occur in the stomach, small intestine or in the colon. Although it is generally considered that only soluble oxalate can be absorbed, the pH range and changes along the GI tract may have a significant impact on the absorption of oxalate. In the stomach the pH is 1.5-2.0, and it is likely that all oxalates are dissolved and potentially available for absorption. In the intestine where the pH ranges from 7-8, some dissolved minerals and oxalate ion will re-precipitate, leading to less oxalate available for absorption. However, it has been shown that oxalate absorption mainly occurs in the small intestine (Holmes *et al*., 1995; Hanes *et al*., 1999; Zimmerman *et al.*, 2005) with the greatest absorption occurring in the lower small intestine

(ileum) (Hanes *et al*., 1999). Furthermore, *in vitro* preparations using isolated, short-circuited segment of rabbit distal colon, Hatch *et al*., (1994) demonstrated that oxalate absorption and secretion could occur in the distal colon. It has been suggested that calcium and phosphate combine with each other in the alkaline conditions leaving free oxalates to be absorbed passively (Jaeger and Robertson, 2004).

Mineral-oxalate binding is pH dependent and binding prevents many minerals (e.g. Ca) from being absorbed in the digestive tract. The free oxalate ion ($C_2O_4^{2-}$) is potentially available to bind to calcium, while the binding capacity is reduced if the semi-dehydro-oxalic acid ($HC_2O_4^-$) or oxalic acid ($H_2C_2O_4$) species are present. The effect of pH on the relative abundance of each oxalate species was discussed by Simpson *et al.,* (2009) and showed that semi-dehydro-oxalic acid is more abundant between pH 2.5 and 4.5 and the oxalate anion is more abundant between pH 4.5 and 6.5. Previous studies have shown that when the relative abundance of the common divalent cations Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+} in foods are considered along with their solubility constants (K_{sp}) , Ca^{2+} oxalate is the least soluble of these salts and is most likely to be the main constituent of the insoluble oxalate concentration of plant foods.

The amount of ingested oxalate absorbed may vary depending on oxalate form, mineral concentration, especially calcium and the presence of fibre in each part of the gastrointestinal tract (Jaeger and Robertson, 2004). Maximum of gastric oxalate absorption for free oxalate is 2.5% (Jaeger and Robertson, 2004) whilst intestinal oxalate absorption ranges between 5-15% depending on the co-ingestion of calcium magnesium and fibre (Holmes *et al*., 1995) or from 2.2 to 18.8% depending on intra-individual oxalate absorption variability (von Unruh *et al*., 2003). A high level of soluble oxalate along with low calcium concentration allows the absorption of oxalate to occur in the stomach. In contrast, the higher the calcium concentration in the stomach, the lower the solution of ingested CaOx the lower the oxalate absorption at this site (Jaeger and Robertson, 2004).

Several intestinal microorganisms have been reported to have ability to degrade oxalate; the most studied species is *Oxalobacter formigenes*, an anaerobic bacterium that inhabits the colon and uses oxalate as its sole source of metabolic energy (Siva *et al.,* 2009). A direct correlation between the number of recurrent kidney stone episodes and the lack of *Oxalobacter* colonization was reported by Sidhu *et al.*, (1999). The absence of *O. formigenes* in the intestinal tract of cystic fibrosis patients was also found this leads to an increase of oxalate absorption, thereby increasing the risk of hyperoxaluria (Sidhu *et al.,* 1998). In

addition to *O. formigenes*, other bacterial species colonizing the intestinal tract may also degrade oxalate including *Lactobacillus acidophilus, Bifidobacterium lactis, Eubacterium lentum, Providencia rettgeri* and *Enterococcus faecalis* (Siva *et al*., 2009). Overall, it has been stated that the lack of intestinal *Oxalobacter* activity can be considered as a risk factor for hyperoxaluria and stone disease, however, the absence of this intestinal bacterium solely is not a direct cause of renal stone formation because of the multifactorial nature of the disease (Hatch and Freel, 2005).

2.3.6.4. Dietary constituents

Oxalate bioavailability depends not only oxalate availability and gastrointestinal factors but also on the composition of the diet in which minerals and dietary fibre has been attracted more interest.

Mineral bioavailability

The crucial role of pH changes within gastrointestinal tract as well as the concentration of key ions, which can bind to oxalate in the gastrointestinal tract such as calcium, magnesium, are important factors that can affect mineral bioavailability (Jaeger and Robertson, 2004). Among intraluminal factors affecting oxalate absorption, calcium availability has a significant effect because of its binding ability with soluble oxalate which therefore leaves less oxalate available for absorption. Encouraging patients with calcium oxalate stones to consume more calcium in the diet has been successful. An inverse relationship between calcium intake and idiopathic oxalate was demonstrated in a cohort study of 45,619 healthy men over four years (Curhan *et al*., 1993). The inverse association was supported directly or indirectly by numerous studies assessing dietary calcium intake and hyperoxaluria symptoms or stone formation. It was demonstrated that when the intraluminal concentration of calcium is increased, the intestinal oxalate absorption is reduced (Liebman and Costa, 2000; Hess *et al*., 1998, Nishiura *et al*., 2002). Likewise, the intestinal absorption and renal excretion of oxalate were enhanced when the intraluminal calcium was lowered (Jaeger *et al*., 1985; Massey *et al*., 1993, Holmes *et al*., 2001). A restriction of dietary calcium intake would result in increases in the relative super saturation of calcium oxalate in the urine (Messa *et al*, 1997). Using both diet and supplements of calcium was shown to be an effective practice for treating enteric hyperoxaluria (Hylander *et al.*, 1980; Curhan *et al*., 2004).

However, it is interesting to note that oxalate absorption was not reduced completely to zero even when an excess of calcium was provided (Liebman and Chai, 1997; Brogren and Savage, 2003; Märtenson and Savage, 2008). It is proposed from these data that there exists a

multitude of important digestive factors which interfere with oxalate absorption. The factors may be intraluminal pH changes that induce oxalate solubility or the existence of other competitive food components that could bind to calcium in higher affinity relative to binding ability between calcium and oxalate.

Dietary fibre

Oxalate absorption is not only affected by the concentration of dietary minerals but also by fibre intake. Increased dietary fibre intake has been attributed to a lower risk of renal stone formation (Hughes and Norman, 1992; Siener *et al*., 2003). Griffith *et al.,* (1981) showed that renal stone patients consumed significantly lower fibre intakes compared to a matched group of healthy people. Fibre has an inverse effect on oxalate absorption by mechanically binding with CaOx crystals or chemically binding with soluble oxalate and then reducing oxalate concentration available for intestinal absorption (Hanson *et al*., 1989). There are many studies that have reported the positive effect of dietary fibre in reducing calcium absorption and urinary calcium excretion in hypercalciuric patients (Shah *et al*., 1980; Tizzani *et al*., 1989; Jahnen *et al*., 1992); however, there has been controversy surrounding the role of dietary fibre in oxalate kidney stone prevention. Several lines of evidence (Ohkawa *et al*., 1984; Ebisuno *et al.,* 1986) showed there was slight increase of oxalate excretion with fibre supplementation from bran. This was attributed to the high oxalate concentration in the bran consumed in the studies (Jahnen *et al*., 1992; Gleeson *et al*., 1990) or binding between fibre and calcium left more oxalate available for absorption (Hughes and Norman, 1992). Meanwhile some studies showed an unchanged urinary oxalate output after oxalate loading (Ala-Opas *et al.,* 1987; Gleeson *et al*., 1990). In contrast, some reported studies showed an association between a higher dietary fibre intake and a lower in urinary oxalate output (Kelsay and Prather, 1983; Strohmaier *et al*., 1988; Hanson, 1989). It has been suggested that reducing gastrointestinal transit time by increasing dietary fibre in the diet shortened the time for oxalate absorption (Assimos and Holmes, 2000). In a study using a modified diet alone to eliminate interfering elements, the positive role of dietary fibre in reducing both urinary oxalate (hyperoxaluria) and urinary calcium (hypercalciuria) was confirmed (Firth and Norman, 1990). Overall, the direct effect of dietary fibre on oxalate absorption is not clearly understood (Hatch and Freel, 2005).

Dietary fibre comprises of water-insoluble fibres (cellulose, hemicelluloses and lignin) and water-soluble fibre (pectin, gum and mucilages) (Dhingra *et al*., 2011). Pectin is a major

structural polysaccharide component of the plant cell walls and used widely as a gelling agent in the food industry. Its molecular structure is principally composed of linearly connected

α 1-4 D-galacturonic acid units and its methyl esters disrupted by some 1-2 linked rhamnose residues (BeMiller, 1986). Pectin with a low degree of esterification (< 50%) can gel in the presence of divalent cations such as calcium via calcium-pectate linkages occurring in the Egg-box Model proposed by Thakur *et al*., (1997). Binding between calcium-pectate and oxalates was reported by Hoagland (1989). Reduced soluble oxalate concentrations after mixing with alcohol insoluble residue comprised primarily of pectin, suggested that oxalates may remain in the calcium-pectate complex of plant tissues. The interaction would result in underestimation of oxalate concentrations in plant samples which contain appreciable pectin concentrations. However, no direct effect of fibre components on oxalate analysis has been reported.

2.4. Summary

Although a number of studies focusing on the determination of antioxidant compounds in fruit and their antioxidant capacity have been carried out over many years, using unapproved and non-specific methods in several studies have produced some unreliable and conflicting data. Therefore, it is important to apply standardised methods to obtain comparable antioxidant data for single fruit types in each geographical area. However, when an advised amount of fruit consumed to reach to healthy diet, which concentrations of oxalates are contributing to which fruit? Does processing and storage of fruit change oxalate concentrations prior to human consumption? Do existing dietary factors affect oxalate availability and therefore oxalate absorption during human digestion? However, to answer these questions, the first important requirement is an accurate analytical method. There are several methods being use to determine oxalates in plant tissues, however, some are very inaccurate as they overlook interfering substances during oxalate extraction and measurement, resulting in under or overestimates of oxalate concentrations. Using a biological method stimulated to be as close as possible to the biological conditions in humans to identify dietary factors influencing oxalate absorption is the second essential requirement. When all these issues are resolved, data released would be more accurate and applicable for giving good dietary advice. Consequently, the prevention and treatment of kidney stones would become more efficient and cost effective.

References

Ader, P., Wessmann, A., & Wolffram, S. (2000). Bioavailability and metabolism of the flavonol quercetin in the pig. *Free Radical Biology and Medicine, 28*(7), 1056-1067.

Ala-Opas, M., Elomaa, I., Porkka, L., & Alfthan, O. (1987). Unprocessed bran and intermittent thiazide therapy in prevention of recurrent urinary calcium stones. *Scandinavian Journal of Urology and Nephrology, 21*(4), 311-314.

Albihn, P. B. E., & Savage, G. P. (2001). The effect of cooking on the location and concentration of oxalate in three cultivars of New Zealand-grown oca (*Oxalis tuberosa* Mol). *Journal of the Science of Food and Agriculture, 81*(10), 1027-1033.

Albihn, P., & Savage, G. (2000). Effect of cooking on the soluble oxalate content of three cultivars of oca (*Oxalis tuberosa*). *Proceedings of the Nutrition Society of New Zealand*, 25, 66–70.

Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences, 90*(17), 7915-7922.

Andrews, J. C. & Viser, E. T. (1951). The oxalic acid content of some common foods. *Food Research, 16*, 306–312.

Antai, S. P., & Obong, U. S. (1992). The effect of felmentation on the nutritent status and on some toxic components of *Icacinia manni. Plant Foods for Human Nutrition, 42*,219-224.

Arts, I. C. W., & Hollman, P. C. H. (2005). Polyphenols and disease risk in epidemiologic studies. *The American Journal of Clinical Nutrition, 81*(1), 317S-325S.

Aruoma, O. (1998). Free radicals, oxidative stress, and antioxidants in human health and disease. *Journal of the American Oil Chemists' Society, 75*(2), 199-212.

Awadalia, M. Z., Bassily, N. S. & Saba, N. A. (1985). Oxalate content of some Egyptian foodstuffs. *Journal of the Egyptian Public Health Association, 60*(3), 41–51.

Bagchi, K., & Puri, S. (1998). Free radicals and antioxidants in health and disease. *East Mediterranean Health Journal, 4*, 350-360.

BeMiller, J. N. (1986). An introduction to pectins: structure and properties. In *Chemistry and Function of Pectins* (310 ed., pp. 2-12): American Chemical Society, New York, USA.

Bielski, B. H. J., Richter, H. W., & Chan, P. C. (1975). Some properties of the ascorbate free radical. *Annals of the New York Academy of Sciences, 258*(1), 231-237.

Bisby, R. H., Brooke, R., & Navaratnam, S. (2008). Effect of antioxidant oxidation potential in the oxygen radical absorption capacity (ORAC) assay. *Food Chemistry, 108*(3), 1002-1007.

Bjelakovic, G., Nikolova, D., Gluud, L. L., Simonetti, R. G., & Gluud, C. (2007). Mortality in randomized trials of antioxidant supplements for primary and secondary prevention. *The Journal of the American Medical Association, 297*(8), 842-857.

Blasa, M., Gennari, L., Angelino, D., & Ninfali, P. (2010). Fruit and vegetable antioxidants in health. In R. R. Watson & V. R. Preedy (Eds.), *Bioactive foods in promoting health: fruits and vegetables* (pp. 37-58). New York: Elsevier Inc.

Borchert, R. (1986). Calcium acetate induces calcium uptake and formation of calcium-oxalate crystals in isolated leaflets of *Gleditsia triacanthos* L. and *Albizia julibrissin Durazz* L. *Planta, 168*(4), 571-578.

Borghi, L., Schianchi, T., Meschi, T., Guerra, A., Allegri, F., Maggiore, U., & Novarini, A. (2002). Comparison of two diets for the prevention of recurrent stones in idiopathic hypercalciuria. *New England Journal of Medicine, 346*(2), 77-84.

Bradbury, J. H., & Nixon, R. W. (1998). The acridity of raphides from the edible aroids. *Journal of the Science of Food and Agriculture, 76*(4), 608-616.

Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews, 56*(11), 317-333.

Brinkley, L. R. D., MgGuire, J. M. D., Gregory, J. M. D., & Pak, C. Y. C. (1981). Bioavailability of oxalate in foods. *Urology, 17*(6), 534-538.

Brogren, M., & Savage, G. P. (2003). Bioavailability of soluble oxalate from spinach eaten with and without milk products. *Asia Pacific Journal of Clinical Nutrition*, *12*, 219-224.

Burton, G. W., & Ingold, K. U. (1984). Beta-carotene: an unusual type of lipid antioxidant. *Science, 224*(4649), 569-573.

Cadenas, E. (1997). Basic mechanisms of antioxidant activity. *BioFactors, 6*(4), 391-397.

Cai, R., Hettiarachchy, N. S., & Jalaluddin, M. (2003). High-performance liquid chromatography determination of phenolic constituents in 17 varieties of cowpeas. *Journal of Agricultural and Food Chemistry, 51*, 1623-1627.

Cai, Y., Luo, Q., Sun, M., & Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences, 74*(17), 2157-2184.

Cao, G., & Prior, R. L. (1998). Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clinical Chemistry, 44*(6), 1309-1315.

Cao, G., Alessio, H. M., & Cutler, R. G. (1993). Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biology and Medicine, 14*(3), 303-311.

Chai, W., & Liebman, M. (2004). Assessment of oxalate absorption from almonds and black beans with and without the use of an extrinsic label. *The Journal of Urology, 172*(3), 953-957.

Chai, W., & Liebman, M. (2005). Effect of different cooking methods on vegetable oxalate content. *Journal of Agricultural and Food Chemistry* 53(8), 3027-3030.

Chang, C.-C., & Beevers, H. (1968). Biogenesis of oxalate in plant tissues. *Plant Physiology, 43*(11), 1821-1828.

Choi, Y. E., Harada, E., Wada, M., Tsuboi, H., Morita, Y., Kusano, T., & Sano, H. (2001). Detoxification of cadmium in tobacco plants: formation and active excretion of crystals containing cadmium and calcium through trichomes. *Planta, 213*(1), 45-50.

Cieslik, E., Greda, A., & Adamus, W. (2006). Contents of polyphenols in fruit and vegetables. *Food Chemistry, 94*(1), 135-142.

Concon, J.M., (1988). Food Toxicology-Principles and Concepts. New York: Marcel Dekker.

Connor, A. M., Luby, J. J., Tong, C. B. S., Finn, C. E., & Hancock, J. F. (2002). Genotypic and environmental variation in antioxidant activity, total phenolic content, and

anthocyanin content among blueberry cultivars. *Journal of the American Society for Horticultural Science, 127*(1), 89-97.

Costantino, L., Albasini, A., Rastelli, G., & Benvenuti, S. (1992). Activity of polyphenolic crude extracts as scavangers of superoxide radicals and inhibitors of xanthine oxidase. *Planta Medica, 58*(04), 342-344.

Curhan, G. C., Willett, W. C., Knight, E. L., & Stampfer, M. J. (2004). Dietary factors and the risk of incident kidney stones in younger women: nurses' health study II. *Archives of Internal Medicine, 164*(8), 885-891.

Curhan, G. C., Willett, W. C., Rimm, E. B., & Stampfer, M. J. (1993). A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones. *New England Journal of Medicine, 328*(12), 833-838.

Dai, J., & Mumper, R.J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules,15*, 7313-7352.

Datta, P.K. & Meeuse, B.J.D. (1955). Moss oxalic acid oxidase: a *flavoprotein. Biochimica et Biophysica* Acta, *17, 602–603.*

De la Rosa, G., Martínez, A., Castillo, H., Fuentes-Ramírez, R. Gardea-Torresdey, J. (2009). Insights into the mechanisms of Cd hyperaccumulation in S. kali, a desert plant species. *Nova Scientia* 2(1), 33-53.

DeBold, S., Hardie, J., Tyerman, S., & Ford, C.M. (2004). Composition and synthesis of raphide crystals and druse crystals in berries of *Vitis vinifera* L. cv. Cabernet Sauvignon: ascorbic acid as precursor for both oxalic and tartaric acids as revealed by radiolabelling studies. *Australian Journal of Grape and Wine Research, 10*, 134–142.

DeKock, P. C., Ohta, Y., Inkson, R. H. E., & Knight, A. H. (1973). The effect of oxalate and ethylenediaminetetraacetic acid on the absorption of calcium into *Lemna*. *Physiologia Plantarum, 28*(3), 379-382.

Dhingra, D., Michael, M., Rajput, H., & Patil, R. Dietary fibre in foods: a review. *Journal of Food Science and Technology*, 1-12.

Ebisuno, S., Morimoto, S., Yoshida, T., Fukatani, T., Yasukawa, S., & Ohkawa, T. (1986). Rice-bran treatment for calcium stone formers with idiopathic hypercalciuria. *British Journal of Urology, 58*(6), 592-595.

Erickson, C., Morton, H. L., Tisdale, E. W., & Zappettini, G. (1952). The present status of the halogeton problem. *Weeds, 1*(4), 321-328.

Erkan, N., Ayranci, G., & Ayranci, E. (2008). Antioxidant activities of rosemary (*Rosmarinus Officinalis* L.) extract, blackseed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesamol. *Food Chemistry, 110*(1), 76-82.

Fassett, D. W. (1973). Oxalates. In *Toxicants occuring naturally in foods,* (2nd ed.) (pp.346-362). Washington, D. C: National Academy of Science.

Finkle, B. J., & Arnon, D. I. (1954). Metabolism of isolated cellular particles from photosynthetic tissues. *Physiologia Plantarum, 7*(4), 614-624.

Finley, D. S. (1999). Patterns of calcium oxalate crystals in young tropical leaves: a possible role as an anti-herbivory defense. *Revista de Biología Tropical, 47*, 27-31.

Firth, W.A., & Norman, R.W. (1990). The effects of modified diets on urinary risk factors for kidney stone disease. *The Journal of the American Dietetic Association, 51*, 404-408.

Folin, O. (1927). Tyrosine and tryptophan determinations in proteins. *The Journal of Biological Chemistry, 73*, 672-649.

Foster, A. S. (1956). Plant idioblasts: remarkable examples of cell specialization. *Protoplasma, 46*(1), 184-193.

Foy, C. D., Chaney, R. L., & White, M. C. (1978). The physiology of metal toxicity in plants. *Annual Review of Plant Physiology, 29*(1), 511-566.

Franceschi, V. R. (1989). Calcium oxalate formation is a rapid and reversible process in *Lemna minor L*. *Protoplasma, 148*(2), 130-137.

Franceschi, V. R., & Nakata, P. A. (2005). Calcium oxalate in plants: formation and function. *Annual Review of Plant Biology, 56*(1), 41-71.

Franceschi, V. R., & Schueren, A. M. (1986). Incorporation of strontium into plant calcium oxalate crystals. *Protoplasma, 130*(2), 199-205.

Franceschi, V., & Horner, H. (1980). Calcium oxalate crystals in plants. *The Botanical Review, 46*(4), 361-427.

Franceschi, V.R. (1987). Oxalic acid metabolism and calcium oxalate formation in *Lemna minor* L. *Plant, Cell and Environment, 10*, 397–406.

Frei, B., England. L., & Ames. B.N. (1989). Ascorbate is an outstanding antioxidant in human blood plasma. *Proceedings of the National Academy of Sciences of the United States of America, 86,* 6377-6381.

Frutos, P., Duncan, A. J., Kyriazakis, I., & Gordon, I. J. (1998). Learned aversion towards oxalic acid-containing foods by goats: does rumen adaptation to oxalic acid influence diet choice? *Journal of Chemical Ecology, 24*(2), 383-397.

Gallaher, R. N. (1975). The occurrence of calcium in plant tissue as crystals of calcium oxalate. *Communications in Soil Science and Plant Analysis, 6*(3), 315 - 330.

Giachetti, E., Pinzauti, G., Bonaccorsi, R., Teresa Vincenzini, M., & Vanni, P. (1987). Isocitrate lyase from higher plants. *Phytochemistry, 26*(9), 2439-2446.

Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., & Kader, A. A. (2002). Antioxidant capacities, phenolic compounds, carotenoids, and vitamin c contents of nectarine, peach, and plum cultivars from California. *Journal of Agricultural and Food Chemistry, 50*(17), 4976-4982.

Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry, 48*(12), 909-930.

Giovanelli, J., & Tobin, N. F. (1964). Enzymic decarboxylation of oxalate by extracts of plant tissue. *Plant Physiology, 39*(2), 139-145.

Gleeson, M. J., Sue Thompson, A., Mehta, S., & Griffith, D. P. (1990). Effect of unprocessed wheat bran oncalciuria and oxaluria in patients with urolithiasis. *Urology, 35*(3), 231-234.

Goldfarb, D. S., Modersitzki, F., & Asplin, J. R. (2007). A randomized, controlled trial of lactic acid bacteria for idiopathic hyperoxaluria. *Clinical Journal of the American Society of Nephrology, 2*(4), 745-749.

Gómez-Ruiz, J. Á., Leake, D. S., & Ames, J. M. (2007). *In vitro* antioxidant activity of coffee compounds and their metabolites. *Journal of Agricultural and Food Chemistry, 55*(17), 6962-6969.

Gorinstein, S., Martin-Belloso, O., Lojek, A., Číž, M., Soliva-Fortuny, R., Park, Y.-S., & Trakhtenberg, S. (2002). Comparative content of some phytochemicals in Spanish apples, peaches and pears. *Journal of the Science of Food and Agriculture, 82*(10), 1166-1170.

Griffith, H. M., O'Shea, B., Kevany, J. P., & McCormick, J. S. (1981). A control study of dietary factors in renal stone formation. *British Journal of Urology, 53*(5), 416-420.

Groenendyk, S., & Seawright, A. A. (1974). Osteodystrophia fibrosa in horses grazing setaria sphacelata. *Australian Veterinary Journal, 50*(3), 131-132.

Hagler, L. & Herman. R.H. (1973). Oxalate metabolism. *The American Journal of Clinical Nutrition, 26*, 758- 765.

Hall, J. L. (2002). Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Experimental Botany, 53*(366), 1-11.

Halladay, S.C., Ryerson, B.A., Smith, C.R., Brown, J.P., & Parkinson, T.M. (1980). Comparison of effects of dietary administration of butylated hydroxytoluene or a polymeric antioxidant on the hepatic and intestinal cytochrome P-450 mixed-function-oxygenase system of rats. *Food and Cosmetics Toxicology, 18*(6), 569-574.

Halliwel, B (2001). Vitamin C and genomic stability. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 475*(1–2), 29-35.

Halliwell, B. (1995). Antioxidant characterization: Methodology and mechanism. *Biochemical Pharmacology, 49*(10), 1341-1348.

Halliwell, B. (1996). Antioxidants in human health and disease. *Annual Review of Nutrition, 16*(1), 33-50.

Halliwell, B. (2006). Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiology, 141*, 312-322

Halliwell, B., & Gutteridge, J. M. C. (1989). Role of free radical and catalytic metals ions in human diseases: An overview. *Methods Enzymologia, 186*, 1–85.

Halliwell, B., & Gutteridge, J.M.C. (Eds.). (2007). Free radicals in biology and medicine (4th ed.). Oxford: Oxford University Press.

Halliwell. (1999). Vitamin C: poison, prophylactic or panacea? *Trends in Biochemical Sciences, 24*(7), 255-259.

Halvorsen, B. L., Carlsen, M. H., Phillips, K. M., Bøhn, S. K., Holte, K., Jacobs, D. R., & Blomhoff, R. (2006). Content of redox-active compounds (ie, antioxidants) in foods consumed in the United States. *The American Journal of Clinical Nutrition, 84*(1), 95-135.

Hanes, D. A., Weaver, C. M., Heaney, R. P., & Wastney, M. E. (1999). Absorption of calcium oxalate does not require dissociation in rats. *The Journal of Nutrition, 129*, 170–173.

Hanson, C. F., Frankos, V. H., & Thompson, W. O. (1989). Bioavailability of oxalic acid from spinach, sugar beet fibre and a solution of sodium oxalate consumed by female volunteers. *Food and Chemical Toxicology, 27*(3), 181-184.

Harborn, J.B .(1980). Plant phenolics. In E.A. Bell & Charlwood.B.V (Eds), *Encyclopedia of Plant Physiology*, volume 8 Secondary Plant Products, (pp.329-395). Berlin Heidelberg New York: Springer-Verlag.

Harborne, J. B., & Williams, C. A. (2000). Advances in flavonoid research since 1992. *Phytochemistry, 55*(6), 481-504.

Hatch, M., & Freel, R. W. (2005). Intestinal transport of an obdurate anion: oxalate. *Urological Research, 33*(1), 1-16.

Hatch, M., Freel, R. W., & Vaziri, N. D. (1994). Mechanisms of oxalate absorption and secretion across the rabbit distal colon. *Pflügers Archiv European Journal of Physiology, 426*(1), 101-109.

Hayaishi, O., Shimazono, H., Katagiri, M., & Saito, Y. (1956). Enzymatic formation of oxalate and acetate from oxaloacetate. *Journal of the American Chemical Society, 78*(19), 5126-5127.

Heilberg, I. P. (2000). Update on dietary recommendations and medical treatment of renal stone disease. *Nephrology Dialysis Transplantation, 15*(1), 117-123.

Heim, K. E., Tagliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *The Journal of Nutritional Biochemistry, 13*(10), 572-584.

Heinonen, I. M., Meyer, A. S., & Frankel, E. N. (1998). Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. *Journal of Agricultural and Food Chemistry, 46*(10), 4107-4112.

Heldt, H.W. (1997). Polysaccharides. In: *Plant Biochemistry & Molecular Biology* (pp. 219-246). New York, USA: Oxford University Press Inc.

Henning, S. M., Seeram, N. P., Zhang, Y., Li, L., Gao, K., Lee, R.-P., Wang, D. C., et al. (2010). Strawberry consumption is associated with increased antioxidant capacity in serum. *Journal of Medicinal Food, 13*(1), 116-122.

Hepler, P. K. (2005). Calcium: a central regulator of plant growth and development. *The Plant Cell Online, 17*(8), 2142-2155.

Herrmann, K. (1972). Über den Oxälsa uregehalt des Obstes und Gemüses. *Zeitschrift Lebensmitteluntersuchung, 148,* 206–210.

Hess, B., Jost, C., Zipperle, L., Takkinen, R., & Jaeger, P. (1998). High-calcium intake abolishes hyperoxaluria and reduces urinary crystallization during a 20-fold normal oxalate load in humans. *Nephrology Dialysis Transplantation, 13*(9), 2241-2247.

Hoagland, P. D. (1989). Binding of dietary anions to vegetable fiber. *Journal of Agricultural and Food Chemistry, 37*(5), 1343-1347.

Holmes, P. R., Goodman, H. O., & Assimos, D. G. (1995). Dietary oxalate and its intestinal absorption. *Scanning Microscopy, 9*, 1109–1120.

Holmes, R. P., & Assimos, D. G. (2004). The impact of dietary oxalate on kidney stone formation. *Urological Research, 32*(5), 311-316.

Holmes, R. P., Goodman, H. O., & Assimos, D. G. (2001). Contribution of dietary oxalate to urinary oxalate excretion. *Kidney Int, 59*(1), 270-276.

Holmes, R.P., & Kennedy, M. (2000)**.** Estimation of the oxalate content of foods and daily oxalate intake. *Kidney International, 57,* 1662–1667.

Hönow, R. & Hesse, A. (2002). Comparison of extraction methods for the determination of soluble and total oxalate in foods by HPLC enzyme-reactor. *Food Chemistry, 78*, 511-521.

Howard, L. R., Clark, J. R., & Brownmiller, C. (2003). Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. *Journal of the Science of Food and Agriculture, 83*(12), 1238-1247.

Hu, C., Cai, Y.-Z., Li, W., Corke, H., & Kitts, D. D. (2007). Anthocyanin characterization and bioactivity assessment of a dark blue grained wheat (*Triticum aestivum* L. cv. Hedong Wumai) extract. *Food Chemistry, 104*(3), 955-961.

Huang, A. S., Lam, S. Y., & Nakayama, T. M. (1994). Microbiological chemical changes in poi stored at 20°C. *Joumal of Agriculture and Food Chemistry*, 42, 45-48.

Huang, A. S., Lam, S. Y., Nakayama, T. M., & Lin, H. (1994). Microbiological and chemical changes in poi stored at 20^0C. *Journal of Agricultural and Food Chemistry, 42*(1), 45-48.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*(6), 1841-1856.

Hughes, J., & Norman, R.W. (1992). Diet and calcium stones. *Canadian Medical Association Journal, 146*(2), 137–143.

Hylander, E., Jarnum, S., & Nielsen, K. (1980). Calcium treatment of enteric hyperoxaluria after jejunoileal bypass for morbid obesity. *Scandinavian Journal of Gastroenterology, 15*(3), 349-352.

Ilarslan, H., Palmer, R. G., & Horner, H. T. (2001). Calcium oxalate crystals in developing seeds of soybean. *Annals of Botany, 88*(2), 243-257.

Ilarslan, H., Palmer, R. G., Imsande, J., & Horner, H. T. (1997). Quantitative determination of calcium oxalate and oxalate in developing seeds of soybean (Leguminosae). *American Journal of Botany, 84*(8), 1042.

Ito, N., Fukushima, S., Hasegawa, A., Shibata, M., & Ogiso, T. (1983). Carcinogenicity of butalated hydroxyanisole in F344 rats. *Journal of National Cancer Institute, 70*, 343-347.

Jaeger, P., & Robertson, W. G. (2004). Role of Dietary Intake and Intestinal Absorption of Oxalate in Calcium Stone Formation. *Nephron Physiology, 98*(2), 64-71.

Jaeger. P., Portmann. L., Jacquet. A.F., & Burckhardt. P. (1985). Influence of the calcium content of the diet on the incidence of mild hyperoxaluria in idiopathic renal stone formers. *American journal of Nephrology, 5*(1), 40-44

Jahnen, A., Heynck, H., Gertz, B., Claßen, A., & Hesse, A. (1992). Dietary fibre: the effectiveness of a high bran intake in reducing renal calcium excretion. *Urological Research, 20*(1), 3-6.

Jakoby, W. B., Ohmura. E., &Hayaishi.O. (1956). Enzymatic decarboxylation of oxalic acid. *The Journal of Biological Chemistry, 222*, 435-446.

James, L. F. (1972). Oxalate toxicosis. *Clinical Toxicology, 5*(2), 231-243.

James, L. F., & Butcher, J. E. (1972). Halogeton poisoning of sheep: effect of high level oxalate intake. *Journal of Animal Science, 35*(6), 1233-1238.

James, L. F., & Cronin, E. H. (1974). Management practices to minimize death losses of sheep grazing halogeton-infested range. *Journal of Range Management, 27*(6), 424-426.

Jáuregui-Zúñiga, D., Angeles Ferrer, M., Calderón, A. A., Muñoz, R., & Moreno, A. (2005). Heavy metal stress reduces the deposition of calcium oxalate crystals in leaves of Phaseolus vulgaris. *Journal of Plant Physiology, 162*(10), 1183-1187.

Jayasinghe, C., Gotoh, N., Aoki, T., & Wada, S. (2003). Phenolics composition and antioxidant activity of sweet basil (*Ocimum basilicum* L.). *Journal of Agricultural and Food Chemistry, 51*, 4442-4449.

Jin, Z. X., Wang, C., Dong, W., & Li, X. (2007). Isolation and some properties of newly isolated oxalate-degrading Pandoraea sp. OXJ-11 from soil. *Journal of Applied Microbiology, 103*(4), 1066-1073.

Jin, Z.-X., Wang, C., Chen, W., Chen, X., & Li, X. (2007). Induction of oxalate decarboxylase by oxalate in a newly isolated Pandoraea sp. OXJ-11 and its ability to protect against Sclerotinia sclerotiorum infection. *Canadian Journal of Microbiology, 53*(12), 1316-1322.

Johansson, S., & Savage, G. P. (2011). The availability of soluble oxalates in stir-fried silver beet (*Beta vulgaris* var. cicla) leaves eaten with yoghurt. *International Journal of Food Science & Technology, 46*(11), 2232-2239.

Jomova, K., Vondrakova, D., Lawson, M., & Valko, M. (2010). Metals, oxidative stress and neurodegenerative disorders. *Molecular and Cellular Biochemistry, 345*(1), 91-104.

Jonassen, J., Kohjimoto, Y., Scheid, C., & Schmidt, M. (2005). Oxalate toxicity in renal cells. *Urological Research, 33*(5), 329-339

Joy, K. W. (1964). Accumulation of oxalate in tissues of sugar-beet, and the effect of nitrogen supply. *Annals of Botany, 28*, 689-701.

Judprasong, K., Charoenkaitkul, S., Sungpang, P., Vasanachitt, K., & Nakjamanong, Y. (2006). Total and soluble oxalate contents in Thai vegetables, cereal grains and legume seeds and their changes after cooking. *Journal of Food Composition and Analysis, 19*, 340-347.

Kader, A. A., Perkins-Veazi, P., & Lester. G.E. (2003). Nutritional quality of fruits, nuts, and vegetables and their importance in human health. Retrieved from http://www.ba.ars.usda.gov/hb66/025nutrition.pdf

Kasidas, G. P. & Rose, G. A. (1980). Oxalate contents of some common foods: determination by an enzymatic method. *Journal of Human Nutrition, 34*, 255–266.

Katalinic, V., Milos, M., Kulisic, T., & Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry, 94*(4), 550-557.

Keates, S. E., Tarlyn, N. M., Loewus, F. A., & Franceschi, V. R. (2000). L-Ascorbic acid and L- galactose are sources for oxalic acid and calcium oxalate in *Pistia stratiotes*. *Phytochemistry, 53*(4), 433-440.

Kelsay, J.L., & Prather, E.S. (1983). Mineral balances of human subjects consuming spinach in a low-fiber diet and in a diet containing fruits and vegetables**.** *American Journal of Clinical Nutrition, 38*, 12-19.

Khambata, S. R., & Bhat, J. V. (1954). Decomposition of oxalate by streptomyces. *Nature, 174*(4432), 696-697.

Khambata, S. R., & Bhat, J. V. (1955). Decomposition of oxalate by mycobacterium lacticola isolated from the intestine of earthworms. *Journal of Bacteriology, 69*(2), 227-228.

Kitchen, J. W., & Burns, E. E. (1965). The Effect of maturity on the oxalate content of spinach (*Spinacia oleraceae* L.). *Journal of Food Science, 30*(4), 589-593.

Kostman, T. A., & Franceschi, V. R. (2000). Cell and calcium oxalate crystal growth is coordinated to achieve high-capacity calcium regulation in plants. *Protoplasma, 214*(3), 166-179.

Kostman, T. A., & Koscher, J. R. (2003). L-galactono-gamma-lactone dehydrogenase is present in calcium oxalate crystal idioblasts of two plant species. *Plant Physiology and Biochemistry, 41*(3), 201-206

Kostman, T. A., Tarlyn, N. M., Loewus, F. A., & Franceschi, V. R. (2001). Biosynthesis of L-ascorbic acid and conversion of carbons 1 and 2 of L-ascorbic acid to oxalic acid occurs within individual calcium oxalate crystal idioblasts. *Plant Physiology, 125*(2), 634-640.

Kozukue, E., Kozukue, N., & Kurosaki, T. (1983). Organic acid, sugar, and amino acid composition of bamboo shoots. *Journal of Food Science, 48*, 935-938.

Kubicek, C.P., Schreferl-Kunar, G., Wöhrer, W., & Röhr, M. (1988). Evidence for a cytoplasmic pathway of oxalate biosynthesis in *Aspergillus niger*. *Applied and Environmental Microbiology, 55*, 633–637.

Larson, R. A. (1988). The antioxidants of higher plants. *Phytochemistry, 27*(4), 969-978.

Leenheer, J. A., Wershaw, R. L., Brown, G. K., & Reddy, M. M. (2003). Characterization and diagenesis of strong-acid carboxyl groups in humic substances. *Applied Geochemistry, 18*(3), 471-482.

Lehr, H.-A., Frei, B., Olofsson, A. M., Carew, T. E., & Arfors, K.-E. (1995). Protection from oxidized LDL–induced leukocyte adhesion to microvascular and macrovascular endothelium *in vivo* by vitamin C but not by vitamin E. *Circulation, 91*(5), 1525-1532.

Leong, L. P., & Shui, G. (2002). An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chemistry, 76*(1), 69-75.

Li,X.X., & Franceschi, V.R. (1990). Distribution of peroxisomes and glycolate metabolism in relation to calcium oxalate formation in *Lemna minor* L. *European Journal of Cell Biology* , *51*, 9–16.

Libert, B. (1987). Genotypic and non-genotypic variation of oxalate and malate content in rhubarb (*Rheum* spp. L.). *Journal of Horticultural Science, 62*(4):513-521

Libert, B., & Creed, C. (1985). Oxalate content of 78 rhubarb cultivars and its relation to some other characters. *Journal of Horticultural Science, 60*(2), 257-261.

Libert, B., & Franceschi, V. R. (1987). Oxalate in crop plants. *Journal of Agricultural and Food Chemistry, 35*(6), 926-938.

Liebman, M., & Chai, W. (1997). Effect of dietary calcium on urinary oxalate excretion after oxalate loads. *The American Journal of Clinical Nutrition, 65*(5), 1453-1459.

Liebman, M., & Costa, G. (2000). Fffects of calcium and magnesium on urinary oxalate excretion after oxalate loads. *The Journal of Urology, 163*(5), 1565-1569.

Liu, R. H. (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phyto-chemicals. *The American Journal of Clinical Nutrition, 78*(3), 517S-520S.

Liu, R. H. (2004). Potential synergy of phyto-chemicals in cancer prevention: mechanism of action. *The Journal of Nutrition, 134*(12), 3479S-3485S.

Loewus, F. A. (1999). Biosynthesis and metabolism of ascorbic acid in plants and of analogs of ascorbic acid in fungi. *Phytochemistry, 52*(2), 193 - 210.

Lotito, S. B., & Frei, B. (2006). Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: Cause, consequence, or epiphenomenon? *Free Radical Biology and Medicine, 41*(12), 1727-1746.

Ma, J. F. (2000). Role of organic acids in detoxification of Aluminum in higher plants. *Plant and Cell Physiology, 41*(4), 383-390.

Ma, J. F., Hiradate, S., & Matsumoto, H. (1998). High Aluminum resistance in buckwheat. *Plant Physiology, 117*(3), 753-759.

Ma, J. F., Hiradate, S., Nomoto, K., Iwashita, T., & Matsumoto, H. (1997). Internal detoxification mechanism of Al in hydrangea (identification of Al form in the leaves). *Plant Physiology, 113*(4), 1033-1039.

Ma, J. F., Ryan, P. R., & Delhaize, E. (2001). Aluminium tolerance in plants and the complexing role of organic acids. *Trends in Plant Science, 6*(6), 273-278.

Ma, Z., & Miyasaka, S. C. (1998). Oxalate exudation by taro in response to Al. *Plant Physiology, 118*(3), 861-865.

MacDonald-Wicks, L. K., Wood, L. G., & Garg, M. L. (2006). Methodology for the determination of biological antioxidant capacity in vitro: a review. *Journal of the Science of Food and Agriculture, 86*(13), 2046-2056.

Macheix, J. J., Fleuriet, A., & Billot, J. (1990). *Fruit Phenolics.* Boca Raton, Fla: CRC Press

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *The American Journal of Clinical Nutrition, 79*(5), 727-747.

Maranz, S., Wiesman, Z., & Garti, N. (2003). Phenolic constituents of shea (*Vitellaria paradoxa*) kernels. *Journal of Agricultural and Food Chemistry, 51,*6268-6273.

Marniemi, J, Hakala, P, Mäki, J & Ahotupa, M (2000). Partial resistance of low density lipoprotein to oxidation in vivo after increased intake of berries. *Nutrition, Metabolism & Cardiovascular Diseases, 10*, 331–337.

Mårtensson, L., & Savage, G. P. (2008). Composition and bioavailability of oxalates in baked taro (Colocasia esculenta var. Schott) leaves eaten with cows milk and cows milk and coconut milk. *International Journal of Food Science & Technology, 43*(12), 2213-2218.

Martin, A., & Frei, B. (1997). Both intracellular and extracellular vitamin C inhibit atherogenic modification of LDL by human vascular endothelial cells. *Arteriosclerosis, Thrombosis, and Vascular Biology, 17*(8), 1583-1590.

Massey, L. K. (2007). Food oxalate: factors affecting measurement, biological variation, and bioavailability. *Journal of the American Dietetic Association, 107*(7), 1191-1194.

Massey, L. K., Roman-Smith, H., & Sutton, R. A. L. (1993). Effect of dietary oxalate and calcium on urinary oxalate and risk of formation of calcium oxalate kidney stones. *Journal of the American Dietetic Association, 93*(8), 901-906.

Massey, L.K. & Kynast-gales.S.A. (1998). Substituting milk for apple juice does not increase kidney stone risk in most normocalciuric adults who form calcium oxalate stone. *Journal of the American Dietetic Association, 98*, 303-308.

Massey, L.K. (2003). Dietary influences on urinary oxalate and risk of kidney stones. *Frontiers in Bioscience, 8*, 584–594.

Mazen, A. M. A. (2004a). Calcium oxalate deposits in leaves of corchorus olitorius as related to accumulation of toxic metals. *Russian Journal of Plant Physiology, 51*(2), 281-285.

Mazen, A. M. A. (2004b). Accumulation of four metals in tissues of *Corchorus olitorius* and possible mechanisms of their tolerance. *Biologia Plantarum, 48*(2), 267-272.

Mazen, A. M. A., Zhang, D., & Franceschi, V. R. (2004). Calcium oxalate formation in *lemna minor*: physiological and ultrastructural aspects of high capacity calcium sequestration. *New Phytologist, 161*(2), 435-448.

Mazza, G., Kay, C. D., Cottrell, T., & Holub, B. J. (2002). Absorption of anthocyanins from blueberries and serum antioxidant status in human subjects. *Journal of Agricultural and Food Chemistry, 50*(26), 7731-7737.

Mc Cord, J.M. (2000).The evolution of free radicals and oxidative stress. *The American Journal of Medicine, 108*, 652-659.

McNair, J. B. (1932). The interrelation between substances in plants: essential oils and resins, cyanogen and oxalate. *American Journal of Botany, 19*(3), 255-272.

Meeuse, J. D. & J. M. Campbell. 1959. An inhibitor of oxalic acid oxidase in beet extracts. *Plant Physiology, 34*, 583-586.

Melino, V., Soole, K., & Ford, C. (2009). Ascorbate metabolism and the developmental demand for tartaric and oxalic acids in ripening grape berries. *BioMed Central Plant Biology, 9*(145), 1-14.

Mendonça, C. d. O. G., Martini, L. A., Baxmann, A. C., Nishiura, J. L., Cuppari, L., Sigulem, D. M., et al. (2003). Effects of an oxalate load on urinary oxalate excretion in calcium stone formers. *Journal of Renal Nutrition, 13*(1), 39-46.

Messa,P., Marangella, M., Paganin, L., Codardini, M., Cruciatti, A., Turrin, D., Filiberto, Z., & Mioni, G. (1997). Different dietary calcium intake and relative supersaturation of calcium oxalate in the urine of patients forming renal stones. *Clinical Science, 93*, 257-263

Michael J, T. (2000). The role of free radicals and antioxidants. *Nutrition, 16*(7–8), 716-718.

Miller, N. J., & Rice-Evans, C. A. (1996). Spectrophotometric determination of antioxidant activity. *Redox Report, 2*, 161–171

Miller, N. J., Rice-Evans, C., Davies, M. J., Gopinathan, V., & Milner, A. (1993). A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical science, 84*(4), 407-412.

Mingfu, W., Simon, J. E., Aviles, I. F., He, K., Zheng, Q., & Tadmor, Y.(2003). Analysis of antioxidative phenolic compounds in artichoke (*Cynara scolymus* L.). *Journal of Agricultural and Food Chemistry, 51*, 601-608.

Molano-Flores, B. (2001). Herbivory and calcium concentrations affect calcium oxalate crystal formation in leaves of sida (*Malvaceae*). *Annals of Botany, 88*(3), 387-391

Monje, P. V., & Baran, E. J. (2002). Characterization of calcium oxalates generated as biominerals in cacti. *Plant Physiology, 128*(2), 707-713.

Morozumi, M., Hossain, R., Yamakawa, K.-i., Hokama, S., Nishijima, S., Oshiro, Y., *et al*. (2006). Gastrointestinal oxalic acid absorption in calcium-treated rats. *Urological Research, 34*(3), 168-172.

Morrison, S. C., & Savage, G. P. (2003). Oxalates. In B. Caballero, L. C. Trugo & P. M. Finglas (Eds), *Encyclopedia of Food Sciences and Nutrition* (2nd ed.) (pp. 4282-4287). London: Academic Press.

Musa. A., Oladiran J.A., Ezenwa .M. I. S., Ogbadoyi. E. O., & Akanya.H.O.(2011). Effect of fruiting on some micronutrients, antinutrients and toxic substances in *Corchorus olitorius* grown in Minna, Niger State, Nigeria. *African Journal of Food Science, 5*(7), 411-416.

Myers, A.T. (1947). Seasonal changes in total and soluble oxalates in leaf blades and petioles of rhubarb. *Journal of Agriculture Research, 74*, 33-47.

Nagahisa, M., & Hattori, A. (1964). Studies on oxalic acid oxidase in green leaves. *Plant and Cell Physiology, 5*(2), 205-215.

Nakagami, T., Nanaumi-Tamura, N., Toyomura, K., Nakamura, T., & Shigehisa, T. (1995). Dietary flavonoids as potential natural biological response modifiers affecting the autoimmune system. *Journal of Food Science, 60*(4), 653-656.

Nakamura, Y., Tsuji, S., & Tonogai, Y. (2003). Method for analysis of tannic acid and its metabolites in biological samples: application to tannic acid metabolism in the rat. *Journal of Agricultural and Food Chemistry, 51*, 331-339.

Nakata, P. A. (2003). Advances in our understanding of calcium oxalate crystal formation and function in plants. *Plant Science, 164*(6), 901-909.

Nataka, P.A. & McConn, M.M. (2000). Isolation of *Medicago truncatula* mutants defective in calcium oxalate cryatsal formation. *Plant Physiology, 124*, 1097-1104.

Nataka, P.A. & McConn, M.M. (2003). Influence of the calcium oxalate defective 4 (cod4) mutation on the growth, oxalate content and calcium content of *Medicago truncatula. Plant Science, 164*, 617-621.

Nataka, P.A. & McConn, M.M. (2007). Genetic evidence for differences in the pathways of druse and prismatic calcium oxalate crystal formation in *Medicago truncatula*. *Functional Plant Biology, 34*, 332-338.

New Zealand Ministry of Health. (2009). The concise New Zealand Food Composition Tables, 8th Edition. Retrieved 25 February, 2011 from http://www.health.govt.nz/publication/concise-new-zealand-food-composition-tables-8th-edition

New Zealand Nutrition Foundation. (2012). Vitamin C. Retrieved March 5, 2012 from http://www.nutritionfoundation.org.nz/nutrition-facts/vitamins/vitamin-c

Niki, E. (2010). Assessment of antioxidant capacity in vitro and in vivo. *Free Radical Biology and Medicine, 49*(4), 503-515.

Niki, E., Noguchi, N., Tsuchihashi, H., & Gotoh, N. (1995). Interaction among vitamin C, vitamin E, and beta-carotene. *The American Journal of Clinical Nutrition, 62*(6), 1322S-1326S.

Nishikimi, M., & Yagi, K. (1991). Molecular basis for the deficiency in humans of gulonolactone oxidase, a key enzyme for ascorbic acid biosynthesis. *The American Journal of Clinical Nutrition, 54*(6), 1203S-1208S.

Nishiura, J. L., Martini, L. A., Mendonça, C. O. G., Schor, N., & Heilberg, I. P. (2002). Effect of calcium intake on urinary oxalate excretion in calcium stone-forming patients. *Brazilian Journal of Medical and Biological Research, 35*, 669-675.

Noll, C. R., Jr., & Burris, R. H. (1954). Nature and distribution of glycolic acid oxidase in plants. *Plant Physiology, 29*(3), 261-265.

Noonan, S.C. & Savage, G.P. (1999). Oxalate content of foods and its effect on humans. *Asia Pacific Journal of Clinical Nutrition, 8*(1), 64-74.

Nuss, R. F., & Loewus, F. A. (1978). Further studies on oxalic acid biosynthesis in oxalate-accumulating plants. *Plant Physiol., 61*(4), 590-592.

Ogawa, Y., Miyazato, T., & Hatano, T. (2000). Oxalate and urinary stones. *World Journal of Surgery, 24*(10), 1154-1159.

Ogawa, Y., Takahashi, S., & Kitagawa, R. (1984). Oxalate content in common Japanese foods. *Acta Urologica Japonica. Hinyokika Kiyo, 30,* 305–310.

Ohkawa,T., Ebisuno, S., Kitagawa, M., Morimoto, S., Miyazaki, Y., Yasukawa, S. (1984). Rice bran treatment for patients with hypercalciuric stones: experimental and clinical studies. *Journal of Urology, 132,* 1140-1145.

Ologhobo, A. D. (1989). Improving the nutritive value of soybean (*Glycine max* (L.) Merr.) through processing: biochemical and protein quality studies. *Tropical Agriculture, 66,* 290-296.

Ozgen, M., Reese, R. N., Tulio, A. Z., Scheerens, J. C., & Miller, A. R. (2006). Modified 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2‘-diphenyl-1-picrylhydrazyl (DPPH) methods. *Journal of Agricultural and Food Chemistry, 54*(4), 1151-1157.

Padayatty, S. J., Katz, A., Wang, Y., Eck, P., Kwon, O., Lee, J.-H., *et al.* (2003). Vitamin C as an antioxidant: evaluation of its role in disease prevention. *Journal of the American College of Nutrition, 22*(1), 18-35.

Perera, C. O., Hallett, I. C., Nguyen, T. T., & Charles, J. C. (1990). Calcium Oxalate Crystals: The irritant factor in kiwifruit. *Journal of Food Science, 55*(4), 1066-1069.

Pérez-Jiménez, J., Arranz, S., Tabernero, M., Díaz- Rubio, M. E., Serrano, J., Goñi, I., and Saura-Calixto, F. (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: extraction, measurement and expression of results. *Food Research International, 41*(3), 274-285.

Poljšak, B., Gazdag, Z., Jenko-Brinovec, Š., Fujs, Š., Pesti, M., Bélagyi, J., et al. (2005). Pro-oxidative vs antioxidative properties of ascorbic acid in chromium(VI)-induced damage: an in vivo and in vitro approach. *Journal of Applied Toxicology, 25*(6), 535-548.

Prior, R. L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C, Lischner, N., Ehlenfeldt, M., Kalt, W., Krewer, G & Mainland, C. M. (1998). Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *Journal of Agricultural and Food Chemistry, 46*(7), 2686-2693.

Prior, R. L., Gu, L., Wu, X., Jacob, R. A., Sotoudeh, G., Kader, A. A., & Cook, R. A. (2007). Plasma antioxidant capacity changes following a meal as a measure of the ability of a food to alter *in vivo* antioxidant status. *Journal of the American College of Nutrition, 26*(2), 170-181.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*(10), 4290-4302.

Pucher, G. W., Wakeman, A. J., & Vickery, H. B. (1939). Organic acid metabolism of the buckwheat plant. *Plant Physiology, 14*(2), 333-340.

Pundir, C. S., & Nath, R. (1984). Occurrence of an oxalate oxidase in *Sorghum* leaves. *Phytochemistry, 23*(9), 1871-1874.

Qian, J.-Y., Liu, D., & Huang, A.-G. (2004). The efficiency of flavonoids in polar extracts of *Lycium chinense* Mill. fruits as free radical scavenger. *Food Chemistry, 87*, 283–288.

Ranson, S. L. (1965). The plant acids. In J. Bonner and J. E. Varner (Eds.). *Plant Biochemistry* (pp. 493-525). New York: Academic Press.

Rassam, M. & Laing, W. (2005). Variation in ascorbic acid and oxalate levels in the fruit of *Actinidia chinensis* tissues and genotypes. *Journal of Agricultural and Food Chemistry, 53*, 2322-2326.

Rassam, M., Bulley, S.M., & Laing, W.A. (2007). Oxalate and ascorbate in *Actinidia* fruit and leaves. *Acta Horticulturae (ISHS) 753*, 479-485

Raven, J.A., Griffiths, H., Glidewell, S.M., & Preston, T. (1982). The mechanism of oxalate biosynthesis in higher plants: Investigationswith the stable isotopes ^{18}O and ^{13}C. *Proceedings of the Royal Society B*: *Biological Sciences, 216*, 87–101.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant capacityapplying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9-10), 1231-1237.

Reichman, S. (2002). The Responses of Plants to Metal Toxicity : A review focusing on Copper , Manganese and Zinc. *Environment*, 1-54. Australian Minerals & Energy Environment Foundation. Retrieved from http://www.plantstress.com/articles/toxicity_i/Metal_toxicity.pdf

Ribera, A. E., Reyes-Diaz, M., Alberdi, M., Zuñiga, G. E., & Mora, M. L. (2010). Antioxidant compounds in skin and pulp of fruits change among genotypes and maturity stages in highbush blueberry (*Vaccinium corymbosum* L.) grown in southern chile. *Journal of Soil Science and Plant Nutrition, 10*, 509-536.

Rice-evans, C. A., Miller, N. J., Bolwell, P. G., Bramley, P. M., & Pridham, J. B. (1995). The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radical Research, 22*(4), 375-383.

Rice-Evans, C., Miller, N., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science, 2*(4), 152-159.

Rietjens, I. M. C. M., Boersma, M. G., Haan, L. d., Spenkelink, B., Awad, H. M., Cnubben, N. H. P., *et al*. (2002). The pro-oxidant chemistry of the natural antioxidants vitamin C, vitamin E, carotenoids and flavonoids. *Environmental Toxicology and Pharmacology, 11*(3–4), 321-333.

Rimm, E. B., Ascherio, A., Giovannucci, E., Spiegelman, D., Stampfer, M. J., & Willett, W. C. (1996). Vegetable, fruit, and cereal fiber intake and risk of coronary heart disease among men. *The Journal of the American Medical Association, 275*(6), 447-451.

Rinallo, C., & Mori, B. (2000). Oxalate and ascorbic acid in kiwifruit during growth and storage. *Italian Journal of Food Science*, 12(4), 435-442

Rivero-Pérez, M. D., González-Sanjosé, M. L., Ortega-Herás, M., & Muñiz, P. (2008). Antioxidant potential of single-variety red wines aged in the barrel and in the bottle. *Food Chemistry, 111*(4), 957-964.

Robert, A, J. (1995). The integrated antioxidant system. *Nutrition Research, 15*(5), 755-766.

Ross, J. A., & Kasum, C. M. (2002). Dietary flavonoids: bioavailability, metabolic effects, and safety. *Annual Review of Nutrition, 22*(1), 19-34.

Rout, G. R., Samantaray, S., & Das, P. (2001). Aluminium toxicity in plants: a review. *Agronomie, 21*(1), 3-21.

Ruiz, N., Ward, D., & Saltz, D. (2002). Calcium oxalate crystals in leaves of pancratium sickenbergeri: constitutive or induced defence? *Functional Ecology, 16*(1), 99-105.

Sakai, W.S., Hanson, M., & Jones, R.C. (1972). Raphides with barbs and grooves in *Xanthosoma sagittifolium* (*Araceae*). *Science*, 178, 314–315.

Salinas, M. L., Ogura, T., & Soffchi, L. (2001). Irritant contact dermatitis caused by needle-like calcium oxalate crystals, raphides, in Agave tequilana among workers in tequila distilleries and agave plantations. *Contact Dermatitis, 44*(2), 94-96.

Saltz, D., Schmidt, H., Rowen, M., Arnon, K., Ward, D., & Schmidt, I. (1999). Assessing grazing impacts by remote sensing in hyper-arid environments. *Journal of Range Management*, *52*(5), 500-507.

Sangketkit, C., Savage, G. P., Martin, R. J., & Mason, S. M. (2001). Oxalate content of raw and cooked oca *(Oxalis tuberosa). Journal of Food Composition and Analysis*, *14*, 389-397.

Savage, G. P. (2002). Oxalates in human foods. *Proceedings of the Nutrition Society of New Zealand,* 27, 4-24.

Savage, G. P., Mårtensson, L., & Sedcole, J. R. (2009). Composition of oxalates in baked taro (*Colocasia esculenta* var. Schott) leaves cooked alone or with additions of cows milk or coconut milk. *Journal of Food Composition and Analysis, 22*(1), 83-86.

Savage, G. P., Vanhanen, L., Mason, S. M., & Ross, A. B. (2000). *Journal of Food Composition* and Analysis, *13*, 201-206.

Savage, G.P., & Mårtensson, L. (2010). Comparison of the estimates of the oxalate content of taro leaves and corms and a selection of Indian vegetables following hot water, hot acid and *in vitro* extraction. *Journal of Food Composition and Analysis, 23* (1), 113-117.

Seal, S. N. & Sen. S.P. (1970). The photosynthetic production of oxalic acid in *Oxalis corniculata*. *Plant and Cell Physiology, 11*, 119-128.

Sefa-Dedeh, S., & Agyir-Sackey, E. K. (2004). Chemical composition and the effect of processing on oxalate content of cocoyam *Xanthosoma sagittifolium* and *Colocasia esculenta cormels*. *Food Chemistry, 85*(4), 479-487.

Shah, P. J. R., Williams, G., & Green, N. A. (1980). Idiopathic hypercalciuria: its control with unprocessed bran. *British Journal of Urology, 52*(6), 426-429.

Shimazono,H ., & Hayaishi. O. (1957). Enzymatic decarboxylation of oxalic acid. *Journal of Biological Chemistry, 227*, 151-159.

Shimokawa, K., Y. Ueda.l, & Z. Kasai. (1972). Decarboxylation of oxalic acid during ripening of banana fruit (*Musa sapientum* L.). *Agricultural and Biological Chemistry, 36*, 2021-2024.

Sidhu, H., Hoppe, B., Hesse, A., Tenbrock, K., Bromme, S., Rietschel, E., *et al*. (1998). Absence of *Oxalobacter formigenes* in cystic fibrosis patients: a risk factor for hyperoxaluria. *The Lancet, 352*(9133), 1026-1029.

Sidhu, H., Schmidt, M.E., Cornelius, J.G., Thamilselvan, S., Khan, S.R., Hesse, A., & Peck. A.B. (1999). Direct correlation between hyperoxaluria/oxalate stone disease and the absence of the gastrointestinal tract-dwelling bacterium *Oxalobacter formigenes*: possible prevention by gut recolonization or enzyme replacement therapy. *Journal of the American Society* of *Nephrology, 10* [Suppl 14], S334-S340

Siener, R., Ebert, D., Nicolay, C., & Hesse, A. (2003). Dietary risk factors for hyperoxaluria in calcium oxalate stone formers. *Kidney International, 63*(3), 1037-1043.

Simpson, T.S., Savage, G.P., Sherlock, R. & Vanhanen, L. (2009). Oxalate content of silver beet leaves at different stages of maturation and the effect of cooking with different milk sources. *Journal of Agricultural and Food Science, 57,* 10804–10808.

Singh, P. P., Kothari, L. K., Sharma, D. C., & Saxena, S. N. (1972). Nutritional value of foods in relation to their oxalic acid content. *The American Journal of Clinical Nutrition, 25*(11), 1147-1152.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*(3), 144-158.

Siva, S., Barrack, E. R., Reddy, G. P. V., Thamilselvan, V., Thamilselvan, S., Menon, M., & Bhandari, M. (2009). A critical analysis of the role of gut *Oxalobacter formigenes* in oxalate stone disease. *British Journal of Urology International, 103*(1), 18-21.

Sleiman, I., Godi, D., Villanacci, V., Pelizzari, G., & Balestrieri, G. P. (2004). Osteitis fibrosa cystica, coeliac disease and Turner syndrome: a case report. *Digestive and Liver Disease, 36*(7), 486-488.

Słupski, J., Lisiewska, Z., Gębczyński, P., & Skoczeń-Słupska, R. (2011). Total and soluble oxalate content in legume vegetables used in the preparation of frozen products for consumption. *International Journal of Food Science & Technology, 46*(9), 1816-1822.

Soong, Y.-Y., & Barlow, P. J. (2004). Antioxidant capacityand phenolic content of selected fruit seeds. *Food Chemistry, 88*(3), 411-417.

Srivastava, S. K., & Krishnan, P. S. (1962). An oxalic acid oxidase in the leaves of Bougainvillea spectabilis. *Biochemical Journal., 85*, 33-30.

Steinmetz, K. A., & Potter, J. D. (1996). Vegetables, fruit, and cancer prevention: a review. *Journal of the American Dietetic Association, 96*(10), 1027-1039.

Storey, R., Wyn Jones, R. G., Schachtman, D. P., & Treeby, M. T. (2003). Calcium-accumulating cells in the meristematic region of grapevine root apices. *Functional Plant Biology, 30*(6), 719-727.

Strohmaier, W. L., Kalchthaler, M., & Bichler, K. H. (1988). Calcium metabolism in normal and in hypercalciuric patients on Farnolith, a dietary fibre preparation. *Urological Research, 16*(6), 437-440.

Stutz, R. E., & Burris, R. H. (1951). Photosynthesis and metabolism of organic acids in higher plants. *Plant Physiology, 26*(2), 226-243.

Sun, T., Powers, J. R., & Tang, J. (2007). Evaluation of the antioxidant activity of asparagus, broccoli and their juices. *Food Chemistry, 105*(1), 101-106.

Sunell, L. A., & Healey, P. L. (1985). Distribution of calcium oxalate crystal idioblasts in leaves of taro (*Colocasia esculenta*). *American Journal of Botany, 72*(12), 1854-1860.

Surveswaran, S., Cai, Y.-Z., Corke, H., & Sun, M. (2007). Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. *Food Chemistry, 102*(3), 938-953.

Suutarinen, J., Honkapää, K., Heiniö, R. L., Autio, K., & Mokkila, M. (2000). The effect of different prefreezing treatments on the structure of strawberries before and after jam making. *LWT - Food Science and Technology, 33*(3), 188-201.

Tang, M., Larson-Meyer, D. E., & Liebman, M. (2008). Effect of cinnamon and turmeric on urinary oxalate excretion, plasma lipids, and plasma glucose in healthy subjects. *The American Journal of Clinical Nutrition, 87*(5), 1262-1267.

Thakur, B. R., Singh, R. K., Handa, A. K., & Rao, M. A. (1997). Chemistry and uses of pectin -A review. *Critical Reviews in Food Science and Nutrition, 37*(1), 47-73.

Thurston, E. L. (1976). Morphology, Fine structure and ontogeny of the stinging emergence of tragia ramosa and t. saxicola (*Euphorbiaceae*). *American Journal of Botany, 63*(6), 710-718.

Tillman-Sutela, E., & Kauppi, A. (1999). Calcium oxalate crystals in the mature seeds of Norway spruce, *Picea abies* (L.) Karst. *Trees - Structure and Function, 13*(3), 131-137.

Tiselius, H-G. (1997). Risk formulas in calcium oxalate urolithiasis. *World Journal of Urology 15*, 176-185.

Tizzani, A., Casetta, G., Piana, P., Vercelli, D. (1989). Wheat bran in the selective therapy of absorptive hypercalciuria: a study performed on 18 lithiasic patients. *Journal of Urology, 142*, 1018-1020.

Tolbert, N. E. (1981). Metabolic pathways in peroxisomes and glyoxysomes [Review]. *Annual Review of Biochemistry, 50*, 133-157.

Tolbert, N. E., & Essner, E. (1981). Microbodies: peroxisomes and glyoxysomes. *The Journal of Cell Biology, 91*(3), 271s-283s.

Toma, R.B., Tabekhia, M.M., & Williams, J.D. (1979). Phytate and oxalate contents in sesame seed (*Sesamum indicum l.*). *Nutrition Reports International*, 20, 25-31.

Toor, R. K., & Savage, G. P. (2005). Antioxidant capacityin different fractions of tomatoes. *Food Research International, 38*(5), 487-494.

Torres, J. L., & Bobet, R. (2001). New Flavanol derivatives from grape (*vitis vinifera*) byproducts. antioxidant aminoethylthio−flavan-3-ol conjugates from a polymeric waste fraction used as a source of flavanols. *Journal of Agricultural and Food Chemistry, 49*(10), 4627- 4634.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology, 39*(1), 44-84.

Valpuesta, V., & Botella, M. A. (2004). Biosynthesis of L-ascorbic acid in plants: new pathways for an old antioxidant. *Trends in Plant Science, 9*(12), 573-577.

Van Duyn, M. A. S., & Pivonka, E. (2000). overview of the health benefits of fruit and vegetable consumption for the dietetics professional: selected literature. *Journal of the American Dietetic Association, 100*(12), 1511-1521.

Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry, 46*, 4113-4117.

Venning, J. A., Burns, D. J. W., Hoskin, K. M., Nguyen, T., & Stec, M. G. H. (1989). Factors influencing the stability of frozen kiwifruit pulp. *Journal of Food Science, 54*(2), 396-400.

Vickery, H.B., & J. K. Palmer. (1956). The metabolism of the organic acids of tobacco leaves. II. Effect of culture of excised leaves in solutions of glycolate at pH 3 to pH 6. *The Journal of Biological Chemistry, 221*, 79-92.

Vieira, K.F.G., da Silva Campelo Borges, G., Copetti, C., Gonzaga, L.V. (2009). Activity and contents of polyphenolic antioxidants in the whole fruit, flesh and peel of three apple cultivars. *Archivos Latinoamericanos de Nutricion, 59*(1), 101-106

Vinson, J. A., Su, X., Zubik, L., & Bose, P. (2001). Phenol antioxidant quantity and quality in foods: fruits. *Journal of Agricultural and Food Chemistry, 49*(11), 5315-5321.

Volk, G. M., Lynch-Holm, V. J., Kostman, T. A., Goss, L. J., & Franceschi, V. R. (2002). The role of druse and raphide calcium oxalate crystals in tissue calcium regulation in *pistia stratiotes* leaves. *Plant Biology, 4*(1), 34-45.

von Unruh, G. E., Voss, S., Sauerbruch, T., & Hess, A. (2003). Reference range for gastrointestinal oxalate absorption measured with a standardized [13C2] oxalate absorption test. *The Journal of Urology,* 169,687-690.

von Unruh, G. E., Voss, S., Sauerbruch, T., & Hesse, A. (2004). Dependence of oxalate absorption on the daily calcium intake. *Journal of the American Society of Nephrology, 15*(6), 1567-1573.

Wagner, G., & Loewus, F. (1973). The biosynthesis of (+)-tartaric acid in pelargonium crispum. *Plant Physiology, 52*(6), 651-654.

Waltner-Toews, D., & Meadows, D. H. (1980). Urolithiasis in a herd of beef cattle associated with oxalate ingestion. *The Canadian Veterinary Journal, 21*(2), 61-62.

Wang, S. Y., & Lin, H.-S. (2000). Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *Journal of Agricultural and Food Chemistry, 48*(2), 140-146.

Wang, S. Y., & Zheng, W. (2001). Effect of plant growth temperature on antioxidant capacity in strawberry. *Journal of Agricultural and Food Chemistry, 49*(10), 4977- 4982.

Ward, D., Spiegel, M., & Saltz, D. (1997). Gazelle herbivory and interpopulation differences in calcium oxalate content of leaves of a desert lily. *Journal of Chemical Ecology, 23*(2), 333-346.

Watanabe, K. & Takahashi, B. (1998). Determination of soluble and insoluble oxalate contents in kiwifruit (*Actinidia deliciosa*) and related species. *Journal* of the *Japanese Society* for *Horticultural Science, 67*(3), 299-305.

Webb, M. A. (1999). Cell-mediated crystallization of calcium oxalate in plants. *The Plant Cell Online, 11*(4), 751-761.

White, P. J. (2000). Calcium channels in higher plants. *Biochimica et Biophysica Acta (BBA) - Biomembranes, 1465*(1-2), 171-189.

Wilson, C. W., Shaw, P. E., & Knight, R. J. (1982). Analysis of oxalic acid in carambola (*Averrhoa carambola L.*) and spinach by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry, 30*(6), 1106-1108.

World Health Organization (WHO). (2003). Fruit and vegetable promotion initiative /a meeting report / 25-27/08/03. Retrieved 2 March, 2012 from http://www.who.int/hpr/NPH/fruit_and_vegetables/fruit_and_vegetable_report.pdf

Xu, H.-W., Ji, X.-M., He, Z.-H., Shi, W.-P., Zhu, G.-H., Niu, J.-K., *et al*. (2006). Oxalate accumulation and regulation is independent of glycolate oxidase in rice leaves. *Journal of Experimental Botany, 57*(9), 1899-1908.

Yang, J., & Loewus, F.A. (1975). Metabolic conversion of L-ascorbic acid in oxalate-accumulating plants. *Plant Physiology, 56,* 283–285.

Yao, L., Jiang, Y., Datta, N., Singanusong, R., Liu, X., Duan, J., *et al*. (2004). HPLC analyses of flavanols and phenolic acids in the fresh young shoots of tea (*Camellia sinensis*) grown in Australia. *Food Chemistry, 84*(2), 253-263.

Yoshihara, T., Sogawa, K., Pathak, M. D., Juliano, B. O., & Sakamura, S. (1980). Oxalic acid as a sucking inhibitor of the brown planthopper in rice (Delphacidae, Homoptera). *Entomologia Experimentalis et Applicata*, *27*(2), 149-155.

Young, I.S., &Woodside, J. V. (2001). Antioxidants in health and disease. *Journal of Clinical Pathology* , *54*,176-86

Yu, L., Jiang, J., Zhang, C., Jiang, L., Ye, N., Lu, Y, *et al*. (2010). Glyoxylate rather than ascorbate is an efficient precursor for oxalate biosynthesis in rice. *Journal of Experimental Botany, 61*(6), 1625-1634.

Zarembski, P. M., & Hodgkinson, A. (1962). The oxalic acid content of English diets. *British Journal of Nutrition, 16*(01), 627-634.

Zbinovsky, V., & R. H. Burris, R.H. (1952). Metabolism of infiltrated organic acids by tobacco

Zheng, S. J., Ma, J. F., & Matsumoto, H. (1998). Continuous secretion of organic acids is related to aluminium resistance during relatively long-term exposure to aluminium stress. *Physiologia Plantarum, 103*(2), 209-214.

Zimmerman, D.J., Hesse, A., & von Unruh, G.E. (2005). Influence of a high-oxalate diet on intestinal oxalate absorption. *World Journal of Urology, 23*, 324-329.

Zindler-Frank, E., Hönow, R., & Hesse, A. (2001). Calcium and oxalate content of the leaves of *Phaseolus vulgaris* at different calcium supply in relation to calcium oxalate crystal formation. *Journal of Plant Physiology, 158*(2), 139-144.

Chapter 3

Total phenolic, ascorbic acid concentrations and antioxidant capacities of fruits available in New Zealand

3.1. Abstract

Biological substances in fruit, especially phenolic compounds, play an important role in preventing chronic diseases such as cancer, cardiovascular disease and diabetes. This is believed to be due, in part, to their antioxidant capacity. For the first time, total phenolic and ascorbic acid concentrations and antioxidant capacities of 41 fruits available in New Zealand were quantified using Folin-Ciocalteu, titration, ABTS and ORAC assays. Total phenolics were extracted, using acid and heating, to ensure bound phenolics were included in this measurement. Fruit contains a wide range of total phenolics, ascorbic acid and antioxidant capacities. Results ranged from 27.4 to 2731.9 mg gallic acid equivalent (GAE)/100 g FW for total phenolic concentrations; from 6.2 to 201.3 mg ascorbic acid (AsA)/100 g FW; from 19.5 to 6045.9 µmol Trolox equivalent (TE)/100 g FW for ABTS; and from 122.3 to 13631.6 µmol TE/100 g FW for ORAC. Berries exhibited the highest antioxidant capacities with ranges from 121.2 to 6045.9 µmol TE/100 g FW for ABTS and from 1121.2 to 13631.6 µmol TE/100 g FW for ORAC. The antioxidant capacities were significantly correlated to total phenolic concentrations but no relationship between AsA concentration and antioxidant capacities was established.

3.2. Introduction

Free radicals are molecules or atoms with a highly chemically reactive free (unpaired) electron. They are formed in living organisms during oxidation reactions including normal metabolism and also under certain circumstances such as environmental stress, wounding and pathogen attack. Overproduction of free radicals may cause various disorders involving disease-inducing oxidant damage such as carcinogenesis, drug associated toxicity, inflammation, atherogenesis, asthma, immune system decline and ageing in aerobic organisms (Lee *et al*., 2004). Compounds that can scavenge free radicals are referred to as antioxidants as they can donate one of their electrons to neutralise radicals, ending the electron-stealing chain reaction (Nag, 2009).

According to the World Health Organisation and the Food and Agricultural Organisation (WHO/FAO) (2003), cardiovascular, diabetes, some kinds of cancer, high cholesterol, high blood pressure and obesity can be significantly reduced with diets rich in fruit and vegetables. The disease preventability of fruit has been associated with several factors, including antioxidants such as phenol compounds, vitamin E, vitamin C, carotenoids, lycopenes and other phyto-chemicals. Phenolic compounds have been identified as the major and most important antioxidants found in plant foods (Dai and Mumper, 2010; Hamid *et al*., 2010). The antioxidant capacity may assist in preventing free radicals from damaging proteins, DNA and lipids and, thus, prevent oxidative damage-induced disease (Halliwell, 1994). Guo *et al*., (1997) reported that fruits which contain more antioxidant substances have high antioxidant capacities. Since fruit are usually consumed fresh, the antioxidants are not lost by heating or oxidative effects during processing, hence, fruit can be considered as a good source of phyto-chemical compounds for daily intake.

Since antioxidant capacity of fruit varies according to environmental growth conditions such as temperature and soil properties (Wang and Lin, 2000; Connor *et al.*, 2002), analysing antioxidants of fruit from certain areas is useful for epidemiological research and setting dietary guidelines. Several databases of antioxidant capacity in fruit have been published in specific areas such as in US (Wu *et al*., 2004b; Mahattanatawee, *et al.*, 2006), China (Fu *et al*., 2010), Singapore (Leong and Shui, 2002; Isabelle *et al*., 2010), Brazil (Rufino *et al*., 2010) and Ecuador (Vasco, *et al*., 2008). However, there is no database for fruit available in New Zealand.

Several methods have been developed to assess antioxidant capacity based on different reaction mechanisms and chemical principles such as 2, 2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), ferric ion reducing antioxidant power (FRAP), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), total radical-trapping antioxidant parameter (TRAP) and oxygen radical absorbance capacity (ORAC). The ORAC is an assay closely related to the biological functions of chain breaking antioxidants (Ou *et al*., 2001) and has been used to evaluate antioxidant capacity in fruit in other databases (Prior *et al*., 1998; Wang and Lin, 2000; Wu *et al*., 2004c).The ABTS assay is a rapid and accurate method that can be used over a wide range of pH values (Lemanska *et al*., 2001) in both aqueous and organic solvent systems (Re *et al*., 1999). Using the ABTS assay along with the ORAC assay would result in a reliable database of antioxidant capacities of phyto-chemical compounds present in certain fruit.

Since phenolic compounds in fruit exist in both free and bound forms, determination of total phenolics requires an alkaline, acid or enzymatic hydrolysis to convert their conjugates to the free forms (Stalikas, 2007). The bound phyto-chemicals may be released in the gastrointestinal tract due to the effects of digestion, such as pH, enzyme and microflora activity and, thereafter, contribute to the health benefits. To sufficiently quantify total phenolic concentration and their antioxidant capacities, phenolic compounds of fruit samples were extracted using acid with heating in the present study.

Ascorbic acid (AsA) is also found abundantly in many fruits. It is considered as one of the most important water-soluble vitamins due to its key role in collagen, carnitine and neurotransmitter biosynthesis as well as preventing and treating scurvy symptoms (Naidu, 2003). Most animals and plants can synthesize AsA, except humans and apes that lack the enzyme gulonolactone oxidase. Moreover, it is believed that several health benefits are attributed to AsA including protecting humans from cancers, heart disease and stress as well as helping to maintain a healthy immune system through scavenging reactive oxygen species via non-enzymatic reactions (Kulikowska-Karpińska and Moniuszko-Jakoniuk, 2004). However, controversially, Wang *et al*., (1996) and Cook *et al*., (2007) reported that AsA showed ambiguous results in its ability to scavenge free radicals. Hence, in this study, AsA concentrations of New Zealand fruits were measured and their contributions to antioxidant capacity were evaluated.

In addition, since the total antioxidant capacity of a given fruit will be the combined action of hundreds of antioxidant compounds in the fruit and not from any single compound (Halvorsen *et al.*, 2006), and because fruit composition varies, regularly consuming a mixture of fruit and vegetable provides better protection than a single supplement (Liu, 2004). Daily taking of a single antioxidant vitamin such as vitamin C, vitamin E or beta carotene as a supplement has been reported not to decrease cancer or cardiovascular risk and could even have negative effects in subjects who are prone to these pathologies (Greenberg *et a*l., 1994; Hercberg *et al*., 1998). New Zealand Food and Nutrition Guidelines for Healthy Adults (2011) recommend people should take at least three servings of vegetables and two servings of fruit daily (New Zealand Ministry of Health, 2011).

By employing commonly used methods for determination of antioxidant capacities, total phenolics and ascorbic acid concentration of fruit available in New Zealand, this study aimed to screen a large of number New Zealand fruits to establish a database of potentially natural

antioxidant sources for New Zealanders who are interested in maintaining their health through diet.

3.3. Materials and methods

3.3.1. Sample preparation

All fruit listed in Table 3.1 were purchased fresh in season as ripe, "ready to eat", from Canterbury (NZ) supermarkets. Edible portions of fruit were pooled and then immediately used to measure ascorbic acid and moisture content; the remainder was frozen and then freeze-dried until a constant weight. Freeze-dried fruit were ground to a powder using a Grindomix GM200 homogenizer (Retsch GmbH, Haan, Düsseldorf, Germany) and kept at -40^{0}C until analysis.

3.3.2. Moisture content

The moisture content of fresh fruit was determined in triplicate by oven drying (Watvic, Watson Victor Ltd., NZ) at 105^{0}C until a constant weight was achieved (AOAC, 2002).

3.3.3. Extraction procedures and measurements

3.3.3.1. Extraction procedure for total phenolics and ABTS assays

The extraction procedure was modified from that of Vinson *et al*., (1998). Freeze-dried sample powders (0.5-1g) were weighed accurately into 50 ml glass screw-cap tubes. Samples were then extracted with 20 ml of 1.2 M HCL in 50% methanol (v/v) (Merck, Darmstadt, Germany) at 60^{0}C in the dark for 2 hours with occasional shaking. The sample extracts were made up to 50 ml with extraction solvent and then centrifuged at 2889 g for 15 minutes at 4^{0}C. The supernatants were kept at - 40^{0}C until measurement of total phenol compounds using the Folin-Ciocalteu and ABTS assays. Triplicates were done for each fruit.

3.3.3.2. Extraction procedure for ORAC

Extractions were performed according to Prior *et al.*, (2003). Freeze-dried powdered samples (1g) were weighed in a 50 ml screw-cap tube with 10 ml of acetone/water/acetic acid mixture (Merck, Darmstadt, Germany), (70: 29.5:0.5, v/v/v). The tube was vortexed for 30 seconds and then sonicated at 37°C for 5 minutes (the tube was inverted once during the sonication step to suspend the samples). The tube was then shaken occasionally at room temperature for 10 minutes and centrifuged at 2889 g for 15 minutes. The pellet was re-extracted with 10 ml of solvent, as above. The combined supernatants were transferred quantitatively to a 25 ml

volumetric flask and made up to volume with the solvent and kept at - 40^0C until analysis could commence.

3.3.3.3. Measurement of ascorbic acid concentrations

L.Ascorbic acid (AsA) was measured using an automated Metrohm titroprocessor with a Pt Titrode connected to a 702 SM Tritrino attached to a 730 sample changer (Metrohm, Ltd., Herisau, Switzerland). Fresh fruit (1 g) was homogenised with 40 ml buffer [(3.84 g sodium acetate (AnalaR grade, BDH Chemicals Ltd., NZ) and 0.808 g oxalic acid dehydrate (AnalaR grade, BDH Chemicals Ltd., NZ) in 1 litre deionised water, pH ≈7.0)] with a hand held homogeniser. The homogenised samples were then titrated against a dye solution [295 mg 2, 6-dichlorophenol indophenol sodium salt hydrate (DPIP) (Sigma-Aldrich, Steinheim, Germany) and 100 mg sodium carbonate (Merck, Darmstadt, Germany)] in 1 litre of deionised water. The end point of the titration was determined potentiometrically by reading the titre volume at the point of inflection of the titration curve generated by the Tiamo software. An AsA standard curve (0.2-1.0 mg/ml) was made from a stock solution of 0.25 g L.ascorbic acid (Sigma-Aldrich, Steinheim, Germany) in 50 ml titration buffer and used to calculate AsA concentration in samples. Results were expressed as mg AsA/100 g FW.

3.3.3.4. Measurement of total phenolic concentration

Total phenolic concentrations (TPCs) of fruit were determined by Folin-Ciocalteu assay based on the method of Singleton and Rosi (1965). The assay involves the reduction of the reagent by the total phenolic compounds and the formation of a blue complex.

A 0.5 ml aliquot of diluted extract was mixed with 2.5 ml of 2 N Folin-Ciocalteu reagent (Sigma-Aldrich, Steinheim, Germany) and 2.0 ml of 7.5% sodium carbonate. The mixture was vortexed and incubated at 50^0C for 5 minutes, then quickly cooled and vortexed again. The absorbance was recorded at 760 nm with a UV-visible spectrophotometer (Unicam Helios Beta, UK). Gallic acid (25-200 µg) (Sigma-Aldrich, Steinheim, Germany) was used to construct a calibration curve and results were expressed as gallic acid equivalents per 100 g fresh weight (mg GAE/100 g FW).

3.3.3.5. Measurement of antioxidant capacity by ABTS assay

The ABTS assay was conducted as a measure of free radical scavenging capacity of fruits. The assay followed the method of Re *et al.*, (1999). First, ABTS (2, 2'- azinobis [3-ethylbenzothiazoline-6-sulphonate]) (Sigma-Aldrich, Steinheim, Germany) is oxidised to intensively-coloured ABTS radical cation ($ABTS^{\bullet+}$). ABTS was dissolved in deionized water to 7 mM concentration and then mixed with 10 ml of a 2.45 mM potassium persulfate

($K_2S_2O_8$) (Sigma-Aldrich, Steinheim, Germany) solution to form $ABTS^{\bullet+}$. The solution was kept in the dark at room temperature for at least 12 hours until the reaction was complete (Stock #1). The $ABTS^{\bullet+}$ solution was diluted with phosphate buffered saline (PBS) (5 mM, pH 7.4) to give an absorbance of ~ 0.7 at 734 nm (Stock #2). Extracted samples (100µl) at appropriate dilution were mixed with 1ml of Stock #2. The absorbance at 734 nm was measured before and 6 minutes after adding the sample with the UV-visible spectrometer. The ABTS radical scavenging capacity was calculated using a Trolox standard curve (25-200 µmol). The results were expressed as µmol Trolox equivalent per 100 g of fresh weight of sample (µmol TE/100g FW). All determinations were conducted in triplicate.

3.3.3.6. Measurement of antioxidant capacity by ORAC assay

The ORAC-fluorescence assay was modified from the method outlined by Ou *et al.*, (2001). The ORAC assay measures oxygen radical absorbance capacity of fruit using a FLUOstar OPTIMA plate reader from BMG Labtech GmbH (Offenburg, Germany) that was equipped with a fluorescent filter (excitation, 485 nm; emission, 520 nm). Reactions were performed in 75 mM phosphate buffer (pH 7) (AnalaR grade, BDH Chemicals Ltd., NZ) and at 37^0C, the optimum temperature for the peroxyl radical to oxidize fluorescein, the fluorescent probe, to produce decay curves. Phosphate buffer was used as blank and to dilute all sample extracts prior to measurement. In detail, 25 µl of diluted fruit extract, blank, Trolox standard (0-200 µmol) (Sigma-Aldrich, Steinheim, Germany) and internal standards were pipetted in triplicate into a 96-well black microplate (Costar 3915, Corning Inc., Corning, NY, USA), followed by addition of 150 µl of 10 nM fluorescein (fluorescein sodium salt, Sigma-Aldrich, Steinheim, Germany) in phosphate buffer. The plate was covered by Parafilm (Pechiney Plastic Packaging Company, Chicago, Illinois, US) to stop evaporation and pre-incubated at 37^0C for 30 minutes. The fluorescence was measured every minute in a kinetic cycle of 60 minutes. Freshly prepared AAPH (2,2'-azobis-2-methyl-propanimidamide dihydrochloride, Sigma-Aldrich, Steinheim, Germany) (25µl) was added using the instrument pump to each well at the start of Cycle 5. The area under the curve (AUC) was obtained by MARS Data analysis software (V1.20 R2, BMG Labtech GmbH, Offenburg, Germany). The final ORAC results of samples were calculated based on the regression equation between the Trolox concentrations and the net AUC. The net AUC was obtained by subtracting the AUC of blank from the AUC of the sample. The ORAC value was expressed as micromoles of Trolox equivalent (µmol TE) per 100 g FW. An example was shown in Appendix 1.

3.3.4. Statistical analysis

Minitab version 16 was used to determine significant correlations between the variables being investigated. Significance was accepted at $p < 0.05$. The data are presented as the mean of three determinations ± S.E.

3.4. Results

In the present study, 41 different fruits available in New Zealand (Table 3.1) were analysed for antioxidant capacities, total phenolic and ascorbic acid concentrations. Some of the fruits selected are well-known and marketed internationally while some are barely known out side New Zealand such as kiwano and kiwiberry. Establishing data on total phenolic concentrations and antioxidant capacity are essential for calculating dietary intakes and for clinical studies to clarify the health benefits of fruit. There are numerous studies focused on commonly consumed fruits and their cultivars worldwide; however, there is lack of data for fruit available in New Zealand.

3.4.1. Effect of extraction temperature on total phenolic concentrations

Besides the effect of solvent choice, there are several factors that affect the efficiency of total phenolic extraction for example pH, temperature and extraction time (Pinelo *et al*., 2005). Jeong *et al*., (2004) reported that temperature can affect the break up the cell membranes and cell walls, increase solubility of the phenolic compounds and decrease viscosity of the solution, thus facilitating their transition through solid fruit mass. Although heating can assist to extract more total phenolic compounds from fruit, excessive temperature may degrade phenolic compounds (Larrauri *et al*., 1997). To investigate the effect of temperature on total phenolic concentrations of fruit under hydrolysis conditions, three different temperatures were tested.

In an attempt to optimize the conditions for extraction of total phenolic compounds, three fruits: golden kiwifruit, blueberry and carambola were used (Figure 3.1). The fruits were extracted at 4^0C, 60^0C and 90^0C for 2 hours with 1.2 M HCL in 50% methanol (v/v). The result (Figure 3.1) showed that the extraction of TPCs was affected significantly ($p < 0.001$) by temperature. For all analysed fruits, TPCs extracted at 60^0C were 2.1- and 1.3-fold higher than those extracted at 4^0C and 90^0C, respectively. Thus, 60^0C was chosen as extraction temperature for TPCs and used for ABTS.

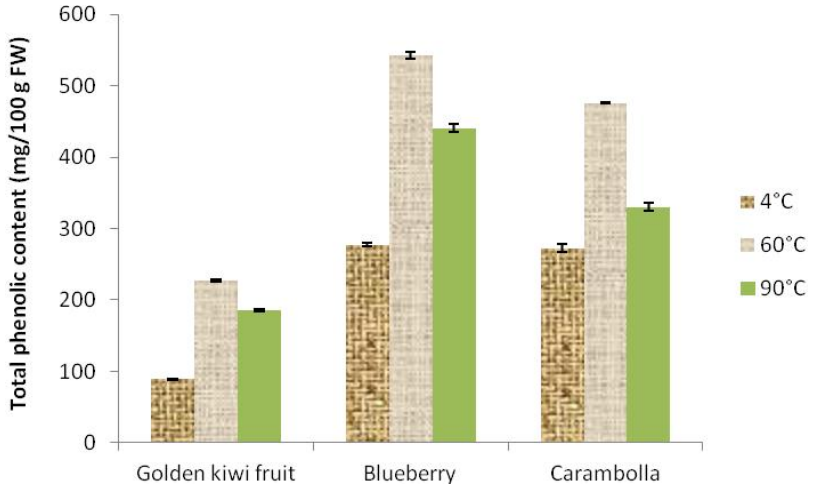


Figure 3.1. Effect of extraction temperature on total phenolic concentrations (mean mg GAE/100 FW ± SE) of three representative fruits during extraction with 1.2 M HCL in 50% methanol (v/v).

3.4.2. Total phenolic concentrations

The TPCs of the 41 analysed fruits available in New Zealand were measured using Folin-Ciocalteu colorimetric method and are presented in Table 3.1. There was a large range of TPCs for the fruit measured, ranging from 39.3 to 2731.9 mg GAE/100g FW. Most of the berries contained relatively high TPCs except red and green gooseberry, at 91.4 and 90.2 GAE/100 g FW, respectively. Indian gooseberry had the highest TPC (2731.9 mg GAE/100 g FW) followed by blackcurrant (855.2 mg GAE/100 g FW) and blueberry (544.4 mg GAE/100 g FW). Moderate concentrations were found in cherries (181.2-277.6 GAE/100 g FW), feijoa (178.3 GAE/100 g FW), grapes (171.3-234.2 GAE/100 g FW), passion fruit (269.6 GAE/100 g FW), pear (153.7 GAE/100 g FW) and plums (171.3-285.3 GAE/100 g FW). Meanwhile, low concentrations of TPC were found in apricot (63.0 mg GAE/100 g FW), kiwano (39.3 mg GAE/100 g FW), green kiwifruit (60.1 GAE/100 g FW), peach (51.3 GAE/100 g FW) and red cherry tomato (70.1 GAE/100 g FW).

3.4.3. Ascorbic acid concentrations

Ascorbic acid (AsA), more commonly known as vitamin C has been considered the major water-soluble vitamin in fruit (Halliwell, 2001). AsA concentrations of the 38 fresh fruits collected were determined and are presented in Table 3.1. AsA ranged from 2.9 to 180.1 mg AsA/100 g FW. Extremely high AsA concentration was found in blackcurrant (180.1 mg AsA/100 g FW) followed by golden kiwifruit (121.3 mg AsA/100 g FW) and red raspberry (110.1 mg AsA/100 g FW). Relatively high values of AsA were found in black raspberry (92.0 mg AsA/100 g FW), kiwiberry (89.8 mg AsA/100 g FW), green kiwifruit (84.2 mg

AsA/100 g FW), cranberry (62.5 mg AsA/100 g FW), strawberry (62.0 mg AsA/100 g FW), persimmon (62.7 mg AsA/100 g FW) and boysenberry (59.6 mg AsA/100 g FW). Low values were obtained for kiwano (2.9 mg AsA/ 100 g FW), rhubarb (6.4 mg AsA/100 g FW) and pear (6.2 mg AsA/100 g FW).

The contribution of AsA to ORAC was calculated (Table 3.1) based on the ORAC value of 1.0 µmol AsA was 0.52 µmol Trolox equivalent (Cao *et al*., 1993). AsA activity was a small proportion of ORAC values in the analysed fruits. The contributions of AsA to the ORAC were less than 20% in almost all fruits analysed except green kiwifruit (40.2%), golden kiwifruit (37.6%), tamarillo (29.7%), persimmon (24%) and pineapple (23.4%).

3.4.4. Antioxidant capacities

Table 3.1 presents the antioxidant capacities of fruits available in New Zealand determined as Trolox equivalent (µmol TE/100 g FW) using ABTS and ORAC assays. The fruits analysed showed a wide range of antioxidant capacities measured by ABTS assay from 19.5 to 6045.9 µmol TE/100 g FW. The highest antioxidant capacities were observed for Indian gooseberry while the lowest capacities were obtained from kiwano. In general, high antioxidant capacities were obtained mostly for berry fruits grown in New Zealand, especially blackcurrant, at 1142.7 µmol TE/100 g FW for ABTS assay and 9047.6 µmol TE/100 g FW for ORAC assay. Most berry fruits scavenged the $ABTS^{\bullet+}$ radical very effectively, except strawberry (258.2 µmol TE/100 g FW) and red raspberry (226.2 µmol TE/100 g FW). Moderate ABTS values were obtained for cherries (219.2 - 285.7 µmol TE/100 g FW), grapes (248.2 - 571.1 µmol TE/100 g FW), plums (311.3 - 511.2 µmol TE/100 g FW), rhubarb (236.2 µmol TE/100 g FW) and tomato (211.7 – 256.4 µmol TE/100 g FW). Much lower antioxidant capacities to these radicals were obtained from apricot, kiwano, kiwiberry, green and golden kiwifruit, passion fruit and tamarillo.

The antioxidant capacity of fruits using the ORAC assay ranged from 122.3 µmol TE/100 g FW for kiwano to 13631.6 µmol TE/100 g FW for Indian gooseberry, which represents a variation of approximately 111-folds. Similar to the radical scavenging ability in the ABTS assay, berry fruits (i.e. blueberry, boysenberry, blackcurrant, cranberry, black raspberry, goji berry, Indian gooseberry and carambola) showed higher oxygen radical absorbance capacity as compared to the other fruits.

Table 3.1. Antioxidant capacities as measured by ABTS and ORAC, total phenolic and AsA concentrations of fruit available in New Zealand.

Fruit	Moisture content (%)	ABTS (µmol TE/100 g FW)	ORAC (µmol TE/100 g FW)	TPC (mg GAE/100 g FW)	Ascorbic acid (mg/100 g FW)	AsA contribution towards ORAC (%)
Apple (Royal gala)	86.4 ± 0.1	162.3 ± 1.1 (30)	1170.4 ± 21.4 (28)	90.2 ± 0.2 (33)	10.5 ± 1.4 (18)	2.7
Apricot	95.0 ± 0.3	33.8 ± 0.5 (40)	615.9 ± 22.1 (39)	63.0 ± 1.0 (36)	14.6 ± 0.4 (20)	7.1
Avocado	77.3 ± 1.3	196.5 ± 11.6 (28)	374.9 ± 20.5 (23)	59.5 ± 3.2 (38)	10.4 ± 1.0 (26)	2.3
Banana	74.5 ± 2.7	354.7 ± 7.5 (14)	853.7 ± 11.5 (34)	119.6 ± 7.4 (25)	8.7 ± 0.5 (29)	3.1
Blueberry	80.1 ± 0.4	852.8 ± 4.0 (05)	5911.7 ± 19.6 (03)	544.4 ± 4.1 (03)	11.9 ± 1.0 (25)	0.6
Boysenberry	85.2 ± 0.2	737.3 ± 5.0 (07)	2463.0 ± 27.5 (12)	480.2 ± 2.1 (05)	59.6 ± 1.7 (09)	7.3
Carambola	89.9 ± 0.4	975.7 ± 1.4 (03)	3017.5 ± 10.4 (06)	475.5 ± 0.8 (06)	n.d.	-[b]
Cherry (black Tartarian)	85.9 ± 0.8	301.1 ± 1.1 (13)	1754.6 ± 19.1 (14)	277.6 ± 0.2 (11)	10.2 ± 2.1 (27)	2.8
Cherry (red Bing)	87.9 ± 2.1	285.7 ± 2.0 (17)	1457.2 ± 10.5 (21)	181.2 ± 1.2 (20)	14.2 ± 1.3 (21)	1.7
Cherry (yellow Royal)	84.5 ± 0.0	269.1 ± 1.1 (18)	1357.3 ± 12.2 (25)	211.2 ± 1.1 (14)	12.6 ± 1.2 (23)	2.9
Cranberry	87.7 ± 0.1	806.2 ± 3.5 (06)	5234.3 ± 18.1 (04)	514.7 ± 3.5 (04)	55.5 ± 0.5 (10)	3.6
Currant (black)	81.3 ± 0.1	1142.7 ± 3.4 (02)	9047.6 ± 26.1 (02)	855.2 ± 2.1 (02)	180.1 ± 1.3 (01)	6.0
Currant (red)	81.1 ± 0.4	678.6 ± 3.6 (09)	2646.4 ± 25.3 (10)	431.9 ± 0.1 (07)	26.9 ± 2.4 (17)	8.7
Feijoa	85.7 ± 0.1	215.3 ± 1.3 (25)	1474.3 ± 20.2 (19)	178.3 ± 12.2 (21)	27.3 ± 1.4 (16)	5.6
Goji berries	83.3 ± 0.1	938.5 ± 0.3 (04)	3255.8 ± 16.4 (07)	215.2 ± 0.6 (16)	n.d.	-[b]
Gooseberry (green)	87.5 ± 0.2	121.2 ± 0.9 (39)	1121.2 ± 14.5 (39)	90.2 ± 1.5 (32)	40.2 ± 0.1 (14)	20.6
Gooseberry (Indian)	81.8 ± 0.9	6045.9 ± 4.9 (01)	13631.6 ± 41.0 (01)	2731.9 ± 4.4 (01)	n.d.	-[b]
Gooseberry (red)	87.3 ± 0.3	135.2 ± 0.7 (32)	1440.7 ± 18.5 (23)	91.4 ± 0.6 (31)	51.2 ± 1.4 (11)	8.6
Grape (black)	81.1 ± 0.1	571.1 ± 1.4 (10)	2745.2 ± 21.1 (09)	234.2 ± 1.2 (15)	13.2 ± 1.8 (22)	1.4
Grape (green)	83.4 ± 0.0	248.2 ± 0.9 (20)	1457.3 ± 25.6 (20)	171.3 ± 0.5 (22)	7.7 ± 0.1 (31)	1.6

Fruit	Moisture content (%)	ABTS (µmol TE/100 g FW)	ORAC (µmol TE/100 g FW)	TPC (mg GAE/100 g FW)	Ascorbic acid (mg/100 g FW)	AsA contribution towards ORAC (%)
Grape (red)	82.3 ± 0.5	305.1 ± 0.9 (16)	1745.3 ± 13.8 (15)	202.7 ± 0.7 (18)	10.1 ± 0.4 (28)	1.7
Kiwano	90.1 ± 0.6	19.5 ± 0.5 (41)	122.3 ± 17.2 (41)	39.3 ± 0.3 (40)	2.9 ± 0.1 (35)	7.0
Kiwi berry	80.6 ± 0.2	74.8 ± 0.9 (35)	1451.2 ± 23.9 (22)	260.5 ± 0.6 (13)	89.8 ± 1.5 (04)	18.6
Kiwi fruit (golden)	84.8 ± 0.0	43.8 ± 0.9 (37)	965.9 ± 5.2 (33)	92.7 ± 1.1 (30)	121.3 ± 1.9 (02)	37.6
Kiwi fruit (green)	87.8 ± 0.1	42.8 ± 0.3 (38)	629.0 ± 12.8 (36)	60.1 ± 0.7 (37)	84.2 ± 2.2 (05)	40.2
Nectarine (white flesh)	83.3 ± 0.5	175.4 ± 1.9 (29)	1071.6 ± 12.2 (32)	102.3 ± 1.3 (27)	7.5 ± 0.2 (32)	2.1
Passion fruit	72.7 ± 0.2	86.2 ± 0.2 (34)	506.7 ± 11.5 (38)	269.6 ± 0.5 (12)	30.2 ± 1.1 (15)	17.9
Peach (white flesh)	87.4 ± 0.4	160.7 ± 0.5 (31)	1117.1 ± 18.5 (31)	51.3 ± 0.8 (39)	8.5 ± 1.1 (30)	2.3
Pear (Taylor's gold)	89.0 ± 0.4	219.2 ± 1.3 (24)	1314.3 ± 22.7 (23)	153.7 ± 2.1 (24)	6.2 ± 1.2 (34)	1.4
Persimmon	81.7 ± 0.8	134.5 ± 0.4 (31)	785.3 ± 12.1 (35)	114.2 ± 0.8 (26)	62.7 ± 1.2 (07)	24.0
Pineapple	87.4 ± 1.5	211.7 ± 1.7 (26)	607.3 ± 10.3 (37)	27.4 ± 0.7 (41)	47.4 ± 1.0 (12)	23.4
Plum (black)	87.1 ± 0.5	511.2 ± 1.1 (11)	2821.6 ± 9.1 (08)	367.1 ± 0.7 (09)	12.5 ± 0.2 (24)	4.1
Plum (cherry)	91.8 ± 0.0	311.3 ± 2.7 (15)	1745.1 ± 21.1 (16)	214.1 ± 1.1 (17)	10.1 ± 0.1 (28)	1.7
Plum (red)	91.6 ± 0.3	356.3 ± 1.0 (11)	1924.3 ± 11.6 (13)	285.3 ± 0.3 (10)	10.2 ± 1.1 (27)	1.8
Raspberry (black)	85.9 ± 0.0	679.0 ± 1.7 (08)	4728.0 ± 17.2 (05)	413.1 ± 0.4 (08)	92.0 ± 0.2 (03)	5.8
Raspberry (red)	84.9 ± 0.3	226.2 ± 0.2 (23)	2516.6 ± 25.5 (11)	198.1 ± 1.0 (19)	110.1 ± 2.7 (04)	13.1
Rhubarb	93.6 ± 0.2	236.2 ± 0.2 (21)	1280.2 ± 17.3 (27)	83.2 ± 1.0 (34)	6.4 ± 0.1 (33)	1.5
Strawberry	91.2 ± 0.1	258.2 ± 3.6 (19)	1684.7 ± 23.7 (17)	99.7 ± 0.4 (28)	62.0 ± 0.4 (08)	11.0
Tamarillo	81.4 ± 0.2	70.6 ± 0.6 (36)	417.3 ± 12.1 (40)	168.1 ± 3.2 (23)	41.3 ± 0.2 (13)	29.7
Tomato	94.4 ± 0.2	256.4 ± 2.2 (20)	1681.2 ± 22.4 (18)	94.3 ± 1.2 (29)	17.4 ± 1.6 (19)	3.1
Tomato (red cherry)	92.4 ± 0.1	211.7 ± 1.5 (27)	1151.4 ± 15.2 (30)	70.1 ± 1.4 (35)	14.2 ± 0.1 (21)	3.7

All data are shown as means ± SE from three replicates. Number in parenthesis is the rank of fruit in the assay. n.d.: not determined.

[a] The ORAC capacity of 1.0 µmol of vitamin C is 0.52 µmol of Trolox equivalent (Cao *et al*., 1993). [b] not calculated

3.4.5. Correlations

To determine the contribution of AsA and TPCs to antioxidant capacities of fruit available in New Zealand, the correlations between AsA and TPCs and antioxidant capacities (ABTS, ORAC) were evaluated (Table 3.2). There was a strong correlation between TPC and antioxidant capacities measured by ABTS and ORAC assays ($r^2 = 0.96$, $p < 0.001$ for ABTS and $r^2 = 0.82$, $p < 0.001$ for ORAC) while AsA concentration showed no correlation with TPC ($r^2 = 0.10$, $p > 0.05$) or ABTS ($r^2 = 0.03$, $p > 0.05$). There was a very weak correlation between AsA and ORAC ($r^2 = 0.13$, $p < 0.05$).

Table 3.2. Correlation coefficients (r^2) between antioxidant capacities (ABTS and ORAC), total phenolic and AsA concentrations in analysed fruits.

	TPCs	ABTS	ORAC
ABTS	0.96^{***}		
ORAC	0.82^{***}	0.77^{***}	
AsA	0.10^{NS}	0.03^{NS}	0.13^{*}

NS: not significant, $*p < 0.05$, $***p < 0.001$.

3.4.6. Fruit serving size

As daily fruit consumption is of benefit in reducing risks of disease, it is useful to evaluate the total antioxidant capacity consumed, based on serving size. Fruit are usually consumed in different amounts depending on their type and consumer behaviour. Definitions of serving size are based on the portion size that people typically eat and on nutritional requirements that people need for maintaining human health (USDA, 2010). One serving of sliced or chopped fruit is equal to one cup; however, a single piece of fruit, such as an apple or two apricots, counts as one serving.

Based on serving size (Table 3.3), blueberry (9695.0 µmol TE/serve), blackcurrant (10676.2 µmol TE/serving), black grape (4721.7 µmol TE/serve), cranberry (4972.6 µmol TE/serve), black raspberry (6430.1 µmol TE/ serve), Indian gooseberry (21537.9 µmol TE/serve) and carrambola (4237.7 µmol TE /serve) provided higher antioxidant capacity per serving as compared to kiwano (122.3 µmol TE/serve), passion fruit (182.4 µmol TE/serve) and tamarillo (500.7 µmol TE /serve). In terms of AsA, a serving of blackcurrant (212.5 mg/serve) supplies the highest amount of AsA, followed by kiwiberry (152.7 mg/serve), red raspberry (149.8 mg/serve), black raspberry (125.1 mg/serve), golden kiwifruit (104.2 mg/serve), strawberry (98.0 mg/serve), redcurrant (90.7 mg/serve), boysenberry (79.2 mg/serve) and green kiwifruit (76.6 mg/serve).

Table 3.3. AsA concentration (mg) and ORAC value (µmol TE) per serving of fruit.

Fruit	Serving size[a] (g)	AsA/serve	ORAC/serve
Apple	130 (1 fruit)	26.7	1521.5
Apricot	108 (2 fruit)	15.7	665.1
Avocado	162 (1 medium fruit)	16.8	2227.3
Banana	128 (1 medium fruit)	11.1	1092.7
Blueberry	164 (1 cup)	19.5	9695.2
Boysenberry	133 (1 cup)	79.2	3275.7
Carambola	128 (1 fruit)	-[b]	4237.7
Cherry (black Tartarian)	150 (1 cup)	18.8	2035.9
Cherry (red Bing)	150 (1 cup)	15.3	2631.8
Cherry (yellow Royal ann)	150 (1 cup)	21.3	2185.7
Cranberry	95 (1cup)	59.3	4972.6
Currant (black)	118 (1 cup)	212.5	10676.2
Currant (red)	118 (1 cup)	90.7	3122.7
Feijoa	60 (2 fruit)	16.4	884.6
Goji berries	120 (1 cup)	-[b]	3930.1
Gooseberries (red)	158 (1 cup)	47.8	696.3
Gooseberries (indian)	158 (1 cup)	-[b]	21537.9
Gooseberry (green)	158 (1 cup)	65.1	2276.3
Grape (black)	172 (1 cup)	22.8	4721.7
Grape (green)	169 (1 cup)	13.1	2462.8
Grape (red)	169 (1 cup)	17.1	2949.5
Kiwano	100 (1 fruit)	2.9	122.3
Kiwiberry	170 (1 cup)	152.6	2467.1
Kiwifruit (golden)	86 (1 fruit)	104.2	830.7
Kiwifruit (green)	91 (1 fruit)	76.6	572.4
Nectarine (white flesh)	143 (1 fruit)	10.7	1532.3
Passion fruit	36 (2 fruits)	10.9	182.4
Peach (white flesh)	138 (1 fruit)	11.8	1541.6
Pear (Taylors gold)	151 (1 fruit)	9.4	1984.6
Persimmon	75 (1 fruit)	47.0	589.0
Pineapple	164 (1 cup, chopped)	23.3	1888.2
Plum (black)	98 (2 fruit)	12.3	903.1
Plum (cherry)	174 (1 cup, sliced)	17.5	3036.5
Plum (red)	90 (2 fruit)	9.2	1552.8
Raspberries (black)	136 (1 cup)	125.1	6430.1
Raspberry (red)	136 (1 cup)	149.8	3422.6
Rhubarb	265 (1 cup, stewed)	17.0	3392.5
Strawberry	158 (1 cup)	98.0	2661.8
Tamarillo	120 (2 fruits)	49.5	500.7
Tomato (red)	190 (1 cup, chopped)	33.1	3194.3
Tomato (red cherry)	190 (1 cup, chopped)	27.0	2187.6

[a] Serving size derived from The Concise New Zealand Food Composition Tables, (2009) and USDA National Nutrient Database for Standard Reference (Release 23). [b] not determined.

3.5. Discussion

3.5.1. Effect of extraction temperatures on total phenolic concentrations

Vinson *et al*., (2001) showed that conjugated phenolics make a major contribution to total phenolic concentrations in fruit, however, most reported studies have only measured the soluble free phenolics and overlooked on bound-phenolic compounds, resulting in underestimation of the total phenolic concentrations. Thus, in this study, TPCs of fruit were extracted using acid and heat. Of the three temperatures tested here, the optimum temperature for total phenolic extraction was found to be 60^0C while 4^0C gave the lowest TPCs. High temperatures could accelerate the permeability of cellular membranes in ground fruit tissue, facilitating extraction of the phenolic compounds during extraction (Spanos *et al*., 1990). Moreover, increasing the extraction temperature could increase extraction of phenolic compounds by increasing solubility and mass transfer rate (Dai and Mumper, 2010). However, too high a temperature along with long extraction time increased the oxidation of phenolic compounds, resulting in their structural degradation into biologically inactive forms and interactions with other non-phenolic components; therefore, decreasing TPCs in the extracts (Shi *et al*., 2005; Liazid *et al*., 2007). This can explain the significantly lower TPCs when extracted at 90^0C compared with those extracted at 60^0C. Therefore, it is important to select an appropriate extraction temperature to maximize extraction of TPCs while maintaining the stability of phenolic compounds.

3.5.2. Total phenolic concentrations

In this study, fruits analysed were arranged into three groups: high, medium and low based on TPCs. These ranges were also used by Vasco *et al*., (2008). Berry fruits were in the high TPCs group. The highest TPCs were found for Indian gooseberry. This could be due to the presence of concentrated hydrolysable tannins comprising ellagic acid, gallic acid, emblicanins A and emblicanins B found in this fruit (Poltanov *et al.,* 2009). Similarly, the relatively high TPCs of carambola may be primarily derived from condensed tannins, i.e. proanthocyanidins, which are considered to be the major phenolic compounds in this fruit (Shui and Leong, 2004).

Within the same fruit species, such as grapes, raspberries and currants, deeper-coloured fruits contained higher TPCs than lighter-coloured fruit (Table 3.1). The concentrations of total phenolics were always higher in black grape, black plum, blackcurrant, black raspberry and black cherry in comparison with red, yellow or green fruits of the same species. This phenomenon can be due to high concentrations of anthocyanin pigments, the major group of

phenolic compounds in dark-coloured fruits (Wang *et al*., 1997). Gavrilova *et al.,* (2011) reported that anthocyanins contributed to 87% and 80% of TPCs in blackcurrant and redcurrant, respectively, and there were ten times higher concentrations of these compounds found in blackcurrant compared to redcurrant. Therefore, it is possible that the large differences of TPCs (855.2 mg GAE/100 g FW vs. 431.9 mg GAE/100 g FW) between blackcurrant and redcurrant determined from this study could be due to differences in the anthocyanins concentrations. Similarly, a significantly higher concentration of anthocyanins in black raspberry (200 mg/ 100 g FW) as compared to red raspberry (less than 60 mg/100 g FW) (Torre and Barritt, 1977) may explain the approximately 2-fold higher TPCs (413.1 mg GAE/100 g FW) of black raspberry as compared to red raspberry (198.1 mg GAE/100 g FW) in this study.

The Folin-Ciocalteu assay estimates the amounts of total phenolic compounds present in an extract. The results are expressed as equivalent to one phenolic compound in this case as gallic acid, which was used as the reference standard, even though there is a mixture of phenolic compounds present in the sample and some of these may be more active than the standard (Prior *et al.,* 2005). However, interfering substances including non-phenolic organic and inorganic substances could react with the reagent at the same time and, consequently, lead to overestimation of phenolic concentrations. In addition, Singleton and Rosi (1965) found that according to differences in molecular structure, phenolic compounds respond differently in the different antioxidant capacity assays. This can explain the outliers in the correlation between TPCs and antioxidant capacities in some fruits analysed in this study. For example, the TPCs of strawberry (99.68 mg GAE/100 g FW) was lower than that of kiwiberry (260.45 mg GAE/100 g FW), whereas, the antioxidant capacity of strawberry (258.19 µmol TE/100g FW) was much higher than that of kiwiberry (74.81 µmol TE/100g FW). Also, while the TPCs of passion fruit (269.62 mg GAE/100 g FW) was approximately three times higher than that of apple (90.17 mg GAE/100 g FW), its ABTS antioxidant capacity (86.19 µmol TE/100g FW) was half that of apple (162.3 µmol TE/100g FW). Similarly, the antioxidant capacities obtained in tamarillo, kiwiberry, and passion fruit were low despite them containing relatively high TPCs. From these observations, it is possible that the potent active phenolic compounds, which have high antioxidant activities, were low in these fruits.

In this study, TPCs were extracted at 60^0C for 2 hours. This completely destroyed vitamin C in the fruit extracts (data not shown). Because Slinkard and Singleton (1977) reported that the Folin-Ciocalteu reagent can be reduced by many non-phenolic compounds, including AsA, some studies corrected total phenolic concentrations by subtracting the contribution of AsA

concentrations (Asami *et al.*, 2003; Isabelle *et al*., 2010). In these studies, TPCs were extracted at room temperature and these would include AsA. In the current study, correcting TPCs for AsA was unnecessary as there was no ascorbic acid detected in the fruit extracts. This was supported by Almeida *et al*., (2011) who eliminated interference of ascorbic acid to TPCs extracts by heating fruit samples in an aqueous mixture of methanol and HCL for two hours.

TPCs of twelve cultivars of blackberry and hybridberry in New Zealand were determined by Connor *et al*., (2005) in which the reported TPCs results for boysenberry ranged from 372-536 mg GAE/100 g FW were comparable with the value obtained from this study (480 mg GAE/100 g FW). Additionally, TPCs of ten apple cultivars in New Zealand including Royal Gala cultivar were also measured in a study of McGhie *et al*., (2005); however, different TPCs assays as well as different reference standards, which were used for the reported results and the current results, make it was difficult to compare TPCs between these studies even for the same fruit cultivar.

3.5.3. Ascorbic acid concentrations

The titration method used in this study determined L.AsA but did not included dehydro-AsA which has been shown to contribute a minor amount to total AsA (Wills *et al*., 1984; Osuna-Garcia *et al*., 1998). AsA concentrations of blackcurrant, kiwifruit, red raspberry and kiwiberry were more dominant than the other fruits analysed in this study (Table 3.1). High concentrations of AsA as well as major contributions of AsA to total antioxidant capacities included ORAC in kiwifruit was supported by Du *et al*., (2009). They also showed that vitamin C concentrations varied greatly between *Actinidia* genotypes. In addition, the high AsA concentration and low TPCs of kiwifruit obtained in this study were in agreement with results obtained by Gil *et al*., (2006), suggesting that kiwifruit can be considered as good source of vitamin C but not TPCs.

AsA concentrations were maintained in the freeze-dried samples that were used for ORAC assay in this study (data not shown). The effect of freeze-drying on AsA concentration has previously been reported. According to Abonyi *et al.,* (2002), there was no significant difference of AsA concentration in strawberry puree before and after being freeze-dried. Similarly, when comparing between freeze-drying and hot-air drying of two tomato varieties, Chang *et al.,* (2006) found that freeze-drying had no significant effect on the AsA concentration of tomatoes. Results from the current study confirmed this.

Generally, in this study, AsA contributions to total antioxidant capacity measured by ORAC were small. AsA made a minor contribution to ORAC values for blackcurrant (6.0%) and red raspberry (13.1%) despite the high AsA concentrations of 180.1 mg/100 g FW and 110.1 mg/100 g FW found, respectively, in these fruit. Low contributions of AsA to ORAC values in fruit were also demonstrated in study of Isabelle *et al*., (2010). Because AsA made a minor contribution to the antioxidant capacity in most of the fruits analysed, suggesting that the primary sources of antioxidant capacity of most fruits available in New Zealand are from antioxidant compounds other than AsA. This suggestion is consistent with earlier reported studies (Wang *et al*., 1996; Prior *et al*., 1998; Kalt *et al*. 1999; Vinson *et al.,* 2001), which indicated that antioxidant capacities in fruit are due primarily to non-vitamin C phyto-chemicals and phenolic compounds are the main phyto-chemicals responsible. In a study of Kalt *et al*. (1999), small fruits were divided into two distinct groups: (1) those with high phenolics, anthocyanins and antioxidant capacity and low AsA; (2) those with low phenolics, anthocyanins, and antioxidant capacity and high AsA. They found that AsA made a small contribution (0.4-9.4%) to the ORAC value of fruits tested and that no correlation between AsA concentration and total antioxidant capacity (ORAC assay) could be established in either group.

The lack of correlation between AsA and ABTS values could be due to heating the extraction at 60^0C in 2 hours for the TPCs and ABTS assays that destroyed AsA, a heat sensitive compounds (Munyaka, *et al*., 2010), to a biologically inactivated form. However, extraction for ORAC assay without heating also resulted in a very weak relationship ($r^2 = 0.13$) between AsA and ORAC values. The low antioxidant capacities of AsA may be due to its dual nature. Poljšak and Raspor, (2008) reported that in the presence of the redox-cycling oxidizing agent such as Cr (VI), antioxidant protection of AsA may change to a pro-oxidative process according to Fenton-like chemistry. Pro-oxidants can generate reactive oxygen species and cause oxidative stress, resulting in various pathological events and/or diseases (Prior and Cao, 1999). In fact, with the presence of free transition metal ions, for example Fe^{3+}, ascorbic acid acts as a pro-oxidant by converting Fe^{3+} into Fe^{2+}, resulting in the formation of superoxide anions and hydroxyl radicals (Rietjens *et al.,* 2002). By using a liposome oxidation method, an antioxidant capacity assay based on inhibition of liposome oxidation, to measure antioxidant capacity of fruits, vegetable and commercial frozen pulps, Hassimotto *et al*., (2005) found that AsA showed high pro-oxidant activities in the presence of copper. The study of Kulkarni *et al*., (2007) on sapota fruit, which are considered rich sources of sugars, proteins, ascorbic acid, phenolics, carotenoids and minerals, reported that the pro-oxidant

capacity of AsA depended on the concentration of the juice samples and suggested that the dual nature of AsA depends on the chemical environment and condition in which the molecule is active.

3.5.4. Antioxidant capacities

The antioxidant capacity of foods and biological systems depends on numerous factors including colloidal properties of the substrates, the type of oxidisable substrate, the condition and stage of oxidation and localisation of antioxidants in different phases as well as the type of reactions between many different antioxidants and free radicals (Frankel and Meyer, 2000). Therefore, it was recommended that at least two different antioxidant assays should be compared to provide a reliable result and comprehensive information about total antioxidant capacity of a given fruit (Pérez-Jiménez *et al*., 2008).

Since ABTS and ORAC are considered to be standardised methods for measuring antioxidant capacity (Prior *et al*., 2005), they were used to quantify antioxidant capacity of fruit in this study. It was interesting that antioxidant capacities of Indian gooseberry were the highest for both ABTS and ORAC assays. Poltanov *et al.*, (2009) found that Indian gooseberry had high concentrations of emblicanins A and emblicanins B of which the $DPPH^{\bullet}$ scavenging activity were 7.86 and 11.20 times higher than ascorbic acid and 1.25 and 1.78 times as much as gallic acid, respectively. Thus, it is possible that the highest antioxidant capacities reported here for Indian gooseberry are due to the high concentrations of these components.

Darker coloured fruits not only contained high TPCs but also high antioxidant capacities as compared to lighter coloured fruits (Table 3.1). High free radical scavenging capacity of deep-coloured fruits such as blueberry, boysenberry, blackcurrant and black raspberry is due to the presence of anthocyanins. Wang and Lin, (2000) found a high concentration of anthocyanin results in a high antioxidant capacity. Prior *et al.*, (1998) recorded a high correlation ($r^2 = 0.77$) between anthocyanins and ORAC in blueberries. Furthermore, Wang and Lin (2000) found a strong correlation between anthocyanins concentration and the ORAC values (r^2 was from 0.84 to 0.98) of strawberries, blackberries and raspberries and there was a significant increase in anthocyanins concentration as the colour changed from green to pink, dark-pink or dark-purple in these fruits. Therefore, different anthocyanins concentrations may explain the large differences of antioxidant capacity among dark and light coloured fruits in the same species in the current study.

Li *et al*., (2007) demonstrated that different extraction solvents and different measurement methods may result in variation of antioxidant capacity measured. This makes it difficult to compare the current results to the literature. However, the study of Wu *et al*., (2004c) that used the same extraction and measurement method for ORAC assay as the present study, reported that ORAC values of six gooseberry cultivars ranged from 20.4 to 41.3 µmol TE/g FW, which were higher as compared with ORAC values for New Zealand gooseberries (11.2 to 14.4 µmol TE/g FW) but lower than Indian gooseberry (136.31 µmol TE/g FW) determined in the current study. The differences in the fruit antioxidant capacity values between this study and the reported literature may be explained by the variations in fruit cultivars (Kim *et al.*, 2003; Scalzo *et al.*, 2005), climate conditions, maturity stage, light intensity, soil characteristics, geography and carbon dioxide concentration in the atmosphere (Wu *et al*., 2004b; Wang, 2006). For example, ORAC value of strawberry in this study (16.8 µmol TE/g FW) was similar to that of Wang *et al.*, (1996) (15.4 µmol TE/g FW). This ORAC value was also within the range reported in Wang and Lin's study (2000) (from 12.2 to 17.4 µmol TE/g FW) but less than that in Kalt *et al*.,'s study (1999) (20.6 µmol TE/g FW) and Wu *et al*.,'s study (2004a) (35.4 µmol TE/g FW). The antioxidant capacity of blackcurrant to peroxyl radicals measured by ORAC assay (90.5 µmol TE/g FW) was in range of the values obtained by Wu *et al*., (2004c) (49.0 to 100.6 µmol TE/g FW). In contrast, Prior *et al*., (1998) quantified the ORAC of different cultivars of blueberry as ranging from 13.9 to 45.9 µmol TE/g FW, meanwhile Wu *et al*., (2004a) found 61.8 µmol TE/g FW and that in this study was determined at 59.1 µmol TE/g FW.

In general, due to the limitation of the sampling scheme, it was not possible to identify the specific reasons for this variance, but genotypes and growing conditions may be the major factors. Therefore, it is necessary to measure antioxidant capacity for particular fruit in many geographic areas over several years using the same standardised assays. The data, which reflect a comparison of fruits, would be useful for consumers and nutritionists who are interested in preventing oxidative stress-induced disease by consuming foods containing natural antioxidant compounds.

3.5.5. Correlations

The high correlation between ABTS and ORAC ($r^2 = 0.77$) found from the current study was supported by previous studies which recorded correlations between these assays ranging from $r^2 = 0.55$ to 0.98 (Stintzing *et al*., 2004; Thaipong *et al*., 2006; da Silva *et al*., 2007; Du *et al*., 2009). This range depends on the hydrophilicity of antioxidant compounds (Zulueta *et al*.,

2009), the extraction solvents and fruit cultivars (Thaipong *et al*., 2006). Each assay gives an estimation of antioxidant capacity that is relevant to specific conditions such as radicals used, reagents of the assay and extraction methods.

Strong correlations were found between TPCs and antioxidant capacities (ABTS and ORAC) suggesting that total phenolic compounds are primarily responsible for free radical scavenging capacity of fruits available in New Zealand. Several previous studies showed similar linear correlations between TPCs and antioxidant capacities of fruits from different areas. For example, strong correlations between ORAC and TPCs were demonstrated for US fruits of *Vaccinium* species (r = 0.85) (Prior *et al*., 1998) and high bush blueberry (r = 0.76) (Ehlenfeldt and Prior, 2001); between ABTS and TPCs for U.S domesticated and wild *Rubus* species (r = 0.67) (Deighton *et al*., 2000), for 18 non-traditional tropical fruits from Brazil (r = 0.92) (Rufino *et al*., 2010) and for 17 Mauritian exotic fruits (r = 0.98) (Luximon-Ramma *et al*., 2003).

3.5.6. The importance of fruit antioxidants

Since reactive oxygen species (ROS) are closely associated with oxidative damage-induced disorders and diseases in the human body (Valko *et al*., 2007) and since the ORAC assay allows the measurement oxygen radical scavenging capacity in biological samples, ORAC values per fruit serving provides an estimate of beneficial phyto-chemicals these fruits may offer. Because the antioxidant defence system of the body is composed of different antioxidant components, of which antioxidant capacity depend on kind of free radicals or oxidants produced in the body (Singh *et al*., 2004), and because fruit contains groups of natural antioxidant compounds instead of a single antioxidant, consuming fruit may meet the demand of the body for antioxidant diversity (Craig, 1997). Liu (2003) reported that due to the additive and synergistic effects of natural antioxidants, the mixing of antioxidant compounds from plant foods, especially fruits, resulted in higher antioxidant activities as compared to an isolated, single compound in a supplement. They suggested that consuming a complex mixture of natural phyto-chemicals through a balanced diet containing fruit would better provide protection for the body than an isolated, single antioxidant. Indeed, many studies have shown that regularly consuming green or yellow fruits and vegetables was capable of preventing cardiovascular disease, cancer and hypertension due to antioxidant compounds such as vitamin C, retinol and β-carotene abundantly present in these plant foods (Steinmetz and Potter, 1996; Halliwell, 2002). There are some controversial discussions about health benefits from taking antioxidant supplements and adverse effects of antioxidants from

single supplements have been shown. Recently, in a systematic review and meta-analysis including 68 randomized trials with almost a quarter of a million participants, Bjelakovic *et al.*, (2007) demonstrated that β-carotene used alone significantly increased mortality. After exclusion of high-bias risks and selenium trials, β-carotene, vitamin A, vitamin E given alone or combined with other supplements significantly increased mortality. They commented that single antioxidant supplements have not shown any beneficial effects on mortality, even worse; these single vitamins supplements increased the risk of death. While the final conclusion about benefits from antioxidant supplement has not been clear, the beneficial effects of consuming fruit and vegetables, however, are well documented, thus, to prevent oxidation stress-induced diseases, people should consume fruit daily instead of adding antioxidants from supplements.

Consuming at least five servings of fruit and vegetables per day has been encouraged worldwide for a healthy diet. The New Zealand recommended daily intake of vitamin C to prevent disease is between 30-60 mg/day (New Zealand Ministry of Health, 2011). By following the recommendation at least two fruit and three vegetable serving per day, in terms of fruit, based on the calculations in the present study (Table 3.3), would supply a range of 12.1- 365.1 mg vitamin C.

3.6. Conclusion

Total phenolic, ascorbic acid concentrations and antioxidant capacities of 41 fruit available in New Zealand were measured. Overall, the data from this study showed that fruits available in New Zealand, especially berries are abundant sources of natural antioxidants. Darker coloured fruits such as blackcurrant, black raspberry, black grape contained higher TPCs and had higher antioxidant capacities compared with lighter coloured fruit of the same species. The strong correlation between TPCs and antioxidant capacities indicated that phenolic compounds are primarily responsible for the ability of fruit to scavenge free radicals. Depending on fruit characteristics and consumer behaviour, antioxidants per serving vary. Indian gooseberry; blackcurrant and blueberry contributed high antioxidants capacity per serve, while blackcurrant, kiwiberry, raspberry and golden kiwifruit are good sources for daily vitamin C intakes.

References

Abonyi, B. I., Feng, H., Tang, J., Edwards, C. G., Chew, B. P., Mattinson, D. S., & Fellman, J. K. (2002). Quality retention in strawberry and carrot purees dried with Refractance Window™ System. *Journal of Food Science, 67*(3), 1051-1056.

Almeida, M. M. B., de Sousa, P. H. M., Arriaga, Â. M. C., do Prado, G. M., Magalhães, C. E. d. C., Maia, G. A., *et al.* (2011). Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil. *Food Research International, 44*(7), 2155-2159.

Asami, D. K., Hong, Y.-J., Barrett, D. M., & Mitchell, A. E. (2003). Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry and corn grown using conventional, organic, and sustainable agricultural practices. *Journal of Agricultural and Food Chemistry, 51*(5), 1237-1241.

Bjelakovic, G., Nikolova, D., Gluud, L. L., Simonetti, R. G., & Gluud, C. (2007). Mortality in randomized trials of antioxidant supplements for primary and secondary prevention. *The Journal of the American Medical Association, 297*(8), 842-857.

Cao, G., Alessio, H. M., & Cutler, R. G. (1993). Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biology and Medicine, 14*(3), 303-311.

Chang, C.-H., Lin, H.-Y., Chang, C.-Y., & Liu, Y.-C. (2006). Comparisons on the antioxidant properties of fresh, freeze-dried and hot-air-dried tomatoes. *Journal of Food Engineering, 77*(3), 478-485.

Connor, A. M., Finn, C. E., & Alspach, P. A. (2005). Genotypic and environmental variation in antioxidant activity and total phenolic content among blackberry and hybridberry cultivars. *Journal of the American Society for Horticultural Science, 130*(4), 527-533.

Connor, A. M., Luby, J. J., Tong, C. B. S., Finn, C. E., & Hancock, J. F. (2002). Genotypic and environmental variation in antioxidant activity, total phenolic content, and anthocyanin content among blueberry cultivars. *Journal of the American Society for Horticultural Science, 127*(1), 89-97.

Cook, N. R., Albert, C. M., Gaziano, J. M., Zaharris, E., MacFadyen, J., Danielson, E., *et al.* (2007). A randomized factorial trial of vitamins C and E and beta carotene in the secondary prevention of cardiovascular events in women: results from the women's antioxidant cardiovascular study. *Archives of Internal Medicine, 167*(15), 1610-1618.

Craig, W. J. (1997). Phyto-chemicals: guardians of our health. *Journal of the American Dietetic Association, 97*(10, Supplement 1), S199-S204.

da Silva, F. L., Escribano-Bailón, M. T., Pérez Alonso, J. J., Rivas-Gonzalo, J. C., & Santos-Buelga, C. (2007). Anthocyanin pigments in strawberry. *LWT - Food Science and Technology, 40*(2), 374-382.

Dai, J., & Mumper, R.J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules, 15*, 7313-7352.

Deighton, N., Brennan, R., Finn, C., & Davies, H. V. (2000). Antioxidant properties of domesticated and wild *Rubus* species. *Journal of the Science of Food and Agriculture, 80*(9), 1307-1313.

Du, G., Li, M., Ma, F., & Liang, D. (2009). Antioxidant capacity and the relationship with polyphenol and vitamin C in *Actinidia* fruits. *Food Chemistry, 113*(2), 557-562.

Ehlenfeldt, M. K., & Prior, R. L. (2001). Oxygen radical absorbance capacity (ORAC) and phenolic and anthocyanin concentrations in fruit and leaf tissues of highbush blueberry. *Journal of Agricultural and Food Chemistry, 49*(5), 2222-2227.

Frankel, E. N., & Meyer, A. S. (2000). The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture, 80*(13), 1925-1941.

Fu, L., Xu, B.-T., Xu, X.-R., Qin, X.-S., Gan, R.-Y., and Li, H.-B. (2010). Antioxidant capacities and total phenolic contents of 56 wild fruits from South China. *Molecules, 15*(12), 8602-8617.

Gavrilova, V., Kajdžanoska, M., Gjamovski, V., & Stefova, M. (2011). Separation, characterization and quantification of phenolic compounds in blueberries and red and blackcurrants by HPLC−DAD−ESI-MSn. *Journal of Agricultural and Food Chemistry*, (in press).

Gil, M. I., Aguayo, E., & Kader, A. A. (2006). Quality changes and nutrient retention in fresh-cut versus whole fruits during storage. *Journal of Agricultural and Food Chemistry, 54*(12), 4284-4296.

Greenberg, E. R., Baron, J. A., Tosteson, T. D., Freeman, D. H., Beck, G. J., Bond, J. H., *et al.* (1994). A clinical trial of antioxidant vitamins to prevent colorectal adenoma. *New England Journal of Medicine, 331*(3), 141-147.

Guo, C., Cao, G., Sofic, E., & Prior, R. L. (1997). High-performance liquid chromatography coupled with coulometric array detection of electroactive components in fruits and vegetables: relationship to oxygen radical absorbance capacity. *Journal of Agricultural and Food Chemistry, 45*(5), 1787-1796.

Halliwell, B. (1994). Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *The Lancet, 344*(8924), 721-724.

Halliwell, B. (2001). Vitamin C and genomic stability. *Mutation Research , 475*(1–2), 29–35.

Halliwell, B. (2002). Effect of diet on cancer development: is oxidative DNA damage a biomarker? *Free Radical Biology and Medicine, 32*, 968-974.

Halvorsen, B. L., Carlsen, M. H., Phillips, K. M., Bøhn, S. K., Holte, K., Jacobs, D. R., & Blomhoff, R. (2006). Content of redox-active compounds (ie, antioxidants) in foods consumed in the United States. *The American Journal of Clinical Nutrition, 84*(1), 95-135.

Hamid, A.A., Aiyelaagbe, O.O., Usman, L. A., Ameen, O. M., & A. Lawal. (2010). Antioxidants: its medicinal and pharmacological applications. *African Journal of Pure and Applied Chemistry, 4*(8), 142-151.

Hassimotto, N. M. A., Genovese, M. I., & Lajolo, F. M. (2005). Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. *Journal of Agricultural and Food Chemistry, 53*(8), 2928-2935.

Hercberg, S., Galan, P., Preziosi, P., Alfarez, M.-J., & Vazquez, C. (1998). The potential role of antioxidant vitamins in preventing cardiovascular diseases and cancers. *Nutrition, 14*(6), 513-520.

Isabelle, M., Lee, B. L., Lim, M. T., Koh, W.-P., Huang, D., & Ong, C. N. (2010). Antioxidant activity and profiles of common fruits in Singapore. *Food Chemistry, 123*(1), 77-84.

Jeong, S.-M., Kim, S.-Y., Kim, D.-R., Jo, S.-C., Nam, K. C., Ahn, D. U., & Lee, S.-C. (2004). Effect of heat treatment on the antioxidant activity of extracts from citrus peels. *Journal of Agricultural and Food Chemistry, 52*(11), 3389-3393.

Kalt, W., Forney, C.F., Martin, A., & Prior, R.L. (1999). Antioxidant capacity, vitamin C, phenolics and anthocyanins after fresh storage of small fruits. *Journal of Agricultural and Food Chemistry, 47*, 4638- 4644.

Kim, D.-O., Jeong, S. W., & Lee, C. Y. (2003). Antioxidant capacity of phenolic phyto-chemicals from various cultivars of plums. *Food Chemistry, 81*(3), 321-326.

Kulikowska-Karpińska, E & Moniuszko-Jakoniuk, J. (2004). The antioxidative barrier in the organism. *Polish Journal of Environmental Studies, 13*(1), 5-13.

Kulkarni, A. P., Policegoudra, R. S., & Aradhya, S. M. (2007). Chemical composition and antioxidant capacityof sapota (*Achras sapota linn.)* fruit. *Journal of Food Biochemistry, 31*(3), 399-414.

Larrauri, J. A., Rupérez, P., & Saura-Calixto, F. (1997). Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. *Journal of Agricultural and Food Chemistry, 45*(4), 1390-1393.

Lee, J., Koo, N., & Min, D. B. (2004). Reactive oxygen species, aging, and antioxidative nutraceuticals. *Comprehensive Reviews in Food Science and Food Safety, 3*(1), 21-33.

Lemanska, K., Szymusiak, H., Tyrakowska, B., Zielinski, R., Soffers, A. E. M. F., & Rietjens, I. M. C. M. (2001). The influence of pH on antioxidant properties and the mechanism of antioxidant action of hydroxyflavones. *Free Radical Biology and Medicine, 31*(7), 869-881.

Leong, L. P., & Shui, G. (2002). An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chemistry, 76*(1), 69-75.

Li, H.-B., Jiang, Y., Wong, C.-C., Cheng, K.-W., & Chen, F. (2007). Evaluation of two methods for the extraction of antioxidants from medicinal plants. *Analytical and Bioanalytical Chemistry, 388*(2), 483-488

Liazid, A., Palma, M., Brigui, J., & Barroso, C. G. (2007). Investigation on phenolic compounds stability during microwave-assisted extraction. *Journal of Chromatography A, 1140*(1-2), 29-34.

Liu, R. H. (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phyto-chemicals. *The American Journal of Clinical Nutrition, 78*(3), 517S-520S.

Liu, R. H. (2004). Potential synergy of phyto-chemicals in cancer prevention: mechanism of action. *The Journal of Nutrition, 134*(12), 3479S-3485S.

Luximon-Ramma, A., Bahorun, T., & Crozier, A. (2003). Antioxidant actions and phenolic and vitamin C contents of common Mauritian exotic fruits. *Journal of the Science of Food and Agriculture, 83*(5), 496-502.

Mahattanatawee, K., Manthey, J. A., Luzio, G., Talcott, S. T., Goodner, K., & Baldwin, E. A. (2006). Total antioxidant activity and fiber content of select florida-grown tropical fruits. *Journal of Agricultural and Food Chemistry, 54*(19), 7355-7363.

McGhie, T.K., Hunt, M., & Barnet, L.E. (2005). Cultivar and growing region determine the antioxidant polyphenolic concentration and composition of apples grown in New Zealand. *Journal of Agricultural and Food Chemistry, 53*, 3065-3070.

Munyaka, A. W., Makule, E. E., Oey, I., Van Loey, A., & Hendrickx, M. (2010). Thermal stability of L-ascorbic acid and ascorbic acid oxidase in broccoli (*Brassica oleracea* var. italica). *Journal of Food Science, 75*(4), C336-C340.

Nag, A. (2009). Role of antioxidants in oxidative stress management. *Research in Environment and Life Sciences, 2*(2), 53-60.

Naidu, K. A. (2003). Vitamin C in human health and disease is still a mystery? An overview. *Nutrition Journal, 2*(1), 2-7.

New Zealand Ministry of Health. (2009). The concise New Zealand food composition tables, 8th Edition. Retrieved 25 February, 2011 from http://www.health.govt.nz/publication/concise-new-zealand-food-composition-tables-8th-edition.

New Zealand Ministry of Health. (2011). Food and nutrition guidelines for healthy adults: a background paper. Retrieved February 18, 2012 from http://www.moh.govt.nz/moh.nsf

Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry, 49*(10), 4619-4626.

Osuna-Garcia, J. A., Wall, M. M., & Waddell, C. A. (1998). Endogenous levels of tocopherols and ascorbic acid during fruit ripening of New Mexican-type chile (*Capsicum annuum* L.). *Journal of Agricultural and Food Chemistry, 46*(12), 5093-5096.

Pérez-Jiménez, J., Arranz, S., Tabernero, M., Díaz- Rubio, M. E., Serrano, J., Goñi, I., & Saura-Calixto, F. (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: extraction, measurement and expression of results. *Food Research International, 41*(3), 274-285.

Pinelo, M., Rubilar, M., Jerez, M., Sineiro, J., & Nunez, 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.

Poltanov, E. A., Shikov, A. N., Dorman, H. J. D., Pozharitskaya, O. N., Makarov, V. G., Tikhonov, V. P., *et al*. (2009). Chemical and antioxidant evaluation of Indian gooseberry (*emblica officinalis* Gaertn., *syn. phyllanthus emblica* L.) supplements. *Phytotherapy Research, 23*(9), 1309-1315.

Prior, R. L., & Cao, G. (1999). *In vivo* total antioxidant capacity: comparison of different analytical methods. *Free Radical Biology and Medicine, 27*(11-12), 1173-1181.

Prior, R. L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C, Lischner, N., Ehlenfeldt, M., Kalt, W., Krewer, G & Mainland, C. M. (1998). Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *Journal of Agricultural and Food Chemistry, 46*(7), 2686-2693.

Prior, R. L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., *et al*. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity ($ORAC_{FL}$) of plasma and other biological and food samples. *Journal of Agricultural and Food Chemistry, 51*(11), 3273-3279.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*(10), 4290-4302.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant capacityapplying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9-10), 1231-1237.

Rietjens, I. M. C. M., Boersma, M. G., HAsAn, L. d., Spenkelink, B., Awad, H. M., Cnubben, N. H. P., *et al*. (2002). The pro-oxidant chemistry of the natural antioxidants vitamin C, vitamin E, carotenoids and flavonoids. *Environmental Toxicology and Pharmacology, 11*(3-4), 321-333.

Rufino, M. d. S. M., Alves, R. E., de Brito, E. S., Pérez-Jiménez, J., Saura-Calixto, F., & Mancini-Filho, J. (2010). Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chemistry, 121*(4), 996-1002.

Scalzo, J., Politi, A., Pellegrini, N., Mezzetti, B., & Battino, M. (2005). Plant genotype affects total antioxidant capacity and phenolic contents in fruit. *Nutrition, 21*(2), 207-213.

Shi, J., Nawaz, H., Pohorly, J., Mittal, G., Kakuda, Y., & Jiang, Y. (2005). Extraction of polyphenolics from plant material for functional foods-engineering and technology. *Food Reviews International, 21*(1), 139 - 166.

Shui, G., & Leong, L. P. (2004). Analysis of polyphenolic antioxidants in star fruit using liquid chromatography and mass spectrometry. *Journal of Chromatography A, 1022*(1-2), 67-75.

Singh, R., Sharad, S., & Kapur, S. (2004). Free radicals and oxidative stress in neurodegenerative diseases: relevance of dietary antioxidants. *Journal of Indian Academy of Clinical Medicine, 5*(3), 218-225.

Singleton, V. L., & Rossi, J. A., Jr. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*(3), 144-158.

Slinkard, K., & Singleton, V. L. (1977). Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture, 28*(1), 49-55.

Spanos, G. A., Wrolstad, R. E., & Heatherbell, D. A. (1990). Influence of processing and storage on the phenolic composition of apple juice. *Journal of Agricultural and Food Chemistry, 38*(7), 1572-1579.

Stalikas, C. D. (2007). Extraction, separation, and detection methods for phenolic acids and flavonoids. *Journal of Separation Science, 30*(18), 3268-3295.

Steinmetz, K. A., & Potter, J. D. (1996). Vegetables, fruit, and cancer prevention: a review. *Journal of the American Dietetic Association, 96*(10), 1027-1039.

Stintzing, F. C., Herbach, K. M., Mosshammer, M. R., Carle, R., Yi, W., Sellappan, S., *et al*. (2004). Color, betalain pattern, and antioxidant properties of cactus pear (*Opuntia spp.*) clones. *Journal of Agricultural and Food Chemistry, 53*(2), 442-451.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Hawkins Byrne, D. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis, 19*(6-7), 669-675.

Torre, L. C., & Barritt, B. H. (1977). Quantitative evaluation of Rubus fruit anthocyanin pigments. *Journal of Food Science, 42*(2), 488-490.

United State Department of Agriculture. (2010). *USDA National Nutrient Database for Standard Reference, Release 23*. Retrieved from http://www.ars.usda.gov

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology, 39*(1), 44-84.

Vasco, C., Ruales, J., & Kamal-Eldin, A. (2008). Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. *Food Chemistry, 111*(4), 816-823.

Vinson, J. A., Hao, Y., Su, X., & Zubik, L. (1998). Phenol antioxidant quantity and quality in foods: vegetables. *Journal of Agricultural and Food Chemistry, 46*(9), 3630-3634.

Vinson, J. A., Su, X., Zubik, L., & Bose, P. (2001). Phenol antioxidant quantity and quality in foods: fruits. *Journal of Agricultural and Food Chemistry, 49*(11), 5315-5321.

Wang, H., Cao, G., & Prior, R. L. (1996). Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry, 44*(3), 701-705.

Wang, H., Cao, G., & Prior, R. L. (1997). Oxygen radical absorbing capacity of anthocyanins. *Journal of Agricultural and Food Chemistry, 45*(2), 304-309.

Wang, S. Y., & Lin, H.-S. (2000). Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *Journal of Agricultural and Food Chemistry, 48*(2), 140-146.

Wang, S.Y. (2006). Effect of pre-harvest conditions on antioxidant capacity in fruits. *Acta Horticulturae* (ISHS*), 712*, 299-306

Wills, R. B. H., Wimalasiri, P., & Greenfield, H. (1984). Dehydroascorbic acid levels in fresh fruit and vegetables in relation to total vitamin C activity. *Journal of Agricultural and Food Chemistry*, *32*(4), 836-838.

World Health Organization & Food and Agricultural Organization (2003). Diet, nutrition and the prevention of chronic diseases. In Report of a joint WHO/FAO expert consultation. Geneva: World Health Organization. Retrieved November 18, 2011 from http://www.who.int/

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2004a). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agricultural and Food Chemistry, 52*(12), 4026-4037.

Wu, X., Gu, L., Holden, J., Haytowitz, D. B., Gebhardt, S. E., Beecher, G., & Prior, R. L. (2004b). Development of a database for total antioxidant capacity in foods: a preliminary study. *Journal of Food Composition and Analysis, 17*(3-4), 407-422.

Wu, X., Gu, L., Prior, R. L., & McKay, S. (2004c). Characterization of anthocyanins and proanthocyanidins in some cultivars of *Ribes, Aronia, and Sambucus* and their antioxidant capacity. *Journal of Agricultural and Food Chemistry, 52*(26), 7846-7856.

Zulueta, A., Esteve, M. J., & Frígola, A. (2009). ORAC and TEAC assays comparison to measure the antioxidant capacity of food products. *Food Chemistry, 114*(1), 310-316.

Chapter 4

The effects of temperature and pH on the extraction of oxalate and pectin from four New Zealand grown fruits

4.1. Abstract

Oxalates and pectin were extracted from four locally grown fruits at three different pH concentrations, pH 6.9, 0.7 and -0.3, and at two different temperatures 21°C and 80°C. The four fruits (green kiwifruit (*Actinidia deliciosa* cv. Hayward), golden kiwifruit (*Actinidia chinensis* cv. Hort16A Zespri), kiwiberry (*Actinidia arguta,* cv. Takaka Green) and persimmon *(Diospyros kaki)*) contained moderate concentrations of oxalates. Total and soluble oxalates were more efficiently extracted at 21°C using 2 M HCL and deionised water, while the highest amounts of pectin were extracted from the fruits at 80°C using 0.2 M HCL. Extraction of the fruits using 0.2 M and 2 M HCL extracted total oxalates which included both insoluble and soluble oxalates. Extraction at 21°C with 2 M HCL gave the highest yields of total oxalates, which ranged from 47.1 to 220.4 mg total oxalates/100 g dry matter (DM) for persimmon and kiwiberry, respectively. Extraction using water at 21°C gave soluble oxalate concentrations ranging from 21.2 mg/100 g DM, for persimmon, to 105.0 mg/100 DM, for kiwiberry fruit. Extraction of pectin was not achieved efficiently using water at either temperature, while extraction using 2 M HCL gave even lower values; optimal extractions of pectin were achieved at 80°C using 0.2M HCL.

4.2. Introduction

Pectins are mixtures of high-molecular-weight cell wall polysaccharides whose complex chemical structures can be classified into proto-pectin, pectin acids and pectinic acids. Pectins make up between 0.5 - 4% of the fresh weight of plant material (Kashyap *et al.,* 2001; Flutto, 2003) and are found in the cell wall of plants where they are closely associated with other structural polysaccharides and positive ions, especially calcium in the form of proto-pectin, the main component of unripe fruit cell walls (Paoletti *et al.*, 1986). During fruit ripening, enzymes in the fruit break down pectin to form soluble pectin (Kashyap *et al*., 2001). HCL extraction of pectin from plant material has been studied by a number of authors (El-Nawawi and Shehata, 1987; Hwang *et al*., 1998; Kalapathy and Proctor, 2001; Kulkani and Vijayanand; 2010), however, the pectin yield varied depending on temperature, pH, extraction time and the plant source (Yeoh *et al.,* 2008).

Calcium in plants is found widely in the cell walls, forming calcium bridges between residues of galacturonic acid belonging to adjacent pectin chains (Neal, 1965). These linkages are associated with the eggbox model or junction zone, in which calcium ion is incorporated into pectin coordination shells with two polyanion oxygens from one pectin chain and three from another chain (BeMiller, 1986; Flutto, 2003). The calcium-pectin complex formed acts as an intracellular cement giving firmness to the plant tissues (Burns and Pressey, 1987). In addition, the calcium makes the structure insoluble and inhibits degradation of the pectin material by polygalacturonase activity (Burns and Pressey, 1987). Kelsay and Prather (1983) suggest that a fibre-mineral-oxalate complex exists that is more difficult to dissolve than a fibre-mineral or oxalate-mineral bond. Since pectin is part of this fibre complex, binding between pectin and oxalate might also occur. This complex may bind with soluble and insoluble oxalates and make them less available for absorption (Hanson *et al*., 1989). Hoagland (1989) used reversed-phase HPLC to determine the nature of the binding between dietary anions and dietary fibre. He found that adding carrot pectin to an oxalate buffer resulted in an extracted material which eluted at the same time as pectin that did not contain any oxalate. Hoagland (1989) showed that soluble oxalate could bind to alcohol insoluble pectin extracted from carrots, cabbage and broccoli in the pH range 5.58 to 6.72. Hoagland (1989) went on to state that it was possible that calcium oxalate could have been precipitated within the pectin fraction as well as being bound to the calcium-pectate complex. This was the main substance found in the alcohol insoluble residue. Since calcium-pectate is widely found in plant tissues (Javis, 1982), oxalate extraction of fruit must involve its release from the calcium-pectate complex. If this extraction does not occur completely then some oxalate will remain in the calcium-pectate complex giving a low estimate of the total oxalate concentrations of the fruit concerned.

This study was carried out to investigate whether pectin found in fruits may interact with oxalate during extraction, which may subsequently lead to a reduction in the amount of oxalate measured in the fruit extract. An understanding of this potential effect would give a clearer understanding about factors relevant to oxalate determination in plant foods.

4.3. Materials and Methods

4.3.1. Sample preparation

Green kiwifruit (*Actinidia deliciosa* cv. Hayward), golden kiwifruit (*Actinidia chinensis* cv. Hort16A Zespri), kiwiberry (*Actinidia arguta*, cv. Takaka Green) and persimmon *(Diospyros kaki)* were purchased fresh from a local supermarket in Christchurch, New Zealand in July

2011. The edible portion of the fruit was chopped into 5 mm slices using a stainless steel knife and the pieces were freeze-dried in a Cuddon freeze dryer (Model E.D. 5.3) and then ground to a fine powder using a Grindomix GM200 homogenizer (Retsch GmbH, Haan, Düsseldorf, Germany) and kept at -24^{0}C until the extraction of the ground powder.

4.3.2. Measurement of pH

The pH was determined in each extraction solution using a pH meter (SevenEasy Mettler, Toledo-GmbH 8603 Schwerzenbanch, Switzerland) that was calibrated using standardised buffers pH 4.0 and pH 7.0.

4.3.3. Oxalate extraction

Quadruplicate extractions of total and soluble oxalates were carried out on 1.0 g of freeze-dried powdered fruit (Savage *et al*., 2000); the extractions were carried out at 21°C and 80°C, using 40 ml deionised water (soluble oxalate extraction) or 40 ml of 0.2 M HCL or 2 M HCL, to extract total oxalates. Extracted oxalates were determined using HPLC chromatography (Savage *et al*., 2000).

4.3.4. Recovery study

The recovery of oxalic acid during extraction was determined by adding 10 mg of oxalic acid (Sigma-Aldrich Co., St Louis, USA) to 1 g freeze-dried kiwi berry powder. These samples were subsequently extracted using deionised water (for soluble oxalates) and 2 M HCL (for total oxalates) and analysed in triplicate at 21^{0}C as outlined above.

4.3.5. Pectin extraction

Pectin was extracted using the method of Kulkarni and Vijayanand (2010). Two concentrations of HCL (0.2 M and 2 M) and two extraction temperatures (21^{0}C and 80^{0}C) were used in quadruplicate. The freeze-dried fruit powder was mixed with an aqueous solution of HCL or deionised water at the ratio 1:40 (w/v). This ratio has also been used previously as an appropriate ratio for the oxalate extraction procedure. The solutions were stirred continuously for 20 min at each temperature and then filtered through four layers of cheese cloth. The filtrate was further filtered through a Whatman No.3 paper using a vacuum filter. The pectin was precipitated by the addition of absolute ethanol (98% purity) in a ratio of 1:2 (w/w) and then kept at room temperature for two hours. The soluble pectin was filtered and washed three times with 75% (v/v), 85% (v/v) and 98% ethanol to remove the soluble impurities. This pellet was vacuum-dried using Thermostat Vacuum Oven (Townson and

Mercer Ltd., Croydon, UK) at 50 ± 1^0C under a vacuum of 30 cm Hg until a constant weight was obtained.

4.3.6. Statistical analysis

Statistical analysis was performed using GenStat version 14 for Windows 7 (VSN International Ltd., Hemel Hempstead, Hertfordshire, UK) to assess effect of temperature and extraction solution on the recovery of oxalate and pectin from the four different fruits. The data were presented as the means of quadruplicate determinations ± S.E.

4.4. Results

The mean recoveries of 10 mg oxalic acid added to 1 g freeze-dried kiwiberry powder, then extracted for 20 minutes using water at 21°C or 2 M HCL at 21°C, were 96.3% ± 1.9 and 98.5% ± 1.6, respectively. Soluble oxalates have been extracted from foods using water while 2 M HCL has been used to extract soluble and insoluble oxalates together to give the total oxalate concentration of a food (Savage *et al*., 2000). Insoluble oxalate was calculated as the difference between the total oxalate and soluble oxalate concentrations (Holloway *et al*., 1989). The extraction of oxalates from the four fruits using water (soluble oxalates) and 0.2M and 2 M HCL (total oxalates) are shown in Table 4.1.

Statistical analysis of the total oxalate extraction data showed that there was a significant interaction ($p < 0.01$) between the extraction solution and temperature. These mean changes of total oxalate concentration at different extraction solution were dependent on the extraction temperatures. Therefore, the means of total oxalate of each given solution and temperatures were considered in this study.

There were significant differences ($p < 0.001$) between soluble oxalate concentrations extracted at 21^0C and 80^0C. At 21^0C, the mean soluble oxalate concentrations of the four fruits were significantly higher than those extracted at 80^0C (54.7 vs. 39.8 mg/100 g DM). The pH of the extraction solutions before adding the samples were 6.9 for deionised water, 0.7 for 0.2 M HCL, -0.3 for 2M HCL. After adding the samples, the pH remained unchanged for both acid solutions, whereas the pH of the water extract changed from the initial value of 6.9 to an average of 6.0 for the two kiwifruits, 6.3 for kiwiberry and 6.5 for persimmon.

2 M HCL was the most effective extraction solution to extract total oxalates (soluble and insoluble oxalates) from the fruit tissue. The mean total oxalate concentration extracted by 2 M HCL at 21^0C and 80°C from all the four fruits combined was 118.8 mg/100 g DM compared to 93.7 mg/100 g DM using 0.2 M HCL. Extraction using 2 M HCL gave a slightly higher yield of total oxalates when the extraction was carried out at 21°C. This effect of temperature was similarly observed for the extractions carried out using 0.2 M HCL.

Overall, extractions using 2 M HCL at 21°C gave the highest overall mean value for total oxalate (mean 123.1 ± 2.5 mg/100 g DW) and was considered to be the optimum extraction condition. Extractions at 21°C using 0.2 M HCL gave a mean value 17.0% lower, while extraction using 0.2 M HCL carried out at 80°C gave a mean value 30.8% lower than the optimum extraction conditions. The mean value for total oxalates extracted by using 2 M HCL at 80°C was 7% lower than the optimum extraction conditions.

Similar to total oxalate statistical analysis, there was also a significant interaction ($p < 0.001$) between the temperature used and the solution used to extract pectin. This suggests that the effect of these two factors on pectin extractions cannot be separated. Both extraction solution and temperature showed significant effects on the amount of pectin extracted from the fruit tissue (Table 4.1). With the same extraction solution, incubating at 80^0C extracted higher amounts of pectin relative to extractions carried out at 21^0C. The highest concentrations of pectin were extracted using 0.2 M HCL at 80°. Overall, the temperature and extraction solution showed significant ($p < 0.001$) effects on pectin yield.

4.5. Discussion

4.5.1. Extracting solutions

The results of the present study showed that the concentrations of pectin extracted by 0.2 M HCL (pH 0.7) were significantly higher ($p < 0.001$) than the values obtained from using 2 M HCL at the same temperature. In contrast, Joye and Luzio (2000) and Liu *et al*., (2006) who analysed orange and lemon peel, showed that extracting pectin below pH 2 was sufficient to release all pectin substances including non-calcium-sensitive pectin and calcium-sensitive pectin. Strong acid conditions gave a higher yield than extractions carried out at pH 5.0 (Liu *et al*., 2006). The influence of HCL concentration on pectin yield was first demonstrated by Conrad (1926) who showed that at a constant temperature, the calcium-pectate yield obtained from potato, onion and parsnip peaked in the range of 0.02 to 0.03 M HCL and then decreased when the HCL concentration increased. Deren'ko and Suprunov (1979) showed that the

amount of pectin substances that could be extracted from rowanberry (*Sornus aucuparia* L.) with aqueous solutions of HCL at 100°C increased when HCL concentration increased from 0.1 M to 0.3 M. However, increasing HCL concentrations above this point resulted in a dramatic reduction in pectin yield giving a 5-fold reduction in yield when rowanberries were extracted with 2 M HCL.

Chang *et al*., (1993) showed that water was the least effective solvent for extracting pectin. This observation was confirmed in this study where 0.2 M HCL was most effective at extracting pectin and extraction with 2 M HCL gave lower yields of pectin. It was well-known that a low acid concentration (pH > 2) was not sufficient to hydrolyse proto-pectin to soluble pectin; however, the fact that a too strong acid concentration resulted in a low pectin yield can be explained, as the too strong acid caused extensive hydrolysis of the high-molecular-weight pectic polysaccharide substances into the soluble smaller molecules and small pectin molecules may not be precipitated following the addition of alcohol (Kalapathy and Proctor, 2001). Consequently, reductions of calcium-pectate linkages as well as reduced gelation of pectin may also have occurred (Conrad, 1926; Sila *et al*., 2009). Since the viscosity of a solution increased with increased pectin concentration and increased molecular weight (Kar and Arslan, 1999), the destruction of pectin may also reduce the viscosity of the solution. Since formation of intermolecular junction zones involved with calcium-pectate linkages required a minimum length of free carboxyl groups of pectin (Kohn, 1975), this destruction may decrease the number of junction zones, resulting in a diminishing number of calcium-pectin linkages, so that the gel-forming ability of the pectin and, consequently, trapping oxalate by calcium-pectate would also be reduced. Reducing the number of junction zones would also release more calcium; however, at strong acid pH < 0.7, binding between free calcium and oxalates cannot occur. It can be seen from pH values in Table 4.1 that total oxalate concentrations extracted by 2 M HCL were always higher than the amounts extracted using 0.2 M HCL, at both temperatures, whereas the pectin amounts extracted by 2 M HCL were the lowest.

Table 4.1. Mean soluble and total oxalate and pectin concentrations extracted at different temperature and using different extraction solution (mg/100 g DW ± standard error).

	Extraction solution											
	Water (pH 6.9)				0.2 M HCL (pH 0.7)				2 M HCL (pH -0.3)			
	21^0C		80^0C		21^0C		80^0C		21^0C		80^0C	
Fruit	Soluble oxalate	Pectin	Soluble oxalate	Pectin	Total oxalate	Pectin	Total oxalate	Pectin	Total oxalate	Pectin	Total oxalate	Pectin
Golden kiwi	49.8 ± 2.2	0.43 ± 0.01	38.7 ± 2.1	1.24 ± 0.01	80.4 ± 3.0	0.48 ± 0.02	65.6 ± 0.9	2.74 ± 0.06	95.9 ± 0.9	0.15 ± 0.01	90.5 ± 1.2	1.21 ± 0.08
Green kiwi	42.9 ± 0.8	0.45 ± 0.02	32.1 ± 0.6	1.45 ± 0.03	102.0 ± 2.1	0.65 ± 0.03	85.6 ± 3.1	3.15 ± 0.03	128.9 ± 0.5	0.20 ± 0.01	121.7 ± 1.6	1.43 ± 0.06
Kiwiberry	105.0 ± 2.1	0.62 ± 0.04	88.2 ± 1.2	2.01 ± 0.02	190.2 ± 1.4	0.61 ± 0.03	170.2 ± 6.6	3.50 ± 0.14	220.4 ± 7.7	0.11 ± 0.01	210.2 ± 4.6	1.70 ± 0.10
Persimmon	21.2 ± 0.4	1.04 ± 0.01	n.d	2.51 ± 0.11	36.2 ± 1.9	1.58 ± 0.04	19.3 ± 1.2	4.03 ± 0.14	47.1 ± 0.7	0.72 ± 0.01	35.6 ± 1.5	1.86 ± 0.04
Means	54.7 ± 1.4	0.64 ± 0.02	39.8 ± 1.0	1.80 ± 0.04	102.2 ± 2.1	0.83 ± 0.03	85.2 ± 3.0	3.36 ± 0.09	123.1 ± 2.5	0.30 ± 0.01	114.5 ± 2.2	1.55 ± 0.07

Analysis of variance	One-way analysis [a]	Two-way analysis [a]	
	Soluble oxalates	Total oxalate	Pectin
Temperature	***	***	***
Extraction solution	-[b]	***	***
Temperature x extraction solution	-	**	***
l.s.d. (5%) within temperature	2.3	3.6	0.2
l.s.d. (5%) between temperatures	-	5.1	0.1

n.d: not detected.

[a]: Using one-way ANOVA (in randomised blocks) for soluble oxalate analysis and two-way ANOVA (in randomised blocks) for total oxalate and pectin analysis.

[b]: not relevant.

Significance: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; NS: not significant. l.s.d: least significant difference.

The oxalate concentrations extracted by 2 M HCL were always higher than the amounts extracted using 0.2 M HCL or by using water, at both temperatures (Table 4.1). It can be concluded from this study that 2 M HCL was too strong to extract intact pectin from plant materials. 2 M HCL may, therefore, reduce chemical and mechanical binding between oxalate and pectin present in the solution. Kelsay and Prather (1983), suggested that a complex fibre-mineral-oxalate structure existed that was more difficult to dissolve than a fibre-mineral complex. Using stronger acid increases oxalates extracted from the samples due to the destruction of the oxalate-calcium-pectin complex. Moreover, using 2 M HCL may reduce the viscosity of the solution because of the structural destruction of the pectin molecules, as explained above, resulting in a good separation of the oxalate peak (the data not shown) because interfering material, eluted with oxalate at the same time under HPLC running conditions, has been reduced. In addition, Hönow and Hesse, (2002) showed that using 2 M HCL completely dissolved all oxalate crystals in plant tissues at room temperature and, thus, this ensured the accuracy of the collected data.

Pectin concentrations of fresh golden kiwifruit extracted using a ratio of sample to water of 1:40 (w/v) in this study were lower than the values obtained by Yuliarti *et al*., (2011) who used a ratio of sample to water of 1:2 (w/v). In contrast, Liu *et al*., (2006) showed that the pectin yield was reduced following a reducing in the ratio of sample to water and was undetected when a ratio of 1:50 was used. In addition, Crandell *et al.*, (1978) showed that a reduction in moisture content of the sample reduced the extraction yield of dry samples compared to fresh samples. Using dried samples in the current study may also account for the lower pectin concentration measured in golden kiwifruit compared to the values reported by Yuliarti *et al*., (2011) for golden kiwifruit also grown in New Zealand.

4.5.2. Extraction temperatures

Overall, higher amounts of pectin were obtained using hot acid and hot water (80°C) compared to using cold acid and cold water, at 21°C (Table 4.1). El-Nawawi and Shehata, (1988) reported that the solubility of pectin increased when the temperature was increased from 50 to 90^0C. However, temperatures over 90^0C caused a decrease in pectin yield due to the large pectin molecules being degraded into smaller molecules that were not precipitated with alcohol (Conrad, 1926; El-Nawawi and Shehata, 1987). In this study, the higher amounts of pectin and lower soluble oxalate collected at the higher extraction temperature (80^0C) could be accounted for by the increased trapping of soluble oxalates into the calcium-pectate complex, as described in extracting solvent section above.

Joye and Luzio, (2000) showed that extracting pectin at lower pH (pH < 2) gave a better result than at a higher pH (pH > 2). However, in this study there was a synergistic effect of extraction pH and temperature on the amount of pectin measured. At 21^0C, water-soluble pectin amounts were not very different from the amounts of pectin extracted using 0.2 M HCL. When the temperature was increased to 80^0C, the amounts of pectin extracted by 0.2 M HCL were twice as high as extractions using water (Table 4.1). These results confirmed the observation of Chang *et al.,* (1993) who showed that hot acid was more efficient at breaking strong molecular bonds. Slightly less pectin was extracted at 80°C with 2M HCL than at 80°C with water. This suggested that the negative effect of the strong acid was greater than the positive effect of higher temperature on the extraction of pectin.

It has been reported that the calcium-pectin linkage in protopectin was thermally-reversible when the incubating temperature ranged from 46-74^0C with an extrinsic calcium source (Fu and Rao, 1999) or from 5-85^0C with an intrinsic calcium source (Gilsenan *et al*., 2000). The samples incubated at a higher temperature in the soluble state gave a larger network structure that was formed by the increasing density of the calcium-pectate linkages (Iijima *et al*., 2002). The explanation is that when fruit samples were extracted at high temperature, the molecular motion of the pectin molecules was enhanced, resulting in structural tangling of molecular chains which would cause more junction zones to be exposed to the free calcium ions, thus enhancing the formation of calcium-pectin cross-linkages (Iijima *et al*., 2002). As high temperatures have been shown to have a greater effect on the release of soluble pectin from insoluble pectin by enhancing hydrolysis of pectin in comparison with low temperature (El-Nawawi and Shehata, 1987), using a high extraction temperature (80^0C) in this study released greater amounts of pectin into the solution (Table 4.1). This may have increased the linkage density due to binding between intrinsic calcium sources and extracted pectin relative to using a low temperature. Since increasing of calcium-pectin cross-linkage density results in an increase of the gel elasticity or viscosity of solution and network strength (Fraeye *et al*., 2009), using high extraction temperature increased the gelatinous property of the solution. It could be proposed that the size of pectin molecules were larger and their gel-forming ability may have been stronger after the samples were extracted at high temperature and therefore may, chemically and mechanically, trap more oxalates inside compared to those extracted at lower temperatures. The formation of these molecules would explain why less oxalate was extracted at the higher temperature in this study.

In summary, stronger acid (2 M HCL) appears to cause extensive hydrolysis of high molecular peptic polysaccharides to smaller soluble molecules that may not be precipitated and therefore measured following addition of alcohol but will release more oxalate for extraction because fewer calcium-pectin bonds occur and because a reduced viscosity of the solution was obtained. However, a higher temperature (80°C) but not > 90°C will lead to a greater extraction of pectin molecules which would then trap oxalates result in less oxalates being extracted and measured by HPLC.

Earlier studies propose that there exists a complex of fibre-mineral-oxalate that is more difficult to dissolve than a fibre-mineral complex (Kelsay and Prather, 1983) and that the fibre could bind chemically or mechanically to oxalates (Hanson *et al*., 1989). However, based on the eggbox model and the fact that both pectin and oxalates are the negatively charged molecules, while the Ca^{2+} ion is positively charged, it is unlikely that chemical binding between pectin and oxalate can occur, therefore, the binding is likely to be physical.

The oxalate data shown in Table 4.2 on a fresh weight basis confirmed that total oxalate concentrations in these four New Zealand grown fruits were quite low and would fall into Group 3, as defined by Noonan and Savage (1999). As the soluble oxalate concentration of the four fruit ranged from 33.3 to 52.1% of the total oxalate concentration, the oxalate concentrations would pose no problems even for people predisposed to form kidney stones. Overall, the values for kiwifruit fell within previously reported values for locally grown fruit and fruit grown and analysed in other parts of the world (Nguyen and Savage, 2012).

Table 4.2. Mean of total, soluble and insoluble oxalate extracted using 2 M HCL at 21^0C (mg/100 g FW ± standard error), values in brackets are the % soluble oxalate of the total oxalate concentration. Pectin values (mg/100 g FW ± standard error) are also shown.

Fruits	Dry matter (%)	Total oxalate	Soluble oxalate	Insoluble oxalate	Pectin extracted at 80°C with 0.2 M HCL
Golden kiwifruit	15.2 ± 0.1	14.6 ± 0.1	7.6 ± 0.3 (52.1%)[1]	7.0 ± 0.3	0.42 ± 0.01
Green kiwifruit	15.8 ± 0.1	20.4 ± 0.1	6.8 ± 0.1 (33.3%)	13.6 ± 0.3	0.49 ± 0.01
Kiwiberry	19.4 ± 0.2	42.8 ± 1.4	20.4 ± 0.4 (47.7%)	21.6 ± 0.7	0.67 ± 0.03
Persimmon	18.3 ± 0.2	8.6 ± 0.1	3.9 ± 0.1 (45.4%)	4.7 ± 0.2	0.73 ± 0.03

[1]: The figures in the brackets are percentages of soluble oxalate concentrations compared with total oxalate concentrations for each fruit

4.6. Conclusions

This is the first study to show a strong inverse relationship between pectin and oxalate concentrations extracted into solution. Although low pH (pH < 2) has been considered the most effective way to extract pectin, the very strong acid concentrations resulted in the lowest amounts of pectin extracted. Extraction temperatures had a small effect on pectin and oxalate extraction, however, higher temperatures resulted in higher pectin yields and, generally, lower recoveries of oxalate in the fruits analysed. Since extraction with a very strong acid concentration at room temperature gave the lowest pectin yield and, in contrast, the highest oxalate yield, it was suggested that extraction for oxalate analyses from fruit samples should be carried out using 2 M HCL at 21^0C to reduce interference from pectin during oxalate analysis.

References

BeMiller, J. N. (1986). An introduction to pectins: structure and properties. In *Chemistry and Function of Pectins* (310 ed., pp. 2-12): American Chemical Society, New York, USA.

Burns, J. K., & Pressey, R. (1987). Ca^{2+} in cell-walls of ripening tomato and peach. *Journal of the American Society for Horticultural Science, 112*(5), 783-787.

Chang, C. Y., Tsai, Y. R., & Chang, W. H. (1993). Models for the interactions between pectin molecules and other cell-wall constituents in vegetable tissues. *Food Chemistry, 48*(2), 145-157.

Conrad, C.M. (1926). A biochemical study of the insoluble pectic substances in vegetables. *American Journal of Botany, 13*(9), 531-547.

Crandall, P. G., Braddock, R. J., & Rouse, A. H. (1978). Effect of drying on pectin made from lime and lemon pomace. *Journal of Food Science, 43*(6), 1680-1682.

Deren'ko, S.A & Suprunov, N.I. (1980). Technology of the production of pectin substances from the fruit of *Sorbus aucuparia*. *Chemistry of Natural Compounds, 15*(6), 686-688.

El-Nawawi, S. A., & Shehata, F. R. (1987). Extraction of pectin from Egyptian orange peel - factors affecting the extraction. *Biological Wastes, 20*(4), 281-290.

El-Nawawi, S. A., & Shehata, F. R. (1988). Effect of the extraction temperature on the quality characteristics of pectin extracted from Egyptian orange peel. *Biological Wastes, 24*(4), 307-311.

Fils-Lycaon, B., & Buret, M. (1990). Loss of firmness and changes in pectic fractions during ripening and overripening of sweet cherry. *HortScience, 25*(7), 777-778.

Flutto, F. (2003). Pectin, properties and determination. In *Encyclopaedia of Food Sciences and Nutrition (Second Edition)* (pp. 4440-4449). Oxford: Academic Press.

Fraeye, I., Doungla, E., Duvetter, T., Moldenaers, P., Van Loey, A., & Hendrickx, M. (2009). Influence of intrinsic and extrinsic factors on rheology of pectin–calcium gels. *Food Hydrocolloids, 23*(8), 2069-2077.

Fu, J. T., & Rao, M. A. (1999). The influence of sucrose and sorbitol on gel-sol transition of low-methoxyl pectin-Ca^{2+} gels. *Food Hydrocolloids, 13*(5), 371-380

Gilsenan, P. M., Richardson, R. K., & Morris, E. R. (2000). Thermally reversible acid-induced gelation of low-methoxy pectin. *Carbohydrate Polymers, 41*(4), 339-349.

Hanson, C. F., Frankos, V. H., & Thompson, W. O. (1989). Bioavailability of oxalic acid from spinach, sugar beet fibre and a solution of sodium oxalate consumed by female volunteers. *Food and Chemical Toxicology, 27*(3), 181-184.

Hoagland, P. D. (1989). Binding of dietary anions to vegetable fiber. *Journal of Agricultural and Food Chemistry, 37*(5), 1343-1347.

Holloway, W. D., Argall, M. E., Jealous, W. T., Lee, J. A., & Bradbury, J. H. (1989). Organic acids and calcium oxalate in tropical root crops. *Journal of Agriculture and Food Chemistry, 37*, 337–341.

Hönow, R. & Hesse, A. (2002). Comparison of extraction methods for the determination of soluble and total oxalate in foods by HPLC enzyme-reactor. *Food Chemistry, 78*, 511-521.

Hwang, J.-K., Kim, C.-J., & Kim, C.-T. (1998). Extrusion of apple pomace facilitates pectin extraction. *Journal of Food Science, 63*(5), 841-844.

Iijima, M., Hatakeyama, T., Nakamura, K., & Hatakeyama, H. (2002). Effect of annealing on calcium pectin gel formation by thermomechanical analysis. *Journal of Thermal Analysis and Calorimetry, 70*(3), 815-824.

Jarvis, M. C. (1982). The proportion of calcium-bound pectin in plant cell walls. *Planta, 54*, 344-346.

Joye, D. D., & Luzio, G. A. (2000). Process for selective extraction of pectins from plant material by differential pH. *Carbohydrate Polymers, 43*(4), 337-342.

Kalapathy, U., & Proctor, A. (2001). Effect of acid extraction and alcohol precipitation conditions on the yield and purity of soy hull pectin. *Food Chemistry, 73*(4), 393-396.

Kar, F., & Arslan, N. (1999). Effect of temperature and concentration on viscosity of orange peel pectin solutions and intrinsic viscosity–molecular weight relationship. *Carbohydrate Polymers, 40*(4), 277-284.

Kashyap, D. R., Vohra, P. K., Chopra, S., & Tewari, R. (2001). Applications of pectinases in the commercial sector: a review. *Bioresource Technology, 77*(3), 215-227.

Kelsay, J.L., & Prather, E.S. (1983). Mineral balances of human subjects consuming spinach in a low-fiber diet and in a diet containing fruits and vegetables**.** *American Journal of Clinical Nutrition, 38*, 12-19

Kohn, R. (1975). Ion binding on polyuronates - alginate and pectin. *Pure and Applied Chemistry, 42*, 371–397

Kulkarni, S. G., & Vijayanand, P. (2010). Effect of extraction conditions on the quality characteristics of pectin from passion fruit peel (*Passiflora edulis f. flavicarpa* L.). *LWT - Food Science and Technology, 43*(7), 1026-1031.

Liu, Y., Shi, J., & Langrish, T. A. G. (2006). Water-based extraction of pectin from flavedo and albedo of orange peels. *Chemical Engineering Journal, 120*(3), 203-209.

Neal, G. E. (1965). Changes occurring in the cell walls of strawberries during ripening. *Journal of the Science of Food and Agriculture, 16*(10), 604-611.

Noonan, S.C. & Savage, G.P. (1999). Oxalate content of foods and its effect on humans. *Asia Pacific Journal of Clinical Nutrition, 8*(1), 64-74.

Nguyen, H.H.V. & Savage, G.P. (2012). Oxalate content of New Zealand grown and imported fruits. Submitted to *Journal of Food Composition and Analysis*.

Paoletti, S., Cesaro, A., Delben, F., & Ciana, A. (1986). Ionic effects on the conformation, equilibrium, properties and rheology of pectate in aqueous solutions and gels. In *Chemistry and Function of Pectins* (310 ed., pp. 73-87): American Chemical Society, New York, USA.

Savage, G. P., Vanhanen, L., Mason, S. M., & Ross, A. B. (2000). Effect of cooking on the soluble and insoluble oxalate content of some New Zealand foods. *Journal of Food Composition and Analysis, 13*(3), 201-206.

Sila, D. N., Van Buggenhout, S., Duvetter, T., Fraeye, I., De Roeck, A., Van Loey, A., & Hendrickx, M. (2009). Pectins in processed fruits and vegetables: Part II-structure-function relationships. *Comprehensive Reviews in Food Science and Food Safety, 8*(2), 86-104.

Yeoh, S., Shi, J., & Langrish, T. A. G. (2008). Comparisons between different techniques for water-based extraction of pectin from orange peels. *Desalination, 218*(1–3), 229-237.

Yuliarti, O., Matia-Merino, L., Goh, K. K. T., Mawson, J. A., & Brennan, C. S. (2011). Effect of celluclast 1.5l on the physicochemical characterization of gold kiwifruit pectin. *International Journal of Molecular Sciences, 12*(10), 6407-6417.

Chapter 5

Oxalate concentration of New Zealand grown and imported fruits

5.1 Abstract

Total and soluble oxalate concentrations of 41 locally grown and 11 imported fruits commonly consumed in New Zealand were determined by extracting with 2M HCL to extract total oxalates or nanopure water to extract soluble oxalates for 20 minutes at 21^0C. The extracted oxalates were analysed by ion exchange HPLC. Total oxalate concentration ranged from 2.9 – 7566.5 mg/100 g fresh weight (FW) while the soluble oxalate ranged from 5 to 56% of the total oxalate concentration. Very high concentrations of oxalate were measured in Indian gooseberry, carambola and rhubarb compared with the other fruits. Total oxalate concentrations in these fruits were 7566.5, 436.1 and 640.2 mg/100 g FW respectively. Twenty-two of the fruits studied contained no detectable oxalates.

5.2 Introduction

Oxalates can be found in relatively small amounts in many plants and may make up from 3 to 80% of the plant's dry weight (Gallaher, 1975). Most vegetables and fruits in a typical Western diet contain low to moderate concentrations of oxalate (Noonan and Savage, 1999; Hönow and Hesse, 2002). The concentrations of oxalate in many leafy plant foods have been well studied but the concentrations of oxalates occurring in fruits and fruit products have not been fully investigated. It is not surprising that oxalate occurs in fruits as oxalates along with carbohydrates are synthesized in the leaves and are then translocated to the fruit.

Early reported studies determined oxalate in fruits using methods such as titration (Allison, 1966; Baker, 1952; Andrews and Viser, 1951; Herrmann, 1972; Awadalia *et al*., 1985), radio-enzymic (Ogawa *et al*., 1984), colourimetric measurements (Zarembski and Hodgkinson, 1962b; Hodgkinson, 1977) or paper chromatography (Blundstonee and Dickson, 1964), which are now known to be unreliable. Enzymic-colourimetric methods (Kasidas and Rose, 1980; Watanabe and Takahashi, 1998) are reliable in experienced hands. HPLC-enzyme-reactor (Hönow and Hesse, 2002) and HPLC methods (Wilson *et al*., 1982; Savage *et al.*, 2000; Rassam and Laing, 2005) are now regarded as the most reliable methods. However, many

studies with fruits have focused on total oxalate concentration and have overlooked the soluble oxalate concentrations.

Noonan and Savage (1999) reviewed the oxalate concentration of many plant foods and they presented the published values available for rhubarb petioles, which ranged from 275-1336 mg/100 g FW. In many publications rhubarb is considered to be a fruit because of the way it is eaten. More recently, Hönow and Hesse (2002) published values for a wider range of fruits commonly available in Europe. This data along with earlier data have been compiled in Table 5.1. Family names and scientific names of each of the analysed fruits are shown in appendix A.1.3. This table shows that there is a wide range of values quoted for common fruits grown in mainly temperate climates. It is also clear that some studies were not able to measure the soluble oxalate concentrations of some fruits. The oxalate concentrations of green and gold kiwifruit have been reported (Watanabe and Takahashi, 1988) and a change in the oxalate concentrations during storage have been reported by Rinallo and Mori (2000).

As people are encouraged to consume more fruit for health reasons it is important to have an accurate knowledge of their total oxalate concentration. It is also important to measure the soluble oxalate concentration of many of these fruits to confirm whether the earlier results quoting only total oxalate concentration were correct. It is possible that some fruits may contribute more oxalates to healthy diets than previously thought and a reduction in the consumption of certain fruits, could well reduce the incidence of kidney stone formation in susceptible individuals. The aim of this study is to build a reliable database of the total, soluble and insoluble oxalate concentration of various fruits available in New Zealand, either grown locally or imported, as people are encouraged to eat more fruit.

Table 5.1. Mean oxalate concentration (mg/100 g FW) of fruit in the published literature.

Fruit	Oxalate concentration		Analytical methods		References	Country
	Total	Soluble				
Apple	1.5	-[1]	Colourimetric method	Whole fresh fruit	Hodgkinson (1977)	England
	1.5	-[1]	Colourimetric method	Whole fresh fruit	Zarembski and Hodgkinson (1962b)	UK
Apricot	6.8	3.4	Titration	Edible portion	Herrmann (1972)	Germany
	2.8	-[1]	Colourimetric method	Fresh fruit	Hodgkinson (1977)	England
	6.8	1.9	HPLC-enzyme- reactor	Edible portion	Hönow and Hesse (2002)	Germany
Banana	0.7	-	Colourimetric method	Raw edible portion	Zarembski and Hodgkinson (1962b)	UK
Blackcurrant	12.4	6.8	Titration	Whole fresh fruit	Herrmann (1972)	Germany
	19.0	3.0	HPLC-enzyme- reactor	Edible portion	Hönow and Hesse (2002)	Germany
	4.3	-[1]	Colourimetric method	Whole fresh fruit	Zarembski and Hodgkinson (1962b)	UK
Red currant	9.9	2.2	Titration	Raw edible portion	Herrmann (1972)	Germany
	4.9-25.5	2.0-9.3	HPLC-enzyme- reactor	Raw edible protion	Hönow and Hesse (2002)	Germany
Carambola	295.4	138.9	HPLC-enzyme- reactor	Raw edible portion	Hönow and Hesse (2002)	Germany
	-[1]	80-730	HPLC	Whole fruit	Wilson *et al*., (1982)	USA
Cherry	7.2	4.3	Titration	Edible portion	Herrmann (1972)	Germany
	2.4	1.5	HPLC-enzyme- reactor	Edible portion	Hönow and Hesse (2002)	Germany
Green gooseberry	19.3	10.1	Titration	mixed	Herrmann (1972)	Germany
	27.0	3.1	HPLC-enzyme- reactor	Edible portion	Hönow and Hesse (2002)	Germany
	2.6	-	Colourimetric method	Stewed	Zarembski and Hodgkinson (1962b)	UK
Red gooseberry	21.6	3.2	HPLC-enzyme- reactor	Edible portion	Hönow and Hesse (2002)	Germany
Golden kiwifruit	23	2.3	HPLC-enzyme- reactor	Edible portion	Hönow and Hesse (2002)	Germany
	19.0	-[1]	Radio enzymatic	Solid materials	Ogawa *et al*., (1984)	Japan
	18-45	-[1]	HPLC-enzyme- reactor	Whole fruit	Rassam and Laing (2005)	NZ
	7.8-10.1	0.6-2.0	Enzymatic colourimetric	Edible protion	Watanabe and Takahashi (1998)	Japan
Green kiwifruit	37.0-65.2	15.8-41.4	HPLC	Edible portion	Perera *et al*., (1990)	NZ
	82.6-84.3	28.0-30.1	Enzymatic colourimetric	Edible portion	Rinallo and Mori (2000)	Italy
	12.7-14.7	2.5-3.0	Enzymatic colourimetric	Edible protion	Watanabe and Takahashi (1998)	Japan

Green grape	7.9	-[1]	-[2]	-[4]	Elmadfa *et al*., (1992/1993)	Germany
	7.8	3.3	Titration	-[4]	Herrmann (1972)	Germany
	1.7	0.6	HPLC-enzyme- reactor	Edible portion	Hönow and Hesse (2002)	Germany
Lemon	276-323	-[1]	Titration	skin	Herrmann (1972)	Germany
	3.1	0.5	HPLC-enzyme- reactor	Edible portion	Hönow and Hesse (2002)	Germany
	46.5	-[1]	Radio enzymatic	Solid materials	Ogawa *et al*., (1984)	Japan
Mandarin	8.5	0.3	HPLC-enzyme- reactor	Edible portion	Hönow and Hesse (2002)	Germany
Orange	6.3	-[1]	-[2]	-[4]	Elmadfa *et al*., (1992/1993)	Germany
	-[1]	-[1]	Titration	Fresh juice squeezed	Herrmann (1972)	Germany
	1.8	0.2	HPLC-enzyme- reactor	Fresh edible portion	Hönow and Hesse (2002)	Germany
	4.0	-[1]	Enzymatic	Fresh juice squeezed	Kasidas and Rose (1980)	England
	17.7	-[1]	Radio enzymatic	Solid material	Ogawa *et al*., (1984)	Japan
	6.2	-[1]	Colourimetric	Fresh squeezed juice	Zarembski and Hodgkinson (1962b)	England
Peach	2.5	0.2	HPLC-enzyme- reactor	Edible portion	Hönow and Hesse (2002)	Germany
Pear	6.2	-[1]	-[2]	-[4]	Elmadfa *et al*., (1992/1993)	Germany
	4.3-6.7	-	Titration	Edible portion	Herrmann (1972)	Germany
	1.3-1.7	-[1]	Colourimetric method	Canned	Hodgkinson (1977)	England
	2.7	0.9	HPLC-enzyme- reactor	Fresh edible portion	Hönow and Hesse (2002)	Germany
	1.7	-[1]	Colourimetric	Fresh edible portion	Zarembski and Hodgkinson (1962b)	UK
Pineapple	1.0	-[1]	Enzymatic	Canned	Kasidas and Rose (1980)	UK
	0-3.7	-[1]	Colourimetric	Canned	Hodgkinson (1977)	UK
	3.0-4.3	0.3-1.6	HPLC-enzyme- reactor	Edible portion	Hönow and Hesse (2002)	Germany
Plum	11.9	-[1]	Titration	Edible portion	Andrews and Viser (1951)	USA
	1.1-3.4	-[1]	-[2]	-[4]	Elmadfa *et al*., (1992/1993)	Germany
	11.9	6.0	Titration	Edible portion	Herrmann (1972)	Germany
	1.7	0.5	HPLC-enzyme- reactor	Edible portion	Hönow and Hesse (2002)	Germany
	1.1-3.4	-[1]	Colourimetric	Stewed	Zarembski and Hodgkinson (1962b)	England
Raspberry	16.4	11.3	Titration	Edible portion	Herrmann (1972)	Germany
	14.9	3.8	HPLC-enzyme- reactor	Edible portion	Hönow and Hesse (2002)	Germany
	2.2	-[1]	Colourimetric method	Whole fresh fruit	Zarembski and Hodgkinson (1962b)	England

Rhubarb	220-760	110-400	Titration	Raw petioles	Allison (1966)	NZ
	438	-[1]	Titration	Raw petioles	Andrews and Viser (1951)	USA
	124-480	83-271	Paper chromatography	Raw petioles	Blundstone and Dickinson (1964)	UK
	537	-[1]	-[2]	-[4]	Elmadfa *et al*., (1992/1993)	Germany
	537	-[1]	Titration	-[4]	Herrmann (1972)	Germany
	260-620	-[1]	Colorimetric method	Stewed petioles	Hodgkinson (1977)	UK
	570-1900	380	HPLC-enzyme- reactor	Raw petioles	Hönow and Hesse (2002)	Germany
	850	-[1]	Enzymatic method	Cooked petioles	Kasidas and Rose (1980)	UK
	3.35-9.48[3]	-[1]	HPLC	Raw petioles	Libert and Creed (1985)	UK
	986.7	287.3	HPLC	Raw petioles	Savage *et al*., 2000	NZ
	756.3	80.7	HPLC	Cooked petioles	Savage *et al*., 2000	NZ
	626[3]	-[1]	Enzymatic GC	Raw petioles	Turner (1980)	NZ
	260-460	-[1]	Colourimetric method	Stewed petioles	Zarembski and Hodgkinson (1962b)	UK
Strawberry	47.0	-[1]	Titration	Whole fresh fruit	Andrews and Viser (1951)	USA
	49.0	-[1]	Titration	Whole fresh fruit	Awadalia *et al*., (1985)	Egypt
	15.8	-[1]	-[2]	-[4]	Elmadfa *et al*., (1992/1993)	Germany
	15.8	9.9	Titration	Whole fruit	Herrmann (1972)	Germany
	1.9-11.5	5.9	Colourimetric method	Whole fresh fruit	Hodgkinson (1977)	UK
	2.9	0.9	HPLC-enzyme- reactor	Whole fresh fruit	Hönow and Hesse (2002)	Germany
	15	-[1]	Enzymatic method	Whole fresh fruit	Kasidas and Rose (1980)	UK
	1.9	-[1]	Colourimetric method	Whole fresh fruit	Zarembski and Hodgkinson (1962b)	UK
	23.4	-[1]	Radio enzymatic	Whole fresh fruit	Ogawa *et al*., (1984)	Japan
Tomato	13	-[1]	Titration	Whole fresh fruit	Andrews and Viser (1951)	USA
	5.3	-[1]	Colorimetric method	Whole fresh fruit	Hodgkinson (1977)	England
	8.5	3.6	HPLC-enzyme- reactor	Whole fresh fruit	Hönow and Hesse (2002)	Germany
	11.0	7.0	HPLC method	Edible portion	Judprasong *et al*., (2006)	Thailand
	2.0	-[1]	Enzymatic method	Whole fresh fruit	Kasidas and Rose (1980)	UK
	5.3	-[1]	Colourimetric method	Whole fresh fruit	Zarembski and Hodgkinson (1962b)	UK
Tamarillo	19.9	3.7	HPLC-enzyme- reactor	Edible portion	Hönow and Hesse (2002)	Germany
Watermelon	0.3	0.3	HPLC-enzyme- reactor	Edible portion	Hönow and Hesse (2002)	Germany

[1]: not determined. [2]: analytical details not given. [3]: dry weight. [4]: details not given

5.3 Materials and methods

5.3.1. Sample preparation

Fresh fruits were purchased in season from different local supermarkets in Christchurch, NZ (Table 5.2). Edible portions as 'ready to eat' of each kind of fruit bought from different stores were pooled. The edible portion of the fruit was chopped into 5 mm slices using a stainless steel knife and the pieces were freeze-dried in a Cuddon freeze dryer (Model E.D. 5.3) and then ground to a fine powder using a Grindomix GM200 homogenizer (Retsch GmbH, Haan, Düsseldorf, Germany) and kept at -24°C until extraction of the ground powder could take place. For the experiment on the kiwifruit fractions, fruit fractions including skin, pulp and seed were separated using a stainless steel knife. The seeds were separated using a laboratory sieve (0.5 mm mesh). Fractions from five fruit were pooled. All samples were immediately frozen in liquid N_2 and were then ground to a fine powder in a coffee mill (Sunbeam, model EM0400, China) then stored until analysis could commence.

5.3.2. Oxalate analysis

The measurement of total and soluble oxalates was performed following the method outlined by Savage *et al*., (2000). Total and soluble oxalate concentrations were determined in triplicate by extracting 1 g of dried powder with 40 mL 2 M HCL or 40 mL nanopure water (Arium 611uv, Sartorius Ltd., Germany), respectively, and incubating in a water bath at 21°C for 20 minutes. The extracts were cooled and filtered through 0.45 µm cellulose acetate filter (Sartorious, Göttingen, Germany) prior to HPLC analysis. Insoluble oxalate was calculated as the difference between total oxalate and soluble oxalate concentrations (Holloway *et al*., 1989).

5.4. Results

Table 5.2 shows the total and soluble oxalate concentration of twenty-one locally grown fruit and Table 5.3 shows the oxalate concentration of nine fruits imported into New Zealand. The results are the mean of three determinations ± SE. Oxalate concentration of fruits are presented on a fresh weight (FW) basis. Twenty-two fruits available in New Zealand including apple (Royal, Eve and Rose varieties), apricot, peach (yellow and white flesh), pear (Taylor's gold), nectarine, banana, avocado, strawberry, cranberry, kiwano, passion fruit, lemon, orange, mandarin, tangelos, tamarillo, black plum, mango and watermelon were found to contain no detectable oxalate concentrations.

For the remainder of the fruits analysed there was a large variation in the oxalate concentrations of the fruits. Total oxalate concentrations ranged from 2.0 to 7566.5 mg/100 g FW while soluble oxalate concentrations ranged from 0.4 - 3855.4 mg/100 g FW and insoluble oxalate concentrations ranged from 0.2-3710.9 mg/100 g FW. Among the fruits analysed in this study, very high concentrations of oxalate were observed in Indian gooseberry, carambola, goji berry and rhubarb. Indian gooseberry had the highest oxalate concentrations (7566.5 mg total oxalate/100 g FW; 3855.4 mg soluble oxalate/100 g FW) followed by rhubarb (640.0 mg total oxalate/100 g FW; 431.3 mg soluble oxalate/100 g FW) and carambola (436.1 mg total oxalate/100 g FW; 301.1 mg soluble oxalate/100 g FW). A relatively high range of total oxalate concentrations (> 26 mg/100 g) were found for redcurrant (30.2 mg/100 g FW), black raspberry (45.7 mg/100 g FW), feijoa (59.9 mg/100 g FW), kiwiberry (42.6 mg/100 g FW) and red gooseberry (28.1 mg/100 g FW). Meanwhile, very low concentrations of oxalates were found for varieties of grape (2.9-3.9 mg total oxalate/100 g FW), tomato (2.0-3.2 mg total oxalate/100 g FW), blueberry (3.2 mg total oxalate/100 g FW), cherry plum (4.1 mg total oxalate/100 g FW), pineapple (5.1 mg total oxalate/100 g FW) and varieties of cherry (4.0-6.3 mg total oxalate/100 g FW).

Table 5.2. Mean oxalate concentration (mg/100 g FW ± SE) of fruits grown in New Zealand.

Fruits	Oxalate concentration		
	Total oxalate	Soluble oxalate	Insoluble oxalate
Blueberry	3.2 ± 0.2	n/d[1]	3.2 ± 0.2
Boysenberry	10.5 ± 0.7	n/d	10.5 ± 0.7
Currant (black)	10.9 ± 0.2	n/d	10.9 ± 0.2
Currant (red)	30.2 ± 1.8	12.3 ± 0.4	17.9 ± 1.6
Cherry (red)	6.3 ± 0.2	0.4 ± 0.0	5.9 ± 0.2
Cherry (yellow)	4.1 ± 0.2	1.4 ± 0.1	2.7 ± 0.1
Cherry (black)	4.0 ± 0.1	1.4 ± 0.1	2.6 ± 0.1
Feijoa	59.9 ± 0.3	17.8 ± 2.3	42. 2 ± 1.8
Gooseberry (green)	19.5 ± 0.2	13.8 ± 0.1	5.7 ± 0.2
Gooseberry (red)	28.1 ± 1.0	23.0 ± 0.6	5.1 ± 0.4
Kiwi berry	42.6 ± 0.1	22.3 ± 0.3	20.3 ± 0.3
Kiwifruit (golden)	15.3 ± 0.9	7.9 ± 1.9	10.1 ± 0.1
Kiwifruit (green)	20.0 ± 0.6	6.8 ± 0.2	14.2 ± 0.1
Raspberry (red)	18.1 ± 0.4	8.1 ± 0.1	10.0 ± 0.3
Raspberry (black)	45.7 ± 1.1	n/d	45.7 ± 1.1
Persimmon	7.4 ± 0.0	3.4 ± 0.1	4.0 ± 0.1
Plum (cherry)	4.1 ± 0.1	3.4 ± 0.1	0.8 ± 0.0
Plum (red)	11.4 ± 0.4	3.5 ± 0.0	7.9 ± 0.5
Rhubarb	640.2 ± 21.6	431.3 ± 5.2	208.9 ± 7.1
Tomato (red cherry)	2.0 ± 0.0	1.6 ± 0.0	0.4 ± 0.0
Tomato (red)	3.2 ± 0.0	2.3 ± 0.0	0.9 ± 0.0

[1]: n/d: none detectable

Table 5.3. Mean oxalate concentration (mg/100 g FW ± SE) of fruits imported into New Zealand.

Country and origin samples	Oxalate concentration		
	Total oxalate	Soluble oxalate	Insoluble oxalate
PHILIPINES			
Pineapple	5.1 ± 0.3	1.2 ± 0.1	3.9 ± 0.4
VIETNAM			
Carambola	436.1 ± 7.4	301.1 ± 6.5	134.9 ± 4.9
CHINA			
Goji berry	138.2 ± 0.6	109.9 ± 0.4	28.3 ± 0.7
USA			
Grape (black)	3.2 ± 0.0	2.9 ± 0.0	0.2 ± 0.0
Grape (red)	3.9 ± 0.0	2.0 ± 0.0	1.9 ± 0.0
Grape (green)	2.9 ± 0.1	2.6 ± 0.1	0.3 ± 0.0
THAILAND			
Indian gooseberry	7566.5 ± 21.5	3855.4 ± 15.0	3710.9 ± 32.0
Mangosteen	8.7 ± 0.1	2.4 ± 0.1	6.3 ± 0.1
Sweet tamarind	6.2 ± 0.2	4.7 ± 0.1	1.5 ± 0.1

Analysis of the oxalate concentrations of the different fractions of fresh ready-to-eat green Hayward (*Actinidia deliciosa* cv Hayward) and golden Hort16A (*A. chinensis* cv Hort16A) kiwifruits grown in New Zealand was carried out to determine the oxalate distribution in the different fruit parts to compare to the oxalate concentration in the edible portion of the fruits. The results (Table 5.4) showed that the pulp, which made up of 90.3 to 90.6% of whole fruit contained the lowest concentrations of all the oxalate forms compared to the seeds and skin fractions. In contrast, the seeds that made up from 0.9 to 2.2% of the whole fruit contained the highest level of total oxalates. As the skin of both the green and golden kiwifruits is a very small proportion of the whole fruit and this fraction contains a very small proportion of soluble oxalate the removal of the skin has very little practical importance.

Table 5.4. The distribution (%) of the three fractions of Hayward and Hort16A kiwifruit cultivars and the mean total, soluble and insoluble oxalate concentration (mg/100 g FW± SE) of these fraction.

	% Fraction		Total oxalate		Soluble oxalate		Insoluble oxalate	
	Hayward	Hort16A	Hayward	Hort16A	Hayward	Hort16A	Hayward	Hort16A
Skin	7.3 ± 0.1	8.6 ± 0.4	47.7 ± 0.2	55.4 ± 1.1	10.8 ± 0.2	11.9 ± 0.3	36.9 ± 0.2	43.6 ± 1.1
Pulp	90.3 ± 0.1	90.6 ± 0.4	19.3 ± 0.3	15.7 ± 0.1	7.6 ± 0.2	8.5 ± 0.1	11.7 ± 0.3	7.2 ± 0.1
Seeds	2.2 ± 0.1	0.9 ± 0.1	116.9 ± 2.8	97.3 ± 1.4	10.2 ± 0.2	12.6 ± 0.3	106.7 ± 2.7	84.7 ± 1.4

From a nutritional point of view soluble oxalate is the most important parameter and it is possible to categorise the fruit analysed in this work into three different groups (Table 5.5). Group 1 contains fruit with soluble oxalate concentrations over 100 mg/100 g FW. Fruit containing soluble oxalates ranging from 20 to 100 mg/100 g FW were classified into Group 2 and fruit containing soluble oxalates lower than 20 mg/100 g FW were catagorised into Group 3.

Table 5.5. Groups of fruit containing different soluble oxalate concentrations (mg/100 g FW).

Group	Soluble oxalate concentration
Group 1	
Carambola	301.1 ± 6.5
Goji berry	109.9 ± 0.4
Indian gooseberry	3855.4 ± 15.0
Rhubarb	431.3 ± 5.2
Group 2	
Kiwiberry	22.3 ± 0.3
Gooseberry (red)	23.0 ± 0.6
Group 3	
Currant (red)	12.3 ± 0.4
Cherry (red)	0.4 ± 0.0
Cherry (yellow)	1.4 ± 0.1
Cherry (black)	1.4 ± 0.1
Feijoa	17.8 ± 2.3
Gooseberry (green)	13.8 ± 0.1
Grape (black)	2.9 ± 0.0
Grape (green)	2.6 ± 0.1
Grape (red)	2.0 ± 0.0
Kiwifruit (green)	7.9 ± 1.9
Kiwifruit (golden)	6.8 ± 0.2
Mangosteen	2.4 ± 0.1
Persimmon	3.4 ± 0.1
Pineapple	1.2 ± 0.1
Plum (cherry)	3.4 ± 0.1
Plum (red)	3.5 ± 0.0
Raspberry (red)	8.1 ± 0.1
Tamarind (sweet)	4.7 ± 0.1
Tomato (cherry red)	1.6 ± 0.0
Tomato (red)	2.3 ± 0.0

5.5. Discussion

Overall, the oxalate concentrations of fruit grown in New Zealand are very low. Blueberry, boysenberry, blackcurrant and black raspberry contained between 3.2 and 45.7 mg of total oxalate/100 g FW, respectively, while no soluble oxalate could be detected. The total oxalate concentrations of cherries, red gooseberry, pineapple, plum and tomato were comparable to previously reported values (Table 5.1). Feijoa contained high concentrations of total oxalates, 59.9 mg/100 g FW but only 30% was soluble oxalate; in contrast, red gooseberry contained lower concentrations of total oxalate, 28.1 mg/100 g FW, but 83% was soluble oxalate. In this study three tropical fruits (carambola, goji berry and Indian gooseberry) and locally grown rhubarb contained very high concentrations of oxalates.

Rhubarb was included in this analysis because it is usually eaten as a fruit even though it is a stalk or petiole (Allison, 1966; Clementi and Misiti, 2010). However, many publications and cookbooks advise that rhubarb should not be consumed regularly as its petioles contain high concentrations of oxalate (Blundstone and Dickinson, 1964; Grieve, 1980). The total and soluble oxalate concentrations found from this study were comparable with the literature, in which total oxalate ranged from 275-1336 mg/100 g FW (Allison, 1966, Noonan and Savage, 1999) and soluble oxalate ranged from 42.4-78.2% of the total oxalate (Allison, 1966). It was reported that the genotype could be the reason for 72% of the variation in oxalate concentration observed in rhubarb (Libert and Creed, 1985). Boiling rhubarb in water and discarding the water reduced total and soluble oxalate concentrations to 23.3% and 71.9%, respectively, compared to the raw tissue (Savage *et al.*, 2000).

Carambola, imported from Vietnam, contained 436.1 and 301.1 mg of total and soluble oxalates/100 g WM, respectively. The soluble oxalate results are within the range reported by Wilson *et al*., (1982) but they did not detect any total oxalate in 15 different cultivars grown in south Florida. The results presented by Hönow and Hesse (2002) were carried out on carambola, which may also have been imported from southern Florida (Wagner *et al*., 1975). The wide differences in oxalate concentrations may have occurred because of differences in the cultivars analysed as the growing conditions in southern Florida and Vietnam are similar. The consumption of one fruit of a high oxalate containing cultivar (which would weigh approximately 148 g) would result in a large intake of oxalates. More recently, carambola fruits have been used to make a fruit juice that contains 21.8 mg soluble oxalate/100 ml juice (Starfruit Juice Drink, Allswell Trading Pte. Ltd., Clementi Arcade, Singapore). The regular

consumption of these fruits or juice extracts would result in a moderate to large intake of oxalates.

Indian gooseberry fruits also contain very high concentrations of total and soluble oxalate that give the fruits a very tart taste. It is fortunate that this means that these fruits cannot be eaten raw in any quantity. However, the fruits are being commercially processed into juice and sold as a functional drink because of its high antioxidant concentrations (Carlsen *et al.*, 2010). The Indian gooseberry juice produced in Thailand contained 1160 and 1162 mg oxalate/100 ml juice of soluble and total oxalate, respectively (Vanhanen *et al.*, 2011). It is interesting that after being processed, the ratio of soluble to total oxalate in the juice increases to 100%, almost twice as much as the ratio in fresh fruit. Although a significant amount of soluble and total oxalate was eliminated during juice processing, individuals susceptible to kidney stone formation should avoid regular consumption of the juice.

Goji berry, considered as a super-fruit, contains high concentrations of antioxidants (Carlsen *et al.*, 2010). However, it also contains moderate to high concentrations of soluble oxalate, which is in contrast to its super-food health image. Consumption of large amounts of these berries would be unwise.

Earlier studies have shown that the total and soluble oxalate concentrations of green kiwifruit are generally higher than those found in golden kiwifruit (Table 5.1). In this study, the oxalate concentrations found in kiwifruit (without the skin) were in the same range as published values but little difference could be seen between the different cultivars. It is interesting to note that analysis of fruits often involves the analysis of the whole fruits while it is common to peel and discard the skin before consumption. Green kiwi fruit have a furry skin that is often discarded while the golden kiwi fruit is smooth and is eaten more often. This led Rassam and Laing (2005), to measure the whole fruit and individual components, skin, outer-pericarp, inner-pericarp and core of six genotypes of gold kiwifruit separately. Highest concentrations of total oxalate were found in the skin (from 20 to 60 mg/100 mg FW) and inner-pericarp (from 25 to 75 mg/100 mg FW) as compared with the outer-pericarp (from 5 to 18 mg/100 mg FW and sometime undetectable) and core (from 2 to 18 mg/100 mg FW).

Most studies of the oxalate concentration of fruit focus on the concentrations of ready-to-eat fruit, however, Watanabe and Takahashi (1998) observed that kiwifruit contained high concentrations of oxalate at the early stage of development and that there was a reduction in oxalate during growth and maturation. This process of overall oxalate reduction in the fruit

increased during post-harvest storage. For example, total oxalate decreased from 35.8 to 12.7 mg/100 g FW for Hayward, from 54.6 to 17.4 mg/100 g FW for Bruno and from 41.7 to 7.8 mg/100 g FW for Yellow Queen. Similarly, Rassam *et al*., (2007) recorded a dramatic fall in total oxalate in the whole fruit of five genotypes from the first week after anthesis (WAA) to 24-29 WAA, reducing from 83-131 mg total oxalate/100 g FW to 12-21 mg total oxalate/100 g FW, respectively. There was also a much lower level of total oxalate concentration at 24-29 WAA than that at 12 WAA of maturity (53-73 mg/100 g FW). Moreover, they found that there was a 4.6 fold variation in oxalate in 134 female siblings of the same genotype of kiwifruit. These studies have shown that the oxalate concentrations of kiwifruit are dependent on maturity stage and cultivar evaluated. The observations could also explain the variation of oxalate concentrations obtained between the literature and the present study.

Herman (1972) reported that no oxalate could be found in freshly squeezed juice from orange, grapefruit and lemons; however, orange and lemon peel contained 59-82 mg oxalate/100 g FW and 276-323 mg oxalate/100 g FW, respectively. These results were supported by the present study where no oxalate was detected in freshly squeezed juice of these citrus fruits and thus, it was reasonable to suggest that Ogawa *et al*., (1985) used whole lemon and orange for their analysis.

The different results presented in the literature and the present study may be due to problems in the older type of extractions and determinations used in these studies. Variations between different cultivars, variation in fruit maturity and differences in fertilization concentrations or climate conditions the plants were grown under may also have contributed (Libert and Franceschi, 1987). The titration method used by Andrews and Viser, (1951) and Awadalia *et al*., (1985), used 5-hour extraction with 3-4 N HCL followed by a 15-hour extraction with diethyl ether could lead to a substantial increase of oxalate concentration due to conversion of oxalogenic compounds to oxalic acid (Zarembski and Hodgkinson, 1962a). Colourimetric methods (Zarembski and Hodgkinson, 1962a; Hodgkingson, 1977; Ogawa, 1984) may suffer from interference during extraction, calcium precipitation, the reduction of oxalate to glycolate or by problems occurring in the colourimetric measurement steps. Enzymatic analysis based on a colourimetric estimation of H_2O_2 can also suffer interference from enzyme inhibitors in the fruit tissue or from pigments extracted from dark-coloured fruits.

Previous advice (National Kidney and Urologic Diseases Information Clearinghouse, 2007; University of Pittsburgh Medical Centre, 2003) to reduce the consumption of berry fruits such as strawberry, cranberry and blueberry is not supported in this study as the analysis of these

fruits showed the absence (strawberry and cranberry) or low amounts (blueberry 3.1 mg total oxalate/100 mg FW) of total as well as soluble oxalates. The oxalate concentration of these fruits is generally very low even when the serving size of each fruit is considered. The most common serving size (The Concise New Zealand Food Composition Tables, 2009) is a cup full. For rhubarb, feijoa and Indian gooseberry the consumption of a cup full would result in the consumption of 1696.5, 145.6, and 9079.6 mg total oxalate, respectively. Indian gooseberries are very tart to taste so the consumption of a cupful is very unlikely but if it was consumed it would result in a very significant intake of oxalate. The consumption of one carambola fruit would involve the consumption of 645 mg of total oxalate. All other fruits contain insignificant oxalate concentrations and, for example for kiwifruit (Table 5.4), it depends on whether the whole fruit is eaten or the skin is discarded.

It is interesting to note that no oxalates could be detected in apple (Royal, Eve and Rose varieties), apricot, peach (yellow and white flesh), pear (Taylor's gold), nectarine, banana, strawberry, lemon, orange, mandarin, mango, tamarillo and watermelon, which is in contrast to earlier studies which report values ranging from zero to 49.0 mg/100 g FW for these fruits. Close inspection showed that apple, avocado, nectarine, banana, mangosteen, have not been measured previously (Table 5.1). Only lemon, orange, mandarine, strawberry, tamarillo and watermelon have previously been reported with total oxalate concentrations over 0.3 mg/100 g FW. The differences of oxalate concentrations between the present study and literature may be due to the differences of cultivars, maturity stage, growing conditions, the part of fruit analysed and the extraction and measurement methods. In fact, the present study set out to quantify total and soluble oxalates in fruit that were collected from local super markets and were often of uncertain age, unknown variety as well as unknown growing conditions. Therefore, it would not be expected to have similar oxalate concentrations between the present study and pulished values.

Overall, this study showed that most of the fruit analysed that was grown locally in New Zealand contained a low concentration of total and soluble oxalates and could be classified into three different groups based on their soluble oxalate concentrations (Table 5.5.).

5.6. Conclusions

The data showed that total oxalate concentrations in most fruits analysed were quite low. This study suggested that all of the fruits analysed in this study should be placed in the low oxalate food group except for feijoa, Indian gooseberries, carambola, rhubarb and goji berries. These five fruits could be classified into Group 1 following the classification suggested by Noonan and Savage (1999). It is suggested that these higher oxalate-containing fruits should be consumed infrequently, and in small amounts, to limit the intake of soluble oxalates.

References

Allison, R. M. (1966). Soluble oxalates, ascorbic and other constituents of rhubarb varieties. *Journal of the Science of Food and Agriculture* 17(12), 554-557.

Andrews, J. C. & Viser, E. T. (1951). The oxalic acid content of some common foods. *Food Research*, *16*, 306–312.

AOAC (2002) method 925.10, Official methods of analysis of AOAC International 17th Edition, Gathersberg, MD, USA: AOAC International.

Awadalia, M. Z., Bassily, N. S. & Saba, N. A. (1985). Oxalate content of some Egyptian foodstuffs. *Journal of the Egyptian Public Health Association*, *60*(3), 41–51.

Baker, C.J.L. (1952). The determination of oxalates in fresh plant material. *Analyst*, *77*, 340–344.

Blundstone, H. A. W., & Dickinson, D. (1964). The chemistry of edible rhubarb. *Journal of the Science of Food and Agriculture* 15, 94-101.

Carlsen, M., Halvorsen, B., Holte, K., Bohn, S., Dragland, S., Sampson, L., et al. (2010). The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. *Nutrition Journal*, *9*(1), 3.

Clementi, E. M., & Misiti, F. (2010). Chapter 27 - Potential Health Benefits of Rhubarb. In W. Ronald Ross and R. P. Victor (Eds.). *Bioactive Foods in Promoting Health* (pp. 407-423). San Diego: Academic Press.

Elmadfa, I., Aign, W., Muskat, E., Frutzsche, D., & Cremer, D.H. (1992/1993). Die große GU Nährwerttabelle. *GU Gräfe und Unzer*, p.64.

Gallaher, R.N. (1975). The occurrence of calcium in plant tissues as crystals of calcium oxalate. *Communication in Soil Science and Plant Analysis 6*, 315-330.

Giachetti, E., Pinzauti, G., Bonaccorsi, R., Vincenzini, M.T. & Vanni, P. (1987). Isocitrate lyase from higher plants. *Phytochemistry*, *26*, 2439–2446.

Grieve, M. (1980). *A model herbal.* London: Penguin Books.

Herrmann, K. (1972). Über den Oxälsa uregehalt des Obstes und Gemüses. *Zeitschrift Lebensmitteluntersuchung, 148*, 206–210.

Holloway, W.D., Argall, M.E., Jealous, W.T., Lee, J.A. & Bradbury, J.H. (1989). Organic acids and calcium oxalate in tropical root crops. *Journal of Agricultural and Food Chemistry, 37*, 337-341.

Hönow, R. and Hesse, A. (2002). Comparison of extraction methods for the determination of soluble and total oxalate in foods by HPLC enzyme-reactor. *Food Chemistry, 78*, 511-521.

Kasidas, G. P. & Rose, G. A. (1980). Oxalate contents of some common foods: determination by an enzymatic method. *Journal of Human Nutrition*, *34*, 255–266.

Libert, B. & Franceschi, V.R. (1987). Oxalate in crop plants. *Journal of Agricultural and Food Chemistry, 35*, 926-938.

Libert, B., & Creed, C. (1985). Oxalate content of 78 rhubarb cultivars and its relation to some other characters. *Journal of Horticultural Science, 60*(2), 257-261.

National Kidney and Urologic Diseases Information Clearinghouse. (2007). *Kidney stones in adults*. Retrieved from http://kidney.niddk.nih.gov/kudiseases/pubs/stonesadults/#oxalate

Noonan, S.C. & Savage, G.P. (1999). Oxalate content of foods and its effect on humans. *Asia Pacific Journal of Clinical Nutrition, 8*(1), 64-74.

Ogawa, Y., Takahashi, S., & Kitagawa, R. (1984). Oxalate content in common Japanese foods. Acta Urologica Japonica. *Hinyokika Kiyo, 30*, 305–310.

Oxalosis and Hyperoxaluria Foundation . (2007). *The oxalate content of foods*. Retrived from http://www.ohf.org/docs/Oxalate2008.pdf

Perera, C. O., Hallett, I. C., Nguyen, T. T., & Charles, J. C. (1990). Calcium oxalate crystals: the irritant factor in kiwifruit. *Journal of Food Science, 55*(4), 1066-1069.

Rassam, M., & Laing, W. (2005). Variation in ascorbic acid and oxalate levels in the fruit of *Actinidia chinensis* tissues and genotypes. *Journal of Agricultural and Food Chemistry, 53*(6), 2322-2326.

Rassam, M., Bulley, S.M., & Laing, W.A. (2007). Oxalate and ascorbate in *Actinidia* fruit and leaves. *ISHS Acta Horticulturae, 753*, 479-484.

Rinallo, C., & Mori, B. (2000). Oxalate and ascorbic acid in kiwifruit during growth and storage. *Italian Journal of Food Science, 12*(4), 435-442.

Savage, G.P., Vanhanen, L., Mason, S., & Ross, A.B. (2000). Effect of cooking on the soluble and insoluble oxalate content of some New Zealand foods. *Journal of Food Composition and Analysis, 13*, 201-206.

Turner, N. A. (1980). Micro-determination of oxalate in crude extracts of plant tissues. *Journal of the Science of Food and Agriculture, 31*(2), 171-176.

University of Pittsburgh Medical Center. (2003). *Low oxalate diet.* Retrieved from http://www.pkdiet.com/pdf/LowOxalateDiet.pdf

Vanhanen, L., Savage, G. P., & Sangketkit, C. (2011). Oxalate content of eleven indigenous Thai plants. *Journal of Food Agriculture and Environment, 9*(3 & 4), 38-40.

Wagner, C. J. Jr., Berry, R. E. & Knight, R. J. (1975). Carambola selection for commercial production. *Proceedings of the Florida State Horticultural Society, 88*, 466–469.

Watanabe, K., & Takahashi, B. (1998). Determination of soluble and insoluble oxalate contents in kiwifruit (*Actinidia deliciosa*) and related species. *Journal of the Japanese Society for Horticultural Science, 67*, 299-305.

Wilson, C. W., Shaw, P. E., & Knight, R. J. (1982). Analysis of oxalic acid in carambola (*Averrhoa carambola L.*) and spinach by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry, 30*(6), 1106-1108.

Zarembski, P. M., & Hodgkinson, A. (1962a). The determination of oxalic acid in food. *Analyst, 87*, 698–702.

Zarembski, P. M., & Hodgkinson, A. (1962b). The oxalic acid content of English diets. *British Journal of Nutrition, 16*, 627–634.

Chapter 6

Effect of storage time and juice processing on the oxalate concentrations of kiwifruit and kiwifruit juice

6.1. Abstract

Oxalate concentrations of the whole fruit and the three fractions skin, pulp and seeds of green and golden kiwifruit following storage at 20^0C for up to 15 days were investigated. Soluble oxalate of the pulp and skin of both cultivars decreased over 15 day storage while total oxalate concentration decreased slightly. The distribution of oxalates of whole fruit was significantly different ($p < 0.001$) between the both cultivars. Significantly higher concentrations of total and soluble oxalates ($p < 0.01$) were found in green kiwifruit juice obtained by enzymatic extraction compared with juice obtained by pressing. Most of the insoluble oxalate from the fresh kiwifruit was retained in the pomace during juice extraction. In the juice, soluble oxalate concentration made up 60.3 - 70.3% of total oxalate concentration compared with 31.7% in the fresh tissue. Pasteurisation temperatures significantly affected the soluble oxalate concentration but did not impact the concentrations of total oxalate in the juice.

6.2. Introduction

Kiwifruit is rich in bioactive compounds such as vitamin C, β-carotene, phenolics minerals such as calcium and potassium, and dietary fibre (Nishiyama *et al*., 2004; Latocha *et al.,* 2010). Kiwifruit has become a significant part of locally grown fruit in the New Zealand domestic market. Furthermore, kiwifruit is currently one of New Zealand's largest horticultural export crops with 380,000 tonnes being harvested in 2009 and 361,066 tonnes being exported to over 55 countries in the same year (USDA, 2010).

Earlier studies found that green kiwifruit contained moderate amounts of total oxalate. Insoluble oxalates in the pulp can cause irritation in the mouth while soluble oxalate may lead to kidney stone formation if consumed in large amounts (Perera *et al*., 1990; Noonan and Savage, 1999). The amount of total oxalate in golden kiwifruit reported in the literature varies from 7.8 to 45 mg/100 g FW, while the values for green kiwifruit range from 12.7 - 84.3 mg/100 g FW (Nguyen and Savage, 2012). When the average dry matter for golden kiwifruit (15.2%) and for green kiwifruit (15.8%) was used (Nguyen and Savage, 2012), the total oxalate

in golden kiwifruit reported in the literature varies from 51.3 to 296 mg/100 g DM, whilst the values for green kiwifruit range from 80.4 – 533.5 mg/100 g DM. The soluble oxalate concentration for green kiwifruit range from 15.8 to 262.0 mg/100 g DM (Perera *et al*., 1990; Watanabe and Takahashi, 1998; Rinallo and Mori, 2000) and the golden kiwifruit values range from 3.9 to 15.1 mg/100 g DM (Watanabe and Takahashi, 1998; Hönow and Hesse, 2002). Although the marked differences may be due to genotypes (Rassam and Laing, 2005), growing conditions (Rinallo and Mori, 2000) and harvest maturity stage (Rassam *et al.*, 2007), using unreliable analytical methods could also result in an under- or overestimation of oxalate concentrations (Nguyen and Savage, 2012).

Commercially grown kiwifruit are harvested when physiologically mature and can be stored for a few months (Hopkirk *et al*., 1986) while locally grown fruit is picked at the 'eating ripeness' stage when its optimum nutrition and sensory features have been reached. Since kiwifruit is a climacteric fruit (Pratt and Reid, 1974), it will ripen further due to biochemical processes which will result in changed physico-chemical properties after harvesting. The changes taking place during storage may affect oxalate concentrations. So far, the oxalate concentration of New Zealand kiwifruit has been reported by Perera *et al*., (1990), Rassam and Laing (2005) and Rassam *et al*., (2007) who reported total oxalate but not soluble oxalate concentration. Moreover, no study has been carried out to follow the changes in total and soluble oxalate during domestic storage conditions.

Kiwifruit that does not reach export standard and is not suitable to sell on the domestic market can be processed into canned slices in syrup, juices, wines and frozen pulp (Venning *et al.*, 1989); juice is the most popular product. These processes may affect not only colour, flavour, vitamin C concentration; antioxidant compounds (Stanley *et al*., 2007) but also the amount and form of oxalate present in the kiwifruit products (Lodge and Perera, 1992). Perera *et al*., (1990) found that pressing kiwifruit to obtain nectar or dehydration for later reconstitution, concentrated the oxalate concentration especially the oxalate crystals released from the edible portion during processing. Making juice by extraction and pasteurisation might also affect the form of oxalate in the juice and this has not been fully investigated. As kiwifruit juice is recommended as an alternative source of vitamins and phyto-chemicals as part of daily fresh fruit consumption (Landon, 2007), measurement of oxalate intake per juice serving is important. The objectives of this study were to determine the changes of oxalate concentration in kiwifruit as they were stored or processed into juice to determine whether the concentration of soluble and insoluble oxalate changes during these treatments.

6.3. Materials and methods

6.3.1. Sample preparation

Green kiwifruit (*Actinidia deliciosa* cv. Hayward) and golden kiwifruit (*Actinidia chinensis* cv. Hort16A) were bought fresh from Growers Direct Market Ltd., Christchurch, NZ. All the fruits (weighing between 80 and 100 g) were carefully inspected and overripe and damaged fruits were excluded. The experiment commenced when a subsample of fruit reached an average total soluble solids (TSS) of 10.8 ^{0}Brix.

6.3.2. Storage conditions

Healthy fruits were stored in the dark in a temperature controlled room at 20 ± 1^{0}C, relative humidity (RH) 50-55%. Two groups of five fruits of each cultivar were sampled on days 0, 5, 10 and 15. Group 1 was analysed whole fruit; Group 2 was divided into three fractions: skin, pulp (comprising outer, inner pericap and core) and seeds, using a stainless-steel knife. The fractions from five fruits were pooled. All samples were immediately frozen in liquid N_2 and then finely ground using a mortar and pestle.

6.3.3. Juice processing

Processing was based on the procedure of Cassano *et al.*, (2007). Selected green kiwifruit, (*Actinidia deliciosa* cv Hayward), weighing between 80 and 100 g, were washed with cold tap water, peeled and then separated into two groups: one was used for making juice by pressing and another one was used for making juice using enzyme extraction.

6.3.3.1. Production of juice by pressing

Ten kg of fruit was peeled, cut into pieces and pressed in a Sunbeam Juice Maker (Model No. JE8600, China). In order to inactivate polyphenol oxidases that can cause browning of the kiwifruit pulp, 30 g sodium sulphite (BDH Chemicals Ltd., Palmerston North, NZ) was added, the juice was filtered through cheese cloth (cotton muslin, Ragtag Ltd., Swanson, Auckland, NZ) and bottled into 100 ml glass screw-cap jars. The jars were pre-pasteurised using a temperature controlled water-bath (Grant OLS200 combined orbital/lined shaking BS/65302-Grant Instruments Ltd., UK). The bottled juice was pasteurized at 70, 80 and 90^{0}C; the juice took 75 seconds to reach 70^{0}C, 83 seconds to reach 80^{0}C and 90 seconds to reach 90^{0}C and was maintained at these temperatures for 10 seconds. The jars were then cooled to 21^{0}C and stored at 4^{0}C until analysis could commence.

6.3.3.2. Production of juice using enzyme extraction

Ten kg of fruit was peeled and crushed using a Cascade Blender (Model No. CE071BR, China). After 20 minutes of processing 100 g pectolase (extracted from *Aspergillus aceleatus*, Brewcraft Ltd., Mt Eden, Auckland, NZ) was added. The puree was incubated for four hours at 21°C before being filtered through cheese cloth and bottled into 100 glass screw-cap jars, pasteurised as outlined above and stored at 4 ± 0.5°C.

6.3.4. Moisture content

The moisture content of each fresh fraction of kiwifruit was determined in triplicate by drying in an oven at 105^0C until a constant weigh was achieved (AOAC, 2002).

6.3.5. Physico-chemical properties

The pH was determined using a pH meter (SevenEasy Mettler, Toledo-GmbH 8603 Schwerzenbanch, Switzerland) that was calibrated using standardised buffers, pH 4.0 and pH 7.0. Total soluble solids (TSS) were measured using a hand refractometer (RHB 32, Syoptek International Limited, Xiamen, China) and the result expressed as ^{0}Brix. Titratable acids (TA) were determined as citric acid by titration to pH 8.2 with 0.1 N NaOH using an automatic titrator (730 Sample Changer, Titrino, Metrohm, Herisau, Switzerland). Firmness was measured using a hand penetrometer (FT327, Effigy Systems, Italy) with a 1cm^2 diameter tip.

6.3.6. Extraction and determination of total and soluble oxalate

Extraction of total and soluble oxalate was conducted at 21^0C using the method of Savage *et al.*, (2000). The insoluble oxalate was calculated as the difference between the total oxalate (acid extract) and soluble oxalate (water extract), according to Holloway *et al*., (1989). Each sample was analysed in triplicate and results are presented as mean of mg oxalate/100 g dry weight (DW) ± standard error (SE).

6.3.7. Statistical analyses

For storage, data were analysed by a three-way ANOVA as a 5 (fraction) by 4 (storage time) by 2 (cultivar) factorial design using the General Treatment Structure in GenStat Version 14 for Windows 7 (VSN International Ltd., Hemel Hempstead, Hertfordshire, UK). The main factors (fractions, storage times and cultivars) and interactions (fractions x storage times; fractions x cultivars; storage times x cultivars; fractions x storage times x cultivars) were considered significant at $p < 0.05$. In the three-way ANOVA, the three-factor interaction was first tested, if the p-value was significant ($p < 0.05$), pairs of two main factors were compared at each level of the third factor while the two-factor interactions and the main factors were ignored. If the three-

factor interaction was not significant, two-factor interactions were considered. If some two-factor interactions were significant and some were not, only significant two-factor interactions and the significant main factors, which had no significant interaction with any other factor, were analysed. If all the three-factor and two-factor interactions were not significant, then the main factors were considered.

Minitab Version 16 (Minitab Ltd., Coventry, UK) was used to assess significant differences between pairs of mean oxalate concentrations of fruit and fruit juice. All data were presented as the mean of five determinations ± S.E with significance accepted at $p < 0.05$.

6.4. Results

6.4.1. Storage conditions

At the commencement of the storage trial the two cultivars of kiwifruit had reached an acceptable stage of ripeness for immediate consumption. The storage trial was intended to follow the changes that might occur in the kiwifruit during normal storage at home. Firmness and total soluble solid were used as primary tools to indicate maturity (Scott *et al*., 1986; Beever and Hopkirk, 1990). These parameters were measured every five days during storage (Table 6.1). By the end of Day 15, the kiwi pericarp was very soft and, therefore, was not fit for further storage. During 15 days of storage at 20^0C, the firmness fell 82.2% for green and 80.6% for golden kiwifruit. Meanwhile, total soluble solids fluctuated through the fifteen days for both cultivars. Total soluble solids reached a peak of 14.7 ^{0}Brix and 14.1 ^{0}Brix on Day 10 followed by a reduction of 10.9 % and 13.5 % by Day 15 for green and golden kiwifruit, respectively.

Table 6.1. Mean of firmness and total soluble solids of green and golden kiwifruit (± SE) during storage at 20^0C.

Storage time (day)	Green kiwifruit		Golden kiwifruit	
	Firmness (kg/cm^2)	Total soluble solids (^{0}Brix)	Firmness (kg/cm^2)	Total soluble solids (^{0}Brix)
0	4.5 ± 0.1^a	10.8 ± 0.2^a	3.1 ± 0.1^a	10.8 ± 0.0^a
5	2.6 ± 0.1^b	12.6 ± 0.1^b	2.4 ± 0.0^b	11.7 ± 0.2^b
10	1.6 ± 0.1^c	14.7 ± 0.2^c	1.3 ± 0.1^c	14.1 ± 0.2^c
15	0.8 ± 0.2^d	13.1 ± 0.1^d	0.6 ± 0.1^d	12.2 ± 0.1^d

Means with different letters in each column are significantly different ($p < 0.05$).

Table 6.2. Dry matter (%) and proportion (%) of different fractions of green and golden kiwifruit.

Fractions	Storage time (days)	Dry matter (%)		Proportion in the whole fruit (% DM)	
		Green kiwifruit	Golden kiwifruit	Green kiwifruit	Golden kiwifruit
Whole fruit	0	17.0 ± 0.2^{a}	16.6 ± 0.3^{a}	100%	100%
	5	18.5 ± 0.4^{b}	18.1 ± 0.3^{ab}		
	10	17.9 ± 0.3^{bc}	17.7 ± 0.2^{bc}		
	15	17.4 ± 0.5^{ac}	17.1 ± 0.3^{ac}		
Skin	0	28.1 ± 0.6^{a}	38.1 ± 0.2^{a}	12.1 ± 0.4	18.4 ± 0.7
	5	30.1 ± 0.4^{ab}	40.4 ± 0.4^{a}		
	10	29.2 ± 0.6^{a}	39.5 ± 0.4^{ab}		
	15	31.7 ± 0.9^{b}	40.8 ± 0.9^{ab}		
Pulp	0	14.4 ± 0.1^{a}	15.2 ± 0.3^{a}	76.1 ± 0.8	77.8 ± 0.7
	5	17.0 ± 0.4^{b}	17.6 ± 0.3^{b}		
	10	16.3 ± 0.6^{b}	17.0 ± 0.2^{b}		
	15	15.4 ± 0.6^{a}	16.0 ± 0.3^{c}		
Seeds	0	82.6 ± 1.1	81.3 ± 2.2	11.1 ± 0.2	3.9 ± 0.0
	5	82.8 ± 0.8	81.8 ± 0.6		
	10	82.1 ± 0.6	81.0 ± 1.5		
	15	82.1 ± 1.0	81.4 ± 0.9		

The values are mean ± SE (n=5).
Means with different letters in each column for each fraction are significantly different ($p < 0.05$)

Table 6.3. Change of oxalate concentrations (mg/100 g DW ± SE) in whole fruit and three fractions of green and golden kiwifruit during storage at 20^0C.

	Time (day)	Total oxalate		Soluble oxalate		Insoluble oxalate	
		Green kiwifruit	Golden kiwifruit	Green kiwifruit	Golden kiwifruit	Green kiwifruit	Golden kiwifruit
Whole fruit	0	150.0 ± 1.6^{aW}	121.7 ± 0.8^{aX}	67.4 ± 1.1^{aW}	57.2 ± 0.6^{aX}	82.6 ± 0.6^{aW}	64.4 ± 1.2^{aX}
	5	148.8 ± 1.9^{abW}	120.9 ± 1.3^{abX}	64.0 ± 0.7^{bW}	51.6 ± 1.4^{bX}	84.9 ± 2.6^{abW}	71.3 ± 2.0^{bX}
	10	147.7 ± 1.4^{abW}	118.1 ± 0.8^{bX}	60.6 ± 0.4^{cW}	43.0 ± 0.8^{cX}	87.1 ± 1.8^{bW}	76.8 ± 1.4^{cX}
	15	145.5 ± 0.6^{bW}	116.1 ± 1.0^{bX}	59.2 ± 1.0^{cW}	41.1 ± 0.7^{cX}	86.2 ± 1.3^{bW}	76.3 ± 1.1^{bcX}
Skin	0	169.7 ± 0.8^{aW}	145.6 ± 2.6^{aX}	38.4 ± 0.8^{aW}	30.7 ± 0.8^{aX}	131.3 ± 0.3^{aW}	114.2 ± 3.8^{aX}
	5	169.0 ± 0.7^{abW}	142.7 ± 3.6^{abX}	34.2 ± 0.6^{bW}	27.4 ± 0.5^{bX}	134.7 ± 0.6^{bW}	126.0 ± 4.3^{abW}
	10	168.7 ± 0.9^{abW}	138.3 ± 0.5^{abX}	30.5 ± 0.5^{cW}	23.3 ± 1.2^{cX}	138.8 ± 1.0^{cW}	122.3 ± 0.9^{abX}
	15	166.1 ± 1.3^{bW}	136.4 ± 1.1^{bX}	28.2 ± 0.3^{dW}	21.5 ± 0.6^{cX}	139.3 ± 1.3^{cW}	122.9 ± 0.9^{bX}
Pulp	0	147.7 ± 1.8^{aW}	116.2 ± 0.8^{aX}	80.5 ± 1.5^{aW}	65.5 ± 0.9^{aX}	67.2 ± 0.3^{aW}	50.7 ± 1.1^{aX}
	5	146.4 ± 2.0^{aW}	115.9 ± 0.9^{abX}	76.5 ± 0.8^{bW}	59.2 ± 1.9^{bX}	69.8 ± 2.8^{abW}	56.7 ± 2.5^{bX}
	10	145.2 ± 2.1^{abW}	113.4 ± 0.8^{bcX}	73.0 ± 0.4^{bcW}	49.1 ± 0.8^{cX}	72.2 ± 2.5^{bW}	64.3 ± 1.6^{cW}
	15	142.9 ± 0.5^{bW}	111.3 ± 0.9^{cX}	71.2 ± 1.3^{cW}	46.9 ± 0.9^{cX}	71.5 ± 1.7^{bW}	64.3 ± 1.4^{cX}
Seeds	0	144.1 ± 3.4^{aW}	119.7 ± 1.7^{aX}	12.6 ± 0.2^{abW}	15.5 ± 0.4^{abX}	131.5 ± 3.2^{aW}	104.1 ± 1.8^{aX}
	5	143.9 ± 4.4^{aW}	118.7 ± 2.1^{aX}	13.4 ± 0.3^{abW}	14.4 ± 0.5^{abW}	130.5 ± 4.1^{aW}	104.3 ± 1.8^{aX}
	10	142.2 ± 1.5^{aW}	115.5 ± 2.4^{aX}	11.7 ± 0.9^{aW}	14.0 ± 0.9^{aW}	131.3 ± 1.7^{aW}	111.1 ± 2.5^{bX}
	15	142.1 ± 2.2^{aW}	117.0 ± 1.7^{aX}	13.9 ± 0.8^{bW}	16.2 ± 0.6^{bW}	128.2 ± 3.0^{aW}	96.5 ± 1.2^{cX}

Means with the different letters (a-d) in each column are significantly different at $p < 0.05$

Means with the different letters (W-X) in each row for each oxalate form between cultivars are significantly different at $p < 0.05$

Table 6.4. Three-way ANOVA statistical analysis using General Treatment Structure for oxalate values of green and golden kiwifruit influenced by fractions, cultivar and storage time.

Source of variation	Degrees of freedom	Significance		
		Total oxalate	Soluble oxalate	Insoluble oxalate
Fractions	3	***	***	***
Cultivars	1	***	***	***
Storage	3	***	***	***
Fractions x cultivars	3	**	***	***
Fractions x storage	9	NS	***	***
Storage x cultivars	3	NS	***	**
Fractions x cultivars x storage	9	NS	***	NS
l.s.d. (5%) within fraction		1.8	0.9	2.1
l.s.d. (5%) within cultivar		1.3	0.6	1.5
l.s.d. (5%) within storage		1.8	0.9	2.1
l.s.d. (5%) fraction x cultivar		2.6	1.2	3.0
l.s.d. (5%) fraction x storage		3.6	1.7	4.2
l.s.d. (5%) storage x cultivar		2.6	1.2	3.0
l.s.d. (5%) fraction x cultivar x storage		5.1	2.4	5.9

Significance: NS not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. l.s.d: least significant difference

To determine the effects of fraction, cultivar and storage times on the oxalate values of the stored kiwifruit, the interactions between these factors were analysed (Table 6.4). A three-way ANOVA was carried out to find possible interactions as well the main factors.
Analysis of variance of total oxalate concentrations including five different fractions of two different cultivars stored for 15 days showed no significant interaction effects between the three factors as well as no significant interactions between fraction and storage and between storage and cultivar, but a significant interaction ($p < 0.001$) between fractions and cultivars was obtained (Table 6.4). Thus, changes of total oxalate concentrations during storage (Table 6.3) were independent of fractions and cultivars. Consequently, it can be concluded that total oxalate concentrations decreased from Day 0 to Day 15 regardless of fractions and cultivars. The overall mean of total oxalate concentration reduced from 133.9 mg/100 g DW (Day 0) to 129.4 mg/100 g DW (Day 15).

Meanwhile, a significant interaction ($p < 0.001$) between fractions and cultivars illustrated that the differences of total oxalate values between fractions were dependent on cultivars. The means of the oxalate concentration of the different fractions of both cultivars are shown in Table 6.5. In green kiwifruit, the skin contained the highest amount of total oxalate, followed by whole fruit, the pulp and the seeds. In the golden kiwifruit, these patterns were the same as those observed in green kiwifruit, except for the pulp and the seeds, the pulp contained a lower amount of total oxalate than the seeds.

Table 6.5. Overall means of total, soluble and insoluble oxalate concentrations (mg/100 g DW ± SE) in different fractions of green and golden kiwifruit, regardless of storage times.

	Green kiwifruit			Golden kiwifruit		
	Total	Soluble	Insoluble	Total	Soluble	Insoluble
Whole fruit	148.0 ± 1.0^{aW}	62.8 ± 1.8^{aW}	85.2 ± 1.0^{aW}	119.2 ± 1.3^{aX}	48.2 ± 3.8^{abX}	72.2 ± 2.9^{aX}
Skin	168.4 ± 0.8^{bW}	32.8 ± 2.2^{bW}	136.0 ± 1.9^{bW}	140.8 ± 2.1^{bX}	25.7 ± 2.1^{cX}	121.4 ± 2.5^{cX}
Pulp	145.5 ± 1.0^{acW}	75.3 ± 2.1^{cW}	70.2 ± 1.1^{cW}	114.2 ± 1.2^{cX}	55.2 ± 4.4^{bX}	59.0 ± 3.3^{dX}
Seeds	143.1 ± 0.5^{cW}	12.9 ± 0.5^{dW}	130.4 ± 0.7^{dW}	117.7 ± 0.9^{acX}	15.0 ± 0.5^{dX}	104.0 ± 3.0^{eX}

Means with the different letters (a-e) in each column are significantly different at $p < 0.05$
Means with the different letters (W-X) in each row for each oxalate form between cultivars are significantly different at $p < 0.05$

Generally, the total oxalate concentrations in kiwifruit decreased over 15 days of storage. The mean of total oxalate concentrations of all green kiwifruit fractions were significantly higher than those in golden kiwifruit (Table 6.5).

Analysis of variance of the soluble oxalate concentrations showed that there are significant interactions ($p < 0.001$) between the three factors as well as significant interactions ($p < 0.001$) between the two factors (Table 6.4). Because the three-factor interaction was significant, the two-factor interactions and the main factors were ignored. The three-way analysis was divided into a two-way analysis of fraction and storage time and then, the two-way analysis was considered for the presence of the third factor, cultivar.
There was a significant ($p < 0.001$) interaction between fractions and storage time for soluble oxalates (Table 6.4). This means that changes of soluble oxalate concentration in the fractions were dependent on storage time for each cultivar. In green kiwifruit, the soluble oxalate concentrations of the whole fruit, skin and pulp significantly ($p < 0.05$) decreased after 15 days of storage, but this did not occur in the seeds (Table 6.3). A similar phenomenon was observed in golden kiwifruit, the soluble oxalate concentrations of all fractions, except for seeds, significantly decreased ($p < 0.05$) from Day 0 until Day 15. No significant change in the soluble oxalate concentrations of the seeds was seen during storage (Table 6.3).
On each storage day, the soluble oxalate concentrations of each fraction, except for the seeds, were significantly different ($p < 0.05$) between green and golden kiwifruit (Table 6.3). The whole fruit, skin and pulp of green kiwifruit showed significantly ($p < 0.05$) higher amounts of soluble oxalates as compared to those in the golden kiwifruit. Meanwhile, in the seeds, soluble oxalate concentrations found in both cultivars were not different during storage (Table 6.3). In both cultivars, the pulp contained the highest mean values of soluble oxalate, followed by whole fruit, the skin and the seeds (Table 6.5).

Overall, soluble oxalate concentrations of kiwifruits reduced after 15 days of storage at 20^0C. There were significant differences ($p < 0.05$) of soluble oxalates in whole fruit, the pulp and skin between two kiwifruit cultivars, green and golden kiwifruit (Table 6.5) in which the pulp contained the highest concentrations of soluble oxalates, whereas, the lowest soluble oxalate concentrations were found in the seeds.

The three-way ANOVA analysis (Table 6.4) showed that there was no interaction between three main factors for insoluble oxalate concentrations. Thus, the two-factor interactions were considered. There were significant two-factor interactions between fraction and cultivar

($p < 0.001$), between fraction and storage ($p < 0.001$) and between storage and cultivar ($p < 0.01$) (Table 6.4). Thus, the three main factors were ignored.

The contribution of insoluble oxalates was significantly different ($p < 0.05$) at all fractions for both cultivars, except for the pulp of green kiwifruit (Table 6.5). Insoluble oxalates were found in the highest amount in the skin, followed by the seeds, the whole fruit and the pulp (Table 6.5). In each fraction, the amounts of insoluble oxalates were significantly ($p < 0.05$) higher in the green kiwifruit cultivar compared to those found in the respective fractions of the golden cultivar (Table 6.5).

Because interaction between fraction and storage time was significant (Table 6.4), the means of insoluble oxalate concentration in these factors were analysed, regardless cultivar.

Table 6.6. Means of insoluble oxalate concentration (mg/100 g DW ± SE) in different fractions of kiwifruit during 15 days of storage.

	Day 0	Day 5	Day 10	Day 15
Whole fruit	73.4 ± 0.9^{aW}	78.1 ± 2.3^{aWX}	82.0 ± 1.6^{aX}	81.3 ± 1.2^{aX}
Skin	122.8 ± 2.1^{bW}	130.4 ± 2.5^{bWX}	130.6 ± 1.0^{bX}	131.1 ± 1.1^{bX}
Pulp	59.0 ± 0.7^{cW}	63.3 ± 2.7^{cWX}	68.3 ± 2.1^{cX}	67.9 ± 1.6^{cX}
Seeds	117.8 ± 2.5^{dWX}	117.4 ± 3.0^{dWX}	121.2 ± 2.1^{dW}	112.4 ± 2.1^{dX}

Means with the different letters (a-e) in each column are significantly different at $p < 0.05$
Means with the different letters (W-X) in each row are significantly different at $p < 0.05$

During storage, insoluble oxalate concentrations of all fractions tended to increase, except in the seeds. These values significantly ($p < 0.05$) increased from Day 0 to Day 5 and remained consistent from Day 10 until the end of storage (Table 6.6). The concentrations of insoluble oxalates were highest in the skin, followed by the seeds and the pulp on any day of storage (Table 6.6).

Due to a significant ($p < 0.01$) interaction between storage and cultivar, the means of insoluble oxalates between these two factors were analysed, regardless to fractions.

Table 6.7. Means of insoluble oxalate concentration (mg/100 g DW ± SE) in different cultivars during 15 days of storage.

	Green kiwifruit	Golden kiwifruit
Day 0	95.9 ± 1.0^{aW}	75.4 ± 1.8^{aX}
Day 5	97.7 ± 2.6^{aW}	81.3 ± 2.5^{abX}
Day 10	100.0 ± 1.8^{aW}	85.7 ± 1.5^{bX}
Day 15	99.0 ± 1.7^{aW}	82.8 ± 1.1^{bX}

Means with the different letters (a-e) in each column are significantly different at $p < 0.05$
Means with the different letters (W-X) in each row between cultivars are significantly different at $p < 0.05$

There has no consistent trend observed for change in insoluble oxalate concentration during storage. For green kiwifruit, although there was an increasing tendency of the means of insoluble oxalate level during 15 days of storage, the differences were not significant. Whereas, for golden kiwifruit, the means of insoluble oxalate concentration significantly ($p < 0.05$) decreased after 15 days of storage (Table 6.7). The green kiwifruit cultivar contains significantly higher concentrations of insoluble oxalate in comparison with those in golden cultivar (Table 6.7).

Overall, the contribution of insoluble oxalates was significantly different in different fractions of kiwifruit. They condensed in the skin and the seeds. After 15 days of storage at 20^0C, insoluble oxalates tended to increase in all fractions except the seeds. The green kiwifruit cultivar contained significant higher insoluble oxalate amounts compared with the golden kiwifruit.

6.4.2. Processing the juice

Treatment of the homogenised kiwifruit for 20 minutes at 21°C with pectolase significantly increased ($p < 0.01$) the yield of extracted juice from 10 kg of pealed kiwifruit. Pressing the homogenised kiwifruit yielded 5.03 ± 0.15 kg juice compared to 6.68 ± 0.21 kg juice recovered after treatment with pectolase. Overall, the efficiency of enzymatic extraction was higher than that of extraction by pressing, 66.8% compared with 50.3%, respectively (Table 6.8). Significantly more juice was extracted from the processed green kiwifruits after they were incubated for four hours with pectolase compared to pressing alone. It can be seen that most of the oxalate concentrations, especially insoluble oxalate, were retained in the pomace (Table 6.9). Most of the insoluble oxalates remained in the pomace with both extraction methods (92.9% in pressed pomace and 86.8% in enzymatic pomace) while a minor amount was found in the juice (7.1% in pressed juice and 13.2 % in enzymatic extracted juice). Total oxalate and soluble oxalate concentrations of the juice extracted following treatment with pectolase (6.64 mg total oxalate/ 100 ml and 4.67 mg soluble oxalate/100 ml) were approximately 2.5 times higher than that of pressed juice (2.72 mg total oxalate/100 ml and 1.64 mg soluble oxalate/100 ml) while insoluble oxalate concentration of the former (1.97 mg/ 100 ml) was twice as high than that of the latter (1.08 mg/100 ml).

The pasteurisation temperature significantly affected soluble oxalate concentrations in both extraction methods while total oxalate concentrations were similar. In enzymatic juice, soluble oxalate concentrations at 70^0C, 80^0C and 90^0C were reduced by 12.4%, 19.9% and 29.1%, respectively, as compared to the concentrations prior to pasteurisation. Likewise, the amounts of soluble oxalate in pressed juice also decreased by 12.2% at 70^0C, 20.7% at 80^0C and 28.7% at 90^0C.

Table 6.8. Physico-chemical properties of enzymatic and pressed green kiwifruit juice.

Parameters	Fresh fruit	Enzymatic juice	Pressed juice
Extraction efficiency (%)		66.8 ± 2.1^a	50.3 ± 1.5^b
pH	3.5 ± 0.4	3.3 ± 0.1	3.4 ± 0.1
Total soluble solids (^{0}Brix)	14.1 ± 0.2	15.2 ± 0.8	13.8 ± 0.5
Titratable acids (%)	1.0 ± 0.0^a	1.3 ± 0.0^b	1.2 ± 0.1^b

Means with different letters in each row are significantly different at $p < 0.05$

Table 6.9. Oxalate changes of green kiwifruit during juice processing.

Portion	Pasteurisation temperature	Total oxalate		Soluble oxalate		Insoluble oxalate	
		Enzyme treatment	Pressed	Enzyme treatment	Pressed	Enzyme treatment	Pressed
Edible portion of fresh fruit (mg/100g FW)		21.81 ± 1.52		6.91 ± 0.35		14.9 ± 0.71	
Pomace (mg/100 g FW)		49.19 ± 1.61	40.02 ± 1.17	12.35 ± 0.25	12.10 ± 0.11	36.83 ± 2.14	27.90 ± 0.84
Juice (mg/100 ml)		6.64 ± 0.04	2.72 ± 0.05	4.67 ± 0.15^{a}	1.64 ± 0.02^{a}	1.97 ± 0.06^{a}	1.08 ± 0.03^{a}
Pasteurised juice (mg/100ml)	70^{0}C	6.65 ± 0.06	2.70 ± 0.10	4.09 ± 0.04^{b}	1.44 ± 0.04^{b}	2.56 ± 0.07^{b}	1.26 ± 0.05^{b}
	80^{0}C	6.65 ± 0.08	2.68 ± 0.05	3.74 ± 0.08^{c}	1.30 ± 0.03^{b}	2.91 ± 0.12^{b}	1.38 ± 0.05^{b}
	90^{0}C	6.67 ± 0.09	2.72 ± 0.10	3.31 ± 0.06^{d}	1.17 ± 0.03^{c}	3.36 ± 0.10^{c}	1.55 ± 0.01^{c}

The values are means ± SE (n=3). Means with different letters in each column for juice are significantly different ($p < 0.05$)

6.5. Discussion

Kiwifruit can be harvested at different maturity stages and stored for several months at low temperature (0 - 4^0C). Before being sold, they are treated with ethylene gas and brought to 20^0C until soft enough to eat (Ritenour *et al*., 1999). Depending on market demand; for domestic consumption, export or processing, there are a range of standardised maturities using total soluble solids as an indicator in which 10.8 ^{0}Brix is listed in a range as indicated by Young and Paterson (1985). Therefore, this study chose the maturity stage at 10.8 ^{0}Brix to begin with, with the aim of providing a comparable and usable data for consumers, wholesalers and producer. The current minimum maturity standard for export kiwifruit from New Zealand is 6.25 ^{0}Brix while that from USA is 6.5 ^{0}Brix (Scott *et al*., 1986). In this study, storing the fruits at 20 ± 1.0^0C was chosen to investigate changes in oxalate concentrations that may occur when the fruits are stored at home.

Firmness decreased during storage along with an increase in total soluble solid concentration in the ripened fruits (Table 6.1). The decline of firmness involved the reduction of proto-pectin to pectin resulting in the release of free Ca^{2+} ions into the tissue, while the change of total soluble solids (^{0}Brix) was due to conversion of insoluble solids such as starch to sugar, glucose and fructose (Esteban *et al.*, 1993; Peleg and Levin, 1998).

On a fresh weight (FW) basis (data not shown), total oxalate concentration in the skin of golden kiwifruit was 54.5 - 58.2 mg/100 g FW in comparison to 20-60 mg/100 mg FW reported for six genotypes of golden kiwifruit (Rassam and Laing, 2005). By microscopic and digital camera observation, Watanabe and Takahashi (1998) and Rassam *et al.*, (2007) recorded that greatest accumulation of oxalate crystals was observed in the inner-pericarp area, the region defined as the tissues adjacent to the seeds. Thus, it was difficult to compare the total oxalate level of the pulp in this study with the values obtained from Rassam and Laing, (2005) because the pulp in the present study included outer-pericarp, inner-pericarp and core but not the skin and seeds; and because the oxalate contribution of each fraction to the whole fruit depends on the genotypes evaluated (Rassam and Laing, 2005). In this study, the higher total oxalate and insoluble oxalate concentrations in the skin rather than in the pulp and the seeds may represent a functional mechanism to protect fruit from insects and herbivores. The downward trend of total and soluble oxalate concentrations of kiwifruit during storage was supported by reported studies in which oxalate degradation occurred during kiwifruit growing and maturation and even at an increased rate during post-harvest

storage (Watanabe and Takahashi, 1998; Rinallo and Mori, 2000; Rassam *et al*., 2007). It has been reported that the biodegradation of oxalates to carbon dioxide and hydrogen peroxide may occur via the enzyme oxalate oxidase (Pan *et al*., 2007) which is found predominantly in higher plants such as beet stems and leaves, sorghum leaves, maize, oats, rice, rye, barley seedling roots and leaves and Amaranthus leaves (Svedruzic *et al.*, 2005), although the presence of this enzyme in kiwifruit has not been reported.

The skin of both cultivars contained high concentrations of insoluble oxalate and low concentrations of soluble oxalates. Soluble oxalate concentrations of the seeds were very low and less than that of the skin whereas the very high concentrations of insoluble oxalates in the seeds were comparable to those of the skin (Table 6. 3). The oxalates may concentrate in the outer cell layers such as the coat or testa of the seeds in the form of small crystal sand (Rassam and Laing, 2005).

Table 6.3 showed that there were significant reductions of soluble oxalate concentration and significant increases of insoluble oxalate concentrations for both kiwifruit cultivars in all fractions, except seeds in green kiwifruit, during storage at 20^0C. Since kiwifruit are considered as a climacteric fruit, being able to continue ripening after harvest may affect the soluble oxalate concentration of kiwifruit during storage. During ripening, there was a conversion of insoluble pectin (proto-pectin) to soluble pectin that decreased fruit firmness as well as increasing pectin solubility in the cell wall and releasing Ca^{2+} from ionic linkages between pectin polysaccharides (MacDougall *et al*., 1995). It is possible that these free calcium ions might combine with soluble oxalates to create insoluble oxalates. This could be an explanation for the decline of soluble oxalate concentrations with the decrease of fruit firmness when the fruit was stored at 20^0C in this study.

Using pectinase during juice processing yielded a significantly larger amount of juice (recovery 24.7%) compared with extraction by pressing. The enzyme was able to facilitate hydrolysis of the esterified pectins, starch and proteins as well as promote fruit maceration faster and more extensively, thereby efficiently releasing juice, flavour components and pigments (Mutlu *et al.*, 1999; Cassano *et al*., 2007). Pectinase degradation of cell walls and middle-lamina pectin (Demir *et al*., 2001) not only increased juice yield but also increased the amounts of total, soluble and insoluble oxalates extracted into the juice (Table 6.9). However, the pomace remaining from the pressed and the enzyme-treated kiwifruit still contained significant amounts of oxalate. It is interesting to note that NEKTA Kiwi fruit juice **(**Nekta

Nutrition Ltd., Auckland, NZ), which is made from 20% condensed juice, contained 2.26 mg/100 ml total oxalate and 1.66 mg soluble oxalate/100 ml which suggests that this juice may be produced by pressing alone. Very little insoluble oxalate (0.60 mg/100 ml) could be found in this juice. It was possible that the insoluble oxalate was retained in the pomace and these oxalate crystals were removed by filtration (Hughes and Norman, 1992; Hatch and Freel, 2005).

The effect of increasing pasteurisation temperature was to decrease the soluble oxalate concentration while the total oxalate concentration remained unchanged. It has been shown that increasing the extraction temperature releases more soluble pectin from insoluble proto-pectin (El-Nawawi and Shehata, 1987). This process then releases bound Ca^{2+} from the polysaccharide which then can combine with soluble oxalate forming insoluble calcium oxalate.

6.6. Conclusions

Overall, oxalate concentrations depended on genotypes and varied between different kiwifruit fractions, however, the skin always contained the highest level of total and insoluble oxalate. Golden kiwifruit contained lower concentrations of oxalates in all fractions, except the seeds, when compared to green kiwifruit. Soluble oxalate concentrations of both cultivars significantly reduced over 15 days of storage at 20^0C. The results from this study confirmed the idea that oxalate concentrations of kiwifruit strongly depended on maturity stage and cultivar. Different extraction methods resulted in different juice recoveries as well as different amounts of oxalate appearing in the juice. While the majority of soluble oxalate was found in the juice, most of the insoluble oxalate and some soluble oxalate were retained in the pomace during juice processing. Further study on the effect of fibre on oxalate concentration could be considered. Pasteurization temperature affected the conversion between the soluble and insoluble forms while total oxalate concentration remained unchanged.

References

AOAC (2002) Method 925.10, Official methods of analysis of AOAC International 17th Edition, Gathersberg, MD, USA: AOAC International.

Beever, D.J. & Hopkirk, G. (1990). Fruit development and fruit physiology. In I. J. Warrington and G.C. Weston. (Eds). *Kiwifruit: Science and Management*. (pp 97-126). Auckland, New Zealand: Ray Richards Publisher and New Zealand Society for Horticultural Science.

Cassano, A., Donato, L., & Drioli, E. (2007). Ultrafiltration of kiwifruit juice: operating parameters, juice quality and membrane fouling. *Journal of Food Engineering, 79*(2), 613-621.

Demir, N., Acar, J., SarIoglu, K., & Mutlu, M. (2001). The use of commercial pectinase in fruit juice industry. Part 3: immobilized pectinase for mash treatment. *Journal of Food Engineering, 47*(4), 275-280.

El-Nawawi, S. A., & Shehata, F. R. (1987). Extraction of pectin from Egyptian orange peel - factors affecting the extraction. *Biological Wastes, 20*(4), 281-290.

Esteban, R. M., Lopez-Andreu, F. J., Martin-Cabrejas, M. A., & Molla, E. (1993). Pectin changes during the development and ripening of eggplant fruits. *Food Chemistry, 46*(3), 289-292.

Hatch, M., & Freel, R. W. (2005). Intestinal transport of an obdurate anion: oxalate. *Urological Research, 33*(1), 1-16.

Hönow, R. & Hesse, A. (2002). Comparison of extraction methods for the determination of soluble and total oxalate in foods by HPLC enzyme-reactor. *Food Chemistry, 78*, 511-521.

Hopkirk, G., Beever. D. J., & Triggs.C.M. (1986). Variation in soluble solids concentration in kiwifruit at harvest. *New Zealand Journal of Agricultural Research, 29*, 475-484.

Hughes, J., & Norman, R.W. (1992). Diets and calcium stones. *Canadian Medical Association Journal, 146*(2), 137-143.

Landon, S. (2007). Fruit nutrition and health. *Food Australia*, *59* (11), 533-588.

Latocha, P., Krupa, T., Wołosiak, R., Worobiej, E., & Wilczak, J. (2010). Antioxidant activity and chemical difference in fruit of different *Actinidia sp*. *International Journal of Food Sciences and Nutrition, 61*(4), 381−394.

Lodge, N., & Perera, C.O. (1992). Processing of kiwifruit. *New Zealand Kiwifruit*, 14-15. Retrieved 12 Feb, 2011 from http://www.hortnet.co.nz/publications/science/lodge2.htm

MacDougall, A. J., Parker, R., & Selvendran, R. R. (1995). Nonaqueous fractionation to assess the ionic composition of the apoplast during fruit ripening. *Plant Physiology, 108*(4), 1679-1689.

Mutlu, M., SarIoglu, K., Demir, N., Ercan, M. T., & Acar, J. (1999). The use of commercial pectinase in fruit juice industry. Part I: viscosimetric determination of enzyme activity. *Journal of Food Engineering, 41*(3-4), 147-150.

Nishiyama, I., Yamashita, Y., Yamanaka, M., Shimohashi, A., Fukuda, T., & Oota, T. (2004). Varietal difference in vitamin C content in the fruit of kiwifruit and other *Actinidia species*. *Journal of Agricultural and Food Chemistry*, 52, 5473−5475

Noonan, S.C. & Savage, G.P. (1999). Oxalate content of foods and its effect on humans. *Asia Pacific Journal of Clinical Nutrition, 8*, 64–74.

Nguyen, H.H.V & Savage, G.P. Oxalate content of New Zealand grown and imported fruits. Submitted to *Journal of Food Composition and Analysis*, 2012

Pan, H.-Y., Whittaker, M. M., Bouveret, R., Berna, A., Bernier, F., & Whittaker, J. W. (2007). Characterization of wheat germin (oxalate oxidase) expressed by *Pichia pastoris*. *Biochemical and Biophysical Research Communications, 356*(4), 925-929.

Peleg, K. & Levin, A. (1998). Detection of the climacteric stage in fruits and vegetables. *Acta Horticulturae* (ISHS), 464, 423-428.

Perera, C. O., Hallett, I. C., Nguyen, T. T., & Charles, J. C. (1990). Calcium oxalate crystals: the irritant factor in kiwifruit. *Journal of Food Science, 55*(4), 1066-1069.

Pratt, H. K., & Reid, M. S. (1974). Chinese gooseberry: seasonal patterns in fruit growth and maturation, ripening, respiration and the role of ethylene. *Journal of the Science of Food and Agriculture, 25*(7), 747-757.

Rassam, M. & Laing, W (2005). Variation in ascorbic acid and oxalate levels in the fruit of *Actinidia chinensis* tissues and genotypes. *Journal of agricultural and food chemistry, 53*, 2322-2326.

Rassam, M., Bulley, S.M., & Laing, W.A. (2007). Oxalate and ascorbate in *Actinidia* fruit and leaves. *Acta Horticulturae (ISHS) 753*, 479-485

Rinallo, C., & Mori, B. (2000). Oxalate and ascorbic acid in kiwifruit during growth and storage. *Italian Journal of Food Science*, 12(4), 435-442

Ritenour, M. A., Crisosto, C. H., Garner, D. T., Cheng, G. W., & Zoffoli, J. P. (1999). Temperature, length of cold storage and maturity influence the ripening rate of ethylene-preconditioned kiwifruit. *Postharvest Biology and Technology, 15*(2), 107-115.

Scott, K. J., Spraggon, S. A., & McBride, R. L. (1986). Two new maturity tests for kiwifruit. *CSIRO Food Research Quarterly, 46*, 25-31.

Stanley, R., Wegrzyn, T., & Saleh, Z. (2007). Kiwifruit processed products. *Acta Horticulturae (ISHS) 753*, 795-800.

Svedruzic, D., Jonsson, S., Toyota, C. G., Reinhardt, L. A., Ricagno, S., Linqvist, Y., & Richards, N. G. J. (2005). The enzymes of oxalate metabolism: unexpected structures and mechanisms. *Archives of Biochemistry and Biophysics, 433*(1), 176-192.

USDA Foreign Agricultural Service. (2010). 2010 Report on state of kiwifruit sector in New Zealand. Retrieved 20 March, 2011 from http://gain.fas.usda.gov/

Venning, J. A., Burns, D. J. W., Hoskin, K. M., Nguyen, T., & Stec, M. G. H. (1989). Factors influencing the stability of frozen kiwifruit pulp. *Journal of Food Science, 54*(2), 396-400.

Watanabe, K. & Takahashi, B. (1998). Determination of soluble and insoluble oxalate contents in kiwifruit (*Actinidia deliciosa*) and related species. *Journal of the Japanese Society* for *Horticultural Science, 67*(3), 299-305.

Young, H., & Paterson, V. J. (1985). The effects of harvest maturity, ripeness and storage on kiwifruit aroma. *Journal of the Science of Food and Agriculture, 36*(5), 352-358.

Chapter 7

Effect of pH on binding activity between oxalate and fibre in raw and cooked rhubarb

7.1. Abstract

Total and soluble oxalate concentrations were measured in raw and cooked rhubarb petioles (*Rheum rhabarbarum* L.). Boiling the rhubarb petioles in water or cooking with trim (low fat) or standard milk significantly ($p < 0.05$) reduced the total and soluble oxalate concentrations of the mixture by dilution (total oxalate mean raw 902.7 mg/100 g fresh weight (FW), mean cooked 454.3 mg/100 g FW). Soluble oxalate concentrations were also reduced by cooking with standard and trim milk leading to a total reduction of 65.9% and 74.5%, respectively when compared to the soluble oxalate concentration of the raw petioles. The potential effect of dietary fibre on decreasing oxalate absorption was demonstrated in the present study by using an *in vitro* digestion technique. Gastric and intestinal available oxalates in fibre and liquid fractions of raw rhubarb and rhubarb cooked with water, cooked with trim milk and cooked with standard milk were measured separately by HPLC. At pH 2.0 the amounts of soluble oxalates binding with fibre were significantly higher ($p < 0.01$) than at pH 7.0 while the amount of insoluble oxalates in the fibre increased when the extractions were carried out at a pH characteristic of the small intestine. High amounts of soluble oxalate trapped in fibre indicate that dietary fibre can reduce oxalate absorption. Less oxalate remained in the dietary fibre after rhubarb was cooked compared with the amount measured in the raw rhubarb stems; suggesting that cooking solubilised dietary fibre releasing more oxalates into solution or allowed a more efficient extraction.

7.2. Introduction

Rhubarb (*Rheum rhabarbarum* L.) belongs to the Polygonaceae family which includes about 60 different species of rhubarb that have been cultivated for thousands of years (Clementi and Misiti, 2010). The rhubarb petiole is an important crop in Europe where large amounts are sold fresh and thousands of tonnes are canned annually (Blundstone and Dickinson, 1964). The petioles of cultivated rhubarb can be eaten raw as a source of minerals, vitamins and fibre or cooked as an acid fruit for desserts and as a filler for the jam-making industry or for pies, tarts and crumbles (Allison, 1966; Clementi and Misiti, 2010). However, many publications

and cookbooks advise that rhubarb should not be consumed regularly as it contains high concentrations of oxalates in the petioles (Blundstone and Dickinson, 1964; Grieve, 1980).

Oxalates are considered as end products in mammalian metabolism existing as water soluble forms with Na^+, K^+ or water insoluble forms with Ca^{2+}, Mg^{2+} (Noonan and Savage, 1999). Calcium oxalate is the most common constituent of kidney stones (Hughes and Norman, 1992). The total oxalate concentration in rhubarb petioles was reported to range from 275 to 1336 mg/100 g fresh weight (FW) (Noonan and Savage, 1999). Soluble oxalate ranged from 42.4-78.2% of the total oxalate (Allison, 1966). An increase of oxalate concentration as the plant aged has been observed (Blundstone and Dickinson, 1964) and different genotypes could be the reason for 72% of the variation in oxalate concentration (Libert and Creed, 1985). Oxalate concentration in raw rhubarb has been well established; however, the concentrations of oxalate in cooked rhubarb, the most common way rhubarb is consumed, are rarely reported. Depending on the cooking method used, the amount of oxalates and the ratio of soluble to total oxalate remaining in cooked food may vary (Savage *et al*., 2000; Chai and Liebman, 2005). Since calcium has the capacity to bind to soluble oxalate to form calcium insoluble oxalate, it is thought that the availability of oxalates in food can be changed by adding extra calcium (Brogren and Savage 2003; Oscarsson and Savage, 2007; Savage *et al*., 2009).

Many chemical methods have been used to measure oxalate in undigested food (Zarembski and Hodgkinson, 1962; Awadalia *et al*., 1985; Savage *et al.*, 2000; Hönow and Hesse, 2002); however, it should not be assumed that the soluble oxalate available for absorption in undigested food is equivalent to that of the same food after biological digestion in the gastrointestinal tract. It is important to use a digestive model that simulates the biological conditions to give a clear understanding of the processes involved. It is believed that factors affecting urinary excretion of oxalate are primary risks for calcium oxalate stone formation (Jaeger and Robertson, 2004). Urinary oxalate consists of endogenous sources derived from ascorbic acid, protein, immediate precursors of oxalate (Hodgkinson, 1977) and dietary sources that are derived mainly from plant foods including fruit, vegetables and cereals (Zarembski and Hodgkinson, 1962). Dietary oxalate absorption occurs throughout the gastrointestinal tract with both paracellular and transcellular uptake mechanisms (Hatch and Freel, 2005) and can contribute up to 50-67% of urinary oxalate output (Holmes *et al.*, 1995; Liebman and Chai, 1997; Holmes *et al.*, 2001). Although the main site of oxalate absorption is usually considered to be the proximal small intestine (Holmes *et al*., 1995; Daugherty and

Mrsny, 1999; Hatch and Freel, 2005), oxalate absorption may also occur in the stomach (Hautmann, 1993). Depending on the amount and form of oxalates, as well as the concentration of other food components such as Ca^{2+}, Mg^{2+} and fibre digested at the same time, oxalate absorption can occur to a small extent in the stomach and to larger extent in the small intestine (Jaeger and Robertson, 2004). The pH changes along the gastrointestinal tract may have a large impact on the form and absorption of oxalate (Jaeger and Robertson, 2004). In the stomach where pH ranges from 1.0 - 2.5, it is likely that all insoluble oxalate in a plant food could be dissolved. The chyme then passes to the alkaline pH of the small intestine with a pH range from 6.5 to 8. At this site some of the oxalate solubilised in the stomach may rebind with minerals such as Ca^{2+} and Mg^{2+} that are also available in the intestine.

Although there has been controversy about the role of dietary fibre intake in kidney stone disease (Hatch and Freel, 2005), by using modified diets with additions of dietary fibre, Firth and Norman (1990) clearly showed significant reductions of oxalate excreted in the urine. Hanson *et al.,* (1989) discovered a lower soluble oxalate bioavailability from sugar beet (0.7%) than from spinach (4.5%) and oxalate solutions (6.5%) even though 120 mg of oxalate was supplied in each case. They suggested that mechanical or chemical binding of fibre with oxalate in sugar beet made less oxalate available for absorption (Hanson *et al.,* 1989). Although digestion of fibre alone did not appear to decrease mineral bioavailability, introducing higher fibre in the diet would also significantly reduce oxalate absorption by producing a fibre-mineral-oxalate complex that may be more difficult to be broken down than an oxalate-mineral or fibre-mineral bond (Kelsay and Prather, 1983). An increase of oxalate in the faeces and a decrease in urinary oxalate concentration when higher fibre was fed, as compared to the lower fibre diet with the same amount of oxalate, suggested that fibre could be one of the important factors in the treatment of patients with hyperoxaluria (Kelsay and Prather, 1983).

By using an *in vitro* digestion method to simulate the physiological environment of the human digestive tract, this study investigated whether changes of pH in the digestive system would result in changes in oxalate form and its potential to bind to dietary fibre. The effect of cooking on the oxalate concentrations of rhubarb stalks were also investigated to give more information on the effects of consuming foods containing high concentrations of oxalates.

7.3. Materials and methods

7.3.1. Sample preparation

Ten rhubarb plants were grown in a local garden (Prebbleton, Christchurch, Canterbury, NZ) and 30 rhubarb petioles were harvested from these plants at the same stage of maturity. Rhubarb petioles were sampled at random from several groups of plants and bulked prior to analysis. Damaged samples were discarded. The petioles were washed with tap water and then frozen at -24^{0}C for further treatment. Analyses were carried out on three groups of rhubarb petioles for each of the four treatments including raw rhubarb, rhubarb cooked with tap water, with trim (low fat) milk (Anchor, Fonterra Brands NZ Ltd**.,** Manurewa**,** NZ) (0.5 g fat; 133 g calcium/100 ml) and with standard milk (Anchor, Fonterra Brands NZ Ltd**.,** Manurewa**,** NZ) (3.3 g fat; 117 g calcium/100 ml). For the cooking treatment, the rhubarb petiole was chopped into 2 cm square pieces and cooked with water, standard milk or trim milk at a ratio of 1:1 (w/v), respectively. The rhubarb mixtures were cooked in stainless steel pots (Zip Elegant, Briscoes Ltd., Christchurch) covered with heavy lids to prevent evaporation. After boiling, the samples were cooked a further 5 minutes to make sure the rhubarb stems were soft when served. All mixtures were then homogenised separately using a Cascade Blender (Model No. CE071BR, China). The mixtures were used for *in vitro* digestion, oxalate analysis and mineral determination.

7.3.2. Digestive juice preparation

Digestive juices, including saliva, gastric, duodenal and bile solution were prepared fresh each day by dissolving the chemicals and reagents listed in Appendix 3 in nanopure water (Arium 611uv, Sartorius Ltd. Germany) and warmed to 37^{0}C prior to use in the digestion experiment.

7.3.3. Gastric oxalate determination

Gastric available oxalates in raw and cooked rhubarb were determined using the method of Savage and Catherwood (2007). In detail, 5 g of raw or cooked rhubarb was weighed accurately into 250 ml conical flask. Nine ml of saliva juice was added and incubated at 37^{0}C for 5 minutes. Then 13.5 ml of gastric juice was added and the flask was then incubated at 37^{0}C for 2 hours. The concentration of the flask was transferred quantitatively to a vacuum filter to separate the pomace (the fibrous residue fraction) and filtered solutions. These were then transferred to a 250 ml volumetric flask and made up to volume with nanopure water for soluble oxalate analysis, or with 2 M HCL for total oxalate analysis and shaken at room temperature for 20 minutes. The extractions were centrifuged at 2889 rcf for 15 minutes and 2

ml of the supernatant was filtered through a 0.45 µm cellulose acetate filter (Sartonius, Goettingen, Germany) prior to injection into the HPLC (Savage *et al*., 2000). Each sample was analysed four times. Insoluble oxalate is calculated as the difference between total oxalate and soluble oxalate concentration (Holloway *et al*., 1989).

7.3.4. Intestinal oxalate determination

Following the gastric digestion as outlined above, 27 ml of duodenal juice and 9 ml of bile juice were added to the flask. The flask was then incubated for a further two hours at 37^0C. The intestinal digested mixture was separated into pomace and solution using a vacuum filter. The filtrates were transferred quantitatively to a 250 mL volumetric flask and made up to volume with nanopure water (soluble oxalate) or 2 M HCL (total oxalate) and then were centrifuged at 2889 rcf for 15 minutes. The supernatants were collected and filtered using a 0.45 µm cellulose acetate filter before the analysis of oxalate by HPLC.

7.3.5. Mineral determination

Mineral concentrations of the raw and three cooked rhubarb treatments were analysed in triplicate. All equipment was soaked in 10% HCL for 48 hours and left to dry prior to analysis. The Teflon microwave digestion vessels were also soaked in Decon solution (Decon Laboratories Ltd., Hove, Sussex, UK) and then rinsed with acid, to eliminate any contamination. A 0.5 g sample was weighed into a 100 mL Teflon microwave digestion vessel. Five mL of 69% nitric acid (Aristar, BDH Chemicals, Ltd., Poole, Dorset, UK) and 2 mL of 30% hydrogen peroxide acid (Aristar, BDH Chemicals, Ltd., Poole, Dorset, UK) were added and the vessels were allowed to stand for 12 hours at room temperature. The samples were then digested for 40 minutes in a microwave digester (Milestone Ethos Sel microwave oven, Sorisole, Italy) with the temperature rising to 200°C at the end of the digestion cycle. The digestion vessel was then cooled and the digested solution was made up to 25 mL with Barnstead Nanopure II water (Thermo Fisher Scientific Australia Pty Ltd., Scoresby Victoria, Australia). The mineral concentration was determined by aspirating the diluted sample into an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES, Varian Inc., Mulgrave, Victoria, Australia) through an ultrasonic nebulizer (Cetac 5000, Varian Inc., Mulgrave, Victoria, Australia. The Varian ICP was calibrated using a multi-element standard solution (Merck & Co. Inc., Whitehouse Station, NJ, USA). A mineral recovery was carried out using 20 mg/kg spike of an ICP Multi-element standard solution containing 23 elements (Merck & Co. Inc., Whitehouse Station, NJ, USA); the recovery

ranged from 92.2-123.4%. Ten cations found in the highest concentrations in the four rhubarb samples were selected and presented as mg/100 g FM.

7.3.6. Statistical analyses

Statistical analyses were performed using Minitab version 16 (Minitab Ltd., Coventry, UK) to assess the effect of cooking treatment on oxalate concentration and oxalate form of rhubarb stems. Data of soluble and insoluble oxalates from *in vitro* digestion were analysed as a 4 (treatment) by 4 (fraction) factorial design using the two-way ANOVA analysis in GenStat version 14 for Windows 7 (VSN International Ltd., Hemel Hempstead, Hertfordshire, UK).

7.4. Results

Oxalate concentrations in undigested raw rhubarb and rhubarb cooked with water, trim milk and standard milk are presented in Table 7.1.

Table 7.1. Mean oxalate concentration of undigested raw and cooked rhubarb (mg/100 g FW ± SE) and the proportion of soluble oxalate to total oxalate concentration (%).

Treatments	pH	Total oxalate	Soluble oxalate	Insoluble oxalate
Raw rhubarb	3.33 ± 0.01	902.7 ± 33.5^{a}	463.2 ± 19.7^{a} (51.5%)	439.5 ± 31.4^{a}
Cooked with water	3.32 ± 0.02	453.4 ± 2.5^{b}	238.0 ± 2.3^{b} (52.5%)	215.4 ± 4.1^{b}
Cooked with trim milk	3.95 ± 0.01	455.2 ± 16.9^{b}	117.7 ± 5.0^{c} (25.9%)	337.6 ± 15.6^{c}
Cooked with standard milk	3.96 ± 0.02	454.2 ± 4.4^{b}	158.1 ± 5.9^{d} (34.9%)	296.2 ± 8.8^{d}
Analysis of variance				
l.s.d. (5%) between treatments	6.7	49.7	28.0	47.8

Different letters in each column indicate significant differences ($p < 0.05$).

Cooking significantly reduced ($p < 0.05$) the total and soluble oxalate concentrations compared to the raw rhubarb. The mean reduction of total oxalate in the three cooking trials was 49.7%. In the three cooking trials, there was a significant effect ($p < 0.05$) of adding different kinds of milk on the soluble oxalate concentration (Table 7.1). While the total

oxalate concentrations in rhubarb petioles cooked with water, cooked with trim milk and cooked with standard milk were not significantly different, the soluble oxalate concentrations of the rhubarb petioles in the two milk cooking treatments were significantly ($p < 0.05$) lower compared to the water treatment. The lowest concentration of soluble oxalate was measured in the trim milk treatment (117.7 mg/100 g FW), a moderate concentration was obtained in the standard milk treatment (158.1 mg/100 g FW) and the highest concentration was observed in the water treatment (238.0 mg/100 g FW). In contrast, in the three cooking treatments, the amount of insoluble oxalates was highest in the trim milk trial and lowest in the water trial.

The oxalate concentrations in the pomace and liquid fractions of the digested raw and cooked rhubarb are shown in Table 7.2. There were significant ($p < 0.01$) interactions between cooking treatments and fractions in both soluble and insoluble oxalates concentration. This indicated that the differences in oxalate concentrations between the fractions depended on the cooking treatments.

It can be seen that relatively high amounts of oxalates remained in the fibre at both the gastric and intestinal incubations. In the four treatments, the proportion of soluble oxalate trapped in the fibre ranged from 16.5 – 30.0% of overall total soluble oxalate concentration found at the pH characteristic of the stomach and from 14.1 - 27.2% of overall total soluble oxalate concentration found at the pH characteristic of the intestine. Meanwhile, the insoluble oxalate concentrations that remained in the fibre were in the range of 35.9-87.4% at pH 2.0 and 22.1-72.4% at pH 7.0 of the total insoluble oxalate concentrations found in these pH values.

The pH values of the digestion media before adding the samples were 1.07 for the gastric juice and 8.0 for the intestinal juice. After adding the samples, the pH of the stomach medium changed to an average of 2.0 and pH of the intestinal mixture changed to an average of 7.0. The pH in the digestive mediums made very clear changes to the oxalate concentrations in both fibre and liquid fractions. In the pomace fraction, the soluble oxalate concentrations were significantly ($p < 0.01$) reduced when the digested rhubarb treatments moved from the stomach to the intestinal media (Table 7.2). The mean reductions of soluble oxalate at pH 7.0 were 68.2% (raw), 50.5% (water cooking), 60.7% (trim milk cooking) and 37.9% (standard milk cooking) in relation to these values observed at pH 2.0. With the change in pH, the mean decreases of soluble oxalate concentrations in the liquid fractions were 17.6%, 32.8%, 70.6% and 61.2% for the raw, cooked with water, cooked with trim milk and cooked with standard milk treatments, respectively.

In contrast to the decreases in soluble oxalate concentrations, when the digestive pH changed from 2.0 to 7.0, the amounts of insoluble oxalate in the fibre and liquid fractions increased significantly ($p < 0.01$). In the fibre fractions, the mean increases in insoluble oxalate concentrations were 49.2% for raw rhubarb, 102.6% for water treatment, 20.5% for trim milk treatment and 35.2% for standard milk treatment. In the liquid fractions, the increase in insoluble oxalate concentrations was much higher than the values obtained in the fibre fractions. These values were 294.4%, 170.1%, 260.0% and 233.1% for raw, cooked with water, cooked with trim milk and cooked with standard milk rhubarb, respectively.

Regarding the cooking treatments, it was clear that the proportions of soluble and insoluble oxalate concentrations remaining in the fibre of the three cooked rhubarbs were much lower than the concentrations obtained for the raw rhubarb (Table 7.2). The proportion of overall total oxalate concentrations remaining in the fibre fraction in the stomach medium was 47.2% for the raw rhubarb compared to 23.2% for water cooking, 35.8% for trim milk cooking and 36.8% for standard milk cooking. Similarly, in the intestine, the fibre fraction of raw rhubarb trapped 45.2% of total oxalate concentration in comparison with 22.5% for the water cooking, 22.2% for the trim milk cooking and 25.6% for the standard milk cooking.

The total calcium and oxalate contents in undigested raw rhubarb and rhubarb cooked with water, trim milk and standard milk are presented in Table 7.3. Raw and cooked rhubarb petioles contain high concentrations of important minerals. Of common minerals found in plant foods, calcium and potassium were in the highest amounts (Table 7.3). Potassium is known to be relevant to soluble oxalate salts and its concentration ranged from 147.6-182.8 mg/100 g FW (Table 7.3). In contrast, calcium, which is considered to be the primary metal cation found in insoluble oxalate salts, showed concentrations ranging from 49.0 – 107.0 mg/100 g FW (Table 7.3). Regardless of dilution, the highest calcium concentration was found in the raw rhubarb, whereas the lowest calcium level was found in the water treatment. Comparable concentrations of calcium were found in the two milk treatments, 63.0 and 62.4 mg/100 g FW.

Table 7.2. Mean oxalate concentration (mg/100 g FW) in different fractions of rhubarb following *in vitro* digestion at pH 2.0 and pH 7.0.

Treatment	Gastric extraction (incubated and separated at pH 2.0)				Intestine extraction (incubated and separated at pH 7.0)			
	Solution		Pomace		Solution		Pomace	
	Soluble oxalate	Insoluble oxalate	Soluble oxalate	Insoluble oxalate	Soluble oxalate	Insoluble oxalate	Soluble oxalate	Insoluble oxalate
Raw rhubarb	441.4 ± 10.1	33.7 ± 2.4	191.1 ± 9.0 (30.0%) [1]	233.7 ± 6.0 (87.4%)	363.9 ± 9.9	132.9 ± 5.2	60.7 ± 1.2 (14.1%)	348.7 ± 10.6 (72.4%)
Cooked with water	288.2 ± 3.5	58.7 ± 5.3	71.6 ± 0.8 (20.0%)	32.9 ± 2.4 (35.9%)	193.6 ± 9.3	158.6 ± 3.0	35.7 ± 2.3 (15.6%)	66.7 ± 3.8 (29.6%)
Cooked with trim milk	255.4 ± 5.0	77.4 ± 3.4	56.8 ± 2.3 (16.5%)	65.6 ± 2.9 (45.9%)	75.2 ± 4.1	279.2 ± 11.5	22.3 ± 1.0 (22.9%)	79.1 ± 2.2 (22.1%)
Cooked with standard milk	263.7 ± 11.4	70.5 ± 3.4	62.4 ± 2.5 (19.1%)	57.1 ± 2.9 (44.7 %)	102.2 ± 2.4	234.7 ± 9.8	38.8 ± 1.0 (27.2%)	77.2 ± 2.4 (24.8%)

Analysis of variance	df	significance of soluble oxalate	significance of insoluble oxalate
Treatment	3	***	***
Fraction	3	***	***
Treatment x fraction	9	***	***
l.s.d. (5%) within treatments		4.1	8.3
l.s.d. (5%) between treatments		8.2	16.7

Significance: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

[1] :The figures in the brackets are percentages of soluble or insoluble oxalate concentrations remaining in pomace compared with total soluble or total insoluble oxalate concentrations respectively for each digestive extraction.

Table 7.3. Mean mineral concentrations (mg/100 g FW ± SE) of raw and three different cooking treatments of rhubarb.

	Ca	K	Mg	Na	P	S	Al	Fe	Mn	Zn
Raw	107.0 ± 4.6^{a}	182.8 ± 4.7^{a}	10.8 ± 0.4^{ac}	2.5 ± 0.1^{a}	20.1 ± 0.9^{a}	8.5 ± 0.4^{a}	0.85 ± 0.03^{a}	0.29 ± 0.02^{a}	0.53 ± 0.02^{a}	0.34 ± 0.01^{a}
Cooked with water	49.0 ± 1.1^{b}	178.2 ± 4.6^{a}	10.6 ± 0.3^{a}	1.0 ± 0.1^{b}	18.6 ± 0.6^{a}	8.0 ± 0.1^{a}	1.94 ± 0.00^{b}	0.18 ± 0.01^{b}	0.44 ± 0.01^{b}	0.23 ± 0.01^{b}
Cooked with trim milk	63.0 ± 1.2^{c}	147.6 ± 2.0^{b}	9.4 ± 0.2^{b}	8.0 ± 0.1^{c}	36.1 ± 0.8^{b}	12.9 ± 0.3^{b}	0.10 ± 0.00^{c}	0.10 ± 0.00^{c}	0.26 ± 0.0^{c}	0.26 ± 0.01^{b}
Cooked with standard milk	62.4 ± 1.4^{c}	158.3 ± 3.3^{b}	11.6 ± 0.3^{c}	8.8 ± 0.2^{d}	41.2 ± 1.0^{c}	14.9 ± 0.4^{c}	0.20 ± 0.01^{d}	0.10 ± 0.01^{c}	0.32 ± 0.01^{d}	0.30 ± 0.01^{c}

Different letters in each column indicate significant differences ($p < 0.05$).

7.5. Discussion

According to The Concise New Zealand Food Composition Tables (2009), rhubarb is considered to be a fruit. Rhubarb contains several interesting phyto-nutrients such as vitamins, polyphenols, minerals and dietary fibre. However, it is also a fruit containing high concentrations of oxalates that can, if consumed regularly, cause kidney stones (Donald, 2009).

Although adding equivalent amounts of water and milk to raw rhubarb diluted the total oxalate concentrations present in the rhubarb matrix to half in comparison to the concentrations measured in raw treatment, cooking rhubarb in milk significantly reduced the ratio of soluble oxalate to the total oxalate concentrations while cooking rhubarb in water did not change this proportion as compared to uncooked rhubarb (Table 7.1). Trim milk was most effective cooking medium in reducing the soluble oxalate concentration of the rhubarb stems, followed by the standard milk; the least effective cooking medium was water. There are several potential explanations for the lowering of soluble oxalates in the milk treatments as compared to the raw and the water treatments. Adding calcium to the cooking media is known to significantly reduce the soluble oxalate concentration in foods containing oxalate (Savage *et al*., 2000; Radek and Savage, 2008). While cooking high oxalate containing food with milk reduces the soluble oxalate concentrations, the calcium bioavailability may also decrease. It can be seen from Table 7.1, that the insoluble oxalates in the three cooked treatment were significantly lower ($p < 0.05$) than the value measured in raw rhubarb but greater with the milk than the water treatments. The expected decreases in the ratio of soluble oxalate to total oxalate concentration of rhubarb stems paralleled the increases in the ratio of insoluble oxalate to the total oxalate concentrations that were cooked in the milk media relative to the values analysed from the raw and water treatments. This suggests that the soluble oxalate concentrations of the rhubarb stems were reduced via the formation of insoluble calcium oxalates in the milk treatment trials. The results of these cooking treatments support the previous studies (Mårtensson and Savage, 2008; Simpson *et al*., 2009; Johansson and Savage, 2011) that indicate cooking a high oxalate food with milk products effectively reduces the soluble oxalate concentration. However, the difference in insoluble oxalate concentrations between the two milk treatments was also significant ($p < 0.05$).

Regardless of the presence of calcium in the media and the dilution of oxalates, the pH of cooking media also affects the ratio of soluble oxalate to total oxalate. Simpson *et al*., (2009) demonstrated that at a pH 3.3, 90% of free oxalates are present as $HC_2O_4^-$; when the pH

reaches 4.0 the proportion of $HC_2O_4^-$ in the solution is reduced to 65%. Adding different types of milk to the cooking medium changed the pH value from 3.3 to 4.0 resulted in a decrease in the concentrations of soluble oxalate in the milk cooking rhubarb (Table 7.1). This possibly is explained by binding between soluble oxalate with free minerals, especially calcium, available in the cooking media to form insoluble oxalates that cannot be absorbed. Cooking rhubarb in water did not lead to a change in the pH thus the proportion of soluble oxalate to total oxalate was not significantly different from the value obtained in the raw rhubarb (Table 7.1).

The digestive pH played a critical role in the change of the oxalate forms and, therefore, the potential for availability of soluble oxalate (Table 7.2). Depending on physiological pH values, oxalate availability was variable. It is thought that some of the soluble oxalates would reform insoluble oxalate complexes, potentially with calcium, magnesium, iron, or other components of the diet, upon movement from the stomach (pH ≈ 2.0) into the intestinal environment, where the pH can range between 6 -7. Indeed, the current study showed that there were significant increases in insoluble oxalate concentrations that occurred at the same time as decreases in soluble oxalate concentrations in both the fibre and liquid fractions (Table 7.2) when the digested rhubarb moved from the pH 2.0 to pH 7.0. If it is assumed that only soluble oxalate in the liquid fraction will be absorbed from the intestinal tract and, according to Hautmann, (1993), if 15-21% of free oxalate load is absorbed in the stomach, the results obtained from this study (Table 7.2) suggest that approximately 66.2 - 92.7 mg oxalate/100 g FW raw rhubarb and 38.3 - 60.5 mg oxalate/100 g FW cooked rhubarb treatments will be absorbed. If 45% of free oxalate load is absorbed in the intestine after 4 hours of digestion (Hautmann, 1993), after ingestion of 100 g FW rhubarb cooked with trim milk, standard milk, or water or raw rhubarb, about 33.8 mg, 46.0 mg, 87.1 mg and 163.8 mg oxalate, respectively, may be absorbed. These values show the combined positive effects of digestive pH and dietary fibre on reducing oxalate absorption. Due to the changes in digestive pH and trapping of oxalate by dietary fibre, the potential oxalate absorptions are significantly lower compared to the initial soluble oxalates in the undigested rhubarb treatments.

Among five dominant mineral cations, calcium (Ca^{2+}), magnesium (Mg^{2+}), iron (Fe^{2+}), manganese (Mn^{2+}) and zinc (Zn^{2+}), considered as possible constituents of insoluble oxalate salts, Fe^{2+}, Mn^{2+} and Zn^{2+} were not considered relevant as insoluble oxalate salts in this study due to the very low concentration of these elements in the four rhubarb treatments (Table 7.3). Although the concentration of Mg^{2+} was considerable (Table 7.3), Simpson *et al*., (2009)

reported that among these mineral cations, calcium oxalate has the lowest solubility product constant (*Ksp*= 2.7 x 10^{-9}) as compared to the other four oxalates, especially Mg^{2+} (*Ksp*= 2.2 x 10^{-5}). Thus, calcium oxalates are the least soluble and are, therefore, precipitated before the other minerals when the calcium concentration was exceeded (Simpson *et al*., 2009). Thus, only the concentration of calcium was relevant in the current study.

As calculated above, the ratio of soluble oxalate to total oxalate concentration in rhubarb cooked with trim milk was lower than the ratio obtained from rhubarb cooked with standard milk (Table 7.1). This difference may be a consequence of the difference in calcium concentration as well as the difference in fat concentration between the two types of milk. However, the results from the mineral determinations (Table 7.3) showed that there were no significant differences in calcium concentrations between rhubarb cooked with trim milk and standard milk. Therefore, the greater reduction of soluble oxalate with trim milk cannot be explained by differences in the calcium concentrations of the two milks. However, it has been reported that consuming food containing fat at the same time as oxalate may increase the oxalate absorption (Andersson and Jagenburg, 1974; Finch *et al*., 1981). It is possible that dietary fat may bind with calcium in the small intestine to form an insoluble soap. This would reduce the intestinal calcium available for binding with soluble oxalate to form insoluble calcium oxalate and, therefore, would leave higher concentrations of free oxalate available for absorption (Bailly *et al*., 2000). During cooking, there may also have been binding between Ca^{2+} and triacylglycerides, which are more abundant in standard milk (Fransson, 1984). This would leave less calcium available to bind with free oxalate. Therefore, the much higher fat concentration in the standard milk than in the trim milk (3.3% vs. 0.5%) could, in part, account for the significantly higher ($p < 0.05$) soluble oxalate concentration from the treatment with standard milk relative to the treatment with trim milk.

When the effect of cooking was considered in the current study, it was interesting to note that after the rhubarb had been cooked, significantly lower proportions of oxalates remained in the pomace fraction (which contained the fibre fraction) than in the uncooked rhubarb (Table 7.2). During cooking the plant tissues were softened due to a disruption of the constituent cell wall matrix (Van Buren, 1979). Cooking increased the solubility of the fibre (Fleury and Lahave, 1991; Guillon and Champ, 2000), breaking more fibre-oxalate bonds resulting in the release of more oxalates into solution.

The effect of dietary fibre in reducing kidney stone formation has yet to be elucidated. However, the amounts of both soluble oxalate and insoluble oxalate remaining in the fibre

fractions following both the *in vitro* gastric and intestinal incubations in this study shows the positive effect of fibre for potentially reducing hyperoxaluria. Moreover, this is the first study clearly showing the binding between dietary fibre and oxalate forms under simulated physiological conditions in the gastrointestinal tract. Most reported studies (Gleeson *et al*., 1990; Hiatt *et al*., 1996; Hess *et al*., 1998) have focused on measuring amounts of oxalate supplemented and urinary oxalate output collected afterwards to draw conclusions about the role of dietary fibre without considering the oxalate availability at any particular pH in the digestive tract. Determining concentrations of oxalate available at each pH characteristic of the stomach and intestine may give a clearer vision that may be useful, at least in part, for clinical treatment for kidney stone patients with surgically disturbed intestinal functions (Tiselius *et al*., 1981). Several previous studies have shown that even though the meals contained an excess of total calcium, not all soluble oxalates are bound to calcium (Savage and Catherwood, 2007; Radek and Savage, 2008; Simpson *et al*., 2009; Johansson and Savage, 2011). This could be explained from the results of the present study which suggest that the binding between oxalates and fibre in the rhubarb stalks would make less oxalates available to bind with free calcium. In general, the overall effect of pH change during digestion and the presence of dietary fibre in the gastrointestinal tract made soluble oxalate less available for absorption. The interaction of dietary fibre and oxalates may partly explain the small amounts (2.4-14.4%) of total oxalate (Barilla *et al*., 1978; Prenen *et al*., 1984) that is absorbed in the gastrointestinal tract. The results of the present study may also be useful for clarifying the hypotheses about the factors affecting oxalate absorption and suggest that supplementing fibre in the diet should be advised widely as a simple treatment to reduce the urinary oxalate that is considered to be a risk for kidney stone formation.

To give comparable nutritional information, it is essential to calculate oxalate and calcium concentrations of rhubarb based on a standard serving size. According to New Zealand Nutrition databases, the standard unit commonly used for rhubarb is 265 g which is a standard cup size (The Concise NZ Food Composition Tables, 2009). From the data in the present study, total calcium concentration per serve was the highest in raw rhubarb with 2.8 g/serve, followed by comparable concentrations found in trim milk and standard milk treatments, 1.67 and 1.65 g/serve, respectively. The proportion of calcium bound to oxalates in raw rhubarb was the lowest (12.7%). In contrast, the highest proportion of bound calcium was found in the trim milk treatment (16.8%). The amount of soluble oxalate/serve in raw rhubarb was 1.23 g, reducing to 0.63 g when cooked with water, 0.31 g when cooked with trim milk and 0.42 g when cooked with standard milk. The proportion of bound calcium in insoluble oxalates in

rhubarb (Table 7.4) was much lower than in other plant foods such as spinach (Brogren and Savage, 2003) and silver beet (Simpson *et al*., 2009). This may be due to pH differences between these plant foods. For example, the pH ranged from 4.9-5.5 in raw and cooked silver beets that contained 32-49% of calcium bound to insoluble oxalate (Simpson *et al*., 2009) while the average pH ranged from 3.3-3.9 in raw and cooked rhubarb (the current study) that contained 12.7 – 16.8% of calcium bound to insoluble oxalate. Siener *et al.*, (2001) showed that the binding capacity between calcium and oxalates increases above pH 4.0 and slows as the pH continues to rise.

Table 7.4. Oxalate and total calcium concentrations per serve (g/standard serving ± SE) of the four rhubarb treatments.

	Oxalate concentration/serve (g/serve)			Calcium/serve (g/serve)		
	Total oxalate	Soluble oxalate	Insoluble oxalate	Total calcium	Bound calcium	% bound calcium[1]
Raw rhubarb	2.39 ± 0.09[a]	1.23 ± 0.05[a]	1.16 ± 0.08[a]	2.84 ± 0.12[a]	0.36 ± 0.03[a]	12.7 ± 0.92[a]
Cooked with water	1.20 ± 0.01[b]	0.63 ± 0.01[b]	0.57 ± 0.01[b]	1.30 ± 0.01[b]	0.18 ± 0.00[b]	13.8 ± 0.45[a]
Cooked with trim milk	1.21 ± 0.04[b]	0.31 ± 0.01[c]	0.89 ± 0.04[c]	1.67 ± 0.03[c]	0.28 ± 0.01[c]	16.8 ± 0.48[b]
Cooked with standard milk	1.20 ± 0.01[b]	0.42 ± 0.01[d]	0.78 ± 0.02[c]	1.65 ± 0.03[c]	0.25 ± 0.01[c]	15.2 ± 0.26[b]
Analysis of variance						
l.s.d. (5%) between treatments	0.13	0.07	0.13	0.18	0.04	1.53

Means with different letters in each column are significantly different ($p < 0.05$)
[1]: refer to appendix 4.2.

7.6. Conclusions

This is the first study to use an *in vitro* digestion method to determine the direct effect of dietary fibre on the partition of oxalate between the soluble and the fibre fractions of a plant to reveal the possible availability of oxalate in the gastrointestinal tract. When a high amount of oxalate is trapped in the fibre fraction this will lead to less oxalate being available for absorption. It would be appropriate to recommend that a high dietary fibre intake should be consumed by people who have a tendency to form oxalate kidney stones.

The pH changes in the gastrointestinal system have a significant effect on the form of oxalates and their potential binding to fibre. Soluble oxalate released following *in vitro* intestinal digestion was always lower than soluble oxalate extracted using water extraction which suggests that less oxalates may be absorbed from the gastrointestinal tract than previously thought. Although cooking released more oxalates from fibre into solution, the concentrations of soluble oxalate were also affected by the presence of calcium and fat. Adding trim milk (rather than full fat milk) to the cooking medium should be used to reduce the total amount of soluble oxalates available for absorption when high oxalate foods are consumed.

References

Allison, R. M. (1966). Soluble oxalates, ascorbic and other constituents of rhubarb varieties. *Journal of the Science of Food and Agriculture* 17(12), 554-557.

Andersson, H., & Jagenburg, R. (1974). Fat-reduced diet in the treatment of hyperoxaluria in patients with ileopathy. *Gut* 15(5), 360-366.

Awadalia, M. Z., Bassily, N. S. & Saba, N. A. (1985). Oxalate content of some Egyptian foodstuffs. *Journal of the Egyptian Public Health Association, 60*(3), 41–51.

Bailly, G.G., Norman, R.W. & Thompson, C. (2000). Effects of dietary fat on the urinary risk factors of calcium stone disease. *Urology* 56, 40–44.

Barilla, D. E., Notz, C., Kennedy, D., & Pak, C. Y. (1978). Renal oxalate excretion following oral oxalate loads in patients with ileal disease and with renal and absorptive hypercalciurias: effect of calcium and magnesium. *American Journal of Medicine, 64*, 579–585.

Blundstone, H. A. W., & Dickinson, D. (1964). The chemistry of edible rhubarb. *Journal of the Science of Food and Agriculture* 15, 94-101.

Brogren, M., & Savage, G. P. (2003). Bioavailability of soluble oxalate from spinach eaten with and without milk products. *Asia Pacific Journal of Clinical Nutrition* 12(2), 219–224.

Chai, W., & Liebman, M. (2005). Effect of different cooking methods on vegetable oxalate content. *Journal of Agricultural and Food Chemistry* 53(8), 3027-3030.

Clementi, E. M., & Misiti, F. (2010). Chapter 27 - Potential Health Benefits of Rhubarb. In *Bioactive Foods in Promoting Health* . (Ed. W. Ronald Ross and R. P. Victor), Pp. 407- 423. San Diego, Academic Press.

Daugherty, A. L., & Mrsny, R. J. (1999). Transcellular uptake mechanisms of the intestinal epithelial barrier Part one. *Pharmaceutical Science & Technology Today, 2*(4), 144-151.

Donald G, B. (2009). Rhubarb and oxalosis (*Rheum species*). *Disease-a-Month, 55*(6), 403-411.

Finch, A.M., Kasidas, G.P., & Rose, G.A. (1981). Urine composition in normal subjects after oral ingestion of oxalate-rich foods. *Clinical Science, 60*, 411-418.

Firth, W.A., & Norman, R.W. (1990). The effects of modified diets on urinary risk factors for kidney stone disease. *The Journal of the American Dietetic Association, 51*, 404-408

Fleury, N., & Lahaye, M. (1991). Chemical and physico-chemical characterisation of fibres from *Laminaria digitata* (kombu breton): a physiological approach. *Journal of the Science of Food and Agriculture, 55*(3), 389-400.

Fransson, G. B., & Lonnerdal, B. (1984). Iron, copper, zinc, calcium and magnesium in human milk fat. *The American Journal of Clinical Nutrition, 39*(2), 185-189.

Gleeson, M.J., Thompson, A.S., Mehta, S., & Griffith, D.P. (1990). Effect of unprocessed wheat on calciuria and oxaluria in patients with urolithiasis. *Urology, 35*, 231–234.

Grieve, M. (1980). *A model herbal.* London: Penguin Books.

Guillon, F., & Champ, M. (2000). Structural and physical properties of dietary fibres and consequences of processing on human physiology. *Food Research International, 33*(3-4), 233-245.

Hanson, C.F., Frankos, V.H. & Thompson, W.O. (1989). Bioavailability of oxalic acid from spinach, sugar beet fibre and a solution of sodium oxalate consumed by female volunteers. *Food Chemistry Toxicology, 27*, 181–184.

Hatch, M., & Freel, R. W. (2005). Intestinal transport of an obdurate anion: oxalate. *Urological Research, 33*(1), 1-16.

Hautmann, R.E. (1993). The stomach: a new and powerful oxalate absorption site in man. *Journal of Urology, 149*, 1401-1404.

Hess, B., Jost, C., Zipperle, L., Takkinen, R., & Jaeger, P. (1998). High-calcium intake abolishes hyperoxaluria and reduces urinary crystallization during a 20-fold normal oxalate load in humans. *Nephrology Dialysis Transplantation, 13*(9), 2241-2247.

Hiatt, R. A., Ettinger, B., Caan, B., Quesenberry, C. P., Duncan, D., & Citron, J. T. (1996). Randomized controlled trial of a low animal protein, high fiber diet in the prevention of recurrent calcium oxalate kidney stones. *American Journal of Epidemiology, 144*(1), 25-33.

Hodgkinson, A. (1977). In J. W. Arrowsmith (Ed.), Oxalic acid in biology and medicine (pp. 159–200). London, New York, San Francisco: Academic Press.

Holloway, W. D., Argall, M. E., Jealous, W. T., Lee, J. A., & Bradbury, J. H. (1989). Organic acids and calcium oxalate in tropical root crops. *Journal of Agriculture and Food Chemistry, 37*, 337–341.

Holmes, P. R., Goodman, H. O., & Assimos, D. G. (1995). Dietary oxalate and its intestinal absorption. *Scanning Microscopy, 9*, 1109–1120.

Holmes, R. P., Goodman, H. O., & Assimos, D. G. (2001). Contribution of dietary oxalate to urinary oxalate excretion. *Kidney International, 59*(1), 270-276.

Hönow, R. & Hesse, A. (2002). Comparison of extraction methods for the determination of soluble and total oxalate in foods by HPLC enzyme-reactor. *Food Chemistry, 78*, 511-521.

Hughes, J., & Norman, R.W. (1992). Diet and calcium stones. *Canadian Medical Association Journal, 146*(2), 137–143.

Jaeger, P., & Robertson, W.G. (2004). Role of dietary intake and intestinal absorption of oxalate in calcium stone formation. *Nephron Physiology, 98*, 64-71

Johansson, S., & Savage, G. P. (2011). The availability of soluble oxalates in stir-fried silver beet (*Beta vulgaris* var. cicla) leaves eaten with yoghurt. *International Journal of Food Science & Technology, 46*(11), 2232-2239.

Kelsay, J.L., & Prather, E.S. (1983). Mineral balances of human subjects consuming spinach in a low-fibre diet and in a diet containing fruits and vegetables. *American Journal of Clinical Nutrition, 38*, 12-19.

Libert, B., & Creed, C. (1985). Oxalate content of 78 rhubarb cultivars and its relation to some other characters. *Journal of Horticultural Science, 60*(2), 257-261.

Liebman, M., & Chai, W. (1997). Effect of dietary calcium on urinary oxalate excretion after oxalate loads. *The American Journal of Clinical Nutrition, 65*(5), 1453-1459.

Mårtensson, L., & Savage, G. P. (2008). Composition and bioavailability of oxalates in baked taro (*Colocasia esculenta* var. Schott) leaves eaten with cows milk and cows milk and coconut milk. *International Journal of Food Science & Technology, 43*(12), 2213-2218.

Noonan, S.C. & Savage, G.P. (1999). Oxalate content of foods and its effect on humans. *Asia Pacific Journal of Clinical Nutrition, 8*, 64–74.

Oscarsson, K. V., & Savage, G. P. (2007). Composition and availability of soluble and insoluble oxalates in raw and cooked taro (*Colocasia esculenta* var. Schott) leaves. *Food Chemistry, 101*(2), 559-562.

Prenen, J.A., Boer, P. & Dorhout Mees, E.J. (1981). Absorption kinetics of oxalate from oxalate-rich food in man. *American Journal of Clinical Nutrition, 40,* 1007–1010.

Radek, M. & Savage, G.P. (2008). Oxalates in some Indian green leafy vegetables. *International Journal of Food Sciences and Nutrition, 59*, 246–260.

Savage, G. P., & Catherwood, D. J. (2007). Determination of oxalates in Japanese taro corms using an *in vitro* digestion assay. *Food Chemistry, 105*(1), 383-388.

Savage, G. P., Mårtensson, L., & Sedcole, J. R. (2009). Composition of oxalates in baked taro (*Colocasia esculenta* var. Schott) leaves cooked alone or with additions of cows milk or coconut milk. *Journal of Food Composition and Analysis, 22*(1), 83-86.

Savage, G. P., Vanhanen, L., Mason, S. M., & Ross, A. B. (2000). Effect of cooking on the soluble and insoluble oxalate content of some New Zealand foods. *Journal of Food Composition and Analysis, 13*(3), 201-206.

Siener, R., Heynck, H. & Hesse, A. (2001). Calcium-binding capacities of different brans under simulated gastrointestinal pH conditions. *In vitro* study with ^{45}Ca. *Journal of Agricultural and Food Chemistry*, *49*(9), 4397-4401.

Simpson, T.S., Savage, G.P., Sherlock, R. & Vanhanen, L. (2009). Oxalate content of silver beet leaves at different stages of maturation and the effect of cooking with different milk sources. *Journal of Agricultural and Food Science, 57*, 10804–10808.

Tiselius, H. G., Ahlstrand, C., Lundström, B., & Nilsson, M. A. (1981). [14C] Oxalate absorption by normal persons, calcium oxalate stone formers, and patients with surgically disturbed intestinal function. *Clinical Chemistry, 27*(10), 1682-1685.

The Concise New Zealand food composition tables (2009), 8th Edition, Plant & Food Research. Wellington: New Zealand Ministry of Health.

Van Buren, J. P. (1979). The chemistry of texture in fruits and vegetables. *Journal of Texture Studies, 10*(1), 1-23.

Zarembski, P.M. & Hodgkinson, A. (1962). The oxalic acid content of English diets. *British Journal of Nutrition, 16*, 627–634.

Chapter 8
General discussion and conclusions

The beneficial health effects of fruit, believed to be associated with a decreased incidence of cardiovascular disease, cancer and other chronic diseases, have been attributed, at least in part, to a complex mixture of antioxidants abundantly present in fruit (Liu, 2003; Liu, 2004). Polyphenols are the major constituent of antioxidant compounds in foods and they have been measured in numerous studies. Concentrations of total phenolic compounds in fruit vary according to geographic areas, genotype variation, concentrations of maturity, environmental conditions the fruit are grown in and the part of fruit sampled (Vieira *et al*., 2009; Wang *et al.*, 2011). The total phenolic concentration of fruit grown in New Zealand ranged from 0.27 – 27.3 mg GAE/g FW which is comparable to values reported for Singapore, 0.02 – 15.9 mg GAE/g FW (Isabelle *et al*., 2010), Ecuador, 0.26 – 21.7 mg GAE/g FW (Vasco *et al*., 2008), Brazil, 5.9 – 116.2 mg GAE/g FW (Rufino *et al*., 2010), Colombia, 0.15 – 10.18 mg GAE/g FW (Contreras-Calderón *et al.*, 2011) and the USA, 0.47 – 7.95 mg GAE/g FW (Wu *et al*., 2004a).

Since fruit contains many different classes and types of antioxidants, analysing the level and antioxidant activity of a single antioxidant will not sufficiently reflect the total antioxidant capacities of the fruit. The total antioxidant capacity of a given fruit is the combined actions of hundreds of antioxidant compounds rather than any single compound (Halvorsen *et al.*, 2006). This concept was investigated and confirmed for the fruit investigated in the current study where contribution from vitamin C, which is a single antioxidant, to the anti-peroxyl radical capacity of most fruit was shown to be very small. For example, vitamin C in blueberry only accounted for 0.6% of total antioxidant capacity of the fruit measured by ORAC assay. Additionally, vitamin C contributed only 5.8 and 6.0% to antioxidant capacity of black raspberry and blackcurrant, respectively, even though their vitamin C concentrations were extremely high, ranking in the first and third positions of the 38 different fruits available in New Zealand. Moreover, there was no correlation between vitamin C and antioxidant capacities measured by the ABTS assay because vitamin C was destroyed by heating, and there was a very weak correlation ($r^2 = 0.13$) between vitamin C and antioxidant capacity measured by ORAC in common fruits in New Zealand. In contrast, strong correlations between total phenolic concentrations and antioxidant capacities measured by the two different types of antioxidant assays, suggest that the antioxidant capacities of fruit come from total phenolic compounds rather than the vitamin C concentration.

The increased consumption of fruit has been encouraged worldwide because of its health benefits (WHO, 2003). However, increasing fruit consumption to achieve these benefits in terms of antioxidants may lead to adverse effects from absorption of oxalates, undesirable substances sometimes found in fruit. There are several methods used to extract and measure oxalate concentrations in plant foods, however, most of them are old, time-consuming and give unreliable data because the methods overlook some substances that may interfere with oxalate determination (Nguyen and Savage, 2012). Depending on the pH and temperature of extraction, the pectin concentration extracted differed and, consequently, oxalate concentrations measured could change due to binding with free pectin in the extraction medium. Extractions carried out at 21^0C resulted in higher soluble oxalate concentrations as compared to extractions conducted at 80^0C. Meanwhile, total oxalate concentrations measured following extraction using 2 M HCL at 21^0C were the highest compared to the values obtained from other extraction solution and temperatures.

Based on the optimised extraction method using 2 M HCL at 21^0C, the oxalate concentration of 41 different fruits available in New Zealand were measured and the data showed that most of the fruits contained low or no oxalate. However, some fruits should not be consumed frequently such as Indian gooseberry, rhubarb, carambola, goji berry because they contain very high concentrations of oxalates. Other fruit also contain relatively high concentrations of total oxalates > 26 mg/100 g FW, such as feijoa, black raspberry, redcurrant, red gooseberry and kiwiberry. Green gooseberry, green and golden kiwifruit were in the medium group with total oxalate concentration < 26 mg/100 g FW.

The present work also showed that oxalate concentrations may vary according to cultivar, maturity stage and part of plant food analysed. Oxalate concentrations were significantly different between two cultivars of kiwifruit; green kiwifruit contained higher amounts of total oxalates, but lower level of soluble oxalates. The variation of total oxalate concentrations in a range of golden kiwifruit genotypes has been reported previously (Rassam and Laing, 2005) but the soluble oxalate concentrations were overlooked. Different fractions of kiwifruit contain significantly different ratios of soluble to insoluble oxalates; considerable amounts of total and insoluble oxalates were found in the skin, while high concentrations of soluble oxalates was detected in the pulp. In general, the level of soluble oxalate is considered of greater importance as it is available for absorption. However, if a low oxalate diet is recommended plant fractions with high concentrations of total and insoluble oxalates, such as kiwifruit skin, should be avoided due to the possibility of increasing solubility of insoluble oxalates following acid pH incubation at pH characteristic of the stomach. After 15 days of

storage of kiwifruit at 20 ± 1^0C and RH 50-55%, soluble oxalate concentration significantly reduced, whereas concentrations of insoluble oxalates increased, suggesting that further oxalate metabolism occurs in post-harvest fruit, especially during fruit ripening.

The way that plant food has been processed prior to human consumption strongly affects the amount of oxalate available for absorption. Soaking, boiling and cooking vegetables have been shown to be potential ways to reduce oxalates due to leaching oxalate from plant foods into the soaking or cooking water (Savage *et al.*, 2000; Chai and Liebman, 2005), however, baking increases oxalate concentration by loss of moisture and results in increasing oxalate availability (Albihn and Savage, 2000; Sangketkit *et al*., 2001). In the present study, making juice reduced the amount of oxalates in the juice compared with the amounts in the fruit because most of the oxalate was retained in the fibre fraction, which was then discarded. Pasteurisation significantly reduced concentrations of soluble oxalates but increased concentrations of insoluble oxalates in the juice suggesting that a conversion between soluble and insoluble oxalate occurs during pasteurisation.

The trapping of oxalate in the fibre fraction during juice processing was investigated further. Along with the effect of cooking medium, digestive pH and dietary fibre resulted in further reductions of oxalate following an *in vitro* digestion which simulated the conditions in the gastrointestinal tract. Although the role of dietary fibre in oxalate absorption has been a controversial subject (Ebisuno *et al*., 1986; Firth *et al*., 1990; Hatch and Freel, 2005), by using an *in vitro* digestion technique, the current study clearly showed that dietary fibre reduced the oxalate potentially available for absorption at different digestive pH values. Due to being trapped by the dietary fibre, concentrations of oxalate available for absorption were reduced by an average of 21.4% and 20.0% of the overall soluble oxalate concentration at pH values characteristic of the stomach and intestine, respectively. This showed that it is unreasonable to assume that all soluble oxalate concentrations measured in undigested foods will be absorbed completely into the body. Being trapped in the fibre fraction makes it less likely that soluble oxalates would bind with free calcium in food even if the amounts of free calcium are in excess of the oxalate concentrations. It is, however, possible that binding to fibre, which is eventually excreted, would reduce soluble oxalate absorption.

Regardless of dilution, adding milk products when cooking further reduced concentrations of soluble oxalates in rhubarb petioles, making less oxalate available at the pHs characteristic of the gastrointestinal tract, as compared with the uncooked petioles. The low fat milk was the most effective cooking medium, which reduced the ratio of soluble oxalates to total oxalates

to 25.9% compared with 52.5% for the water medium and 34.9% for the full fat milk medium. Cooking foods containing high oxalate with milk has been reported as a potential way to reduce oxalate in food due to binding between calcium in milk products and soluble oxalate in the food to form insoluble calcium oxalate, which is then excreted without absorption (Savage and Catherwood, 2007; Radek and Savage, 2008; Simpson *et al*., 2009; Johansson and Savage, 2011). Although soluble oxalate concentration of rhubarb cooked in the trim milk medium was significantly ($p < 0.05$) lower than that of rhubarb cooked in the standard milk, the mineral determination in this study showed that there was no significant difference in calcium concentration between the rhubarb cooked with low fat milk compared with full fat milk. The other minerals that may potentially form insoluble salts due to binding with soluble oxalates were considered unimportant because of their very small concentrations in the cooking media or in the rhubarb tissue. The effect of fat on increasing oxalate availability has been previously reported (Andersson and Jagenburg, 1974; Finch *et al*., 1981). It is possible that the much higher fat concentration of the standard milk than that of the trim milk (3.3% vs. 0.5%) resulted in the significantly higher ($p < 0.05$) soluble oxalate concentration of the standard milk cooking mix relative to the trim milk cooking mix.

Recommendation for further studies

In this study, the measurement of total phenolic compounds in New Zealand-available fruit and their activity in scavenging free radicals was determined. The quantification of oxalate in a wide range of fruit, as well as in different fruit fractions and at different fruit maturities, together with the optimisation of pH and extraction temperature of a practical extraction method for oxalate has been reported. The interactions between fibre and oxalate observed suggest a potential solution for the reduction of oxalate consumed in the diet which may then reduce the occurrence of oxalate kidney stones in susceptible people. However, some aspects of the current work need further study.

- Applying an *in vitro* digestion to find out how digestive factors affect antioxidant concentrations as well as antioxidant capacities of fruit would help to make it clear whether dietary and digestive factors would enhance the release of more un-extracted antioxidants or break them down into other non-antioxidant compounds.

- Determining oxalate concentration in other fruit products such as fruit nectar, fruit leather, fruit pickles, fruit chutneys, fruit chips and instant fruit drink powders

needs to be carried out to give a complete understanding about the effect of each fruit processing method on oxalate concentration of the final product.

- Studying potential plant breeding and selection methods which can be applied to reduce oxalate concentration of fruits containing high oxalate concentrations, for example, Indian gooseberry and carambola fruit, would be valuable.

- Expanding an *in vitro* digestion method to an *in vivo* study through feeding natural foods containing high oxalate concentrations with supplements of different types of dietary fibre would confirm the *in vitro* data observed in this study. Measuring urinary oxalate each two hour interval after feeding is required to enable to identification of the exact level of oxalates absorbed at each defined segment of the gastrointestinal tract. This should be carried out on kidney stone and non-kidney stone forming individuals to give more comparable and practical results. Feeding experiments to assess the interaction effects of calcium, fat and fibre on urinary oxalate output would also be useful. Biological research into the effect of other dietary factors such as protein, ascorbic acid and intestinal microorganisms is necessary to evaluate the overall effects of dietary factors on oxalate absorption from a range of different oxalate containing foods commonly eaten in the diets of different people in many parts of the world.

References

Albihn, P. B. E., & Savage, G. P. (2001). The effect of cooking on the location and concentration of oxalate in three cultivars of New Zealand-grown oca (*Oxalis tuberosa* Mol). *Journal of the Science of Food and Agriculture, 81*(10), 1027-1033.

Andersson, H., & Jagenburg, R. (1974). Fat-reduced diet in the treatment of hyperoxaluria in patients with ileopathy. *Gut* 15(5), 360-366.

Chai, W., & Liebman, M. (2005). Effect of different cooking methods on vegetable oxalate content. *Journal of Agricultural and Food Chemistry* 53(8), 3027-3030.

Contreras-Calderón, J., Calderón-Jaimes, L., Guerra-Hernández, E., & García-Villanova, B. (2011). Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia. *Food Research International, 44*(7), 2047-2053.

Ebisuno, S., Morimoto, S., Yoshida, T., Fukatani, T., Yasukawa, S., & Ohkawa, T. (1986). Rice-bran treatment for calcium stone formers with idiopathic hypercalciuria. *British Journal of Urology, 58*(6), 592-595.

Finch, A.M., Kasidas, G.P., & Rose, G.A. (1981). Urine composition in normal subjects after oral ingestion of oxalate-rich foods. *Clinical Science, 60*, 411-418.

Firth, W.A., & Norman, R.W. (1990). The effects of modified diets on urinary risk factors for kidney stone disease. *The Journal of the American Dietetic Association, 51*, 404-408

Halvorsen, B. L., Carlsen, M. H., Phillips, K. M., Bøhn, S. K., Holte, K., Jacobs, D. R., & Blomhoff, R. (2006). Content of redox-active compounds (ie, antioxidants) in foods consumed in the United States. *The American Journal of Clinical Nutrition, 84*(1), 95-135.

Hatch, M., & Freel, R. W. (2005). Intestinal transport of an obdurate anion: oxalate. *Urological Research, 33*(1), 1-16.

Hönow, R. & Hesse, A. (2002). Comparison of extraction methods for the determination of soluble and total oxalate in foods by HPLC enzyme-reactor. *Food Chemistry, 78*, 511-521.

Isabelle, M., Lee, B. L., Lim, M. T., Koh, W.-P., Huang, D., & Ong, C. N. (2010). Antioxidant activity and profiles of common fruits in Singapore. *Food Chemistry, 123*(1), 77-84.

Johansson, S., & Savage, G. P. (2011). The availability of soluble oxalates in stir-fried silver beet (*Beta vulgaris* var. cicla) leaves eaten with yoghurt. *International Journal of Food Science & Technology, 46*(11), 2232-2239.

Liu, R. H. (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *The American Journal of Clinical Nutrition, 78*(3), 517S-520S.

Liu, R. H. (2004). Potential synergy of phytochemicals in cancer prevention: mechanism of action. *The Journal of Nutrition, 134*(12), 3479S-3485S.

Nguyen, H.H.V & Savage, G.P. Oxalate content of New Zealand grown and imported fruits. Submitted to *Journal of Food Composition and Analysis*, 2012

Radek, M. & Savage, G.P. (2008). Oxalates in some Indian green leafy vegetables. *International Journal of Food Sciences and Nutrition, 59*, 246–260.

Rassam, M. & Laing, W (2005). Variation in ascorbic acid and oxalate levels in the fruit of *Actinidia chinensis* tissues and genotypes. *Journal of Agricultural and Food Chemistry, 53*, 2322-2326.

Rufino, M. d. S. M., Alves, R. E., de Brito, E. S., Pérez-Jiménez, J., Saura-Calixto, F., & Mancini-Filho, J. (2010). Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chemistry, 121*(4), 996-1002.

Sangketkit, C., Savage, G. P., Martin, R. J., & Mason, S. M. (2001). Oxalate content of raw and cooked oca *(Oxalis tuberosa). Journal of Food Composition and Analysis, 14*, 389-397.

Savage, G. P., & Catherwood, D. J. (2007). Determination of oxalates in Japanese taro corms using an *in vitro* digestion assay. *Food Chemistry, 105*(1), 383-388.

Savage, G. P., Vanhanen, L., Mason, S. M., & Ross, A. B. (2000). Effect of cooking on the soluble and insoluble oxalate content of some New Zealand foods. *Journal of Food Composition and Analysis, 13*(3), 201-206.

Simpson, T.S., Savage, G.P., Sherlock, R. & Vanhanen, L. (2009). Oxalate content of silver beet leaves at different stages of maturation and the effect of cooking with different milk sources. *Journal of Agricultural and Food Science, 57*, 10804–10808.

Vasco, C., Ruales, J., & Kamal-Eldin, A. (2008). Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. *Food Chemistry, 111*(4), 816-823.

Vieira, K.F.G., da Silva Campelo Borges, G., Copetti, C., Gonzaga, L.V. (2009). Activity and contents of polyphenolic antioxidants in the whole fruit, flesh and peel of three apple cultivars. *Archivos Latinoamericanos de Nutricion, 59*(1), 101-106

Wang, S. Y., Seeram, N. P., & Stoner, G. D. (2011). Correlation of antioxidants and antioxidant enzymes to oxygen radical scavenging activities in berries. In G.D. Stoner & N.P. Seeram (Eds.), *Berries and Cancer Prevention* (pp. 79-97).New York: Springer.

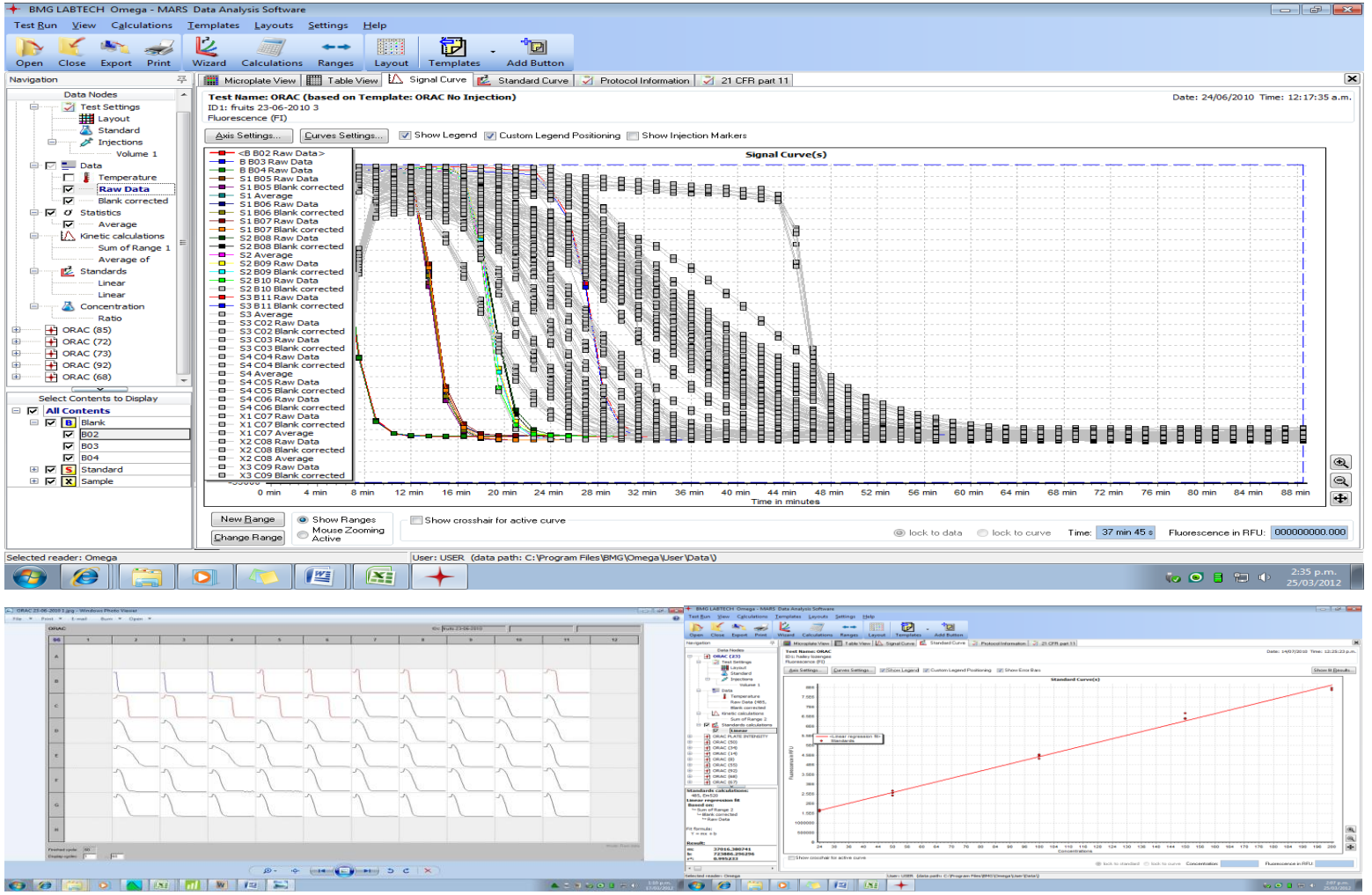
World Health Organization (WHO). (2003). Fruit and vegetable promotion initiative /a meeting report / 25-27/08/03. Retrieved 2 March, 2012 from http://www.who.int/hpr/NPH/fruit_and_vegetables/fruit_and_vegetable_report.pdf

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2004a). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agricultural and Food Chemistry, 52*(12), 4026-4037.

Appendix 1. Antioxidant capactity determination by plate reader

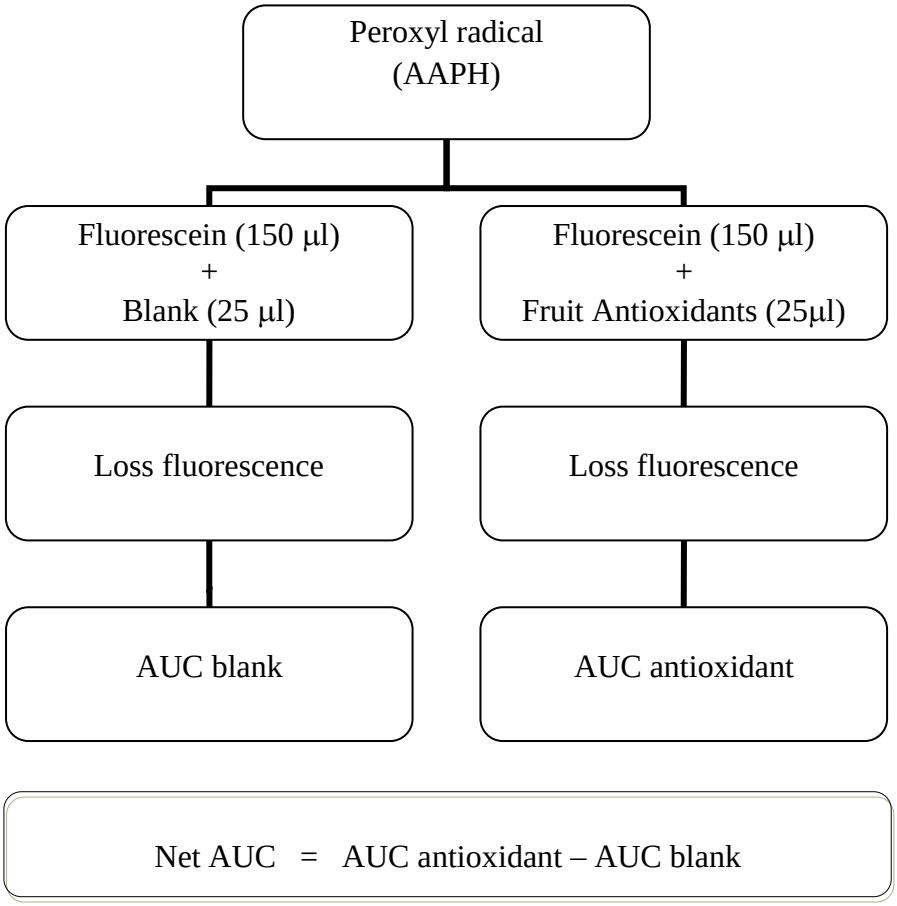
A.1.1. An example of an ORAC output from the plate reader

The curves were plotted by FLUOstar OPTIMA software for a series of standards, phosphate buffer (blank) and diluted fruit extractions, which were prepared as described in the chapter 3. Each line presents the output from a fruit extraction.



A.1.2. Schematic depiction of the ORAC assay

Reactive oxygen species generator (AAPH) was added to parallel reactions that contain equal amounts of fluorescein (150 μl) added either to a buffer blank or extraction samples and standards. The antioxidant capacity of a sample is the net difference between the area under the curve (AUC) of the sample and that of the blank.



Antioxidant capacity was calculated from the net AUC by comparison to a standard curve prepared with trolox as presented in A.1.1.

Appendix 2. English, family and scientific name of analysed fruit

English name	Family name	Scientific name
Apple	Rosaceae	*Malus domestica* Borkh.
Apricot	Rosaceae	*Prunus armeniaca* L.
Avocado	Lauraceae	*Persea americana* Mill.
Banana	Musaceae	*Musa* L.
Blueberry	Ericaceae	*Vaccinium corymbosum* Rydb.
Boysenberry	Rosaceae	*Rubus ursinus* × *idaeus*
Carambola	Oxalidaceae	*Averrhoa carambola* L.
Cherry (Red Bing)	Rosaceae	*Prunus avium* L.
Cherry (Yellow Royal)	Rosaceae	*Prunus avium* L.
Cherry (Black Tartarian)	Rosaceae	*Prunus avium* L.
Cranberry	Ericaceae	*Vaccinium oxycoccos*
Currant (black)	Grossulariaceae	*Ribes nigrum* L.
Currant (red)	Grossulariaceae	*Ribes rubrum* L.
Feijoa	Myrtaceae	*Feijoa sellowiana* (O.Berg) O.Berg
Goji berry	Solanaceae	*Lycium chinense* Mill.
Gooseberry (green)	Grossulariaceae	*Ribes uva-crispa* L.
Gooseberry (red)	Grossulariaceae	*Ribes uva-crispa* L.
Gooseberry (Indian)	Phyllanthaceae	*Phyllanthus emblica* L.
Grape (black)	Vitaceae	*Vitis vinifera* L.
Grape (green)	Vitaceae	*Vitis vinifera* L.
Grape (red)	Vitaceae	*Vitis vinifera* L.
Kiwano	Cucurbitaceae	*Cucumis metuliferus* E. Mey
Kiwi berry	Actinidiaceae	*Actinidia arguta* Planch. ex Miq.
Kiwifruit (golden)	Actinidiaceae	*Actinidia chinensis* L.
Kiwifruit (green)	Actinidiaceae	*Actinidia deliciosa* L.
Mangosteen	Clusiaceae	*Garicina mangostana* L.
Nectarine	Rosaceae	*Prunus persica var. nectarina*
Passion fruit	Passifloraceae	*Passiflora edulis* Sims
Peach	Rosaceae	*Prunus persica* (L.) Batsch
Pear	Rosaceae	*Pyrus communis* L.
Persimmon	Ebenaceae	*Diospyros kaki*
Pineapple	Bromeliaceae	*Ananas comosus* (L.) Merr.
Plum (black)	Rosaceae	*Prunus nigra* Aiton.

Plum (red)	Rosaceae	*Prunus domestica* L.
Plum (cherry)	Rosaceae	*Prunus cerasifera* Ehrh.
Raspberry (red)	Rosaceae	*Rubus idaeobatus* L.
Raspberry (black)	Rosaceae	*Rubus occidentalis* L.
Rhubarb	Polygonaceae	*Rheum rhabarbarum* L.
Strawberries	Rosaceae	*Fragaria* × *ananassa* Duchesne
Tamarillo	Solanaceae	*Solanum betaceum* Cav.
Tomato (cherry red)	Solanaceae	*Solanum lycopersicum* var. *cerasiforme*
Tomato (red)	Solanaceae	*Solanum lycopersicum* L.
Tamarind	Fabaceae	*Tamarindus indica* L.

Appendix 3. Oxalate determination by HPLC

A.3.1. Standard calibration

Oxalic acid standards were prepared by dissolving 99.99% oxalic acid (Sigma-Aldrich Co., St Louis, USA) in either 2M HCL (Arista, BDH Chemicals, Ltd., Poole, Dorset, UK) or deionised water (Arium 611uv, Sartorius Ltd. Germany) and to make the following concentrations: 0.05, 0.1, 0.5, 1.0, 2.5, 5.0 and 10.0 mg/100 ml. After filtering through a 0.45 μm cellulose nitrate filter (Sartorious AG, Gottingen, Germany), standards were measured by HPLC.

A.3.2. HPLC analysis

The equipment consists of a ternary Spectra-Physics, SP 8800 HPLC pump (Spectra-Physics, San Jose, CA, USA), a Waters, U6K injector (Waters Inc., Marlborough, MA, USA), a UV⁄ VIS detector Spectra-Physics SP8450 (Spectra- Physics, San Jose, CA, USA) set at 210 nm and an autosampler (Hitachi AS-2000, Hitachi Ltd, Kyoto, Japan). The chromatographic separation was carried out at 25^0C through a 300 x 7.8 mm ion exclusion column (Rezex ROA Organic acid 8%) combined with a cation H+ guard column (Bio-Rad, Richmond, California, USA). Data capture was facilitated through a PeakSimple chromatography data system (SRI model 203, SRI Instrument, California, USA) and data analysis was performed via PeakSimple version 3.59 (SRI Instruments, Torrance, California, USA).
The mobile phase used was an aqueous solution of 0.025 M sulphuric acid (HPLC grade). Before use, the mobile phase was filtered and degassed using a 0.45 μm membrane filter combined with a vacuum pump. A 20 μL aliquot of extracted sample was injected into the column and eluted at a flow rate of 0.6 ml/min. The oxalate peak of sample is identified by comparison of retention time with standards of oxalic acid and concentration was calculated from the standard curve.

A.3.3. HPLC limit of detection (LOD)

The limit of detection was assessed by using an adaptation of the ICH Harmonised Tripartite Guidelines (Validation of Analytical Procedures, ICH, Q2 (R1), 1995). Briefly, reference chromatograms of water and 2 M HCL were recorded. Afterwards, progressively smaller concentrations of standard oxalic acid in deionised water or in 2 M HCL were injected. The concentrations of the standards were recorded for the area that was three times higher than the area of the noise in the corresponding reference chromatograms. Each standard solution was injected and compared six times to ensure consistent results. The LOD was determined to be 0.01 mg oxalate/100 ml in both water and acid.

Appendix 4. An *in vitro* digestion method and determination of bound calcium concentration

A.4.1. Chemicals used the preparation of digestive fluid for digestive juice used in *in vitro* digestion proceduce

a. The saliva solution used during the *in vitro* digestion assay			pH = 6.5 ± 0.2
Inorganic:			
KCl	8.96 g/100 ml	-	10 ml
KSCN	2 g/100 ml	-	10 ml
$NaH_2PO_4.H_2O$	10.2 g/100 ml	-	10ml
Na_2HPO_4	5.68 g/100 ml	-	10 ml
NaCl	17.53 g/100 ml	-	1.7 ml
NaOH	4 g/100 ml	-	1.8 ml
			Make up to 500 ml with H_2O
Organic:			
Urea	2.5 g/100 ml	-	8 ml
			Make up to 500 ml with H_2O
Add 50 ml inorganic and 50 ml organic and heat to 37^0C			
Then add: 14.5 mg α-amylase			
1.5 mg uric acid			
5 mg mucin			

b. The gastric juice solution used during the *in vitro* digestion assay			pH = 1.07 ± 0.07
Inorganic:			
NaCl	17.53 g/100 ml	-	15.7 ml
$NaH_2PO_4.H_2O$	10.2 g/100 ml	-	3.0 ml
KCl	8.96 g/100 ml	-	9.0 ml
$CaCl_2.2H_2O$	2.22 g/100 ml	-	18.0 ml
HCL	37% g/g	-	0.83 ml
NH_4Cl	3.06 g/100 ml	-	10 ml
			Make up to 500 ml with H_2O
Organic:			
Glucose	6.5 g/100 ml	-	10 ml
Glucuronic acid	0.2 g/100 ml	-	10 ml
Urea	2.5 g/100 ml	-	3.4 ml
Glucosamine hydrochloride	3.3 g/100 ml	-	10 ml
			Make up to 500 ml with H_2O
Add 60 ml inorganic and 60 ml organic and heat to 37^0C			
Then add: 0.12 g bovine serum albumin			
0.12 g pepsin			
0.36 g mucin			

c. The duodenal juice solution used during the *in vitro* digestion assay			pH = 7.8 ± 0.2
Inorganic:			
NaCl	17.53 g/100 ml	-	40 ml
$NaHCO_3$	8.47 g/100 ml	-	40 ml
KH_2PO_4	0.8 g/100 ml	-	10 ml
KCl	8.96 g/100 ml	-	6.3 ml
$MgCl_2.6H_2O$	1.01 g/100 ml	-	10 ml
HCL	37% g/g00 ml	-	180 µl
			Make up to 500 ml with H_2O
Organic:			
Urea	2.5 g/100 ml	-	4 ml
			Make up to 500 ml with H_2O
Add 120 ml inorganic and 120 ml organic and heat to 37^0C			
Then add: $CaCl_2.2H_2O$	2.22 g/100 ml	-	2.16 ml
0.24 bovine serum albumin			
0.72 g pancreatin			

d. The bile solution used during the *in vitro* digestion assay			pH = 8.0 ± 0.2
Inorganic:			
KCl	8.96 g/100 ml	-	4.2 ml
NaCl	17.53 g/100 ml	-	30 ml
$NaHCO_3$	8.47 g/100 ml	-	68.3ml
HCL	37% g/g	-	200 µl
			Make up to 500 ml with H_2O
Organic:			
Urea	2.5 g/100 ml	-	8 ml
			Make up to 500 ml with H_2O
Add 50 ml inorganic and 50 ml organic and heat to 37^0C			
Then add: $CaCl_2.2H_2O$	2.22 g/100 ml	-	2.16 ml
0.18 g bovine serum albumin			
0.72 g bile			

A.4.2. Calculation for bound calcium concentration

Insoluble oxalate can be assumed to be calcium oxalate

Molecular weight of calcium = 40.078 g/mol

Molecular weight of calcium oxalate = 128.097 g/mol

Calcium in calcium oxalate = (40.078 *100)/128.097 = 31.28 %

For example: a food might have 1.55 g insoluble oxalate/100 g DM

(1.55 x 31.287)/100 = 0.4849 g bound Ca/100 g DM

Appendix 5. Papers submitted from this thesis

1. Ha, V.H. Nguyen., and G.P. Savage. (2011). Effect of pH on binding activity between oxalate and fibre in raw and cooked rhubarb. *Abstract proceeding of Nutrition Society of Australia*, 2011.

2. Ha, V.H. Nguyen., and G.P. Savage. (2012). Oxalate availability in raw and cooked rhubarb. *Proceeding of the Nutrition Society of New Zealand*. (In press).

3. Ha, V.H. Nguyen., and G.P. Savage. (2012). Oxalate content in New Zealand and imported fruit. Submitted to *Journal of Food Composition and Analysis*.

4. Ha, V.H. Nguyen., and G.P. Savage. (2012).The effects of temperature and pH on the extraction of oxalate and pectin from green kiwifruit (*Actinidia deliciosa* L.), golden kiwifruit (*Actinidia chinensis* L.), kiwiberry (*Actinidia arguta*) and persimmon (*Diospyros kaki*) (IJFST-2012-10889). Submitted to *the International Journal of Food Science and Technology* Manuscript number: IJFST-2012-10888.

5. Ha, V.H. Nguyen., and G.P. Savage. (2011). Effect of pH on binding activity between oxalate and fibre in raw and cooked rhubarb. Submitted to *Plant Foods for Human Nutrition.* Manuscript number: QUAL2632R2.