An investigation of the stability of yacon storage roots under commercial conditions and the feasibility of preparing blackcurrant-yacon juice mixtures for the retail market

A thesis

submitted in partial fulfilment of

the requirements for the degree of

Master of Science in Chemistry

at the

University of Waikato

by

Maria Teresa Revell

University of Waikato

2012

Abstract

The stability of fructooligosaccharides (FOS) was investigated in yacon (*Smallanthus sonchifolius* Poepp. & Endl., Asteraceae) storage roots and blackcurrant-yacon juice mixtures. The roots and syrup used in this study are from yacon plants grown for NZ Biotechnologies, the only company in New Zealand which grows yacon commercially for export.

The hydrolysis of FOS in storage roots packaged in a semi-permable polymer was significantly reduced compared to unpackaged roots from the same plant, stored in the same conditions (5 °C for up to 72 days). The effectiveness of the packaging was dependent upon a complete seal around the root, indicating the need for selection of relatively straight and smooth roots for successful packaging.

Blackcurrant-yacon juice mixtures offer the prebiotic effect of FOS combined with the antioxidant activity of blackcurrant juice. This study was designed to assess the effect of pasteurisation conditions on carbohydrate and polyphenol concentrations and antioxidant activity. The reduction in active ingredients per mL can then be compensated for by the initial formulation before pasteurisation.

The rates of hydrolysis and release of FOS, 1-kestose, sucrose, D-glucose and D-fructose were calculated and agreed with previous studies with the exception of 1-kestose. The initial concentrations of polyphenols (330.67-524.40 gallic acid equivalent mg L^{-1}) and anthocyanins (1013.11-1362.25 cyanidin-3-glucoside equivalent mg L^{-1}) were also similar to other studies. The concentration of both polyphenols and anthocyanins decreased with heating time, but this difference was statistically significant at a 99.95 % confidence level only for anthocyanins.

The decrease of anthocyanin concentration with time in blackcurrant-yacon juice without pH stabilisation was correlated with the rate of FOS hydrolysis. This indicates that the presence of FOS stabilizes the anthocyanins, possibly by formation of a complex or compound. This is supported by evidence from elsewhere, that dietary fibre-type molecules can transport antioxidants to the large bowel, with associated health effects.

Acknowledgements

Many thanks are due to my supervisor at the University of Waikato, Merilyn Manley-Harris. For the time you have made for me, and the patience you have had with me, I am extremely grateful.

To Dr. Robert Welch from NZ Biotechnologies, I am honoured to have worked for you on this project. The commitment you have to providing products with proven concentrations of bioactive components is admirable, and the nutraceutical industry would benefit greatly if everyone had such integrity.

To the Ministry of Science and Innovation, thank you for the financial support towards this project and for providing funding for students to undertake scientific study with commercial applications that potentially benefit the New Zealand economy.

I would like to thank Cuisine Resources for producing the initial juice analysed in this study and for providing me with the information needed to work out the best course of action for further research. In particular I would like to thank Ray Walker for showing me all aspects of yacon syrup production, letting me sort through mountains of boxes in the factory and for always smiling no matter what. I was also glad to get to know Caroline, one smart woman also juggling chemistry and young children, who was very helpful whenever I had questions.

At the University of Waikato there are so many people to thank. Hopefully I have thanked them as I went along for each service! Thank you (in no particular order), Simon, Megan, Jess MacAskill, Jess King, Tiffany, Johnathan, Aaron, Sam, Wendy, Pat, Amu, Annie, Jenny, Kerry, John Little, John McDonald Wharry, and Jolene!!

The last and let’s face it, it has to be the largest, thanks goes to my family. Mum, Dad and Rebecca thank you so much for putting up me for the last two years. Finishing this thesis without your help with the kids, would have been impossible. Brayden and Lia, thank you for trying your hardest to understand that sometimes Mummy really did need to write when you wanted to play

Table of Contents

3. Results 32

List of Tables

List of Equations

Table of Figures

List of abbreviations, symbols and units

α	alpha (anomeric configuration)
α(1→2)	alpha glycosidic bond from carbon 1 to carbon 2 of different monosaccharide residues
β	beta (anomeric configuration)
β(2→1)	beta glycosidic bond from carbon 2 to carbon 1 of different monosaccharide residues
ε	epsilon, symbol for molar absorptivity
λ	lambda, symbol for wavelength in spectrophotometry
λ_{max}	the wavelength at which the analyte absorbs the maximum fraction of visible or ultra violet light as detected by spectrophotometry
(1↔2)	glyciosidic bond in which each monosaccharide supplies a hemiacetal group to the bond resulting in a non-reducing sugar
A_{510}	absorbance at 510 nanometres
AA	ascorbic acid
AAE	ascorbic acid equivalent
Abs	absorbance
AD	Anno Domini
ANOVA	analysis of variance
AOAC	Association of Analytical Communities
BMI	body mass index
CAD	coronary artery disease
COPD	chronic obstructive pulmonary disease
cyn-3-glu	cyanidin-3-glucoside
DF	dilution factor
DM	dry matter
DP	degree of polymerisation
DPPH•	2,2-diphenyl-1-picrylhydrazyl free radical
EC	Enzyme Commission
ELSD	evaporative light scattering detection

Endl.	Endlicher
ENDO	European Commission project on non-digestible oligosaccharides
F, Fru	fructose
FC	Folin Ciocalteu
FEH	fructan exohydrolase
FFT	fructan:fructan 1-fructosyltransferase
FRAP	ferric reducing antioxidant capacity
FOS	fructooligosaccharide
G, Glu	glucose
GAE	gallic acid equivalent
GC-MS	gas chromatography-mass spectrometry
GF	sucrose
GF_2	1-kestose
GF_3	nystose
GF_4	fructofuranosylnystose
GF_n	general formula for fructooligosaccharides
g FOS kg BW^{-1} day^{-1}	grams of FOS per kilogram of body weight per day
GmBH	Gesellschaft mit beschränkter Haftung
H.	Harold
HAT	hydrogen atom transfer
HOMA-IR	homeostasis model assessment of insulin resistance
HPAE-PAD	high performance anion exchange chromatography with pulsed amperometric detection
HPLC	high performance liquid chromatography
HPLC-UV	high performance liquid chromatography with ultra violet light detection
HPLC-RI	high performance liquid chromatography with refractive index detection
IBS	Irritable Bowel Syndrome
LC-MS	liquid chromatography-mass spectrometry
L mol^{-1} cm^{-1}	litres per mole per centimetre, unit of molar absorptivity
MAC	monomeric anthocyanin concentration
MOA	mode of action

MRSA	methicillin-resistant *Staphylococcus aureus*
MSSA	methicillin-susceptible *Staphylococcus aureus*
NDO	non-digestible oligosaccharide
NZ	New Zealand
ORAC	oxygen radical absorbance capacity
PDA	photodiode array
PD	percentage deviation
Poepp.	Poeppig
RP	relative percentage
RPC	relative percentage change
SCFA	short chain fatty acids
SET	secondary electron transfer
SST	sucrose:sucrose 1-fructosyltransferase
STZ	Streptozotocin
TLC	thin layer chromatography
TPTZ	2, 4, 6-tripyridyl-*s*-triazine
UV-Vis	ultraviolet and visible light

1. Introduction

1.1 Yacon

Yacon (*Smallanthus sonchifolius* Poepp. & Endl.) H. Robinson is a perennial herb native to South America and a distant relative of the sunflower. The storage roots, often incorrectly called tubers, contain high concentrations of fructooligosaccharide (FOS), and have been consumed in the Andes for a number of centuries. The leaves have been used to make tea and as fodder for ruminant animals.[1] Introduced into New Zealand as a garden curiosity in the 1980's, yacon has been established as a commercial crop in this country, for almost ten years. Much of the current interest in yacon was generated by the investigation into the lost crops of the Incas by the National Research Council.[1]

1.2 Taxonomy

Yacon was originally classified as *Polymnia* (Compositae, Heliantheae, subtribe Melanpodinae) by De Candolle in 1831[2]. Yacon is often referred to as *Polymnia sonchifolia* as classified by Poepping and Endlicher in 1845 or *Polymnia edulis*, a synonym attributed by Weddell[2]. Robinson re-established the *Smallanthus* species as morphologically distinct from *Polymnia* in 1978. *Smallanthus* includes only native South American plant species and the current literature refers to yacon mainly as *Smallanthus sonchifolius* or *Polymnia sonchifolia.*[2]

1.3 Common names

In some South American countries yacon has a number of common names associated with its physical characteristics (i.e. watery), whereas other countries have adopted names attributed by researchers or growers.[1]

There are two main native South American languages, both with different names for yacon. The Quechua names are *yacó* and *llakuma* whereas *aricoma* and *aricona* are used in Aymara. In Spanish there are many names including *yacón, jacón, llacón, llamón, arboloco, puhe, jícama jíquima, jíkima, and jiquimílla*. English growers use *yacon, yacon strawberry,* or *leaf cup* whereas in France the name *poir de terre Cochet* is used. The German word is *Erdbirne* and in Italy the name is derived from the original classification of *Polymnia*; *polimnia.*[1]

1.4 Distribution

The most likely origin of *Smallanthus sonchifolius* is on the eastern slopes of the Andes from northern Bolivia to central Peru. It is this area which contains the most diverse variety of clones in the present day.[2] Yacon grows in the Andes at elevations of up to 3,300 metres[1-3] but is successfully grown at sea level in New Zealand and the United States.[2] Flowering of wild yacon has diminished, particularly in Northern Argentina, and even in areas where there is abundant flowering such as Bolivia and Peru there is low seed viability. This is probably due to intensive characteristic selection through cultivation.[2]

1.5 Description

Yacon is a perennial herb which can grow anywhere between 0.7 - 1.5 metres in height.[2] The stems and leaves are pubescent and dark green, often with some purple colouration that is also seen in the storage roots. The leaves are broadly ovate and can be as large as 30 x 20 cm. Each plant grows between 4 - 25 storage roots associated with an extensive fibrous root system and fleshy meristematic buds also known as turiones or rhizophores. Storage roots vary in size depending on the time of year and age of the plant, but can reach up to 25 cm in length and 10 cm in diameter and resemble the NZ kumara (*Ipomea batatas*).[2, 4] The shape is dependent on pressure from surrounding stones or root, ranging from round or oblong to irregular (**Figure 1.1**).

The fructoligosaccharides (FOS) accumulated in the storage roots is utilised as a carbohydrate reserve after the aerial part of the plant dies in winter. They also protect against desiccation in drought and frost conditions, through adjustment of osmotic pressure when FOS is hydrolysed.[5] For maximal FOS content the best time for harvesting is early-mid winter, that is mid-May to early June in New Zealand.[4]

Figure 1. 1 Yacon flower (left) and yacon storage roots (right)

1.6 Historical use of yacon

Evidence of yacon utilisation by Andean people over centuries can be seen by the use of yacon root fibres in Nazcan textiles and images of yacon on clay pots (500 - 1200 AD). Yacon remains have also been found in archaeological sites associated with the Candelaria culture (1 - 1000 AD) in Argentina.[3]

Yacon storage roots are considered a fruit in many areas of South America even though it is well known as a low energy food source and this misconception is due to the preparation of the storage roots[2]. They are often dug up and sun-dried (known as *ckochascca* or *soleado*) before consumption and this increases the concentration of fructose as the FOS degrades.[2-3, 6] Yacon storage roots are a traditional fare on the feast of Corpus Christi in South America and the dried leaves are made into tea or used as animal fodder.[1] The relatively low caloric value of yacon meant that it was not as widely cultivated as other Andean crops, such as the potato (*Solanum tuberosum*), in times of famine.[2]

1.7 Storage root composition

The main component of yacon storage roots is water. The general composition of fresh yacon is shown in **Table 1.1**

Table 1. 1 Constituents of yacon storage root[7]

Mean storage root composition per 100 g fresh matter	
Water	81.30 g
Saccharides	13.80 g
Fibre	0.90 g
Proteins	1.00 g
Lipids	0.10 g
Ash	1.10 g
Mean mineral contents per 100 g fresh matter	
Potassium	334.00 mg
Phosphorus	34.00 mg
Calcium	12.00 mg
Magnesium	8.40 mg
Sodium	0.40 mg
Iron	0.20 mg
Mean vitamin contents per 100 g fresh matter	
Vitamin B_1	0.07 mg
Vitamin B_2	0.31 mg
Vitamin C	5.00 mg
β-carotene	0.13 mg
Polyphenols	203.00 mg

Not all of the carbohydrates are FOS or free sugars, so a percentage of the fresh weight is likely to be cellulose or insoluble fibre. Yacon has high moisture and calcium, moderate iron and low nitrogen and phosphorus content compared with similar root crops such as potato, taro, Jerusalem artichoke and Japanese yam.7

1.7.1 Fructooligosaccharides

Plants of the Asteraceae, Liliaceae, Amaryllidaceae and Gramineae families produce short-chain fructose based carbohydrates known as fructooligosaccharides (FOS).[8] A number of years of research have established FOS as a functional food and a prebiotic[9-13] that is a dietary supplement which will promote the ideal balance of intestinal microflora.[14-15]

1. 7.2 FOS structure and definition

FOS are short chain carbohydrates consisting of a primary sucrose unit (GF) with an (1↔2) glycosidic bond between D-glucopyranose and D-fructofuranose moiety, with *n* attached D-fructofuranosyl moieties linked by β (2→1) bonds, forming a non-reducing sugar,[16] **Figure 1.2**.

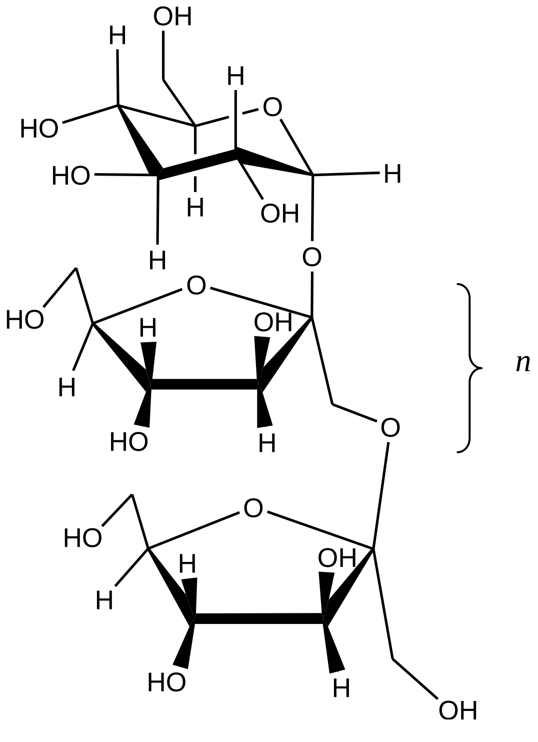


Figure 1. 2 Molecular formula for FOS (n=1-9)

An oligosaccharide is separated in definition from a polysaccharide in that there are a discrete number of attached fructose units to give a defined structure.[17] GF_3 to GF_{10} are generally the range considered as FOS but some literature includes molecules with higher degrees of polymerisation (DP).[14] Inulin is the corresponding polysaccharide (up to DP 70), hence FOS is often referred to as inulin-type oligosaccharide or inulooligosaccharides.[8-9, 18] Throughout the primary research in this study, FOS is the carbohydrate portion in a size exclusion chromatogram that has DP 3 or greater and no upper limit is implied.Other terms that are interchangeable with FOS throughout the literature include oligofructose, fructose-oligosaccharide and oligofructan.

1.7.3 FOS biosynthesis

A vast array of plant species (approximately 36,000) produce inulin including Jerusalem artichoke, bananas, chicory, garlic, leek, onions and wheat[19-20] and all contain FOS in trace amounts.

The average DP of FOS produced by the Jerusalem artichoke is 6 as elucidated by Edelman & Jefford [19] and this is very similar to the FOS found in yacon. Yacon and other plants which produce FOS and inulin have been investigated and the mechanism for biosynthesis of these saccharides is the same.[6, 17] The three main enzymes are sucrose:sucrose 1-fructosyltransferase (SST, *EC* 2.4.1.99), fructan:fructan 1-fructosyltransferase (FFT, *EC* 2.4.1.100) and fructan exohydrolase (FEH, *EC* 3.2.1.80).[18]

The first step is the production of 1-kestose (GF_2) by SST through the transfer of a fructose from one sucrose molecule to another, **Equation 1.1**. The irreversible, enzyme-mediated pathway forms β (2→1) bonds and does not synthesise higher DP molecules.

$$\mathrm{GF} + \mathrm{GF} \rightarrow \mathrm{GF}_2 + \mathrm{G} \qquad \textbf{1.1}$$

Higher DP FOS is produced when FFT reversibly transfers fructose from one FOS molecule ($GF\text{-}F_n$) to sucrose (GF) or another FOS molecule ($GF\text{-}F_m$). β (2→1) bonds are formed between the fructose molecules resulting in a linear chain configuration, **Equation 1.2**.

$$\text{GF-F}_n + \text{GF-F}_m \rightleftharpoons \text{GF-F}_{n-1} + \text{GF-F}_{m+1} \qquad \textbf{1.2}$$

FOS molecules can also be produced by the successive hydrolysis of the terminal fructose residue of inulin or higher DP FOS, **Equation 1.3**, by fructan hydrolase (FEH).

$$\text{GF}_n \rightarrow \text{GF}_{n-1} + \text{F} \qquad \textbf{1.3}$$

It is FEH action that allows FOS, which has been stored, to be utilised as a carbohydrate store and allows for adjustment of osmotic pressure in yacon storage roots. The rapid enzymatic depolymerisation of FOS results in a large number of small carbohydrate molecules which promote the absorption of water, preventing desiccation in extreme temperature conditions.[18]

1.7.4 Industrial sources of FOS

There are two current industrial sources of FOS, extraction of inulin from plant sources followed by hydrolysis and enzymatic synthesis utilising fungal and bacterial transferases. FOS syrups such as RAFTILOSE® are produced by the hydrolysis of chicory inulin *via* a mould endo-inulase enzyme[21] or through acid hydrolysis[22] and powdered FOS such as NeoSugar®, can be obtained by spray drying the syrup.[23] The enzymatic method produces an average DP 4 FOS with about 5 % mono- and disaccharide content.[23] Oligofructase (Beldem, Belgium), a commercial endo-inulase was employed by Ronkart *et al.*[24] for hydrolysis of globe artichoke inulin and only GF_2-GF_4 FOS molecules were produced. Acid hydrolysis is pH and temperature dependent and less specific than enzymatic hydrolysis producing a higher average DP FOS but lower total FOS yield and high fructose content.[22]

Products such as Neosugar® are produced by enzymatic synthesis. Fructosyltransferase isolated from *Aspergillus* spp. resulted in yields between 60-90 %[25- 28] with higher yields in an airlift reactor, particularly with *Aspergillus niger*.[27] The product of fungal enzyme synthesis is primarily 1-kestose (GF_2). Chiang *et al.*[29] were able to immobilise β-fructofuranosidases from two *Aspergillus* species on to methacrylamide beads and achieved a yield of 60 %, which is comparable to more conventional enzymatic synthesis. The concept of immobilisation was expanded upon by Chien, Lee and Lin[30] who encapsulated *Aspergillus japonicus* cells in gluten and utilised a column reactor thereby achieving a yield of 61 % at a rate of 173 g FOS per hour. *Aspergillus japonicus* immobilised in calcium alginate yielded 61.28 %.[31] A solid support consisting of agricultural residues, which also acts as a nutrient source, using *Aspergillus japonicus* shows some promise; 128 g L^{-1} FOS was produced with coffee silverskin[*] as a support.[32]

Bacterial enzymes produce lower and more variable yields of FOS (between 23-56 %) but this may be dependent on the species investigated to date. *Rhodotorula* sp. was trialled as a source of β-fructofuranosidase and the FOS produced was successfully separated from monosaccharides using activated charcoal, fixed-bed columns (80 % and 97 % separation from glucose and sucrose respectively).[33] Cycloinulooligosaccharide fructanotransferase isolated from *Bacillus circulans*[34] produced higher DP products, primarily GF_5.

The byproduct of industrial processes, spent osmotic sugar solution has been utilised by Aachary and Prapulla[35] to synthesise FOS *via* transfructosylation with *Aspergillus oryzae* fructosyl transferase. The FOS produced this way was also reported to be golden brown in colour and had a more appealing appearance than FOS produced directly from sucrose.

---

[*] Silverskin is the thin membrane between the coffee bean and the pulp of the coffee cherry. It is a waste product of coffee bean roasting

1.7.5 Health effects associated with the consumption of FOS

A majority of the research into the health benefits of FOS has been focused on its use as a prebiotic. A prebiotic is classified as a substance which is less than 90 % digestible by the human alimentary tract, allowing it to be available selectively to probiotic bacteria in the colon.[14- 16, 36] FOS has proved to be a more effective prebiotic than the polysaccharide inulin[13,37-39], shown by preferential fermentation of lower DP FOS molecules.

Probiotic bacteria are purported to benefit an animal host by improving the microbial balance of the intestine.[16] This “balance” is the ratio of different microbial genera that consistently inhabit different niches of the bowel in mammalian hosts, allowing for optimal function of the colon. Modern diet and lifestyle can increase the numbers of deleterious bacteria such as *Clostridium* and sulphate-reducing species increasing the risk of cancer, ulcerative colitis and inflammatory disease.[11] Bengmark[40] notes that antibiotics are being over prescribed, not only leading to resistant strains of pathogenic bacteria but a reduction in the numbers of protective microflora, such as *Bifidobacterium* and *Lactobacillus* in the gut. The occurrence of antibiotic–associated diarrhoea due to *Clostridium difficile* infection is prevented by the pH regulation of short chain fatty acids produced by FOS fermentation by *Bifidobacterium* spp.[40]

Both probiotic bacterial species and use of prebiotics have been shown to protect against *Salmonella* infections in chicken.[41] Evidence for the probiotic properties of *Bifidobacterium* spp. also comes from studies into the microflora of breast-fed versus formula-fed babies. *Bifidobacterium* is the main bacterial genus in breast-fed infants with less than 1 % enterobacteria present in the colon. In formula-fed babies the bacterial population is much more complex and is associated with a higher degree of infection and possibly reduced immunity in these infants.[39, 42-43]

In the late 1990s the ENDO project (DGXII AIRII-CT94-1095) was funded by the European commission to establish a consensus on the status of non-digestible oligosaccharides (NDO) as functional foods. A number of experts in the field of pro- and prebiotics collaborated on the project and established NDO as functional foods, with FOS being the most widely studied and accepted, as prebiotics.[14, 39]

There are two main reasons that prebiotic supplementation is preferable to consuming live probiotics alone. There is a loss of live bacteria through the course of packaging capsules and the acid conditions of digestion. Only a small percentage of the original probiotic bacterial count will actually make it to the colon. The other problem is that probiotic supplementation without prebiotic to feed the existing and introduced probiotic bacteria results in competition between the species. This competition means that there is no increase in total probiotic count.[42] Delivery of prebiotics suffers minimal breakdown through digestion and provides fuel for the existing (or associated if a probiotic species is delivered with a prebiotic) probiotic population. Long term supplementation with prebiotics will lead to a healthy, sustained population of probiotic microflora.[16, 44]

The use of yacon extract as a prebiotic has been investigated by a number of researchers. Bibas Bonet *et al*.[36] investigated the effect of yacon flour on mice and reported that initially bifidobacteria count doubled and lactobacilli increased significantly. By the end of the 75 day study however the bifiobacteria count had dropped to the same level as that present in control animals, but the lactobacilli counts remained raised. After 60 days, even though the bifidobacteria count was reduced, the enterobacteria (pathogenic) bacterial count was significantly less in the gastrointestinal tract of mice fed yacon flour. The health benefits of probiotic bacteria are summarised in **Table 1.2**

Table 1.2 Health benefits associated with the probiotic effect. *Studies carried out in vivo* are referenced in bold

Health benefit	**Mechanism of action (MOA)**
Improvement of lactose malabsorption**[45]**	Probiotic bacteria produce β-galctosidase to compensate for inactive human lactase
Prevention of intestinal infectio**[46]**	Possible MOA include: • Nutrient competition • Production of antimicrobial secretions • Adhesion site blockage • Immune stimulation**[36, 47, 48,49]** • Suppression of toxin production
Suppression of cancer[14, 50, 51]	*Lactobacillus* and *Bifidobacteria* effective in blocking ulcer and polyp formation with possible MOA: • Binding, blocking or removal of carcinogen/procarcinogen from diet[52] • Suppression of the bacteria which produce enzymes that convert procarcinogens to carcinogens • Stimulation of the immune system[52]
Digestive aid [42, 44]	• An established probiotic colony can utilise undigested nutrients
Reduced risk of kidney stone formation**[53,]**	• Some *Lactobacillus* and *Bifidobacteria* spp. utilise dietary oxalate in the intestinal tract reducing the urinary concentrations which contribute to the formation of kidney stones

Table 1.2 Health benefits associated with the probiotic effect. *In vivo* are referenced in bold

Health benefit	Mechanism of action (MOA)
Decrease in the incidence of heart disease [14, 44]	Potential MOA: • Reduction of intestinal cholesterol absorption • Direct utilisation of cholesterol • Bacterial metabolites may affect blood lipid levels systemically[52]
Immune stimulation[20]	• Increased γ-interferon levels • Stimulation of local and systemic antibody production • Enhanced macrophage activity • Increased concentration of natural killer cells
Reduced Irritable Bowel Syndrome (IBS)[20]	• The biofilm barrier protecting the epithelium is fortified by probiotic bacteria • The production of pro- and anti-inflammatory cytokines by probiotic bacteria can help stabilise the inflammation associated with IBS
Nutritional effects*	• Mineral absorption particularly iron, calcium and magnesium[**54**, 55, **56**, **57**, **58**, **59**] • Short chain fatty acid (SCFA) production[16, 44, **58**]

*Sakai *et al.*[57] found FOS reduced post-gastrectomy (removal of part of the stomach) anaemia in rats by was prevented by increasing iron absorption by the gut.

In vitro and *in vivo* studies have shown that FOS is available as a food source selectively for *Bifidobacteria* and *Lactobacillus* species[9, 14, 36-39, 60- 61] and this can be contrasted with lactulose, lactose and other short chain oligosaccharides which are available to a wide range of microflora.[44] The β (2→1) bonds are not cleaved by mammalian digestive enzymes, leaving FOS molecules intact for fermentation by those colonic bacteria that are able to do so with their β-fructosidase enzyme. There have been genetic studies to identify the operon associated with FOS utilisation in *Lactobacillus paracasei*[62] and *Lactobacillus plantarum.*[63] *Lactobacillus* spp. are beneficial because they utilise ammonia and amines that could otherwise be toxic to the host.[43] The bifidobacteria; *B. longum, B. infantitis, B. adolescentis, B. breve, B. cantenulum* and *B. pseudocatenulatum* all preferentially metabolised FOS over lactose and inulin.[39, 60] As well as being prebiotic NDO such as FOS, also act as soluble dietary fibre which can lead to regulation of bowel habit i.e. daily defaecation. The consensus from the ENDO project was that a 1.5-2 g increase in faecal bulk per gram of NDO consumed was a consistent result over a number of studies.[14]

1.8 Medicinal properties of yacon

1.8.1 Antidiabetic properties

Yacon storage roots are commonly consumed in Bolivia by diabetics and people with kidney disease. Habib *et al*.[64] investigated treatment of STZ-induced diabetic mice with yacon flour (made from dried storage roots). Blood glucose levels did not change even though small clusters of insulin positive pancreatic cells were formed in the treated rats. However blood lipid levels (triacylglycerol and low density lipoprotein) decreased and glucagon-like peptide levels increased. To date the only reported human clinical trial into the health benefits of yacon syrup with consideration of antidiabetic effect is by Genta *et al.*[65] A study was undertaken in which obese women took a daily dose of 0.14 g FOS kg body weight $(BW)^{-1}$ day^{-1} for three months and they achieved very significant results in terms of weight loss, body mass index improvement, waist circumference decrease and satiation after eating.

Satiety is a feature of the low glycaemic index (slow release of energy) of FOS, which has been found to be associated with a healthy blood lipid profile, better base levels of glucose and insulin and a lower demand for insulin after eating.[65] The significant loss in abdominal fat (seen as decrease of waist circumference in **Table 1.3**) is thought to be due to FOS modulation of three specific proteins (glucagon-like protein, glucose-dependent insulinotropic protein and gherlin) which suppress glucagon secretion, delay stomach emptying and regulate food intake respectively. The dose was chosen as it is well tolerated by people in terms of reduced side-effects such as diarrhoea and flatulence.[65] Since this was a double blind study, and the main difference was yacon syrup in the diet, it can be concluded that yacon was effective, that is unless the results are biased by one or two people in the yacon group having excessive weight loss. An initial group of 20 women receiving 0.29 g FOS kg BW^{-1} day^{-1} discontinued in the study due to uncomfortable side-effects leaving 35 women in the study (the numbers in treatment and cont. Markers for insulin resistance of fasting glucose levels and homeostasis model assessment of insulin resistance (HOMA-IR) were raised in all women before the study but reduced to normal and near normal levels after 120 days.[65]

Table 1. 3 Clinical results from Genta *et al*.[58] The difference between women taking yacon and control syrups is noteworthy. Yacon syrup group received a dose of 0.14 g FOS kg BW-1day-1.

	Yacon syrup		**Placebo**	
	Pre-treatment	**120 days**	**Pre-treatment**	**120 days**
Mean body weight, kg	91.2 ± 8.4	76.2 ± 6.1[a]	90.7 ± 10.3	92.3 ± 10.1
Mean BMI, kg m^{-2}	34 ± 2	28 ± 3[a]	33 ± 3	32 ± 4
Mean waist circumference, cm	105.1 ± 5.0	95.2 ± 4.8[a]	101.4 ± 3.1	101.9 ± 2.4
Mean frequencies of defecation, time day^{-1}	0.28 ± 0.08	0.99 ± 0.05[a]	0.30 ± 0.10	0.30 ± 0.10

[a] Significant difference using paired Student's *t* test between pre-treatment and experimental values, $p < 0.05$.

This study agrees with other studies that show FOS (from non-yacon sources) reduces the blood glucose levels of diabetic patients but without effect on non-diabetics. It is important to note that the recommended daily dose[11, 66] of FOS for prebiotic effect is 5 g day^{-1} whereas these results were obtained at mean initial doses of 12.8 g day^{-1} down to 10.8 g day^{-1} by the end of the study. The other use of yacon syrup contemplated through the results of this study is an appetite suppressant because many of the current appetite suppressants on the market e.g. sibutramine and phentermine have risks and adverse side-effects which outweigh the benefits for some patients.[65] In a recent review of toxicity of traditional South American medicinal plants[67] aqueous and hexane yacon storage root extract proved to be non-toxic in a conventional toxicity test on shrimp.

In Argentina, the dried leaves are made into a tea as a treatment for diabetes.[68] In a study by Aybar *et al.*[68] when diabetic rats drank 2 % yacon leaf tea instead of water for thirty days, a significant improvement in diabetic and renal symptoms was observed, **Figure 1.3**.

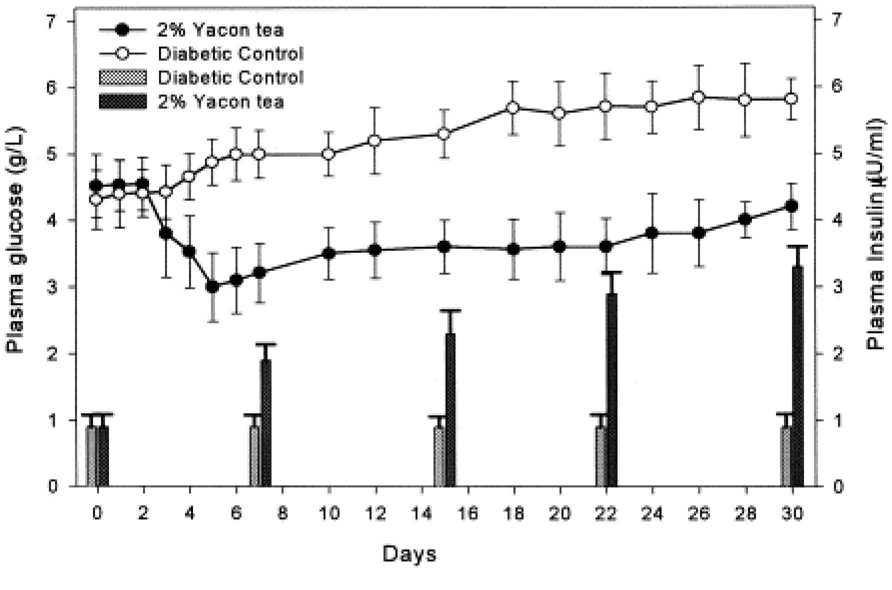


Figure 1. 3 Plasma glucose and plasma insulin results from Aybar *et al.*[68]

Plasma glucose is the upper scatter plot where it can be seen that mice drinking 2 % yacon tea have reduced values compared with control diabetic mice. The plasma insulin in the lower bar chart significantly increases over time with yacon tea compared to the constant value in control mice.

Dou *et al.*[69] tested yacon leaf extract on alloxan-induced diabetic mice.
100 mg kg^{-1} day^{-1} showed a significant decrease in plasma glucose compared with control at 95 % significance, while a dose of 200 mg kg^{-1} day^{-1} showed a significant decrease in blood glucose at a 99 % level of significance. In 2008, Dou *et al.*[70] characterised 4 novel diterpenes as well as finding five already characterised molecules from yacon leaf extract, but the individual components were not tested in the 2010 trial.

Genta *et al.*[71] tested six different components (chlorogenic, caffeic and three dicaffeicoilquinic acids, plus the sesquiterpene lactone enhydrin) of their yacon leaf extract (in methanol, butanol and chloroform) and all extracts were significantly effective in decreasing the hyperglycaemic peak after meals in diabetic rats as well as increasing plasma insulin levels after 8 weeks of administration. Work by Narita & Inouye[72] shows that the chlorogenic and caffeic acids isolated from yacon leaf have an inhibitory affect on pancreatic α-amylase enzymes effectively reducing blood glucose levels by reducing the hydrolysis of carbohydrates such as starch and amylose. A recent toxicity assessment on yacon leaf extract[73] reported renal damage in rats that had been treated with yacon leaf extract. The authors attribute the toxicity to the sesquiterpene lactone component of the leaf. This finding suggests that the best course of action is extraction of the less toxic therapeutic compounds from the leaf.

1.8.2 Antioxidant properties

Antioxidants prevent the oxidation of biological tissues and are indicated in protection against inflammatory diseases such as cardiovascular disease,[74-82] some cancers,[74-75, 80, 83-84] rheumatoid arthritis,[74-75] type 2 diabetes,[74, 76, 85-86] liver disease[84, 87-88] and chronic obstructive pulmonary disease (COPD).[74]

Chlorogenic and caffeic acids are not only responsible for the antidiabetic properties of yacon, they have also been shown to have antioxidant properties. Park *et al.*[89] found that chlorogenic acid was more effective at free radical scavenging than vitamin E. Caffeic acids appeared to exhibit antioxidant properties by dose-dependently inhibiting the production of nitrous oxide.

Polyphenols are antioxidant compounds found in fruit and vegetables, derived from phenylalanine or shikimic acid *via* the plant shikimic acid pathway.[74]
Many of the studies into protective effects of antioxidants have focussed on total polyphenol intake or specifically on a class of polyphenols such as anthocyanins.

1.8.3 Antibacterial effects

Joung *et al.*[90] discovered that a *n*-hexane extract of yacon leaf exposed to 4000 lux light for 18 h was active against six strains of methicillin-resistant *Staphlyococcus aureus* (MRSA) and a strain of methicillin-susceptible *S. aureus* (MSSA). There was a synergistic antibiotic effect when the light treated yacon extract was mixed with conventional antibiotic drugs. In a review on the antimicrobial activity of medicinal plants, Rios & Recio[91] identified phenols as the principle antibacterial components (especially against Gram positive bacteria) in the yacon extract, whereas all the antimicrobial substances reported to date, extracted from yacon storage roots and leaves have been melampolide-type sesquiterpene lactones and *ent*-kaurane derivatives without aromatic moieties.[92-93]

1.8.4 Anticancer effects

Pilot studies are being conducted into the effectiveness of components of yacon leaf extract on human cancer cell lines. Siriwan, Naruse & Tamura[94] found that three hexane extracts of yacon sesquiterpene lactones successfully induced significant levels of apoptosis in cervical cancer cells. A synergistic effect of these compounds with enhydrin (present in the leaf) was also reported.

1.9 Methods of extraction and analysis of FOS

There are a number of FOS extraction and analysis methods described throughout the literature. The greatest variation is seen in extraction methods. The aim of extraction is to halt the action of enzymes which hydrolyse carbohydrates while concentrating water soluble FOS, sucrose and glucose in the extraction solvent.

Wong[4] compared water extraction of freeze dried yacon with methanol and ethanol (70 % v/v; each with a final water extraction cycle) extraction methods *via* HPLC with refractive index detection (HPLC-RI). Dark green discolouration occurred in the water extractions indicating that polyphenol oxidase was not inhibited. The mean FOS concentrations of water extraction were significantly lower than methods utilising organic solvents. This is also supported by studies comparing oligosaccharide extraction with different ethanol concentrations[95-96] including the finding that mid range DP (448 - 500 amu) molecules are extracted at higher ethanol percentages.[95] Muir *et al.*[97] were unable to detect any FOS in bananas with a hot water extraction and analysis with HPLC-ELSD, however water only extractions carried out with enzyme assays[98] and HPAE-PAD[99] detection have obtained similar results to organic solvent extractions of the same plant material.[100]

Wong[4] found that methanol extraction was not as effective as ethanol whereas Downes & Terry[101] found the reverse to be true for onion powder. This may be because of the different structure of the plant, with methanol being a superior solvent for fleshy tissue and ethanol better for fibrous tissue. Studies into the concentration of FOS in yacon, onions, burdock, wheat and grapes involving a single 5 – 10 minute extraction cycle in 80 % aqueous ethanol[6, 102-103] were carried out on fresh tissue whereas other studies on a range of plant tissues employed multiple ethanol or both water and ethanol (70-99 % v/v) extraction cycles on fresh or freeze-dried tissue.[96, 101, 104-107] A direct comparison of different FOS extraction methods with a range of detection methods on identical samples has not been carried out to date. The ENDO recommendation for FOS separation from polysaccharides is extraction in 80 % aqueous ethanol for 30 minutes at 0 °C with enzymatic assay or HPAE-PAD for analysis.[14] The extraction method lacks a water extraction step to maximise the yield of water soluble carbohydrates[96] as do some high temperature methods reported.[105, 108-109]

Early studies used the spectrophotometric phenol-H_2SO_4 method to measure the concentrations of FOS separated by TLC.[102, 110] Isocratic and gradient HPAE-PAD detection with different NaOH:sodium acetate eluent mixtures have been utilized[6, 90, 99-100, 103-104, 107] and the detection limit of this technique is reported as $\mu g\ g^{-1}$. Commercial FOS standards are not readily available resulting in difficulty identifying and quantifying FOS peaks with this method, although Fukai *et al.*[6] were able to derive a linear relationship between response factor and DP. Enzyme assays were utilised in a number of studies, where the concentrations of fructose produced by hydrolysis of the higher molecular weight fraction of the extract, were compared with relative sucrose and glucose concentrations.[96-98, 111] HPLC-RI was favoured by studies into plants with high FOS concentrations.[106, 108] The limit of detection for FOS is higher with this detection method, but the isocratic water mobile phase means it is a very inexpensive technique and juice samples do not require extraction or derivatisation before analysis. Also the time required for HPLC-RI is half that required for HPAE-PAD[112] so sample throughput is faster. The extraction method of Jaime *et al.*[106] was modified by Wong[4] for the purpose of sugar extraction of yacon storage roots and this method has been used for a number of years for sugar analysis of yacon storage roots and juice at the University of Waikato.

1.10 Stability of FOS

1.10.1 Heat

A number of storage trials have been performed on FOS producing fruit and vegetables. Some studies reported that low temperatures resulted in increased activity of the FEH enzyme and associated decrease in FOS content.[98 105, 108] The optimal storage temperature appears to be 10 °C with a number of studies reporting the lowest rates of FOS degradation at this temperature. Wong[4] reported that FOS reached undetectable levels in yacon after 8 weeks of storage at 5 °C and Benkeblia *et al*.[104] found that FOS degradation in onions was faster at 15 °C and room temperature. Benkeblia *et al*.[104] also reported that the higher DP FOS molecules were preferentially hydrolysed but this was found to be species dependent in another study.[106] FOS accumulation in ripening bananas was also more effective at 10 °C (compared with 19 °C).[100] It appears that plants of different genera have metabolic enzymes operating at different temperatures. Burdock (*Arctium lappa* L.) roots steadily accumulated FOS over 12 weeks at 10 °C, with final concentrations of 2.7 to 3 times the intial concentrations.[105, 108] The effect of higher temperatures was investigated when yacon root flour was made by heating storage roots at 60 °C for 24-48 hours for a study by Bibas Bonet *et al.*[36] A significant decrease in DP 3-5 and DP 7 FOS molecules and doubling of both the fructose and glucose content was observed in the flour in comparison with fresh storage roots (**Figure 1.4**).

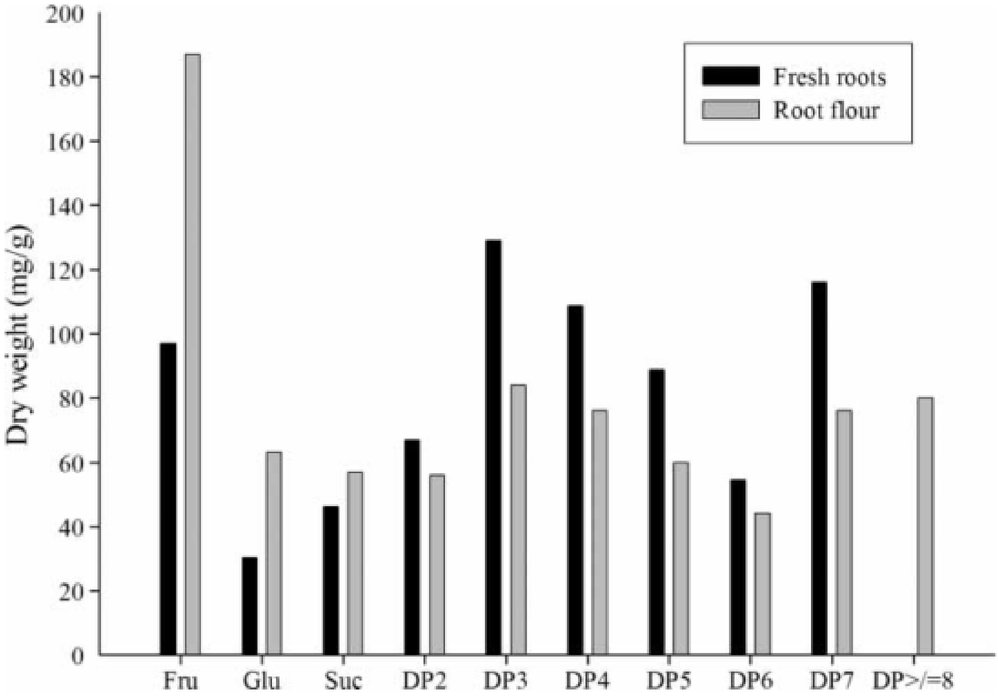


Figure 1. 4 Changes in DP of FOS with heating at 60 °C for 24-48 hours during the production of yacon flour[36]

1.10.2 Acid

The acid hydrolysis of inulin derived FOS was investigated by Blecker *et al.*[113] and the rate of fructose production was found to be directly proportional to hydrogen ion concentration [H^+]. L'homme *et al.*[114] compared rates of hydrolysis at pH 4, 7 and 9 and found that the higher the pH, the slower the hydrolysis of FOS.

1.11 Bioactive components of blackcurrant juice

The main bioactive components of blackcurrant juice are antioxidants, primarily from the wide class of compounds classified as polyphenols. Polyphenols are a large class of antioxidants which have been shown to inhibit lipid peroxidation and protect low-density lipoproteins (the beneficial form of cholesterol) from oxidation *in vitro*. *In vitro* studies have also shown that polyphenols can also prevent platelet aggregation and increase vasodilation, thus providing protection against cardiovascular disease. A number of epidemiological studies comparing normal dietary intake of polyphenols and disease have been conducted with showing a possible preventative effect in coronary artery disease (CAD),[74, 79] stroke,[115-116] rectal cancer[74] and asthma.[115] Case control and prospective studies on specific lignans (a type of polyphenol) also indicate a possible protective effect against CAD[117-118] and breast,[119-120] endometrial[121] and ovarian[122] cancers. These effects may not be due to the antioxidant properties alone and that is why it is important to measure polyphenol concentrations as well as determining antioxidant capacities.[89] The most common method of polyphenol analysis reported for blackcurrant juices is the Folin-Ciocalteu (FC) assay (described in full in **Section 2.5.1**) although the extraction methods, reagent ratios and choice of λ_{max} wavelength varies between studies (**Table 1.2**). The FC assay reports a general polyphenol concentration in gallic acid equivalents (GAE) because the response of a wide range of polyphenols is relatively close to that of the gallic acid standards.[123] The FC method is vulnerable to interference by reducing sugars. Fukushima *et al.*[124] utilised reversed phase chromatography to separate the polyphenol fraction from the sugars in green tea and coffee, and this might be utilised with blackcurrant juice. HPLC with UV detection has also been employed for more detailed analysis of polyphenol content. Heinonen *et al.*[125] reported almost double the concentration of blackcurrant polyphenols detected by HPLC, compared with FC assay, mostly due to anthocyanin content.

Table 1. 4 Folin-Ciocalteu methods reported for analysis of polyphenol content of blackcurrant berries and juice

Reference	Extraction method	Analysis method	Results
Da Silva Pinto *et al.*[126]	5 g fruit homogenised in 100 mL distilled water	0.5 mL extract , 0.5 mL 95 % ethanol, 5 mL distilled water, 0.5 mL 50 % (v/v) FC reagent and 1 mL sodium carbonate	13.5 GAE mg g^{-1} fresh weight Absorbance read at 725 nm
Bakowska-Barczak & Kolodziejczyk[127]	10 g frozen berries extracted in two cycles of 70 mL 80 % methanol (0.1% formic acid)	1 mL extract, 1 mL FC reagent and 10 mL sodium carbonate solution, made up to 100 mL with deionised water	6.24 - 7.42 GAE mg g^{-1} Absorbance read at 765 nm
Moyer *et al.*[128]	Homogenised fruit extracted in acetone and then two cycles of 70:30 acetone/water (v/v)	200 µL 1:500 or 1:1000 diluted extract, 200 µL diluted FC reagent and 2 mL sodium carbonate, made up to 20 mL with distilled water	4.98 - 8.15 GAE mg g^{-1}
Attonen & Karjalainen[129]			6.67 - 8.00 GAE mg g^{-1} Absorbance read at 765 nm
Benvenuti *et al.*[130]	20 g frozen berries soaked in 20 mL methanol/2 % HCl (95:5 v/v) for 1 hour before homogenisation and another extraction cycle for filtrate	1 mL extract, 5 mL FC reagent and 10 mL sodium carbonate made up to 100 mL with deionised water	5.31 - 8.89 mg g^{-1} GAE fresh weight Absorbance read at 750 nm
Mitic *et al.*[131]	10 mL juice sonicated with 25 mL 1 % HCl in methanol	1 mL of 1:5 extract/methanol (v/v), 0.5 mL 1:2 FC reagent/water (v/v) and 2 mL sodium carbonate	512.73 mg L^{-1} GAE Absorbance read at 765 nm

The red colour of blackcurrant juice is due to a class of polyphenols called anthocyanins (**Figures 1.4**). In acidic conditions the sugar moieties are cleaved at C_3 to give anthocyanidins (**Figure 1.5**) Anthocyanidins are present in different forms at different pH. At acid pH the predominant form is the flavylium cation in oxonium form and the conjugated system of this cation gives rise to the red colour seen in the juice (pH 2.7).

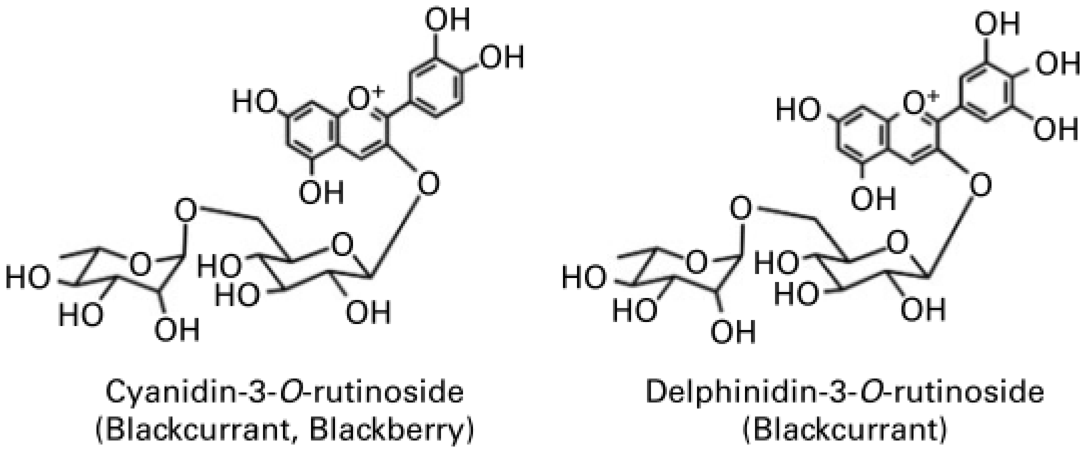
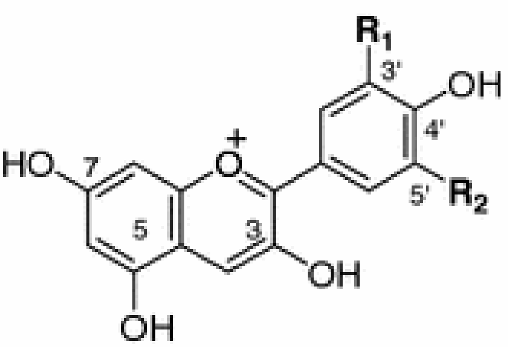


Figure 1. 5 Structures of predominant anthocyanins in blackcurrants[132]



R1	R2	Anthocyanidin
H		Pelargonidin
OH	H	Cyanidin
OH	OH	Delphinidin
OH	OCH3	Petunidin
OCH3	H	Peonidin
OCH3	OCH3	Malvidin

Figure 1. 6 General structure of anthocyanidins[132]

The majority of studies reporting blackcurrant anthocyanin concentrations analysed whole fruit, reporting results in mg of cyn-3-glu per mass of fruit.

Table 1. 2 Published blackcurrant anthocyanin concentrations

Reference	Anthocyanin concentration
Bakowska-Barczak & Kolodziejczyk [127]	1.97 - 3.94 mg g^{-1} fresh weight
Bordonaba & Terry [133]	3.49 - 9.24 mg g^{-1} dry matter
Kahkonen *et al.*[134]	2.14 - 2.36 mg g-1 fresh weight
Benvenuti *et al.*[130]	1.53 - 2.81 mg g^{-1} fresh weight
Mitic *et al.*[131]	757.36 - 920.92 mg L^{-1} juice

Kahkonen *et al.*[134] detected a total of 2356 mg kg^{-1} (2.356 mg g^{-1}) of anthocyanins with reversed phase HPLC and photodiode array detection compared with 2137 mg kg^{-1} (2.137 mg g^{-1}) by the pH differential spectrophotometry method. It is not possible to classify one anthocyanin as predominant in blackcurrant juice to replace cyanidin-3-glucoside as the reference compound. This is because anthocyanin content varies between species and with growing, harvesting and processing conditions although rutinosides are present in greater concentrations than glucosides.[127]

2. Experimental

2.1. Materials

MilliQ water (resistivity >17 MΩ) was sourced from a Barnstead E-Pure water filter. Absolute ethanol (Univar) was used for all FOS extractions. Analytical grade sucrose and D-(-)-fructose from Aldrich and D-(+)-glucose (BDH) were used as HPLC standards.

For anthocyanin analysis the 0.025 M potassium chloride buffer (pH 1), 0.4 M sodium acetate buffer (pH 4.5) and bisulfite solution (2×10^{-4} g L^{-1}) were made to the specifications outlined by Guisti & Wrolstad[135] using KCl (Sigma-Aldrich), concentrated HCl (Univar), MilliQ deionised water, sodium acetate trihydrate (Sigma-Aldrich) and $K_2S_2O_5$ (≥ 98 %; Sigma-Aldrich).

25 % Folin-Ciocalteu reagent (Sigma Aldrich) and concentrated sodium carbonate (ReagentPlus® ≥ 99 %; Sigma-Aldrich) solutions, made up with MilliQ deionised water, were used for polyphenol assays. The sodium carbonate solution was made by boiling sodium carbonate (200 g) in MilliQ water (800 mL). A few crystals of sodium carbonate were added to the cooled solution and it was allowed to stand at room temperature for 24 hours. After filtration through Whatman No. 1 filter paper the solution was made up to 1 L with MilliQ water and stored in a Schott bottle for use as needed; this solution remains stable at room temperature indefinitely.[123]

For antioxidant assays working ferric reducing antioxidant power (FRAP) reagent was a 10:1:1 ratio of 300 mM acetate buffer, pH 3.6 [sodium acetate trihydrate (Sigma-Aldrich) and glacial acetic acid (Univar)]: 10 mM 2, 4, 6-tripyridyl-*s*-triazine (TPTZ; Fluka) in 40 mM HCl (Univar): 20 mM $FeCl_3.6H_2O$ (Sigma-Aldrich). Fresh reagent was prepared when required. 2, 2-diphenyl-1-picrylhydrazyl free radical (DPPH•) was obtained from Aldrich and reaction solutions were made up with HPLC grade methanol (Ajax). Aqueous solutions of

$FeSO_4.7H_2O$ (Sigma-Aldrich) and ascorbic acid (Supelco) were made with MilliQ water as standards for ferric reducing antioxidant power (FRAP) analysis.

Aqueous solutions of ascorbic acid supplied by Sigma Aldrich, were used to produce a calibration curve to give ascorbic acid equivalent (AAE) values for antioxidant activity of the samples *via* DPPH• assay

2.2 General methods

Storage root samples were extracted in 7 mL Supelco glass vials with Teflon lined screw tops. Extracts were filtered with Millex®-HN 13 mm, 0.45 µm syringe filters (Millipore). Heating for extraction and kinetic studies was carried out with a Labnet AccuBlock Digital Dry Bath heating block. All samples were centrifuged before HPLC and spectrophotometry (Eppendorf centrifuge 5702/Heraeus Sepatech Medifuge). Extract solutions were concentrated under reduced pressure using an Eyela rotary evaporator at 40 °C. Fresh storage roots were freeze dried in a Labconco Freezone Plus Six freeze dry system with bulk tray drier.

2.2.1 Sample preparation

Cuisine Resources Ltd. (Pukekohe, NZ) vacuum sealed between four and six cleaned roots of varying size from individual plants in semi-porous plastic polymer. The same numbers of roots from each plant were stored unpackaged in cardboard boxes. The roots were sampled over a number of weeks to determine the FOS, sucrose, glucose and fructose levels. The levels of sugars between packaged and unpackaged were compared to determine if any protective effect could be attributed to the use of the packaging. Roots were peeled and thinly sliced. Single layers of slices were frozen with liquid nitrogen on aluminium foil prior to freeze-drying. The operating pressure of the freeze drier condenser was approximately 58×10^{-3} mbar and the temperature was -78 °C. Dried samples were crushed with a mortar and pestle to form a fine powder, and handling was kept to a minimum to avoid moisture uptake. All homogenised samples were placed in sealed plastic bags and stored at -20 °C until required for extraction.

Individual blackcurrant juice and yacon syrup mixtures were packaged in 150 mL pouches and stored in the dark at two different temperature conditions; room temperature and 5 °C. At monthly intervals for a total of three months, a 20 g subsample was collected and stored at -20 °C until required for extraction and analysis. The monthly results were for comparison with the results obtained from freshly packed juice mixtures to assess the stability of FOS over time in the juice mixtures. The storage trial was ceased when the serving per pouch was found to be between 1.8-3.4 g which was significantly lower than the calculated 5 g per serving. A kinetic study of the changes in sugar composition over time at high temperatures was conducted. 1.0 ± 0.1 mL aliquots of juice were heated at 135 ± 5 °C for up to 5 min with sampling every 15 s and at 95 ± 5 °C for up to 30 mins with sampling every 30 s. On removal from the heating block the vial was immediately immersed in an ice bath to prevent further decomposition.

2.3 FOS Methods

The extraction method of Jaime *et al.*[106] was modified by Wong[4] for the purpose of sugar extraction of yacon storage roots and this method has been used for a number of years for sugar analysis of yacon storage roots and juice at the University of Waikato. This method has been shown to avoid enzymatic degradation of the sugars as well having low variability.[4]

Samples of ground yacon or freeze-dried beverage (0.1000 ± 0.0050 g) were mixed with 70 % (v/v) ethanol/MilliQ water (5.0 ± 0.5 mL) in sealed glass vials and immediately placed in a heating block (100 ± 5 °C). The samples were incubated for ten minutes with shaking every five minutes. After incubation samples were immediately centrifuged (3000 x *g*, 15 min) and the supernatant from each vial was collected into 100 mL round-bottom flasks. The pellet was mixed with 70 % (v/v) ethanol/MilliQ water (5.0 ± 0.5 mL) and the extraction procedure was repeated for the 2nd-5th extraction steps. The 6th extraction was with MilliQ water (5.0 ± 0.5 mL; 80 ± 5 °C). The pooled supernatant for each sample was evaporated to dryness. The samples were taken to complete dryness in the freeze-drier overnight. The dried extracts were made up to 5.0 ± 0.5 mL with MilliQ water and care was taken to rinse the entire surface of the flask.
The extracts were filtered into glass vials and stored frozen until analysis.

Sample extracts were thawed to room temperature before manual injection onto the HPLC. Each replicate sample extract was analysed once.

The juice samples did not require extraction, however it was necessary to centrifuge samples and inject supernatant to avoid accumulation of potassium carbonate (used to adjust pH of the juice) in the columns. Analysis of yacon storage root extract and blackcurrant-yacon juice was performed using a high performance liquid chromatography (HPLC) system with size exclusion columns (Shodex KS-801 and KS-802, internal diameter 8 mm; length 300 mm, strong cation-exchange resin gel packing, minimum 17,000 theoretical plate separation per column, molecular weight range of 50-1000 amu for KS-801 and 50-10,000 amu for KS-802) fitted in series to Waters model 2414 refractive index detector (internal temperature 30 °C; Waters module column heater). An in-line degasser (Populaire Degasys^{TM}) degassed the deionised water mobile phase before reaching the HPLC pump (Waters model 515) at a flow rate of 1 mL min^{-1}. A manual HPLC injector (Rheodyne model 7725i) was utilised and data was collected *via* Empower Software. The data acquisition time for each manual injection was 25 minutes.

External calibration curves of sucrose, D-glucose and D-fructose (1, 2, 5, 10 and 15 mg mL^{-1}) were created with Microsoft® Excel (**Appendix 5.1.1**). The response was calculated as a ratio of integrated area to standard concentration in mg mL^{-1} (**Appendix 5.1.2**).

2.4 Total monomeric anthocyanin content

2.4.1 pH-differential method

The samples were thawed to room temperature. The appropriate dilution factor (DF) for each sample was obtained by diluting individual samples with the KCl buffer (pH 1.0) until the absorbance of the sample at $\lambda_{vis\text{-}max}$ was less than 1.2 (i.e. within the linear range of the detector). The final volume/initial volume gave the dilution factor. The dilution factors ranged from 40-80 depending on the sample.

Two dilutions of each sample were prepared, one in the KCl buffer (pH 1.0) and one in the sodium acetate buffer (pH 4.5) and allowed to equilibrate for 15 minutes. The absorbance of each dilution was measured at $\lambda_{\text{vis-max}}$ of cyanidin-3-glucoside (cyn-3-gly) at 510 nm and corrected for haze with subtraction of absorbance at 700 nm.[135] Anthocyanin concentration is given as cyn-3-gly equivalent mg L^{-1}.

2.5 Total polyphenol concentrations

2.5.1 Folin-Ciocalteu polyphenol assay

The Folin-Ciocalteau (FC) reagent is a mixture of tungsten and molybdenum oxides. Polyphenols reduce these oxides and the intensity of the blue product (at 765 nm) is proportional to the concentration of polyphenolic compounds in the solution. The method used was adapted from that reported by Waterhouse.[123] Samples were thawed to room temperature and 20 ± 1 µL of diluted sample (or MilliQ water in the case of blanks) was mixed with 1580 ± 50 µL of MilliQ water and 100 ± 5 µL of FC reagent, in a 2 mL cuvette. The mixture was left to stand for approximately 3 min (and never longer than 8 min), before 300 ± 15 µL of sodium carbonate solution was added. The mixture was mixed well, left to stand for 2 hours at room temperature and the absorbance at 765 nm was recorded. The absorbance of blank samples was subtracted from the absorbance obtained for samples, and this value was compared with a gallic acid calibration curve to give polyphenol concentration as gallic acid equivalent (GAE).

2.5.2 FC Calibration

Standards of 0 mg L^{-1}, 50 mg L^{-1}, 100 mg L^{-1}, 250 mg L^{-1} and 500 mg L^{-1} of gallic acid were prepared in 10 % aqueous ethanol with the same concentrations of sucrose, D-glucose and D-fructose as the pre-heat treatment juice matrix of each sample. These sugar solutions account for the interference of reducing sugars which react with the FC reagent. The gallic acid standards were used to generate a calibration curve [absorbance vs concentration] each time a set of samples was analysed (**Appendix 5.2.1**). The gradient for gallic acid from the calibration curve was used to calculate the concentration (GAE) of polyphenols in all beverage samples.

2.6 Total antioxidant capacity

2.6.1 Ferric reducing antioxidant power (FRAP) assay

Samples were thawed to room temperature and diluted 1:10 with MilliQ water. Aliquots (6 ± 0.5 µL) of the diluted juice and FRAP reagent (990 ± 10 µL) were added to individual wells of a 96-well microtitre plate. Readings at 600 nm were recorded every minute for six minutes after the plate was shaken at 150 rpm for 1 min before the first reading (Fluostar plate reader BMG Laboratories, GmBH). Blanks samples and standards were added along the middle and around the edges of the plate to account for any difference in activity due to temperature variation across the plate (**Figure 2.1**).

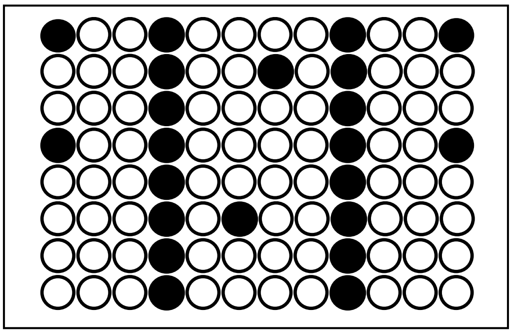


Figure 2. 1 Placement of blank samples and standards on microtitre plate. The two straight lines of wells across the plate contained blanks, and the other wells contained standards

2.6.2 FRAP calibration

Aqueous $FeSO_4.7H_2O$ solutions at 100, 250, 500 and 1000 µM (0.0278 g L^{-1}, 0.0695 g L^{-1}, 0.1390 g $L^{-1}$and 0.2780 g L^{-1} respectively) and ascorbic acid solutions of 600, 750, 800 and 1000 µM (0.1057 g L^{-1}, 0.1321 g L^{-1}, 0.1409 g L^{-1} and 0.1761 g L^{-1} respectively) were used as calibration standards. The absorbance readings were taken as for samples above. The Fe(II) reactions represent a one electron exchange reaction, therefore a blank-corrected 100 µM Fe(II) standard is equivalent to a FRAP value of 100 µM. The constant stochiometric factor of 2.0 for ascorbic acid to FRAP value, means that a 1000 µM ascorbic acid standard corresponds to a FRAP value of 2000 µM.

Reactions of FRAP reagent with aqueous solutions of known Fe (II) concentrations and freshly prepared aqueous (MilliQ) ascorbic acid solutions were used to produce calibration curves (**Appendix 5.2.2**) from 100-2000 µM FRAP. Seven points per sample (over 0-6 min) were recorded and compared with the calibration curve to determine the FRAP of the sample. Raw data from BMG Optima was converted into Microsoft® Excel spreadsheets and blank absorbance values were subtracted to calculate FRAP values for standards and samples.

2.6.3 2, 2-diphenyl-1-picrylhydrazyl free radical (DPPH•) assay

5.0 ± 0.5 µL of supernatant of centrifuged juice was added to 195 ± 10 µL of 50 µM (0.0197 g L^{-1}) DPPH• solution in individual wells of a 96 well microtitre plate sampler. Samples were kept at 37 °C and absorbance at 517 nm was recorded five times over 40 minutes.

2.6.4 DPPH• Calibration

Aqueous solutions of 50 µM (8.806 mg L^{-1}), 100 µM (17.612 mg L^{-1}), 150 µM (26.418 mg L^{-1}), 200 µM (35.224 mg L^{-1}), 250 µM (44.033 mg L^{-1}), 300 µM (52.836 mg L^{-1}) and 350 µM (61.642 mg L^{-1}) of ascorbic acid were used as calibration standards. As for samples, 5.0 ±0.5 µL of standard solution was added to 195 ± 10 µL of 50 µM DPPH• solution and the absorbance at 517 nm was recorded over 40 minutes at 37 °C.

The ascorbic acid standards were used to generate a calibration curve [percentage inhibition (% I) versus standard concentration] each time a set of samples was analysed. The percentage inhibition of each sample as AAE was determined using this calibration curve.

3. Results

3.1 Evaluation of the effect of packaging upon the FOS concentrations of storage roots

3.1.1 Determination of relative percentages of sugar

FOS concentrations were determined by size exclusion HPLC with refractive index detection. The external calibration curves for FOS/sucrose, D-glucose and D-fructose (**Appendix 5.1.1**) were used to calculate the concentration in mg g^{-1} or mg mL^{-1} (**Appendices 5.1.2 and 5.1.3**) of these sugars in all storage root and juice mixture samples. Approximate retention times were 14.1 minutes for sucrose, 16.3 minutes for D-glucose and 17.6 minutes for D-fructose standards (**Figure 3.1**). In general the variation in retention time was ± 0.05 minutes.

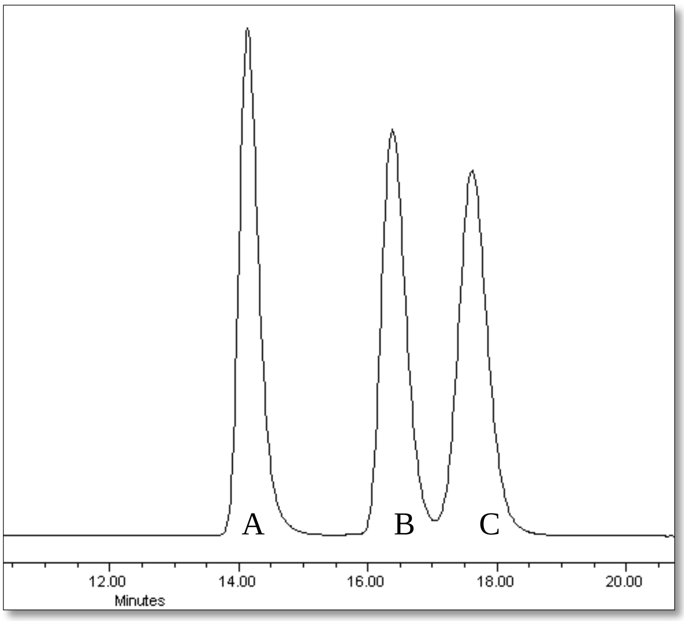


Figure 3. 1 Chromatogram of a standard solution containing (A) sucrose, (B) D-glucose and (C) D-fructose. Chromotography conditions can be found in Section 2.3

To control for sources of error and allow for comparison between different sized storage roots with different initial FOS concentrations, the concentration of each sugar measured *via* HPLC was converted into relative percentage ($\mathrm{RP_{sugar}}$) of total measured carbohydrate concentration, **Equation 3.1**.

$$\mathrm{RP_{sugar\ x}} = \frac{[\mathrm{sugar_x}]}{[\text{all sugars}]} \qquad \mathbf{3.1}$$

[all sugars] = ([FOS]+[1-kestose]+[sucrose]+[glucose]+[fructose])

In juice mixtures the relative percentage of each sugar was calculated at each time and related to the relative percentage of the sugar in the juice before heating, to give relative percentage change in sugar composition ($\mathrm{RPC_{sugar}}$), **Equation 3.2**.

$$\mathrm{RPC_{sugar\ x}} = \left(\frac{[\text{sugar x}]_{\text{time y}}/[\text{all sugars}]_{\text{time y}}}{[\text{sugar x}]_{\text{time 0}}/[\text{all sugars}]_{\text{time 0}}}\right) \times 100 \qquad \mathbf{3.2}$$

The percent deviation (PD) of each set of replicates was calculated (**Equation 3.2**). Any results that produced a PD greater than 5 % were repeated until the PD was reduced, unless there was an inadequate amount of sample for re-extraction. PD is the equivalent of coefficient of variance, for data sets that are too small for standard deviation to be a meaningful statistic.

$$\mathrm{PD} = \left(\frac{(\text{Maximum }\ \mathrm{RPC_{sugar}} - \text{Average }\mathrm{RPC_{sugar}})}{\text{Average }\ \mathrm{RPC_{sugar}}}\right) \times 100 \qquad \mathbf{3.3}$$

3.1.2 Changes in relative percentage sugar composition in storage roots over time

The region from 10.5 - 12.5 minutes on the chromatogram of storage root extract (**Figure 3.2**) arises from higher DP FOS (GF_8-GF_3). The peak at 13 minutes (C) is 1-kestose (GF_2) and the resolution was sufficient to integrate it separately from higher DP molecules.

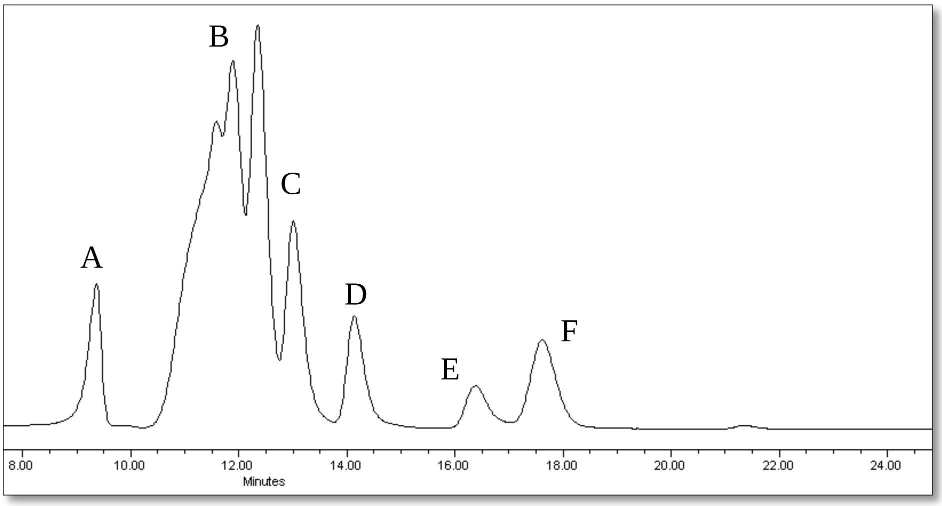


Figure 3. 2 Chromatogram of storage root extract (A) excluded material, (B) GF_8-GF_3 FOS, (C) GF_2 1-kestose, (D) sucrose, (E) D-glucose and (F) D-fructose

The RP of FOS, 1-kestose, sucrose, glucose and fructose of storage roots vacuum packed in semi-permeable polymer were compared with the RP of the same sugars in unpackaged storage roots from the same plant, stored at 5 °C for up to 72 days. The effectiveness of the packaging was dependent upon a complete seal around the root. This meant that a number of samples were lost to rot during the course of storage, especially if the roots were particularly small or misshapen. Three sets of samples (from three different plants, 10 roots per plant) were prepared at Cuisine Resources (Pukekohe) at the beginning of June 2011 (early season storage roots). A sufficient number of these roots were successfully sealed in the packaging to allow testing at 4, 28, 49 and 72 days after the roots were harvested and they comprise the results included in this study (**Appendix 5.3**). The sugar concentrations of unpackaged roots after 49 days were not able to be measured accurately due to extent of degradation or desiccation of the roots. Those that did not develop mould or rot were too shrivelled to obtain adequate sample for freeze drying past this point. An earlier yacon root storage trial[4] without packaging, conducted at the University of Waikato found that a number of roots stored at 5 °C decomposed and developed mould throughout the storage period and the last storage root still in adequate condition for sampling was at week 8 (comparable with day 49 in this study). This type of decomposition was not seen at 10 °C in the earlier trial, but this temperature was not considered for the current study, because the standard refrigeration temperature of shipping containers is 5 °C.

Four sets of samples were prepared in July 2011, at the end of the harvesting season (late season storage roots), however no data could be analysed for these samples. The sugar concentrations in the unpackaged storage roots decreased at rapid rate, resulting in degradation of the unpackaged roots before the first monthly analysis. This occurrence can be explained by increased activity of the fructan exohydrolase enzyme (FEH), which is stimulated by cold weather[5-6] as a mechanism to maintain the osmotic pressure within storage roots over winter. **Figure 3.3** is the sugar composition of a late season packaged storage root compared with an unpackaged storage root from the same plant showing the significant loss of FOS and 1-kestose and the release of glucose and fructose. The increased glucose concentrations and lack of change in the sucrose peak also indicate that sucrose is being hydrolysed at approximately the same rate that it is being formed *via* 1-kestose hydrolysis.

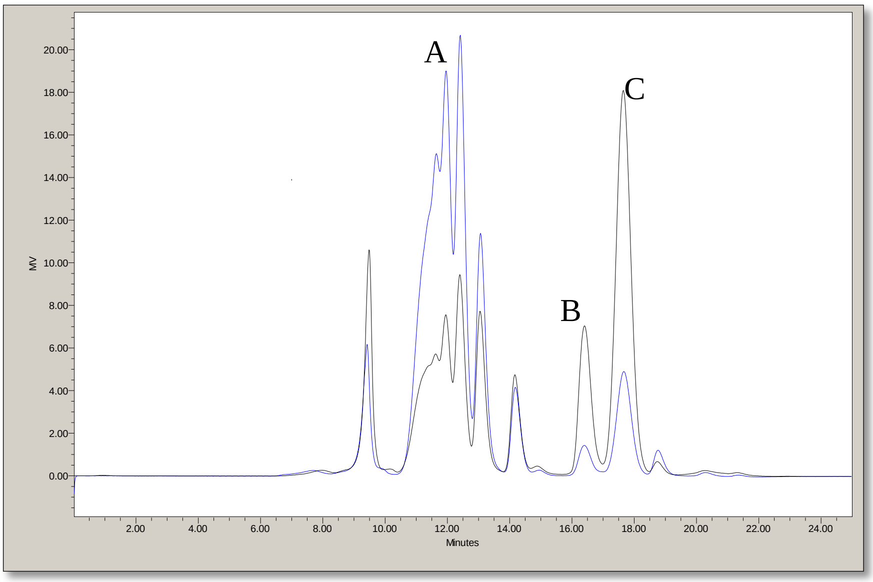


Figure 3. 3 Differences in (A) GF$_8$-GF$_3$, (B) D-glucose and (C) D-fructose between packaged (blue trace) and unpackaged (black trace) late season storage roots on day 2

The early season storage root results are less dramatic than the late season, but the effectiveness of packaging is evident. The chromatograms of packaged and unpackaged roots from two plants on days 4 and 49 are shown in **Figure 3.4** and

Figure 3.5. The mean RP sugar values and results of statistical analysis are given in **Table 3.1**. The black trace on each chromatogram arises from the storage root extracted on day 4. The blue trace arises from the storage root that has been stored at 5 °C for 49 days. In packaged storage roots the concentration of low DP FOS remains relatively constant with a slight decrease in higher DP FOS and an increase in sucrose, D-glucose and D-fructose (**Figure 3.4**). In unpackaged storage roots the concentration of all FOS and sucrose significantly decreases and D-glucose and especially D-fructose concentrations increase (**Figure 3.5**).

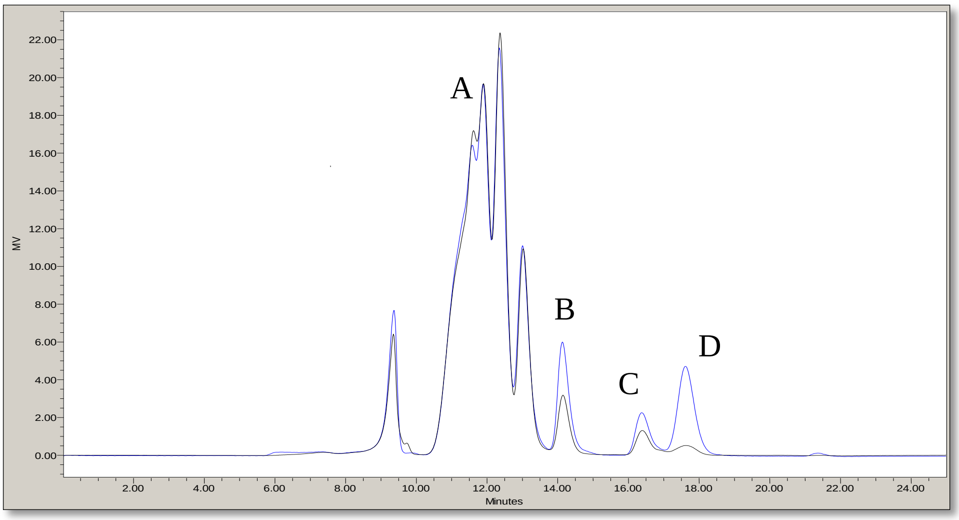


Figure 3. 4 Changes in sugar composition of packaged storage root extract between day 4 (black trace) and day 49 (blue trace)

(A) slight decrease in long chain FOS, (B) increase in sucrose,
(C) increase in D-glucose, and (D) increase in D-fructose

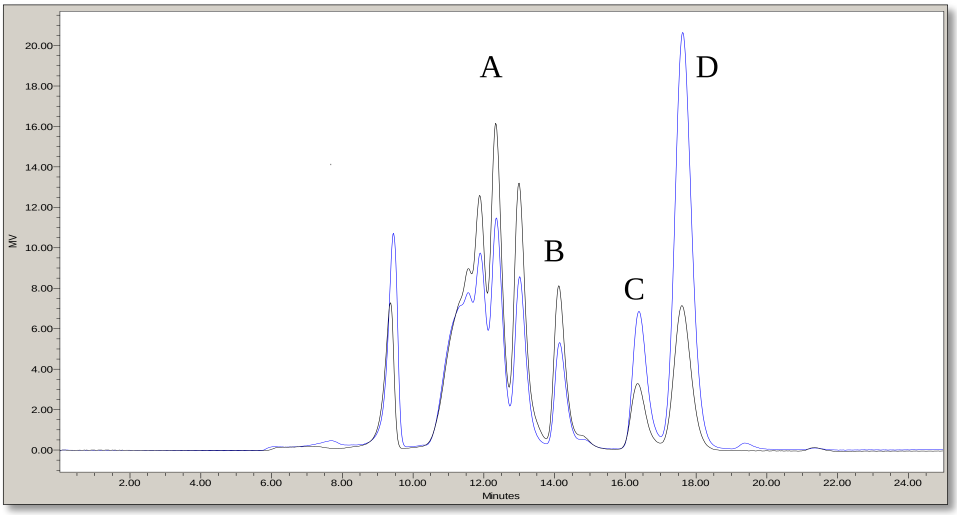


Figure 3. 5 Changes in sugar composition of unpackaged storage root extract between day 4 (black trace) and day 49 (blue trace)

(A) decrease in GF_8-GF_2, (B) decrease in sucrose,

(C) increase in D-glucose, and (D) increase in D-fructose

The two-way ANOVA results (**Table 3.1**) show that the differences in mean FOS, D-glucose and D-fructose between packaged and unpackaged storage roots are statistically significant at a 99.95 % confidence level. The difference between each sugar over time appears statistically significant in the two-way ANOVA, but when the packaged and unpackaged values are considered separately with one-way ANOVA tests (**Table 3.2**), it is clear that the difference in these sugars is due to the unpackaged roots only. This indicates that the packaging successfully reduces the degree of FOS hydrolysis that occurs in the storage roots over time.

Table 3. 1 Relative sugar percentage changes in yacon storage roots over time

Analyte	Day 4		Day 28		Day 49		Day 72		Two way ANOVA P-values	
	Packaged	Unpackaged	Packaged	Unpackaged	Packaged	Unpackaged	Packaged	Unpackaged	Day	Type
FOS	79.01	76.02	72.90	53.45	70.81	61.00	66.63	N/A	≤0.001	≤0.001
(Standard error)	(0.72)	(1.44)	(3.39)	(3.82)	(0.19)	(0.25)	(0.35)			
1-kestose	12.83	14.37	13.35	13.01	11.60	9.39	13.03	N/A	0.008	0.648
(Standard error)	(0.41)	(1.76)	(0.43)	(0.76)	(0.28)	(0.73)	(0.50)			
Sucrose	3.79	4.43	4.81	6.37	5.84	5.60	6.10	N/A	0.003	0.069
(Standard error)	(0.23)	(0.15)	(0.67)	(0.49)	(0.37)	(0.25)	(0.05)			
Glucose	2.40	2.52	3.50	5.88	4.63	6.92	3.44	N/A	≤0.001	≤0.001
(Standard error)	(0.39)	(0.26)	(0.28)	(0.52)	(0.71)	(0.54)	(6.83)			
Fructose	1.98	2.65	5.43	16.30	7.12	17.09	10.81	N/A	≤0.001	≤0.001
(Standard error)	(0.38)	(0.84)	(2.36)	(2.57)	(0.31)	(0.68)	(0.27)			

Table 3. 2 P-values for one-way ANOVA of sugar concentrations relative to packaging type versus day of storage

Type	FOS	1-kestose	Sucrose	Glucose	Fructose
Packaged	0.063	0.042	0.053	0.050	0.096
Unpackaged	0.004	0.059	0.016	≤0.001	≤0.001

There are a small number of storage and post-harvest treatment studies that have been carried out on yacon. The investigations by Graefe *et al.*[136] into the sun drying procedure commonly used in South America and by Scher utilising hot air drying[137] confirm that FOS is rapidly hydrolysed at higher temperatures. The reduction of FOS hydrolysis by storage at lower temperatures has been investigated by a small number of researchers. Three different yacon accessions (plants grown in the same area) stored at 4 °C, were monitored by Cisneros-Zevallos *et al.*[138] The same cultivar (strain of yacon plant grown by propagation) used in the current study was analysed after storage at 10 °C by Wong.[4] Ohyama *et al.*[102] measured the sugar composition in Japanese grown roots stored at unspecified cool temperatures for 96 days. The mean daily relative percentage change in sugars within yacon storage roots from the storage studies, which had adequate reporting of values, is outlined in **Figure 3.6**.

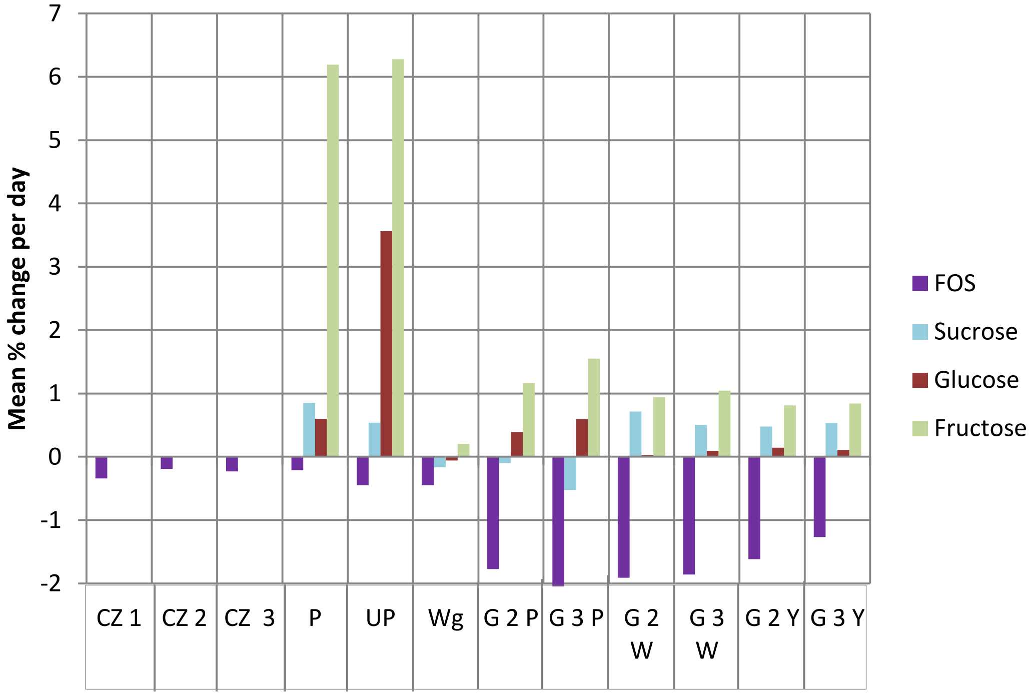


Figure 3. 6 Mean changes in percentage sugar composition per day. CZ = one of 3 different cultivars of Peruvian yacon root investigated by Cisneros-Zevallos *et al.*,[138] for which no monosaccharide data is available. P and UP are the results of packaged and unpackaged roots from the current study. Wg = results from the storage trial at 10 °C by Wong.[4] G = Sun-drying study by Graefe *et al.*[136] where 2 and 3 = 2000 and 3000 metres above sea level respectively and P = purple cultivar, W = white cultivar and Y=yellow cultivar

There is a very noticeable difference between the amount of fructose measured relative to the degree of FOS hydrolysis, between the roots analysed in this study and all of the others reported in the literature. This is an artefact due to the presentation of sugar concentrations in the graph. RPC (percentage change relative to the individual initial concentrations of each sugar) was the standard format for reporting FOS hydrolysis in yacon in the literature, because it allows comparison within the storage period of each trial. In the present study the initial relative percentage of FOS is significantly higher and the initial relative percentage of D-glucose and D-fructose is significantly lower than in other yacon root storage studies. Comparison with the literature generally means that different accessions and cultivars are being compared, as well as different extraction techniques. However the study by Wong[4] used the same cultivar as the current study and the same extraction technique. **Table 3.2** allows for direct comparison of RP (percentage of each sugar relative to the total sugar concentrations at the time of analysis) and demonstrates that the unpackaged root in this study behaved in the same way as the roots in the study by Wong[4] with respect to hydrolysis of FOS, whereas the packaged roots exhibited noticeably less FOS hydrolysis.

Table 3. 3 Comparison of the relative percentage (RP) of sugars in yacon storage roots of the same cultivar over seven weeks of storage. The storage temperature was 10 °C in the study by Wong[4] compared with 5 °C for the unpackaged and packaged roots in this study.

RP_{sugar}	Wong[4]	Unpackaged	Packaged
Initial RP_{FOS}	56.25	90.39	91.84
Final RP_{FOS}	26.18	70.39	82.41
Initial $RP_{Sucrose}$	6.43	4.43	3.79
Final $RP_{Sucrose}$	3.55	5.60	5.84
Initial $RP_{Glucose}$	3.49	2.52	2.40
Final $RP_{Glucose}$	13.46	6.92	4.63
Initial $RP_{Fructose}$	13.16	2.65	1.98
Final $RP_{Fructose}$	34.77	17.09	7.12

Ohyama *et al.*[102] also reported sugar composition results in mg g^{-1} for yacon roots stored for 96 days, but the initial concentrations and exact storage temperature were not reported. It was however, possible to calculate the RP of each sugar to allow for comparison with the storage roots in this trial (**Figure 3.7**). All mean relative percentage FOS values over the course of the current study were included to illustrate that the extent of FOS hydrolysis seen in the trial by Ohyama *et al.*[102] would not occur in the same time frame for the packaged roots, assuming the rate of hydrolysis is dependent on storage temperature.

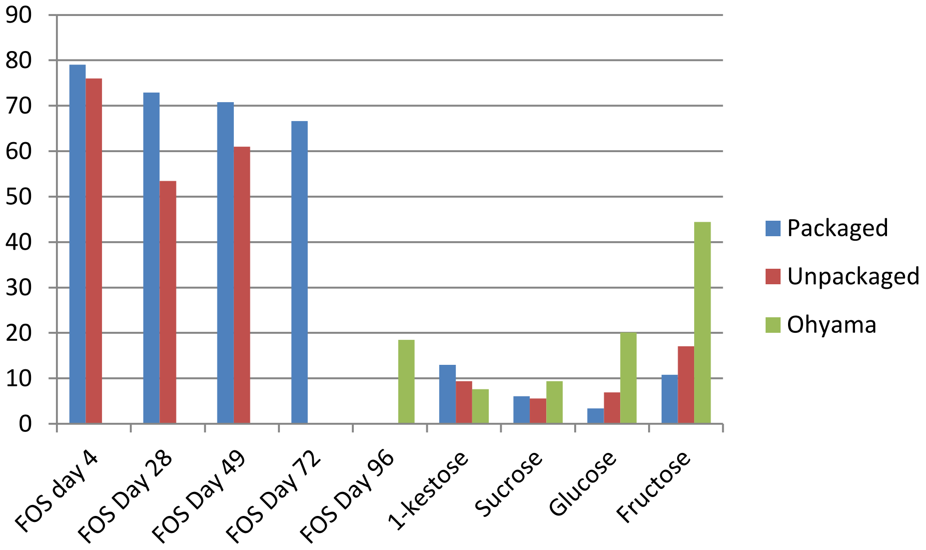


Figure 3. 7 Comparison of the relative percentage sugar composition of yacon roots, stored in cold conditions. Packaged and unpackaged refer to the roots from this study (stored at 5 °C), whereas the green bars are the results reported by Ohyama *et al.*[102] for roots stored in cool conditions for 96 days. The individual sugars show values for the final day of analysis; day 49 for unpackaged, day 72 for packaged and day 96 for Ohyama

Comparison of the extent of FOS hydrolysis in yacon storage roots in this study with data from the few storage trials published in the literature show that the rate of hydrolysis is slowed by successful packing in the semi-permeable polymer.

3.2 Evaluation of the effect of processing conditions upon blackcurrant-yacon juice mixtures

The amount of yacon syrup added to the blackcurrant juice was determined by the FOS concentration in mg g^{-1} of the syrup (calculation in **Appendix 5.1.4**) to give 5 g of FOS per 150 g serving. The syrup which was used, had the highest FOS (and consequently lowest fructose) concentration of those available.
The blackcurrant juice was a 10 % blackcurrant concentrate supplied by Just the Berries Ltd. A large volume of juice (approximately 150 L) was filled into pouches at Cuisine Resources Ltd and a number of pouches were collected and stored at room temperature and 5 °C for monitoring of FOS, polyphenols, anthocyanins and antioxidant activity with storage. The FOS concentration of the juice from these samples was found to be lower than the predicted 5 g per serve (1.8 - 3.4 g), and this was attributed to the high temperature pouch filling. During this process the juice was heated to 135 °C for 5 minutes, and then reduced to 90-95 °C while the pouches were filled. This is the standard procedure for sterilisation of juice for pouch filling.

A preliminary study heating juice (95 ± 5 °C) from a pouch showed a significant decrease in FOS with heating (**Figure 3.8**). A detailed kinetic study of small-scale formulated juice without added sucrose was designed to monitor changes in the sugars, total polyphenols, anthocyanins and antioxidant activity of the juice that can then be compensated for during mass production of juice.

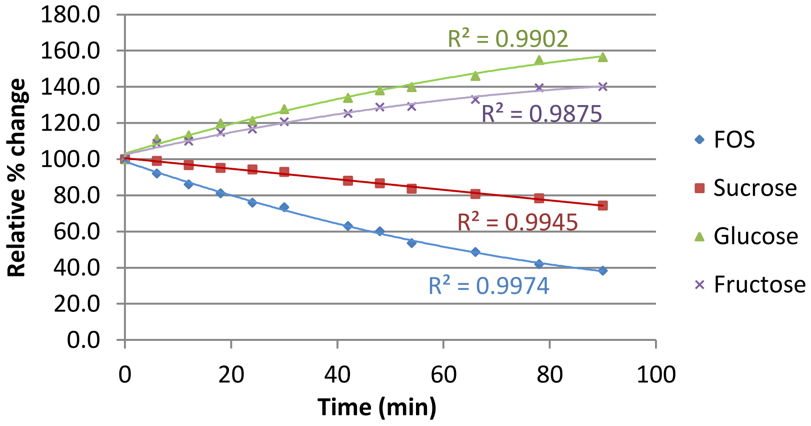


Figure 3. 8 Preliminary review of relative change in sugar composition (where FOS is GF_8-GF_2) with heating at 95 °C with juice from a pouch

The first formulation of juice (A) was pH adjusted with potassium carbonate (K_2CO_3) until the juice was at pH 4.2. The change in sugar concentrations were monitored on heating the juice at 95 ± 5 °C for 30 minutes (sampling every 30 s). It was observed that appreciable amounts of solid K_2CO_3 precipitate out of solution on heating and the purple colour of the juice is retained in this solid. Because the colour of blackcurrant juice is attributed mainly to anthocyanins, it was surmised that the anthocyanin concentrations detected by the pH-differential method would be reduced compared to the concentrations actually present in the juice. Another formulation of blackcurrant-yacon juice was prepared with the minimum amount of K_2CO_3 to adjust the pH to 4 ± 0.2 and analysed in triplicate at 95 ± 5 °C for 30 minutes with sampling every 30 s (B) to compare with the original juice and at 135 ± 5 °C for 15 minutes with sampling every 15 seconds (D). A blackcurrant-yacon juice without K_2CO_3 (C) and a blackcurrant juice without yacon or K_2CO_3 added (D) were prepared and heated under the same conditions (in triplicate) to allow for comparison to gauge the effect of different variables (**Appendix 5.4**). As expected, heating the blackcurrant-yacon juice to 135 °C resulted in rapid hydrolysis of FOS (**Figure 3.9**) and release of fructose (**Figure 3.10**) in the juice.

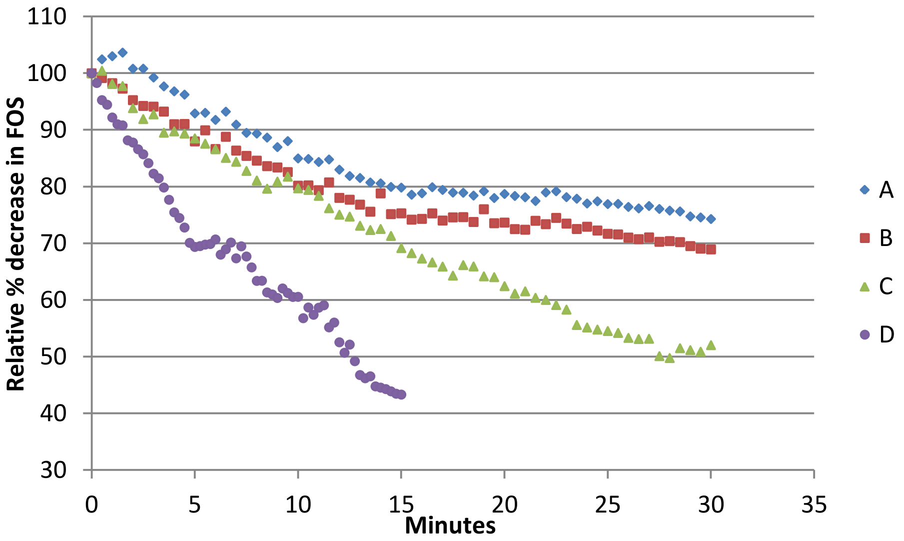


Figure 3. 9 Relative percentage decrease in FOS concentration with heating (A) excess K_2CO_3 juice pH > 4 (B) minimum K_2CO_3 juice pH 4 ± 0.2 (C) juice without pH adjustment, all heated at 95 °C and (D) minimum K_2CO_3 juice heated at 135 °C.

The relative percentage of 1-kestose drops initially (between 0 - 5 minutes) for all juices (**Figure 3.11**). In all juices with K_2CO_3 added, the relative percentage of 1-kestose stabilises, presumably as an equilibrium is reached between FOS hydrolysis to form 1-kestose and hydrolysis of 1-kestose to give sucrose and fructose. The relative percentage of 1-kestose continues to decline in the juice without pH stabilisation, at a slower rate after 5 minutes. In all cases, a linear increase in the relative percentage of sucrose (**Figure 3.12**) occurred with heating, indicating that sucrose is a zero order reactant, which corresponds to its resistance to hydrolysis compared with FOS. The pattern of relative percentage change in glucose concentration (**Figure 3.13**) varied between juices.

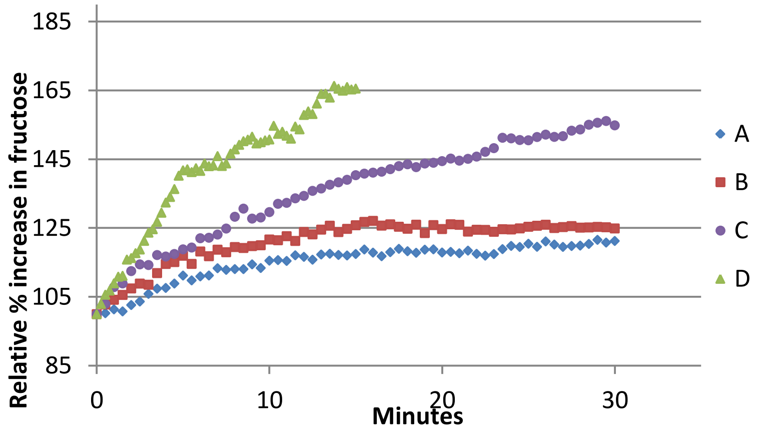


Figure 3. 10 Relative percentage increase in fructose concentrations with heating (A) excess K_2CO_3 juice pH > 4 (B) minimum K_2CO_3 juice pH 4 ± 0.2 (C) juice without pH adjustment, all heated at 95 °C and (D) minimum K_2CO_3 juice heated at 135 °C

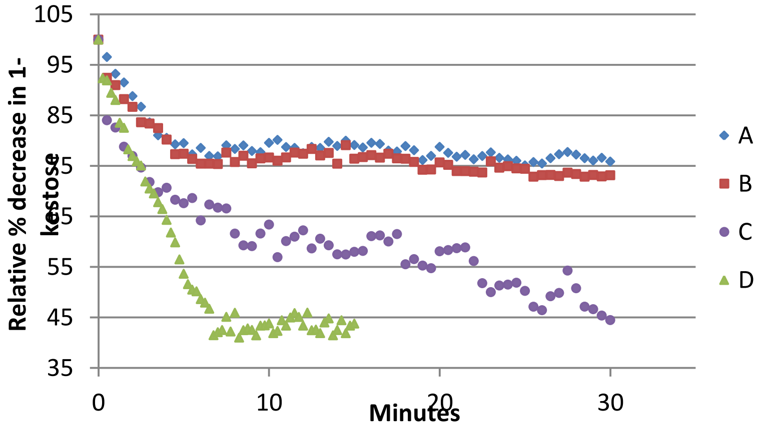


Figure 3. 11 Relative percentage decrease in 1-kestose concentrations with heating (A) excess K_2CO_3 juice pH > 4 (B) minimum K_2CO_3 juice pH 4 ± 0.2 (C) juice without pH adjustment, all heated at 95 °C and (D) minimum K_2CO_3 juice heated at 135 °C

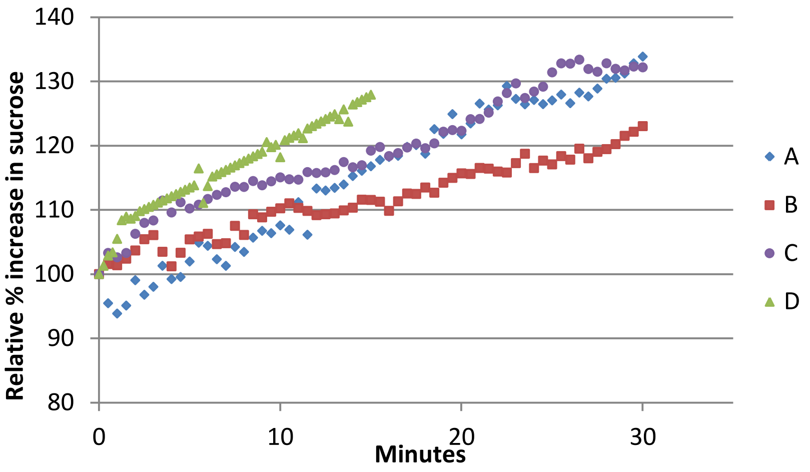


Figure 3. 12 Relative percentage increase in sucrose concentrations with heating (A) excess K_2CO_3 juice pH > 4 (B) minimum K_2CO_3 juice pH 4 ± 0.2 (C) juice without pH adjustment, all heated at 95 °C and (D) minimum K_2CO_3 juice heated at 135 °C

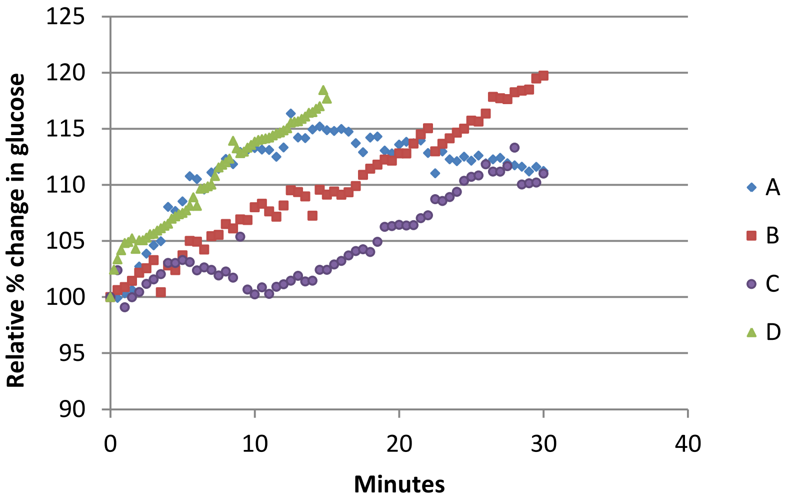


Figure 3. 13 Relative percentage changes in glucose concentration with heating (A) excess K_2CO_3 juice pH > 4 (B) minimum K_2CO_3 juice pH 4 ± 0.2 (C) juice without pH adjustment, all heated at 95 °C and (D) minimum K_2CO_3 juice heated at 135 °C

The hydrolysis of FOS under acidic conditions is a pseudo first order reaction, at higher pH it is second order in FOS and acid, which can be explained as follows, **Figure 3.14**:

1. A preliminary equilibrium occurs in which the FOS becomes protonated.

$$\mathrm{FOS} + \mathrm{H}^+ \xrightleftharpoons{\mathrm{K}} \mathrm{FOSH}^+$$

2. The equilibrium constant is proportional to the concentration of the protonated form of FOS.

$$\mathrm{K} = \frac{[FOSH^+]}{[FOS][H^+]}$$

3. Rearrangement of the equation for the equilibrium constant in 2. gives an expression for the concentration of protonated FOS.

$$[\mathrm{FOSH}^+] = \mathrm{K}[\mathrm{FOS}][\mathrm{H}^+]$$

4. Substitution into the rate equation for the second, rate determining step gives a reaction that is second order in FOS and acid concentration.

$$\mathrm{Rate} = -\frac{d[FOS]}{dt} = \mathrm{k}\ [\mathrm{FOSH}^+] = \mathrm{kK}\ [\mathrm{FOS}][\mathrm{H}^+]$$

5. At low pH acid will be present in excess so the reaction is pseudo first order at higher pH acid is limiting and the reaction becomes second order

Figure 3. 14 Summary of the proposed kinetics of FOS hydrolysis

For the pseudo first order reactions a plot of the natural log of the relative percentage change (proportional to $[\mathrm{FOS}]\mathrm{t}/\ [\mathrm{FOS}]\mathrm{t}_0$ in conventional kinetic plots) versus time for FOS hydrolysis (**Figure 3.15**) allowed for estimation of some kinetic parameters.

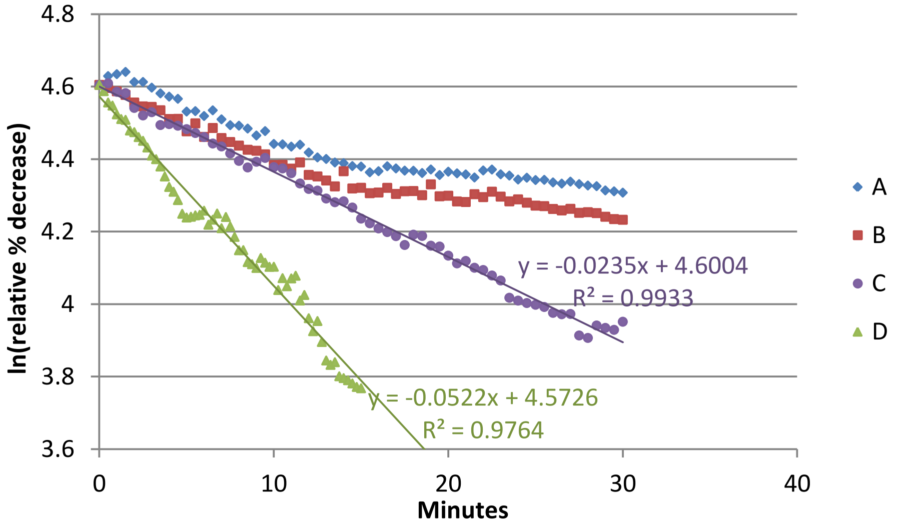


Figure 3. 15 Log of the relative percentage decrease in FOS against time. Linear relationships are seen for C (no K_2CO_3 added, pH 2.8, heated at 95 °C) and D (minimal K_2CO_3, pH 4 heated at 135 °C). Juices A and B, both with pH $\geq$ 4 heated at 95 °C generate curves that are not linear indicating that they are not first order

The rate constant (k_{obs}) of FOS hydrolysis is 23.5 x 10^{-3} min^{-1} for C (pH 2.8, heated at 95 °C) and 52.2x10^{-3} min^{-1} for D (pH 4, heated at 135 °C) with half lives ($t_{1/2}$) of 29.5 and 13.3 minutes respectively. The kinetics of FOS hydrolysis using aqueous solutions of FOS standards up to GF_5 have been investigated by L'homme.[114] The rate constants reported for first order hydrolysis of nystose (GF_4) and fructofuranosylnystose (GF_5) were 12.2x10^{-3} min^{-1} and 11.9x10^{-3} min^{-1} respectively at 100 °C (pH 4). Although not directly comparable, these are fairly similar to those found in this study. More acidic conditions were not studied by the latter and it was found that increasing the pH, decreased the rate of hydrolysis, to the point that when FOS solution was heated for 24 hours at pH 9, no hydrolysis was detected for temperatures between 80 - 120 °C[114]. The slowing rate with increasing pH indicates pseudo first order kinetics of FOS hydrolysis in acidic conditions becoming second order in less acidic conditions.

pH adjustment appears to be an effective means of stabilising FOS in blackcurrant juice, but this affects the stability of antioxidant components of the juice that are more stable in acidic conditions. Whether this will actually impact on the antioxidant capacity of the juice will be assessed in the following sections.

3.3 Evaluation of the effect of processing conditions upon polyphenols in blackcurrant-yacon juice mixtures

3.3.1 Folin-Ciocalteu method for polyphenol analysis

The Folin-Ciocalteu (FC) method is the most widely used assay for total phenolic determination. The FC reagent is a mixture of molybdenum and tungsten oxides (bright yellow) which are reduced by phenolic compounds to give a bright blue product. The absorbance at 765 nm is proportional to phenol concentration. This method has been standardised by Waterhouse[123] for use particularly in wine analysis, but it is able to be used for other juices as long as interferences such as ascorbate and reducing sugars are taken into account. There is ascorbic acid in the juice mixtures studied but the concentrations are not significant enough to warrant correction. The concentrations of reducing sugars are known as a result of FOS analysis and therefore can be accounted for in the matrix of standards. The method outlined in **Section 2.5.1** is the micro scale method reported by Waterhouse.[123]

3.3.2 Effect of heating upon polyphenol concentrations

The correlation between polyphenol concentration, in gallic acid equivalent (GAE) mg L^{-1}, and heating time (**Figure 3.16**) was very low in juices with K_2CO_3 added (R^2 values for A and B are 0.005 and 0.011 respectively). The analysis of variance values for these juices show no statistically significant relationship between polyphenol concentration and heating time (P-values for A and B are 0.263 and 0.205 respectively). Although the correlation is poor for the other juices (C $r^2 = 0.248$ and D $r^2 = 0.297$) there is a statistically significant relationship between heating time and polyphenol concentration ($P<0.001$). This indicates that polyphenol concentrations decrease with heating, but the results obtained in this study show a large degree of scatter. The polyphenol concentrations recorded, range from 128.19 - 524.4 GAE mg L^{-1}.The maximum value arises from barely heated (2.5 min at 95 °C) blackcurrant juice without K_2CO_3 or yacon syrup added. This is similar to the 512.73 mg L^{-1} reported by Mitic *et al.*[2] for commercially available blackcurrant juice. Accounting for the fact that juice is 10 %

blackcurrant concentrate and assuming a density of 1 mg mL^{-1}, the concentration range of polyphenols in the concentrate can be calculated as 1.28-5.24 mg g^{-1} (**Appendix 5.2.3**). Comparing these results with fresh berries (4.98-13.5 GAE mg g^{-1})[3-5] it would appear that polyphenol concentrations are significantly reduced with processing. It is important to note however that fresh berry readings may also be higher due to protein reacting with the FC reagent.[123]

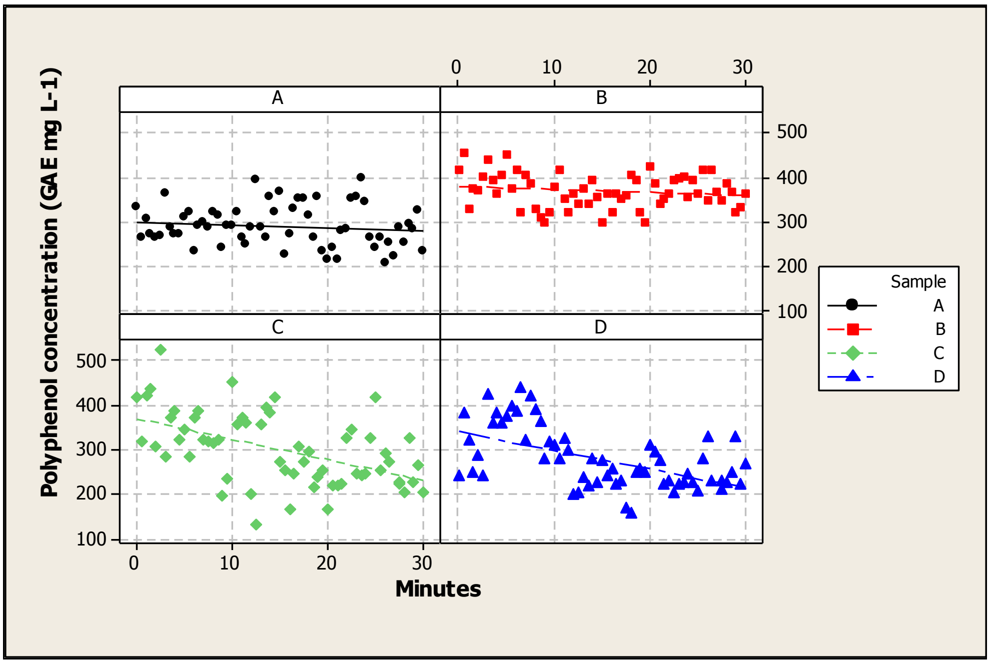


Figure 3. 14 Change in polyphenol concentration with heating (A) excess K_2CO_3 blackcurrant-yacon juice pH > 4, (B) minimum K_2CO_3 blackcurrant-yacon juice pH 4 ± 0.2, (C) blackcurrant juice without yacon syrup and (D) blackcurrant-yacon juice without pH adjustment, all heated at 95 °C

For the small changes expected with heating, in overall polyphenol concentration, a more sensitive method such as HPLC with UV detection, utilising standards targeted at the main polyphenol components in blackcurrant juice would provide a more accurate result. This type of analysis is also free from sugar and ascorbate interference. However the FC assay was an inexpensive and rapid route for screening the polyphenol content, that did not require expensive acetonitrile solvent or an extraction and purification step.

3.4 Effect of processing on anthocyanin content of blackcurrant-yacon juices

3.4.1 pH differential method

Although commonly referred to as the pH differential method for anthocyanin concentration, the assay actually measures anthocyanidin concentration, once the sugar moiety has been cleaved from the anthocyanin and then converts this to anthocyanin concentrations using physical constants from a reference anthocyanin. Anthocyanidins are present in different forms at different pH. At pH 1 the predominant form is the flavylium cation in oxonium form, which can range in colour from orange/red to purple[135] (**Figure 3.17**). At pH 4.5 the colourless hemiketal form is the main form of an anthocyanidin. By measuring the absorbance at $\lambda_{\text{vis-max}}$ (510 nm) and 700 nm for the predominant anthocyanidin in pH 1 and pH 4.5 solutions of the same sample, the concentration of total monomeric anthocyanidins (MAC) can be calculated, even with polymerised degraded products and other interfering compounds in solution.[135] The MAC was calculated (assuming 1 cm cuvette path length) using the standard AOAC method[139] (**Equation 3.4)** in which A is difference between the corrected A_{510} at pH 1 and pH 4.5, MW is 449.9 g mol^{-1}; the molecular weight of the reference anthocyanin, cyanidin-3-glucoside, DF is the dilution factor of the sample (between 40-80) and ε is 26,900 L mol^{-1} cm^{-1}; the molar absorptivity of cyanidin-3-glucoside.

$$MAC\ (cyn-3-glu\ mg\ L^{-1}) = \left(\frac{A\ \times MW \times DF \times 1000}{\varepsilon}\right) \qquad \mathbf{3.4}$$

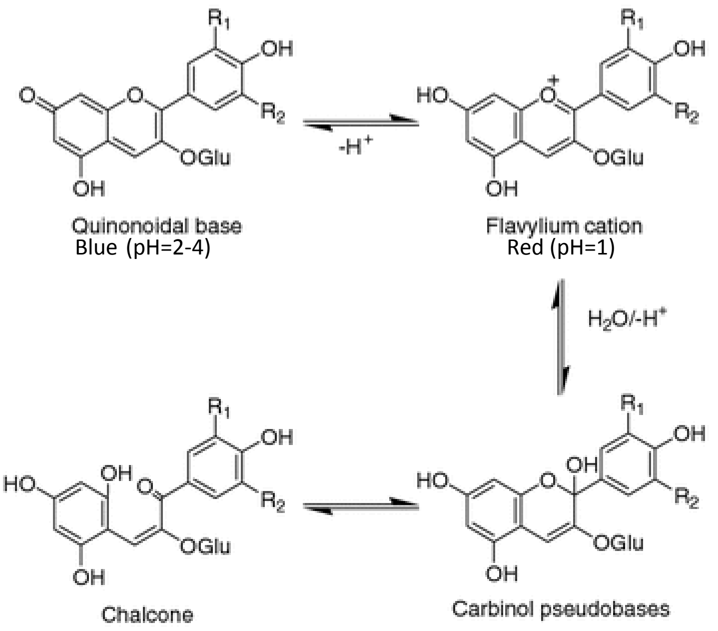


Figure 3. 17 pH-dependant forms of anthocyanidins and their associated colours

3.4.2 Effect of heating on anthocyanin concentrations

The pH of the blackcurrant-yacon juice needs to be adjusted to at least 4 to maintain the FOS content. The temperature when filling pouches with juice is required to be high for pasteurisation. Ascorbic acid is added to the yacon syrup to preserve it.[7] Anthocyanins are reported to be sensitive to oxidation with high pH, raised temperature and in the presence of ascorbic acid (as well as in the presence of sulphur dioxide, metal ions and UV radiation).[3-4] Therefore a decrease in MAC in blackcurrant-yacon juice, when compared with pure blackcurrant juice without pH adjustment was expected. It was also observed that a significant amount of potassium carbonate precipitates out of solution with heating, and it retains the purple colour of the juice. This indicates that anthocyanidins are removed from the supernatant that is tested in the pH-differential method. The MAC in cyanidin-3-glucoside (cyn-3-glu) equivalent mg L^{-1} obtained in this study (**Figure 3.18**), showed a statistically significant dependence on the length of time the juice was heated ($P < 0.001$). For these results to be compared with the results of this study to be compared with the commonly reported anthocyanin levels in fresh blackcurrants the reported concentration range of 1361.24 – 573.51 mg L^{-1} cyn-3-glu is equivalent to 13.61-5.73 mg g^{-1} cyn-3-glu assuming a density of

1 g mL^{-1} (**Appendix 5.2.3**), which compares favourably with freeze-dried extract (3.49 - 9.24 mg g^{-1}) obtained by Bordonaba & Terry.[133] The anthocyanin concentrations in blackcurrant juice reported by Mitic *et al.*[131] are within the range measured in this study assuming the same degree of dilution of concentrate.

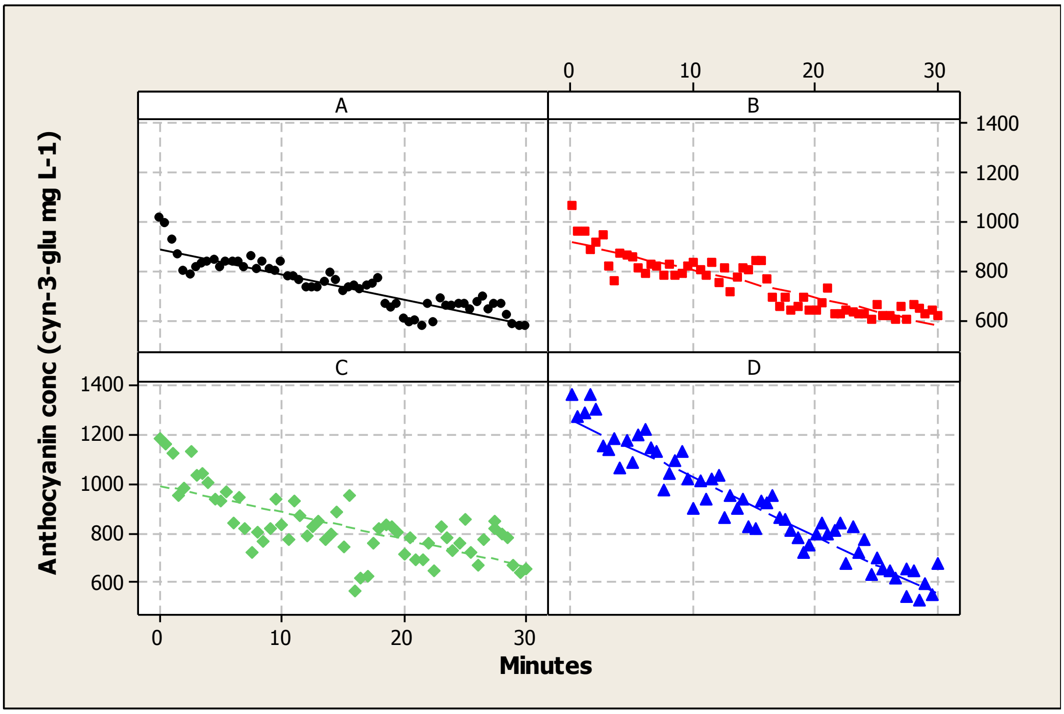


Figure 3. 18 Changes in MAC with heating at 95 °C (A) excess K_2CO_3 juice pH > 4, (B) minimum K_2CO_3 juice pH 4 ± 0.2, (C) blackcurrant juice without yacon syrup and (D) blackcurrant-yacon juice without pH adjustment

The blackcurrant-yacon juice without pH adjustment had the largest correlation coefficient (r^2 = 0.915) for reduction in MAC with increasing time, and the fastest apparent rate of anthocyanin degradation. Both the juices with excess K_2CO_3 (r^2 = 0.804) and minimum K_2CO_3 (r^2 = 0.872) had better correlation of values and a slower decrease in MAC than pure blackcurrant juice (r^2 = 0.501), shown by shallower slopes in **Figure 3.18**. A number of different dilution factors were utilised with the pure blackcurrant juice, to obtain readings within the range of the spectrophotometer and this contributes to scatter in the data. The initial MAC before heating in all juices (1013.11-1362.25 cyn-3-glu mgL^{-1}) is higher than the 757.36 – 920.92 cyn-3-glu mg L^{-1} reported by Mitic *et al.*[131] showing that a pouch filling process with limited heating time, would still produce a juice with significant MAC. Howard *et al.*[140] reviewed polyphenolic degradation in blueberries, blackberries and black raspberries and found that storage after processing had a greater impact on anthocyanin degradation than processing itself, and there is the possibility that anthocyanins will be protected from the effects of a raised pH if they are bound to the K_2CO_3 in the blackcurrant-yacon juice mixtures. Neutralisation *via* K_2CO_3 also reduces the impact of ascorbic acid oxidation on MAC. Brownmiller *et al*.[141] reported 72 % retention of anthocyanins after processing in non clarified juice (the same concentrations retained by whole berries canned in syrup) compared with only 41 % retention of anthocyanins in clarified juice. This illustrates the extent to which anthocyanins are retained by solids in the juice.

The faster rate of anthocyanin degradation in the blackcurrant-yacon juice that is not pH stabilised, compared with the other juices, appears to be associated with the rate of FOS hydrolysis. A regression analysis of anthocyanin concentration versus FOS relative percentage change gives $r^2 = 0.917$, $P \leq 0.001$. As discussed in **Section 3.4.1**, the pH differential method cleaves anthocyanidin moieties from agylcones to obtain an absorbance reading. A review by Sauro-Calixto[142] concludes that an essential function of compounds resistant to hydrolysis by human enzymes and which ferment in the large colon is the transport of antioxidant molecules to the large intestine. Not only is this associated with adjustment of the colonic environment in favour of *Bifidobacterium* and *Lactobacillus* but it potentially protects against colon cancer.[74] Sauro-Calixto[142] defines hydrolysis resistant compounds as dietary fibre (DF) but this definition is inclusive of oligosaccharides and other smaller molecules with glycosidic linkages or hydroxyl groups available for hydrogen bonding to antioxidant molecules. As such FOS could function as an aglycone for anthocyanidin molecules. When hydrolysis of FOS occurs, released anthocyanidins are vulnerable to factors which facilitate their oxidation, such as heat or polymerisation with other components of the juice which are less labile in the pH 1 buffer. This results in decreasing MAC with heating time. This also explains why the initial MAC detected in blackcurrant-yacon juice without pH adjustment is higher than that of pure blackcurrant juice in which there is no possibility of anthocyanin-FOS polymerisation. In the review[142], the harsh thermal and acidic conditions that are required to measure what is described as the non-extractable polyphenol component bound to DF, is suggested as the only means to determine the concentration of antioxidant molecules associated with DF. FOS represents the one of the simplest forms of DF as defined by Saura-Calixto[142] and anthocyanidins are relatively small polyphenols and as such the extraction process is simplified compared with for example, resistant starch and lignans. The glycosidic linkages in FOS are labile in acid, and the samples prepared in buffer for pH differential analysis were stored for up to a week before the assays were carried out which gave ample time for the FOS to hydrolyse, when it was not protected by the potassium carbonate. When FOS degrades in the pH 1 buffer the anthocyanidins released are at no risk of degradation, hence the MAC measured is higher than other juices. When FOS degrades while heating and anthocyanidins can no longer bind, the reduction in MAC is proportional to the degree of FOS hydrolysis. These results explained by the hypothesis of Sauro-Calixto[142] warrant further investigation into the combined effect of FOS and antioxidants.

3.5 Evaluation of the effect of processing conditions upon the antioxidant capacity of blackcurrant-yacon juice

Throughout the literature the most biologically relevant antioxidant capacity measure is reported to be oxygen radical absorbance capacity (ORAC), but at present the cost of this assay is extremely high and it is not without problems (discussed in **Section 3.5.4**). If it is decided that further investigations will be carried out on blackcurrant-yacon juice mixtures, ORAC analysis of the juice before and after processing would be beneficial. Notwithstanding this, the antioxidant assays employed in this study are widely reported in investigations into antioxidant capacity of whole fruits and juices.

3.5.1 2, 2-diphenyl-1-picrylhydrazyl free radical (DPPH•) antioxidant assay

The DPPH• antioxidant assay uses a stable free radical to test the levels of antioxidants. Some antioxidants act by donating a hydrogen to the free radical; in this assay DPPH• is reduced to DPPHH. DPPH• in solution is a violet colour with a strong absorbance at 517 nm which fades when reduction occurs. The decrease in intensity of absorbance at 517 nm can be related to the antioxidant power of the sample.[87] Antioxidant power *via* the DPPH• assay is reported as the percentage inhibition (% I) of oxidation of the sample using **Equation 3.5**.

$$\% I = \left(\frac{(Abs_{blank} - Abs_{sample})}{Abs_{blank}}\right) \times 100 \qquad \textbf{3.5}$$

3.5.2 Ferric Reducing/Antioxidant Power FRAP assay

Antioxidants prevent the oxidation of biological molecules by rapidly reducing the oxidant (i.e. reactive oxygen species) before the opportunity for biological oxidation arises. This redox reaction means that “total antioxidant power” can be considered the equivalent of total reducing power. The FRAP assay utilises a direct redox-linked colorimetric method in which antioxidants act as reductants on an easily reduced oxidant, present in excess. The reduction of ferric tripyridyltriazine (Fe^{III} - TPTZ) complex forms the intense blue ferrous form at low pH, the formation of which can be monitored by change in the absorption at 593 nm, and converted to FRAP value in µM using the standard calibration curve (**Appendix 5.2.2)**. It is important to note that FRAP results relate to one aspect of antioxidant capacity, so a low FRAP value does not mean that the sample has no antioxidant properties. For example Pantelidis *et al*.[8] reported that red currants and gooseberries had the lowest FRAP values compared to raspberries, blackberries and cherries, yet a number of studies[3, 9-10] found that these berries had high activities against DPPH• and singlet oxygen free radicals.

3.5.3 Effect of heating on antioxidant capacity of blackcurrant-yacon juices

The change in % I of DPPH• with heating monitored in this study (**Figure 3.19**) do not show a relationship between time heated and % I (R^2 values between 0-0.053; P-values 0.041-0.751), with the exception of A (blackcurrant-yacon juice with excess K_2CO_3). Regression analysis of A for decrease in % I in against time in minutes, gave an $r^2 = 0.616$ and analysis of variance was signicant ($P<0.001$), but this trend is insufficiently stong to confirm any affect of processing conditions on antioxidant activity. The trend may relate to the observation that K_2CO_3 precipitated out with heating (see **Section 3.2**).

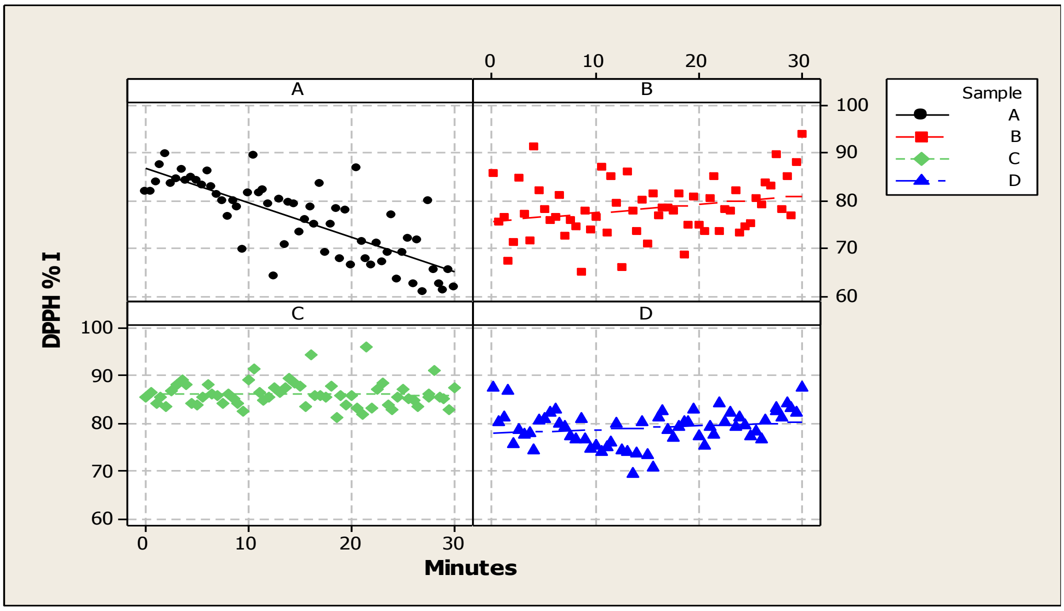


Figure 3. 15 Changes in percentage inhibition of DPPH• with heating at 95 °C (A) excess K_2CO_3 juice pH > 4 (B) minimum K_2CO_3 juice pH 4 ± 0.2 (C) blackcurrant juice without yacon syrup and (D) blackcurrant-yacon juice without pH adjustment

FRAP value (**Figure 3.20**) was not found to relate to heating time for any of the juices (R^2 values of 0-0.122 and P-values of 0-0.373).

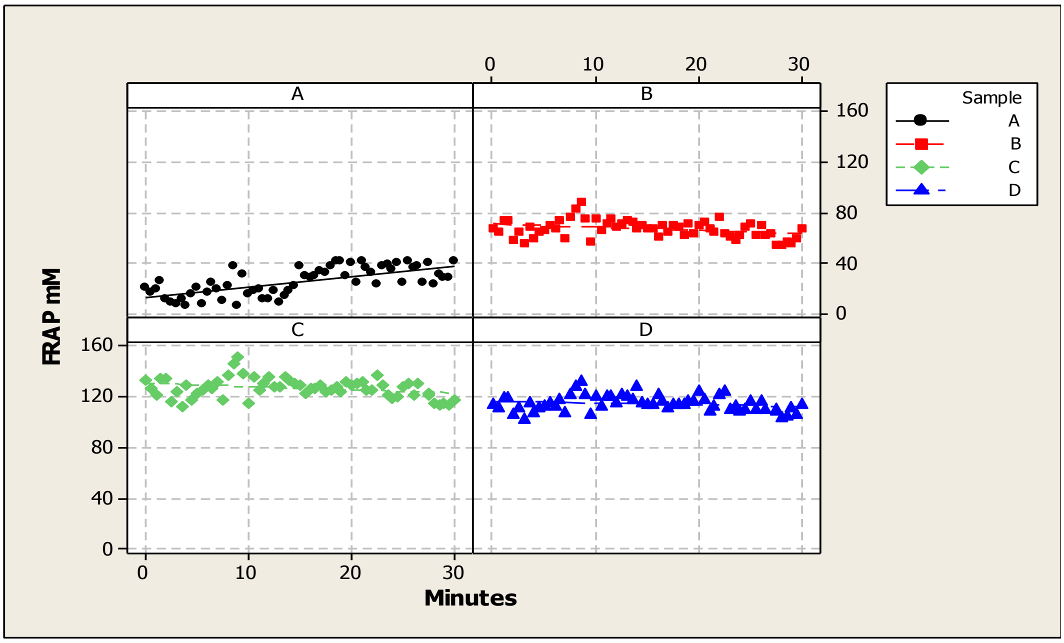


Figure 3. 16 Changes in FRAP value with heating at 95 °C (A) excess K_2CO_3 juice pH > 4 (B) minimum K_2CO_3 juice pH 4 ± 0.2 (C) blackcurrant juice without yacon syrup and (D) blackcurrant-yacon juice without pH adjustment

It was difficult to obtain reproducible triplicate results in the FRAP assay and there are a number of reasons for this. The mixing of FRAP reagent and samples for 1 minute at 37 °C before readings as specified in the standard method,[143] were taken may be insufficient to allow for equilibration and consequently rapid reduction of TPTZ and this can be tested by incubating both the reagent and sample at 37 °C before the assay in any future investigations. There was considerable temperature variance across the plate which was somewhat accounted for by placement of blanks, but had a significant impact over the 6 minute acquisition time. The standard concentrations used in this study for both DPPH• and FRAP assays were based on the accurate absorbance range of conventional spectrophotometers. It was discovered that the plate reader produced a much shallower gradient than that obtained with a conventional UV-Vis spectrophotometer. This can be seen by a comparison of calibration curves of the same concentration standards recorded on each instrument (**Figure 3.21**). Therefore higher concentration standards can be utilised with a plate reader with less dilution of the sample, resulting in less scatter. This also means that % I values are not directly comparable between studies. It is however, a useful measure of change within a study, easily converted to µM of a reference standard with the use of a calibration curve.

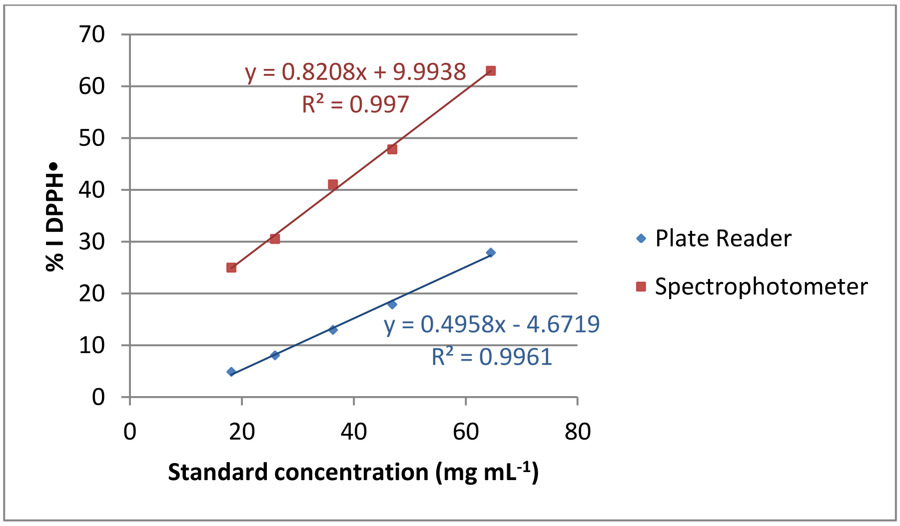


Figure 3. 21 Comparison of ascorbic acid DPPH calibration curves obtained on a Varian 100 Scan UV-Vis spectrophotometer and a BMG Fluostar microtitre plate reader

3.5.4 Evaluation of the relevance of antioxidant capacity

Review of literature concerning the effect of processing on antioxidant capacity shows that there is no clear definition of the term in common usage. Many researchers have applied any convenient method as was the case in this study.
A number of factors affect the reliability and applicability of the results of antioxidant capacity assays. The most difficult issue is that there are many different kinds of free radical within biological systems e.g. superoxide, singlet oxygen, peroxyl and nitric oxide free radicals and each antioxidant has a different ability to quench different radicals.[144-146] For example, carotenoids are very efficient at quenching singlet oxygen but not peroxyl free radicals[145] whereas the opposite is true for phenolic compounds. Huang *et al*.[147] point out that the activity (interchangeable with the term capacity in the literature) is only a meaningful measure when considered in the context of specific reaction conditions. The activity reported for a single antioxidant assay only demonstrates the activity under the conditions of the assay, which will not be the same as the complex conditions of the human body.

4. Conclusions and Recommendations

4.1 Conclusions

The main purpose of this study was to investigate the effect of processing conditions on carbohydrate composition, polyphenol concentrations and antioxidant capacity of blackcurrant-yacon juice, and make recommendations for future mass production of this juice. To minimise the amount of syrup require to fulfil the 5 g of FOS per serving of juice, the pouch-filling process needs to have as short a heating time as possible. FOS hydrolysis was slowed in juice that is adjusted with K_2CO_3 to pH 4, but this may negatively impact the amount of polyphenols and antioxidant capacity of the juice. The results of this study can be used to estimate the concentration of FOS after a pre-determined heating time, as long as the initial concentrations of FOS including 1-kestose are known. Any syrup with significantly higher % FOS content will have a faster initial rate of FOS hydrolysis and fructose release. Any syrup with a significantly lower % FOS content will hydrolyse at a slower rate initially than reported in this study. Analysis of the syrup before mass production of the juice is essential, and a shorter heating trial, based on the actual pouch filling heating time could be undertaken to determine the rate for any syrup with significantly different sugar composition. Without adequate temperature control throughout the pouch filling process the final FOS content will not be able to be estimated using the kinetic data from this study as rate is temperature dependant. The faster rate of monomeric anthoyanin decrease associated with FOS hydrolysis indicates that some kind of protective effect is being exerted by unhydrolysed FOS, for example by formation of some kind of complex. This is supported by the evidence from elsewhere that dietary fibre-type molecules can transport antioxidants to the large bowel, with associated health effects.

4.2 FOS-anthocyanin interactions

The stabilising relationship between FOS and anthocyanins in blackcurrant-yacon juice needs to be further investigated. Not only should the experiments from this study be repeated but purified FOS and anthocyanin model systems should be assessed, including replicating digestive conditions. Obviously the ultimate goal is *in vivo* testing if the results of preliminary studies support the findings of the present study.

4.3 Selection of storage roots

Due to the rapid hydrolysis of FOS in storage roots that were harvested later in the season, only roots picked and immediately packaged before the middle of June, should be considered eligible for export. It is not only because of the risk of losing FOS content before packaging, but also the fact that the FEH enzyme will begin to act immediately upon the opening of the packaging, resulting in rapid discolouration of the flesh of the root from cream to orange-brown on the release of fructose. This is likely to be considered undesirable by consumers compared to the slower hydrolysis which occurs in unpackaged early season roots. The shape of roots selected for packaging must also be carefully considered to ensure that a complete seal is formed, so that spoiling of the root will not occur during shipping.

4.4 Juice formulation considerations

The effectiveness of K_2CO_3 as an additive for pH stabilisation needs to be assessed to determine if it reduces the concentration of anthocyanins in the blackcurrant-yacon juice by oxidation or polymerisation, or if in fact the anthocyanins that come out of solution with the K_2CO_3 precipitate are consequently protected from heat degradation. Acid digestion of the precipitate after heating could be used to investigate this, but requires the amount of K_2CO_3 added to each vial to be known beforehand. If there is a protective effective effect then instructions to “shake the pouch well, before drinking”, should be added to the packaging of the pouch.

4.5 Improvement of juice analysis

The hydrolysis of 1-kestose in blackcurrant could be further investigated by the addition of 1-kestose to the external calibration curves. Currently the sucrose gradient is used to determine the concentration of FOS, whereas a 1-kestose standard could account for any difference in response between sucrose and 1-kestose. The discrepancy between glucose release in juices should also be investigated by repeating the heating experiment.

4.6 Improvement of polyphenol analysis in blackcurrant-yacon juice

The Folin-Ciocalteu (FC) method of polyphenol analysis is significantly affected by reducing sugars in solution, even when these are accounted for by making up standards in similar concentration sugar solutions. Separation of the polyphenol and sugar fractions of juice by reverse phase HPLC before FC assay or HPLC with UV detection (HPLC-UV) of common blackcurrant polyphenols would provide more accurate results, if cost is not a restriction. The detection of anthocyanins is also improved with HPLC - UV compared with the pH-differential method but the preparation and run time involved make it an impractical technique for the many samples collected in this study. HPLC analysis of 5 - 10 samples over the 15 or 30 minute sampling period could be used in comparison with the pH-differential results to provide a more accurate estimation of the change in anthocyanin concentration with heating.

References

1. Popenoe, H.; King, S. R.; Leon, J.; Kalinowski, L. S.; Vietmeyer, N. D.; Dafforn, M. *Lost crops of the Incas: Little-known plants of the Andes with promise for worldwide cultivation*; National Research Council: Washington DC, **1989**.

2. Grau, A.; Rea, J., Yacon. *Smallanthus sonchifolius* (Poepp. & Endl.) H. Robinson. In *Andean root and tubers: Ahipa, arracacha, maca and yacon.* , 21 ed.; Hermann, M.; Heller, J., Eds. International Plant Genetic Resources Institute: Rome Italy, **1997**; Vol. 3.

3. Zardini, E., Ethanobotanical notes on "Yacon", *Polymnia sonchifolia* (Asteracae). *Economic Botany* **1991,** *45* (1), 72-85.

4. Wong, N. A. A study of fructooligosaccharides in yacon (*Smallanthus sonchifolius* Poepp. and Endl.) during growth and storage. The University of Waikato, Hamiton, NZ, **2003**.

5. Itaya, N. M.; Machado de Carvalho, M. A.; Figueiredo-Ribeiro, d. C. L., Fructosyl transferase and hydrolase activities in rhizophores and tuberous roots upon growth of *Polymnia sonchifolia* (Asteraceae). *Physiologia Plantarum* **2002,** *116*, 451-459.

6. Fukai, K.; Ohno, S.; Goto, K.; Nanjo, F.; Hara, Y., Seasonal fluctuations in fructan content and related enzyme activities in yacon *(Polymnia sonchifolia). Journal of Soil Science & Plant Nutrition* **1997,** *43* (1), 171-177.

7. Asami, T.; Kubota, M.; Minamisawa, K.; Tsukihashi, T., Chemical composition of yacon, a new root crop from the Andean Highlands. *Journal of Soil Science & Plant Nutrition* **1989,** *62* (6), 621-627.

8. Grizard, D.; Barthomeuf, C., Non-digestible oligosaccharides used as prebiotic agents: mode of production and beneficial effects on animal and human health. *Reproduction Nutrition Development* **1999,** *39*, 563-588.

9. Roberfroid, M., Dietary fiber, inulin, and oligofructose: a review comparing their physiological effects. *Crictical Reviews in Food Science and Nutrition* **1993,** *33* (2), 103-148.

10. Alles, M. S.; Hautvast, J. G. A. J.; Nagengast, F. M.; Hartemink, R.; van Laere, K. M. J.; Jansen, J. B. M. J., Fate of fructooligosaccharides in the human intestine. *British Journal of Nutrition* **1996,** *76*, 211-221.

11. Fooks, L. J.; Fuller, R.; Gibson, G. R., Prebiotics, probiotics and human gut microbiology. *International Dairy Journal* **1999,** *9*, 53-61.

12. Ojansivu, I.; Ferreira, C. L.; Salminen, S., Yacon, a new source of prebiotic oligosaccharides with a history of safe use. *Trends in Food Science & Technology* **2011,** *22*, 40-46.

13. Rossi, M.; Corradini, C.; Amaretti, A.; Nicolini, M.; Pompei, A.; Zanoni, S.; Matteuzzi, D., Fermentation of fructooligosaccharides and inulin by bifidobacteria: a comparative study of pure and fecal cultures *Applied and Environmental Microbiology* **2005,** *71* (10), 6150-6258.

14. Van Loo, J.; Cummings, J.; Delzenne, N.; Englyst, H.; Franck, A.; Hopkins, M.; Kok, N.; MacFarlane, G.; Newton, D.; Quigley, M.; Roberfroid, M.; van Vliet, T.; van den Heuvel, E., Functional food properties of non-digestible oligosaccharides: a consenus report from the ENDO project (DGXII AIRII-CT94-1095). *British Journal of Nutrition* **1999,** *81*, 121-132.

15. Sarkar, S., Potential of prebiotics as functional foods - a review. *Nutrition & Food Science* **2007,** *37* (3), 168-177.

16. Gibson, G. R.; Willems, A.; Reading, S.; Collins, M. D., Symposium 2. Fermentation of non-digestible oligosaccharides by human colonic bacteria. *Proceedings of the Nutrition Society* **1996,** *55*, 899-912.

17. Yun, J. W., Fructooligosaccharides-Occurence, preparation, and application. *Enzyme and Microbial Technology* **1996,** *19*, 107-117.

18. Edelman, J.; Jefford, T. G., The mechanism of fructosan metabolism in higher plants as exemplified in *Helianthus tuberosus*. *New Phytologist* **1968,** *67*, 517-531.

19. Flamm, G.; Glinsmann, W.; Kritchevsky, D.; Prosky, L.; Roberfroid, M., Inulin and oligofructose as dietary fiber: a review of the evidence. *Critical Reviews in Food Science and Nutrition* **2010,** *41* (5), 353-362.

20. Looijer-van Langen, M. A. C.; Dieleman, L. A., Prebiotics in chronic intestinal inflammation. *Inflammatory Bowel Diseases* **2009,** *15* (3), 454-462.

21. De Leenheer, L., Production and use of inulin: Industrial reality with a promising future. In *Carbohydrates as Raw Materials III*, van Bekkum, H.; Roper, H.; Voragen, A. G. J., Eds. VCH Verlagsgesellschaft mbH: Weinheim, Germany, **1996**; Vol. 3.

22. Kim, K.; Hamdy, M. K., Acid hydrolysis of Jersualem artichoke for ethanol formation. *Biotechnology and Bioengineering* **1986,** *XXVIII*, 138-141.

23. Franck, A., Technological functionality of inulin and oligofructose. *British Journal of Nutrition* **2002,** *87* (Suppl. 2), S287-S291.

24. Ronkart, S. N.; Blecker, C. S.; Fourmanoir, H.; Fougnies, C.; Deroanne, C.; Herck, J.-C. V.; Paquot, M., Isolation and identification of inulooligosaccharides resulting from inulin hydrolysis. *Analytica Chimica Acta* **2007,** *604*, 81-87.

25. Lachman, J.; Fernandez, E. C.; Orsak, M., Yacon [*Smallanthus sonchifolia* (Poepp. et Endl.) H. Robinson] chemical composition and use - a review. *Plant, Soil and Environment* **2003,** *49* (6), 283-290.

26. Caicedo, L. A.; Silva, E.; Sanchez, O. F., Semibatch and continuous fructooligosaccharides production by *Aspergillus* sp. in a mechanically agitated airlift reactor. *Journal of Chemical Technology & Biotechnology* **2009,** *84*, 650-656.

27. Bakowska-Barczak, A. M.; Kolodziejczyk, P., Evaluation of saskatoon berry (*Amelanchier alnifolia* Nutt.) cultivars for their polyphenol content, antioxidant properties, and their storage stability. *J. Agri. Food Chem.* **2008,** *56*, 9933-9940.

28. Nemukula, A.; Mutanda, T.; Wilhelmi, B. S.; Whiteley, C. G., Response surface methodology: Synthesis of short chain fructooligosaccharides with a fructosyltransferase from *Aspergillus aculeatus*. *Bioresource Technology* **2009,** *100*, 2040-2045.

29. Chiang, C.-J.; Lee, W.-C.; Sheu, D.-C.; Duan, K.-J., Immobilization of β-fructofuranosides from *Aspergillus* on methacrylamide-based polymeric beads for production of fructooligosaccharides. *Biotechnology Press* **1997,** *13* (5), 577-582.

30. Chien, C.-S.; Lee, W.-C.; Lin, T.-J., Immobilization of *Aspergillus japonicus* by entrapping cells in gluten for production of fructooligosaccharides. *Enzyme and Microbial Technology* **2001,** *29*, 252-257.

31. Cruz, R.; Cruz, V. D.; Belini, M. Z.; Belote, J. G.; Vieira, C. R., Production of fructooligosaccharides by the mycelia of *Aspergillus japonicus* immobilized in calcium alginate. *Bioresource Technology* **1998,** *65*, 139-143.

32. Mussatto, S. I.; Teixeira, J. A., Increase in the fructooligosaccharides yield and productivity by solid-state fermentation with *Aspergillus japonicus* using agro-industrial residues as support and nutrient source. *Biochemical Engineering Journal* **2010,** *53*, 154-157.

33. Kuhn, R. C.; Filho, F. M., Purification of fructooligosaccharides in an activated charcoal fixed bed column. *New Biotechnology* **2010,** *27* (6), 866-869.

34. Kawamura, M.; Matsuda, N., Synthesis of a series of fructooligosaccharides with sucrose and cycloinulohexaose extending over ten degrees of polymerization using cycloinulooigosaccharide fructanotransferase from *Bacillus circulans* OKUMZ 31B. *Bioscience, Biotechnology, and Biochemistry* **2008,** *72* (4), 1119-1121.

35. Aachary, A. A.; Prapulla, S. G., Value addition to spent osmotic sugar solution (SOS) by enzymatic conversion to fructooligosaccharide (FOS), a low calorie prebiotic. *Innovative Food Science and Emerging Technologies* **2009,** *10*, 284-288.

36. Bibas Bonet, M. E.; Meson, O.; de Moreno de LeBlanc, A.; Dogi, C. A.; Chaves, S.; Kortsarz, A.; Grau, A.; Perdigon, G., Prebiotic effect of yacon (*Smallanthus sonchifolius*) on intestinal mucosa using a mouse model. *Food and Agricultural Immunology* **2010,** *21* (2), 175-189.

37. Gibson, G. R.; Wang, X., Bifidogenic properties of different types of fructo-oligosaccharides. *Food Microbiology* **1994,** *11* (6), 491-198.

38. Gibson, G. R.; Beatty, E. R.; Wang, X.; Cummings, J., Selective stimulation of bifidobacteria in the human colon by FOS and inulin. *Gastroenterology* **1995,** *108*, 975-982.

39. Biedrzycka, E.; Bielecka, M., Prebiotic effectiveness of fructans of different degrees of polymerization. *Trends in Food Science & Technology* **2004,** *15*, 170-175.

40. Bengmark, S.; Martindale, R., Prebiotics and synbiotics in clinical medicine. *Nutrition in Clinical Practice* **2005,** *20* (2), 244-261.

41. Dunkley, K. D.; Callaway, T. R.; Chalova, V. I.; McReynolds, J. L.; Hume, M. E.; Dunkley, C. S., Foodborne *Salmonella* ecology in the avian gastrointestinal tract. *Anaerobe* **2009,** *15*, 26-35.

42. Kolida, S.; Tuohy, K.; Gibson, G. R., The human gut flora in nutrition and approaches for its dietary modulation. *British Nutrition Foundation* **2000,** *25*, 223-231.

43. Chierici, R.; Fanaro, S.; Saccomandi, D.; Vigi, V., Advances in the modulation of the microbial ecology of the gut in early infancy. *Acta Paediatrica Supplement* **2003,** *91*, 56-63.

44. Nadeau, D. A., Intestinal warfare: the role of short-chain fructooligosaccharides in health and disease. *Nutrition in Clinical Care* **2000,** *3* (5), 266-273.

45. Marteau, P.; Flourie, B.; Pochart, P.; Chastang, C.; Desjeux, J.-F.; Rambaud, J.-C., Effect of the microbial lactase (*EC* 3.2.1.23) activity in yoghurt on the intestinal absorption of lactose: an in vivo study in lactase-deficient humans *British Journal of Nutrition* **1990,** *64* (71-79).

46. Nisbet, D., Defined competitive exclusion cultures in the prevention of enteropathogen colonisation in poultry and swine. *Antonie van Leeuwenhoek* **2002,** *81* (481-486).

47. Fukushima, Y.; Kawata, Y.; Hara, H.; Terada, A., Effect of a probiotic formula on intestinal immunoglobulin A production in healthy children. *International Journal of Food Microbiology* **1998,** *42* (39-44).

48. Fukushima, Y.; Kawata, Y.; Mizumachi, K.; Kurisaki, J.; Mitsuoka, T., Effect of bifidobacteria feeding on fecal flora and production of immunoglobulins in lactating mouse. *International Journal of Food Microbiology* **1999,** *46*, 193-197.

49. Roller, M.; Rechkemmer, G.; Watzl, B., Prebiotic inulin enriched with oligofructose in combination with the probiotics *Lactobacillus rhamnosus* and *Bifidobacterium lactis* modulates intestinal immune functions in rats. *The Journal of Nutrition* **2004,** *134*, 153-156.

50. Bouhnik, Y.; Flourie, B.; Riottot, M.; Bisetti, N.; Gailing, M. F.; Guibert, A., Effects of fructo-oligosaccharides ingestion on fecal bifidobacteria and selected metabolic indexes of colon carcinogenesis in healthy humans. *Nutrition and Cancer* **1996,** *26*, 21-29.

51. Buddington, R. K.; Williams, C. H.; Chen, S. C.; Witherly, S. A., Dietary supplement of neosugar alters the fecal flora and decreases activities of some reductive enzymes in human subjects. *American Journal of Clinical Nutrition* **1996,** *63*, 709-716.

52. Akalin, A. S.; Tokusoglu, O.; Gonc, S.; Aycan, S., Occurrence of conjugated linoleic acid in probiotic yoghurts supplemented with fructooligosaccharide. *International Dairy Journal* **2007,** *17*, 1089-1095.

53. Ferraz, R. R. N.; Marques, N. C.; Froeder, L.; Menon, V. B.; Siliano, P. R.; Baxmann, A. C.; Heilberg, I. P., Effects of *Lactobacillus casei* and *Bifidobacterium breve* on urinary oxalate excretion in nephrolithiasis patients. *Urology Research* **2009,** *37*, 95-100.

54. Coudray, C.; Bellanger, J.; Castiglia-Delavaud, C.; Remesy, C.; Vermorel, M.; Rayssignuier, Y., Effect of soluble or partly soluble dietary fibres supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. *European Journal of Clinical Nutrition* **1997,** *51*, 375-380.

55. Mineo, H.; Hara, H.; Kikuchi, H.; Sakurai, H.; Tomita, F., Various indigestible saccharides enhance net calcium transport from the epithelium of the small and large intestine of rats in vitro. *Journal of Nutrition* **2001,** *131*, 3243-3246.

56. Van den Heuvel, E.; Muys, T.; van Dokkum, W.; Schaafsma, G., Oligofructose stimulates calcium absorption in adolescents. *American Journal of Clinical Nutrition* **1999,** *69* (3), 544-48.

57. Sakai, K.; Ohta, A.; Takasaki, M.; Tokunaga, T., The effect of short chain fructooligosaccharides in promoting recovery from post-gastrectomy anemia is stronger than that of inulin. *Nutrition Research* **2000,** *20* (5), 403-412.

58. Chen, H.-L.; Lu, Y.-H.; Lin, J.-J.; Ko, L.-Y., Effects of fructooligosaccharide on bowel function and indicators of nutritional status in constipated elderly men. *Nutrition Research* **2000,** *20* (12), 1725-1733.

59. Delzenne, N.; Aertssens, J.; Verplaetse, H.; Roccaro, M.; Roberfroid, M., Effect of fermentable fructo-oligosaccharides on mineral, nitrogen and energy digestive balance in the rat. *Life Sciences* **1995,** *57* (17), 1579-1587.

60. Mitsuoka, T., Intestinal flora and human health. *Asia Pacific Journal of Clinical Nutrition* **1996,** *5*, 2-9.

61. Hopkins, M.; Cummings, J.; MacFarlane, G., Inter-species difference in maximum specific growth rates and cell yields of bifidobacteria cultured on oligosaccharides and other simple carbohydrate sources. *Journal of Applied Microbiology* **1998,** *41*, 85-101.

62. Yong, J. G.; Lee, J.-H.; Hutkins, R. W., Functional analysis of the fructooligosaccharide utilization operon in *Lactobacillus paracasei* 1195. *Applied and Environmental Microbiology* **2007,** *73* (18), 5716-5724.

63. Saulnier, D. M.; Molenaar, D.; de Vos, W. M.; Gibson, G. R.; Kolida, S., Identification of prebiotic fructooligosaccharide metabolism in *Lactobacillus plantarum* WCFS1 through microarrays. *Applied and Environmental Microbiology* **2007,** *73* (6), 1753-1765.

64. Habib, N. C.; Honore, S. M.; Genta, S. B.; Sanchez, S. S., Hypolipidemic effect of *Smallanthus sonchifolius* (yacon) roots on diabetic rats: Biochemical approach. *Chemico-Biological Interactions* **2011,** *194*, 31-39.

65. Genta, S. B.; Cabrera, W.; Habib, N.; Pons, J.; Carillo, I. M.; Grau, A.; Sanchez, S., Yacon syrup: Beneficial effects on obesity and insulin resistance in humans. *Clinical Nutrition* **2009,** *28*, 182-187.

66. Gibson, G. R.; Wang, X., Regulatory effects of bifidobacteria on the growth of other colonic bacteria. *Journal of Applied Microbiology* **2008**.

67. Bussmann, R. W.; Malca, G.; Glenn, A.; Sharon, D.; Nilsen, B.; Parris, B.; Dubose, D.; Ruiz, D.; Saleda, J.; Martinez, M.; Carillo, L.; Walker, K.; Kuhlman, A.; Townesmith, A., Toxicity of medicinal plants used in traditional medicine in Northern Peru. *Journal of Ethanopharmacology* **2011,** *137*, 121-140.

68. Aybar, M. J.; Sanchez Riera, A. N.; Grau, A.; Sanchez, S. S., Hypoglycemic effect of the water extract of *Smallanthus sonchifolius* (yacon)

leaves in normal and diabetic rats. *Journal of Ethanopharmacology* **2001,** *74*, 125-132.

69. Dou, D.-Q.; Tian, F.; Qiu, Y.-K.; Xiang, Z.; Xu, B. X.; Kang, T. G.; Dong, F., Studies on chemical constituents of the leaves of *Smallanthus sonchifolius* (yacon): Structures of two new diterpenes. *Natural Product Research* **2010,** *24* (1), 40-47.

70. Dou, D.-Q.; Tian, F.; Qiu, Y.-K.; Kang, T. G.; Dong, F., Structure elucidation and complete NMR spectral assignments of four new diterpenoids from *Smallanthus sonchifolus*. **2008**.

71. Genta, S. B.; M., C. W.; Mercado, M. I.; Grau, A.; Catalan, C. A.; Sanchez, S. S., Hypoglycemic activity of leaf organic extract *Smallanthus sonchiflius*: Constituents of the most active fractions. *Chemico-Biological Interactions* **2010,** *185*, 143-152.

72. Narita, Y.; Inouye, K., Kinetic analysis and mechanism on the inhibition of chlorogenic acid and its components against porcine pancreas α-amylase isozymes I and II. *Journal of Agricultural and Food Chemistry* **2009,** *57*, 9218-9225.

73. de Oliveira, R. B.; de Paula, D. A. C.; Rocha, B. A.; Franco, J. J.; Gobbo-Neto, L.; Uyemura, S. A.; dos Santos, W. F.; Da Costa, F. B., Renal toxicity caused by oral use of medicinal plants: The yacon example. *Journal of Ethanopharmacology* **2011,** *133*, 434-441.

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

75. Ames, B. N.; Shigenaga, M. K.; Hagen, T. M., Oxidants, antioxidants and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences of the United States of America* **1993,** *90*, 7915-7922.

76. Andersen, L. F.; Jacobs, D. R. J.; Carlsen, M. H.; Blomhoff, R., Consumption of coffee is associated with reduced risk of death attributed to inflammatory and cardiovascular diseases in the Iowa women's health study. *American Journal of Clinical Nutrition* **2006,** *83*, 1039-1046.

77. Bhattacharya, S. K.; Bhattacharya, A.; Sairam, K.; Ghosal, S., Effect of bioactive tannoid principles of *Emblica officinalis* on ischemia-reperfusion-induced oxidative stress in rat heart. *Phytomedicine* **2002,** *9* (2), 171-174.

78. Criqui, M. H.; Ringel, B. L., Does diet or alcohol explain the French paradox? *Lancet* **1994,** *344* (1719-1723).

79. Hertog, M. G. L.; Kromhout, D.; Aravanis, C.; Blackburn, H.; Buzina, R.; Fidanza, F.; Giampaoli, S.; Jansen, A.; Menotti, A.; Nedelijkovic, S.; Pekkarinen, M.; Simic, B. S.; Toshima, H.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B., Flavonoid intake and long term risk of coronary heart disease and cancer in the seven countries study. *Archives of Internal Medicine* **1995,** *155*, 381-386.

80. Joshipura, K. J.; Hu, F. B.; Manson, J. E.; Stampfer, M. J.; Rimm, E. B.; Speizer, F. E.; Colditz, G.; Ascherio, A.; Rosner, B.; Spieglman, D.; Willett, W. C., The effect of fruit and vegetable intake on risk for coronary heart disease and cancer in the seven countries study. *Archives of Internal Medicine* **2001,** *155*, 381-386.

81. Ness, A. R.; Powles, J. W., Fruit and vegetables, and cardiovascular disease: A review. *International Journal of Epidemiology* **1997,** *26*, 1-13.

82. Samman, S.; Sivarajah, G.; Man, J. C.; Ahmad, Z. I.; Petocz, P.; Caterson, I. D., A mixed fruit and vegetable concentrate increases plasma antioxidant vitamins and folate and lowers plasma homocysteine in men. *The Journal of Nutrition* **2003,** *133* (7), 2188-2193.

83. Giovanucci, E., Meta-analysis of coffee consumption and risk of colorectal cancer. *American Journal of Epidemiology* **1998,** *147*, 1043-1052.

84. Inoue, M.; Yoshimi, I.; Sobue, T.; Tsugane, S., JPHC study group. Influence of coffee drinking on subsequent risk of hepatocellular carcinoma: a prospective study in Japan. *Journal of the National Cancer Institute* **2005,** *97,* 293-300.

85. Van Dam, R. H.; Hu, F. B., Coffee consumption and risk of type 2 diabetes. *Journal of the American Medical Association* **2005,** *294*, 97-104.

86. Yamaji, T.; Mizoue, T.; Tabata, S.; Ogawa, S.; Yamaguchi, K.; Shimizu, E.; Mineshita, M.; Kono, S., Coffee consumption and glucose tolerance status in middle-aged Japanese men. *Diabetologica* **2004,** *47*, 2145-2151.

87. Ruhl, C. E.; Everhart, J. E., Coffee and tea consumption are associated with a lower incidence of chronic liver disease in the United States. *Gastroenterology* **2005,** *129* (6), 1928-1936.

88. Tverdal, A.; Skurtveit, S., Coffee intake and mortality from liver cirrhosis. *Annals of Epidemiology* **2003,** *13*, 419-423.

89. Park, J. S.; Yang, J. S.; Hwang, B. Y.; Yoo, B. K.; Han, K., Hypoglycemic effect of yacon tuber extract and its constituent, chlorogenic acid, in streptozotocin-induced diabetic rats. *Biomolecules & Therapeutics* **2009,** *17* (3), 256-262.

90. Joung, H.; Kwon, D.-Y.; Choi, J.-G.; Shin, D.-Y.; Chun, S.-S.; Yu, Y.-B.; Shin, D.-W., Antibacterial and synergystic effects of *Smallanthus sonchifolius* leaf extracts against methicillin-resistant *Staphylococcus aureus* under light intensity. *Journal of Natural Medicines* **2010,** *64*, 212-215.

91. Rios, J. L.; Recio, M. C., Medicinal plants and antimicrobial activity. *Journal of Ethanopharmacology* **2005,** *100*, 80-84.

92. Lin, F.; Hasegawa, M.; Kodama, O., Purification and identification of antimicrobial sesquiterpene lactones from Yacon *(Smallantus sonchifolius)* leaves. *Bioscience, Biotechnology, and Biochemistry* **2003,** *67* (10), 2154-2159.

93. Coll Araoz, M. V.; Mercado, M. I.; Catalan, C. A. N., Ent-kaurane derivatives from the root cortex of yacon and three other *Smallanthus* species (Heliantheae, Asteraceae) *Biochemical Systematics and Ecology* **2010,** *38* (5), 1042-1048.

94. Siriwan, D.; Naruse, T.; Tamura, H., Effect of epoxides and α–methylene-γ-lactone skeleton of sesquiterpenes from yacon (*Smallanthus sonchifolius*) leaves on capase-dependent apoptosis and NF-κB inhibition in human cervical cancer cells. *Fitoterapia* **2011,** *82*, 1093-1101.

95. Wichienchot, S.; Jatupornpipat, M.; Rastall, R. A., Oligosaccharides of pitaya (dragon fruit) flesh and their prebiotic properties. *Food Chemistry* **2010,** *120*, 850-857.

96. Kerepesi, I.; Toth, M.; Boross, L., Water-soluble carbohydrates in dried plant. *Journal of Agricultural and Food Chemistry* **1996,** *44*, 3235-3239.

97. Muir, J. G.; Rose, R.; Rosella, O.; Liels, K.; Barrett, J. S.; Shepherd, S. J.; Gibson, P. R., Measurement of short-chain carbohydrates in common Australian vegetables and fruits by high-performance liquid chromatography (HPLC). *Journal of Agricultural and Food Chemistry* **2009,** *57*, 554-565.

98. Leroy, G.; Grongnet, J. F.; Mabeau, S.; Corre, D. L., Changes in inulin and soluble sugar concentration in artichokes (*Cynara scolymus* L.) during storage. *Journal of Agricultural and Food Chemistry* **2010,** *90*, 1203-1209.

99. L'homme, C.; Peschet, J. L.; Puigserver, A.; Biagini, A., Evaluation of fructans in various fresh and stewed fruits by high-performance anion-exchange chromatography with pulsed amperometric detection. *Journal of Chromatography A* **2001,** *920*, 291-297.

100. Ghedini der Agopian, R.; Purgatto, E.; Cordenunsi, B. R.; Lajolo, F. M., Synthesis of fructooligosaccharides in banana *'Prata'* and its relation to invertase activity and sucrose accumulation. *Journal of Agricultural and Food Chemistry* **2009,** *57*, 10765-10771.

101. Downes, K.; Terry, L. A., A new acetonitrile-free mobile phase method for LC-ELSD quantification of fructooligosaccharides in onion (*Allium cepa* L.). *Talanta* **2010,** *82*, 118-124.

102. Ohyama, T.; Ito, O.; Yasuyoshi, S.; Ikarashi, T.; Minamisawa, K.; Masatsugu, K.; Tsukihashi, T.; Asami, T., Composition of storage carbohydrate in tubers of yacon *(Polymnia sonchifolia)*. *Soil Science & Plant Nutrition* **1990,** *36* (1), 167-171.

103. Blanch, M.; Sanchez-Ballesta, M. T.; Escribano, M. I.; Merodio, C., Fructo-oligosaccharides in table grapes and response to storage. *Food Chemistry* **2011,** *129*, 724-730.

104. Benkeblia, N.; Shiomi, N.; Osaki, M., Kinetics and hydrolysis parameters of total fructooligosaccharides of onion bulbs: effects of temperature regimes and cultivars. *Journal of Food Biochemistry* **2006,** *31*, 14-27.

105. Ishiguro, Y.; Onodera, S.; Benkeblia, N.; Shiomi, N., Variation of total FOS, total IOS, inulin and their related-metabolizing enzymes in burdock roots (*Arctium lappa* L.) stored under different temperatures. *Postharvest Biology and Technology* **2010,** *56*, 232-238.

106. Jaime, L.; Martinez, F.; Martin-Cabrejas, M. A.; Molla, E.; Lopez-Andreu, F. J.; Waldron, K. W.; Esteban, R. M., Study of the total fructan content in different onion tissues. *Science of Food and Agriculture* **2000,** *81*, 177-182.

107. Narai-Kanayama, A.; Tokita, N.; Aso, K., Dependence of fructooligosaccharide content on activity of fructooligosaccharide-metabolizing enzymes in yacon *(Smallanthus sonchifolius)* tuberous roots during storage. *Journal of Food Science* **2007,** *72* (6), S381-S387.

108. Imahori, Y.; Kitamura, N.; Kobayashi, S.; Takihara, T.; Ose, K.; Ueda, Y., Changes in fructooligosaccharide composition and related enzyme activities of burdock root during low-temperature storage. *Postharvest Biology and Technology* **2010,** *55*, 15-20.

109. Kahane, R.; Vialle-Guerin, E.; Boukema, I.; Tzanoudakis, D.; Bellamy, C.; Chamaux, C.; Kik, C., Changes in non-structural carbohydrate composition during bulbing in sweet and high-solid onions in field experiments. *Environmental and Experimental Botany* **2001,** *45*, 73-83.

110. Tomasic, J.; Jennings, H. J.; Glaudemans, C. P. J., Evidence for a single type of linkage in a fructofuran from *Lolium perenne*. *Carbohydrate Research* **1978,** *62*, 127-133.

111. (a) Kocsis, L.; Liebhard, P.; Praznik, W., Effect of seasonal changes on content and profile of soluble carbohydrates in tubers of different varieties of Jerusalem artichoke (*Helianthus tuberosus* L.). *Journal of Agricultural and Food Chemistry* **2007,** *55*, 9401-9408; (b) Vidanarachchi, J. K.; Iji, P. A.; Mikkelsen, L.

L.; Sims, I.; Choct, M., Isolation and characterization of water-soluble prebiotic compounds from Australian and New Zealand plants. *Carbohydrate Polymers* **2009,** *77*, 670-676.

112. Campbell, J. M.; Bauer, L. L.; Fahey Jr., G. C.; Hogarth, A. J. C. L.; Wolf, B. W.; Hunter, D. E., Selected fructooligosaccharide (1-kestose, nystose and 1^F-β-fructofuranosylnystose) composition of foods and feeds. *Journal of Agricultural and Food Chemistry* **1997,** *45*, 3076-3082.

113. Blecker, C.; Fougnies, C.; Van Herck, J.-C.; Chevalier, J.-P.; Paquot, M., Kinetic study of the acid hydrolysis of various oligofructose samples. *Journal of Agricultural and Food Chemistry* **2002,** *50*, 1602-1607.

114. L'homme, C.; Arbelot, M.; Puigserver, A.; Biagini, A., Kinetics of hydrolysis of fructooligosaccharides in mineral-buffered aqueous solutions: influence of pH and temperature. *Journal of Agricultural and Food Chemistry* **2003,** *51*, 224-228.

115. Knekt, P.; Kumpulainen, J. T.; Jarvinen, R.; Rissanen, H.; Heliovaara, M.; Reunanen, A.; Hakulinen, T., Flavonoid intake and risk of chronic disease. *American Journal of Clinical Nutrition* **2002,** *76*, 560-568.

116. Keli, S. O.; Hertog, M. G. L.; Feskens, E. J. M.; Kromhout, D., Dietary flavonoids, antioxidant vitamins, and incidence of stroke: the Zutphen study. *Archives of Internal Medicine* **1996,** *156*, 637-642.

117. Vanharatam, M.; Voutilainen, S.; Rissanen, T. H.; Adlercreutz, H.; Salonen, J. T., Risk of cardiovascular disease-related and all-cause death according to serum concentrations of enterolactone: Kuopio Ischaemic Heart Disease Risk Factor Study. *Archives of Internal Medicine* **2003,** *163*, 1099-1104.

118. Vanharatam, M.; Voutilainen, S.; Lakka, T.; Van der Lee, M.; Adlercreutz, H.; Salonen, J. T., Risk of acute coronary events according to serum concentrations of enterolactone: a prospective population-based case-control study. *Lancet* **1999,** *354*, 2112-2115.

119. Pietinen, P.; Stumpf, K.; Mannisto, S.; Kataja, V.; Uusitupa, M.; Adlercreutz, H., Serum enterlactone and risk of breast cancer: a case control study in Eastern Finland. *Cancer Epidemiology, Biomarkers and Prevention* **2001,** *10*, 339-344.

120. Ingram, D.; Sanders, K.; Kalybaba, M.; Lopez, D., Study of phytoestrogens and breast cancer. *Lancet* **1997,** *350*, 990-994.

121. Horn-Ross, P. L.; John, E. M.; Canchola, A. J.; Stewart, S. L.; Lee, M. M., Phytoestrogen intake and endometrial cancer risk. *Journal of the National Cancer Institute* **2003,** *95*, 1158-1164.

122. McCann, S. E.; Freudenheim, J. L.; Marshall, J. R.; Graham, S., Risk of human ovarian cancer is related to dietary intake of selected nutrients, phytochemicals and food groups. *Journal of Nutrition* **2003,** *133*, 1937-1942.

123. Waterhouse, A. L., Determination of total phenolics. *Current Protocols in Food and Analytical Chemistry* **2002,** *Supplement 6* (I1), 1.1-1.8.

124. Fukushima, Y.; Ohie, T.; Yonekawa, Y.; Yonemoto, K.; Aizawa, H.; Mori, Y.; Watanabe, M.; Takeuchi, M.; Hasegawa, M.; Taguchi, C.; Kondo, K., Coffee and green tea as a large source of antioxidant polyphenols in the Japanese population. *J. Agri. Food Chem.* **2009,** *57*, 1253-1259.

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

126. da Silva Pinto, M.; Kwon, Y.-I.; Apostolidis, E.; Lajolo, F. M.; Genovese, M. I.; Shetty, K., Evaluation of red currants (*Ribes rubrum* L.), black currants (*Ribes nigrum* L.), red and green gooseberries (*Ribes uva-crispa*) for potential management of type 2 diabetes and hypertension using *in vitro* models. *Journal of Food Biochemistry* **2010,** *34*, 639-660.

127. Bakowska-Barczak, A. M.; Kolodziejczyk, P., Black currant polyphenols: Their storage stability and microencapsulation. *Industrial Crops and Products* **2011,** *34*, 1301-1309.

128. Moyer, R. A.; Hummer, K. E.; Finn, C. E.; Frei, B.; Wrolstad, R. E., Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: *Vaccinium*, *Rubus* and Ribes. *Journal of Agricultural and Food Chemistry* **2002,** *50*, 519-525.

129. Anttonen, M. J.; Karjalainen, R. O., High-performance liquid chromatography analysis of black currant (Ribes nigrum L.) fruit phenolics grown either conventionally or organically. *Journal of Agricultural and Food Chemistry* **2006,** *54*, 7530-7538.

130. Benvenuti, S.; Pellati, F.; Melegari, M.; Bertelli, D., Polyphenols, anthocyanins, ascorbic acid and radical scavenging activity of *Rubus, Ribes* and *Aronia*. *Journal of Food Science* **2004,** *69* (3), 164-169.

131. Mitic, M. N.; Obradovic, M. V.; Kostic, D. A.; Naskovic, D. C.; Micic, R. J., Phenolics content and antioxidant capcity of commercial red fruit jices. *Hemijska Industrija* **2011,** *65* (5), 611-619.

132. Del Rio, D.; Borges, G.; Crozier, A., Berry flavonoids and phenolics: bioavailability and evidence of protective effects. *British Journal of Nutrition* **2010,** *104*, S67-S90.

133. Boronaba, J. G.; Terry, L. A., Biochemical profiling and chemometric analysis of seventeen UK-grown black currant cultivars. *Journal of Agricultural and Food Chemistry* **2008,** *56*, 7422-7430.

134. Kahkonen, M. P.; Heinamaki, J.; Ollilainen, V.; Heinonen, M., Berry anthocyanins: isolation, identification and antioxidant activities. *Journal of the Science of Food and Agriculture* **2003,** *83*, 1403-1411.

135. Giusti, M. M.; Wrolstad, R. E., Pigments and colorants. In *Handbook of food analytical chemistry: pigments, colorants, flavours, texture, and bioactive food components*, Wrolstad, R. E.; Acree, T. E.; Decker, E. A.; Penner, M. H.; Reid, D. S.; Schwartz, S. J.; Shoemaker, C.; Smith, D.; Sporns, P., Eds. Wiley-Interscience: Hoboken, NJ, **2000**.

136. Graefe, S.; Hermann, M.; Manrique, I.; Golombek, S.; Buerkert, A., Effects of post-harvest treatments on the carbohydrate composition of yacon roots in the Peruvian Andes. *Field Crops Research* **2004,** *86*, 157-165.

137. Scher, C. F.; de Oliveira Rios, A.; Norena, C. P. Z., Hot air drying of yacon (*Smallanthus sonchifolius*) and its effect on sugar concentrations. *International Journal of Food Science & Technology* **2009,** *44*, 2169-2175.

138. Cisneros-Zevallos, L.; Nunez, R.; Campos, D.; Noratto, G.; Chirinos, R.; C., A., Characterisation and evaluation of fructooligosaccharides in yacon roots (*Smallanthus sonchifolia* Poepp & End.) during storage. In *Nutraceuticals and functional foods*, Food Expo: Anaheim, CA, **2002**.

139. Communities, A. o. A., Total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants and wines. AOAC International, **2005**; http://www.aoac.org/omarev1/2005_02.pdf.

140. Howard, L. R.; Prior, R. L.; Liyange, R.; Lay, J. O., Processing and storage effect on berry polyphenols: challenges and implications for bioactive properties. *Journal of Agricultural and Food Chemistry* **2012,** *Article in Press*.

141. Brownmiller, C.; Howard, L. R.; Prior, R. L., Processing and storage effects on procyanidin composition and concentration of processed blueberry products. *Journal of Agricultural and Food Chemistry* **2009,** *57*, 1896-1902.

142. Saura-Calixto, F., Dietary fiber as a carrier of dietary antioxidants: an essential physiological function. *Journal of Agricultural and Food Chemistry* **2011,** *59*, 43-49.

143. Benzie, I. F. F.; Strain, J. J., Ferric reducing/ antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. In *Methods in Enzymology*, Packer, L., Ed. Academic Press: San Deigo, CA, **1999**; Vol. 299.

144. Gulcin, I., Antioxidant activity of food constituents: an overview. *Archives of Toxicology* **2012,** *86,* 345-391.

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

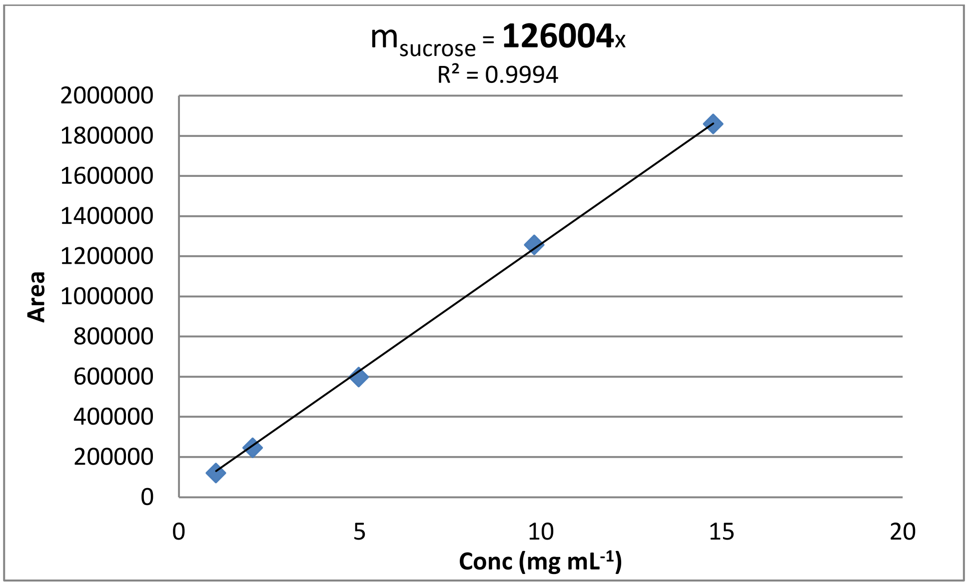
146. Ou, B.; Hampsch-Woodill, M.; Flanagan, J.; Deemer, E. K.; Prior, R. L.; Huang, D., Novel fluorometric assay for hydroxyl radical prevention capacity using fluorescein as the probe. *Journal of Agricultural and Food Chemistry* **2002,** *50,* 2772-2777.

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

5. Appendices

5.1 FOS analysis calibration and calculations

5.1.1 Example of sucrose calibration curve



5.1.2 Concentration of DP ≥ 2 sugars in storage root extract in mg mL^{-1}

$$[\mathrm{sugar_x}]\mathrm{mg\ mL^{-1}} = \frac{\mathrm{Area_{sugar_x}}}{\mathrm{m_{sucrose}}\ (\mathrm{mg\ mL^{-1}\ area^{-1}})}$$

5.1.3 Concentration of DP ≥ 2 sugars in storage root extract in mg g^{-1} dry matter

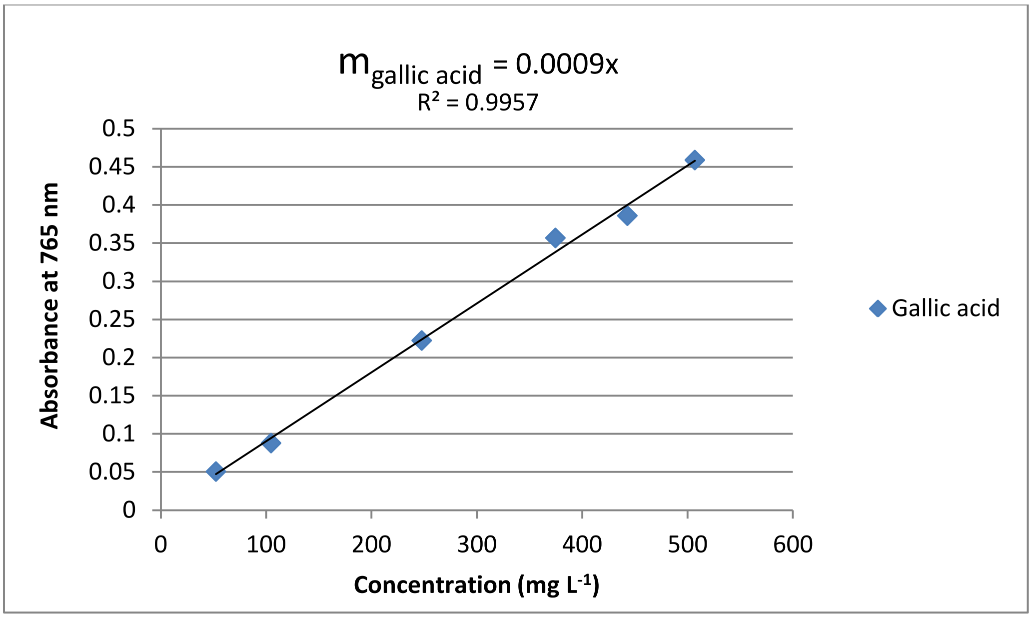
$$[\mathrm{sugar_x}]\ \mathrm{mg\ g^{-1}} = \frac{[\mathrm{sugar_x}](\mathrm{mg\ mL^{-1}})\ \times \mathrm{volume\ of\ extract\ (mL)}}{\mathrm{mass\ of\ freezedried\ storage\ root\ (g)}}$$

5.1.4 Concentration of DP ≥ 2 sugars in yacon syrup in mg FOS g^{-1}

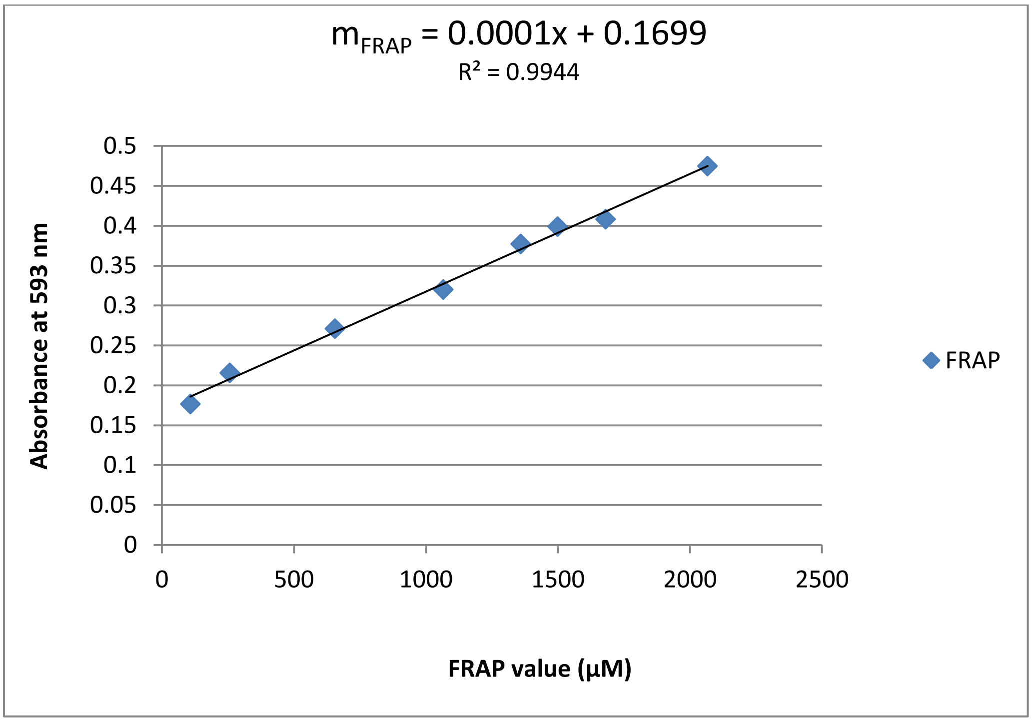
$$[\mathrm{sugar_x}]\mathrm{mg\ g^{-1}} = \frac{[\mathrm{sugar_x}]\ \mathrm{mg\ mL^{-1}} \times\ \mathrm{dilution\ factor_{volumetric\ flask}(mL)}}{\mathrm{mass\ ofsyrup(g)}}$$

5.2 Other calibration curves and calculations

5.2.1 Example of gallic acid calibration curve for micro-method



5.2.2 Example of FRAP calibration curve



5.2.3 Calculation for comparison of polyphenols or anthocaynins between juice and other blackcurrant preparations

Blackcurrant juice is 10 % concentrate

$\therefore$ mg L^{-1} concentrate = mg L^{-1} of juice x 10

Assuming a density of 1 g mL^{-1} gives concentration in mg kg^{-1}

Dividing mg kg^{-1} by 1000 gives mg g^{-1}

$\therefore$[polyphenols as GAE or anthocyanins as cyn-3-glu] in concentrate (mg g^{-1}) =

[polyphenols as GAE or anthocyanins as cyn-3-glu] in juice (mg L^{-1}) / 100

5.3 Individual yacon storage root data

Packaged root plant 1					
		Av mg g^{-1}	PD	Av RP	PD
Day 4	FOS	584.399	0.593	77.609	0.018
	1-kestose	94.451	1.154	12.543	0.543
	Sucrose	31.230	1.149	4.147	0.538
	Glucose	23.115	0.251	3.070	0.863
	Fructose	19.811	1.287	2.631	1.899
Day 28	FOS	572.882	0.793	77.599	0.018
	1-kestose	92.591	1.354	12.541	0.543
	Sucrose	30.615	1.348	4.147	0.538
	Glucose	22.718	0.052	3.077	0.862
	Fructose	19.458	1.088	2.636	1.898
Day 49	FOS	567.970	0.263	70.499	1.650
	1-kestose	90.054	2.373	11.174	0.987
	Sucrose	45.430	9.020	5.646	10.393
	Glucose	41.389	14.956	5.127	13.598
	Fructose	60.980	13.837	7.554	12.474
Day 72	FOS	507.285	1.367	66.517	0.290
	1-kestose	104.647	2.628	13.719	0.971
	Sucrose	46.208	3.825	6.056	2.169
	Glucose	26.063	0.323	3.418	1.334
	Fructose	78.477	1.402	10.290	0.255

Unpackaged root plant 1					
		Av mg g^{-1}	PD	Av RP	PD
Day 4	FOS	587.042	0.405	73.899	0.202
	1-kestose	125.406	0.780	15.786	0.173
	Sucrose	37.579	2.064	4.730	1.457
	Glucose	22.828	1.290	2.874	0.683
	Fructose	21.538	1.835	2.711	1.228
Day 28	FOS	473.019	4.828	62.521	0.909
	1-kestose	86.793	1.545	11.487	2.377
	Sucrose	41.659	3.396	5.509	0.526
	Glucose	43.178	3.226	5.711	0.695
	Fructose	111.659	2.388	14.773	1.534
Day 49	FOS	451.843	7.249	61.488	5.286
	1-kestose	79.636	0.143	10.853	2.113
	Sucrose	44.694	6.707	6.083	4.743
	Glucose	42.711	14.323	5.837	16.248
	Fructose	115.198	13.070	15.739	15.001

Packaged root plant 2

		Av mg g^{-1}	PD	Av RP	PD
Day 4	FOS	642.296	1.195	79.976	0.436
	1-kestose	109.558	2.932	13.638	1.303
	Sucrose	26.986	3.922	3.359	2.293
	Glucose	13.757	4.393	1.712	2.765
	Fructose	10.572	5.169	1.315	3.541
Day 28	FOS	574.096	0.433	74.775	0.340
	1-kestose	103.710	1.831	13.507	1.058
	Sucrose	31.801	1.575	4.142	0.802
	Glucose	30.958	2.225	4.032	1.452
	Fructose	27.215	1.329	3.544	0.556
Day 49	FOS	582.382	0.579	71.147	0.253
	1-kestose	94.032	1.094	11.487	0.768
	Sucrose	43.630	0.225	5.330	0.551
	Glucose	45.218	1.644	5.524	1.970
	Fructose	53.296	1.676	6.511	2.002
Day 72	FOS	522.234	3.613	66.078	0.312
	1-kestose	105.166	2.639	13.311	0.663
	Sucrose	49.021	3.279	6.203	0.022
	Glucose	27.569	1.839	3.490	1.463
	Fructose	86.263	2.699	10.918	0.603

Unpackaged root plant 2

		Av mg g^{-1}	PD	Av RP	PD
Day 4	FOS	590.225	2.605	78.767	0.012
	1-kestose	81.442	2.077	10.870	0.516
	Sucrose	31.636	2.409	4.222	0.184
	Glucose	20.102	2.525	2.683	0.068
	Fructose	25.924	4.228	3.458	1.636
Day 28	FOS	395.248	3.348	50.818	0.671
	1-kestose	107.482	2.661	13.822	0.017
	Sucrose	55.934	1.755	7.195	0.923
	Glucose	53.322	1.244	6.860	1.434
	Fructose	165.642	1.861	21.306	0.816
Day 49	FOS	436.902	0.518	60.763	0.413
	1-kestose	62.372	0.327	8.675	0.223
	Sucrose	37.989	0.527	5.283	0.631
	Glucose	53.714	0.796	7.470	0.900
	Fructose	128.050	0.850	17.809	0.955

Packaged root plant 3

		Av mg g^{-1}	PD	Av RP	PD
Day 4	FOS	765.644	0.956	79.436	0.311
	1-kestose	118.590	0.347	12.304	0.299
	Sucrose	37.258	1.178	3.866	1.824
	Glucose	23.218	3.511	2.410	4.155
	Fructose	19.126	1.354	1.985	1.999
Day 28	FOS	506.818	2.319	66.318	0.351
	1-kestose	107.142	4.349	14.012	1.681
	Sucrose	46.974	3.063	6.145	0.393
	Glucose	25.979	1.660	3.400	1.010
	Fructose	77.378	2.743	10.124	0.073
Day 49	FOS	561.139	0.178	70.778	0.011
	1-kestose	96.171	0.193	12.130	0.360
	Sucrose	51.957	0.347	6.553	0.180
	Glucose	25.680	3.668	3.239	3.501
	Fructose	57.869	1.055	7.299	1.222
Day 72	FOS	532.605	0.009	67.276	1.581
	1-kestose	95.579	4.676	12.064	3.089
	Sucrose	47.791	2.330	6.035	0.741
	Glucose	27.014	1.084	3.412	0.506
	Fructose	88.876	7.498	11.213	5.916

Unpackaged root plant 3

		Av mg g^{-1}	PD	Av RP	PD
Day 4	FOS	598.561	1.016	75.406	0.322
	1-kestose	130.630	1.192	16.456	0.146
	Sucrose	34.501	5.996	4.344	4.662
	Glucose	15.985	2.717	2.013	1.381
	Fructose	14.143	3.364	1.781	2.028
Day 28	FOS	478.106	1.136	62.007	1.645
	1-kestose	105.783	2.002	13.720	2.511
	Sucrose	49.373	4.924	6.401	4.416
	Glucose	39.088	14.184	5.065	13.685
	Fructose	98.770	3.544	12.807	3.035
Day 49	FOS	443.113	0.605	60.741	0.212
	1-kestose	63.044	0.891	8.642	0.074
	Sucrose	39.687	0.622	5.440	0.195
	Glucose	54.434	1.579	7.461	0.762
	Fructose	129.243	1.247	17.716	0.430

5.4 Blackcurrant-yacon juice FOS results tables

Excess K_2CO_3, pH 4.2, heated at 95 °C

Sugar concentrations in mg g^{-1}					
Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
0	32.590	12.326	9.894	16.063	29.127
0.5	33.393	11.903	9.445	16.051	29.208
1	33.571	11.493	9.286	16.117	29.534
1.5	33.778	11.282	9.409	16.169	29.362
2	32.851	10.948	9.799	16.501	29.900
2.5	32.856	10.689	9.575	16.685	30.195
3	32.346	10.308	9.698	16.803	30.845
3.5	31.837	9.993	10.022	16.863	31.286
4	31.553	9.930	9.817	17.354	31.346
4.5	31.363	9.773	9.849	17.295	31.720
5	30.282	9.802	10.087	17.433	32.396
5.5	30.316	9.528	10.376	17.793	31.987
6	29.910	9.686	10.329	17.752	32.324
6.5	30.383	9.489	10.122	17.602	32.403
7	29.638	9.479	10.021	17.847	33.014
7.5	29.165	9.749	10.311	17.899	32.876
8	29.123	9.660	10.235	18.041	32.942
8.5	28.894	9.746	10.455	17.966	32.940

Relative percentage change in sugar composition					
Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
0	100.00	100.00	100.00	100.00	100.00
0.5	102.46	96.57	95.46	99.92	100.28
1	103.01	93.24	93.86	100.33	101.40
1.5	103.64	91.53	95.10	100.66	100.81
2	100.80	88.83	99.05	102.72	102.65
2.5	100.81	86.72	96.78	103.87	103.67
3	99.25	83.63	98.02	104.61	105.90
3.5	97.69	81.07	101.29	104.98	107.41
4	96.82	80.56	99.22	108.04	107.62
4.5	96.23	79.29	99.55	107.67	108.90
5	92.92	79.52	101.95	108.53	111.22
5.5	93.02	77.30	104.88	110.77	109.82
6	91.77	78.58	104.40	110.51	110.98
6.5	93.23	76.99	102.31	109.58	111.25
7	90.94	76.91	101.29	111.11	113.35
7.5	89.49	79.09	104.22	111.43	112.87
8	89.36	78.37	103.45	112.31	113.10
8.5	88.66	79.07	105.67	111.84	113.09

Sugar concentrations in mg g^{-1}

Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
9	28.346	9.610	10.560	18.140	33.344
9.5	28.691	9.581	10.524	18.168	33.036
10	27.694	9.809	10.644	18.196	33.658
10.5	27.664	9.881	10.576	18.174	33.705
11	27.490	9.707	11.002	18.169	33.633
11.5	27.634	9.686	10.500	18.071	34.109
12	27.049	9.557	11.211	18.204	33.978
12.5	26.683	9.708	11.181	18.690	33.738
13	26.570	9.684	11.221	18.347	34.178
13.5	26.312	9.834	11.273	18.339	34.242
14	26.261	9.733	11.404	18.463	34.140
14.5	26.055	9.861	11.484	18.505	34.095
15	26.017	9.756	11.554	18.452	34.221
15.5	25.609	9.696	11.652	18.441	34.602
16	25.689	9.810	11.697	18.469	34.335
16.5	26.039	9.782	11.715	18.431	34.034
17	25.884	9.620	11.853	18.266	34.377
17.5	25.729	9.597	11.880	18.137	34.657
18	25.720	9.730	11.747	18.346	34.456
18.5	25.560	9.629	12.126	18.361	34.325
19	25.810	9.389	12.053	18.160	34.587
19.5	25.418	9.491	12.357	18.120	34.613
20	25.652	9.711	12.045	18.246	34.346
20.5	25.536	9.565	12.213	18.285	34.400

Relative percentage change in sugar composition

Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
9	86.98	77.97	106.73	112.93	114.48
9.5	88.04	77.73	106.37	113.10	113.42
10	84.98	79.58	107.58	113.28	115.55
10.5	84.88	80.17	106.90	113.14	115.72
11	84.35	78.75	111.20	113.11	115.47
11.5	84.79	78.58	106.13	112.50	117.10
12	83.00	77.54	113.32	113.33	116.66
12.5	81.87	78.76	113.01	116.35	115.83
13	81.53	78.57	113.42	114.22	117.34
13.5	80.74	79.79	113.95	114.16	117.56
14	80.58	78.96	115.26	114.94	117.21
14.5	79.95	80.00	116.08	115.20	117.06
15	79.83	79.15	116.79	114.87	117.49
15.5	78.58	78.67	117.78	114.80	118.80
16	78.82	79.59	118.23	114.98	117.88
16.5	79.90	79.36	118.41	114.74	116.85
17	79.42	78.05	119.81	113.71	118.02
17.5	78.95	77.87	120.08	112.91	118.99
18	78.92	78.94	118.73	114.21	118.30
18.5	78.43	78.12	122.56	114.30	117.85
19	79.20	76.17	121.83	113.05	118.75
19.5	77.99	77.01	124.90	112.80	118.83
20	78.71	78.79	121.75	113.59	117.92
20.5	78.35	77.61	123.45	113.83	118.10

Sugar concentrations in mg g^{-1}

Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
21	25.461	9.470	12.520	18.265	34.284
21.5	25.247	9.516	12.428	18.304	34.505
22	25.741	9.408	12.493	18.125	34.233
22.5	25.807	9.488	12.790	17.835	34.081
23	25.469	9.576	12.592	18.148	34.215
23.5	25.378	9.444	12.508	18.035	34.635
24	25.105	9.406	12.575	18.009	34.906
24.5	25.230	9.372	12.511	18.073	34.814
25	25.070	9.259	12.569	18.015	35.088
25.5	25.081	9.340	12.658	18.089	34.832
6	24.906	9.305	12.525	17.978	35.285
26.5	24.822	9.434	12.688	18.035	35.021
27	24.959	9.531	12.630	18.056	34.823
27.5	24.790	9.585	12.750	17.979	34.895
28	24.694	9.523	12.902	17.949	34.932
28.5	24.647	9.434	12.917	17.929	35.073
29	24.362	9.377	12.982	17.861	35.418
29.5	24.302	9.444	13.140	17.927	35.187
30	24.212	9.353	13.243	17.869	35.324

Relative percentage change in sugar composition

Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
21	78.13	76.83	126.54	113.71	117.70
21.5	77.47	77.20	125.62	113.95	118.46
22	78.98	76.33	126.27	112.84	117.53
22.5	79.18	76.98	129.27	111.03	117.01
23	78.15	77.69	127.27	112.98	117.47
23.5	77.87	76.62	126.42	112.27	118.91
24	77.03	76.31	127.10	112.11	119.84
24.5	77.42	76.03	126.46	112.51	119.53
25	76.92	75.12	127.04	112.15	120.46
25.5	76.96	75.78	127.94	112.61	119.59
26	76.42	75.50	126.60	111.92	121.14
26.5	76.16	76.54	128.24	112.27	120.24
27	76.59	77.33	127.66	112.41	119.55
27.5	76.07	77.77	128.87	111.93	119.80
28	75.77	77.26	130.41	111.74	119.93
28.5	75.63	76.54	130.56	111.61	120.41
29	74.75	76.08	131.22	111.19	121.60
29.5	74.57	76.62	132.81	111.60	120.81
30	74.29	75.88	133.86	111.24	121.28

Minimal K_2CO_3, pH 4.0, heated at 95 °C

Sugar concentrations in mg g^{-1}

Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
0	32.273	11.372	9.766	15.030	31.559
0.5	32.000	10.513	9.919	15.124	32.444
1	31.709	10.349	9.901	15.164	32.877
1.5	31.397	10.034	10.001	15.247	33.321
2	30.742	9.860	10.125	15.356	33.917
2.5	30.412	9.513	10.297	15.416	34.363
3	30.373	9.485	10.359	15.524	34.258
3.5	30.092	9.383	10.105	15.095	35.325
4	29.374	9.124	9.884	15.454	36.164
4.5	29.385	8.795	10.091	15.391	36.338
5	28.394	8.805	10.294	15.589	36.918
5.5	29.026	8.688	10.338	15.782	36.167
6	27.960	8.582	10.379	15.772	37.308
6.5	28.655	8.582	10.222	15.667	36.874
7	27.872	8.574	10.236	15.845	37.473
7.5	27.568	8.831	10.499	15.863	37.239
8	27.298	8.619	10.361	16.008	37.713
8.5	26.986	8.760	10.675	15.949	37.629

Relative percentage change in sugar composition

Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
0	100.00	100.00	100.00	100.00	100.00
0.5	99.15	92.45	101.56	100.62	102.81
1	98.25	91.00	101.38	100.89	104.18
1.5	97.29	88.23	102.40	101.45	105.58
2	95.26	86.70	103.67	102.17	107.47
2.5	94.23	83.65	105.43	102.57	108.89
3	94.11	83.40	106.07	103.29	108.56
3.5	93.24	82.50	103.47	100.43	111.94
4	91.02	80.23	101.21	102.82	114.59
4.5	91.05	77.34	103.32	102.40	115.14
5	87.98	77.43	105.40	103.72	116.98
5.5	89.94	76.39	105.85	105.00	114.60
6	86.63	75.46	106.27	104.94	118.22
6.5	88.79	75.46	104.67	104.24	116.84
7	86.36	75.39	104.81	105.43	118.74
7.5	85.42	77.65	107.51	105.54	118.00
8	84.58	75.79	106.09	106.51	119.50
8.5	83.62	77.03	109.31	106.12	119.24

Sugar concentrations in mg g^{-1}					
Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
9	26.911	8.589	10.628	16.070	37.802
9.5	26.646	8.700	10.714	16.062	37.878
10	25.875	8.720	10.766	16.233	38.406
10.5	25.890	8.648	10.842	16.280	38.339
11	25.616	8.722	10.773	16.177	38.711
11.5	26.061	8.831	10.727	16.106	38.276
12	25.178	8.805	10.662	16.256	39.098
12.5	25.073	8.913	10.676	16.460	38.878
13	24.795	8.767	10.689	16.436	39.314
13.5	24.391	8.823	10.735	16.378	39.673
14	25.434	8.581	10.776	16.120	39.089
14.5	24.254	8.998	10.898	16.467	39.383
15	24.299	8.693	10.894	16.402	39.712
15.5	23.939	8.730	10.867	16.443	40.022
16	23.983	8.773	10.728	16.400	40.115
16.5	24.296	8.717	10.872	16.433	39.681
17	23.889	8.805	10.992	16.517	39.798
17.5	24.068	8.702	10.985	16.667	39.579
18	24.082	8.692	11.082	16.749	39.394
18.5	23.807	8.621	11.004	16.803	39.765
19	24.531	8.445	11.156	16.871	38.997
19.5	23.736	8.451	11.229	16.857	39.727
20	23.773	8.610	11.298	16.954	39.366
20.5	23.401	8.555	11.289	16.953	39.802

Relative percentage change in sugar composition					
Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
9	83.38	75.53	108.83	106.92	119.78
9.5	82.56	76.51	109.70	106.87	120.02
10	80.17	76.68	110.24	108.00	121.70
10.5	80.22	76.05	111.02	108.32	121.49
11	79.37	76.70	110.31	107.63	122.67
11.5	80.75	77.65	109.83	107.16	121.28
12	78.02	77.42	109.17	108.16	123.89
12.5	77.69	78.37	109.31	109.52	123.19
13	76.83	77.09	109.45	109.36	124.57
13.5	75.58	77.58	109.91	108.97	125.71
14	78.81	75.46	110.34	107.25	123.86
14.5	75.15	79.12	111.58	109.56	124.79
15	75.29	76.44	111.54	109.13	125.84
15.5	74.18	76.76	111.27	109.40	126.82
16	74.31	77.14	109.85	109.12	127.11
16.5	75.28	76.66	111.33	109.34	125.74
17	74.02	77.43	112.55	109.89	126.11
17.5	74.58	76.51	112.48	110.89	125.41
18	74.62	76.43	113.47	111.44	124.83
18.5	73.77	75.81	112.67	111.80	126.00
19	76.01	74.26	114.23	112.25	123.57
19.5	73.55	74.31	114.97	112.16	125.89
20	73.66	75.71	115.68	112.81	124.74
20.5	72.51	75.23	115.59	112.80	126.12

Sugar concentrations in mg g^{-1}

Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
21	23.365	8.416	11.380	17.086	39.754
21.5	23.874	8.414	11.369	17.210	39.132
22	23.682	8.400	11.325	17.291	39.302
22.5	24.039	8.383	11.310	16.981	39.286
23	23.715	8.635	11.454	17.083	39.114
23.5	23.406	8.492	11.596	17.156	39.351
24	23.536	8.525	11.378	17.232	39.329
24.5	23.322	8.476	11.491	17.285	39.426
25	23.133	8.466	11.434	17.392	39.575
25.5	23.100	8.289	11.560	17.381	39.670
26	22.921	8.324	11.506	17.488	39.762
26.5	22.828	8.328	11.673	17.711	39.460
27	22.930	8.304	11.527	17.693	39.547
27.5	22.678	8.379	11.625	17.681	39.637
28	22.717	8.349	11.666	17.773	39.496
28.5	22.660	8.289	11.742	17.794	39.515
29	22.433	8.327	11.868	17.809	39.563
29.5	22.288	8.295	11.930	17.957	39.529
30	22.247	8.322	12.015	17.995	39.420

Relative percentage change in sugar composition

Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
21	72.40	74.00	116.52	113.68	125.97
21.5	73.98	73.99	116.41	114.51	124.00
22	73.38	73.87	115.96	115.04	124.54
22.5	74.49	73.71	115.81	112.98	124.49
23	73.48	75.93	117.28	113.66	123.94
23.5	72.52	74.67	118.73	114.14	124.69
24	72.93	74.96	116.51	114.65	124.62
24.5	72.26	74.53	117.66	115.00	124.93
25	71.68	74.45	117.07	115.72	125.40
25.5	71.58	72.89	118.37	115.64	125.70
26	71.02	73.20	117.82	116.35	125.99
26.5	70.73	73.23	119.52	117.84	125.04
27	71.05	73.02	118.03	117.72	125.31
27.5	70.27	73.68	119.03	117.64	125.60
28	70.39	73.41	119.45	118.25	125.15
28.5	70.21	72.89	120.23	118.39	125.21
29	69.51	73.22	121.52	118.49	125.36
29.5	69.06	72.94	122.16	119.48	125.26
30	68.93	73.17	123.03	119.73	124.91

No K_2CO_3, pH 2.8, heated at 95 °C

Sugar concentrations in mg g^{-1}

Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
0	32.729	11.012	10.392	15.294	30.573
0.5	32.874	9.256	10.734	15.660	31.475
1	32.109	9.097	10.663	15.156	32.975
1.5	31.992	8.680	10.734	15.292	33.303
2	30.715	8.478	11.044	15.363	34.400
2.5	30.085	8.226	11.220	15.474	34.995
3	30.357	7.910	11.260	15.535	34.938
3.5	29.298	7.687	11.583	15.607	35.825
4	29.374	7.785	11.389	15.760	35.693
4.5	29.245	7.524	11.554	15.759	35.919
5	28.968	7.448	11.453	15.799	36.331
5.5	28.661	7.561	11.515	15.772	36.492
6	28.343	7.074	11.607	15.658	37.318
6.5	27.843	7.420	11.674	15.699	37.365
7	27.620	7.352	11.717	15.665	37.647
7.5	27.092	7.334	11.805	15.589	38.181
8	26.540	6.787	11.802	15.643	39.229
8.5	26.064	6.530	11.897	15.559	39.950

Relative percentage change in sugar composition

Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
0	100.00	100.00	100.00	100.00	100.00
0.5	100.44	84.05	103.29	102.39	102.95
1	98.11	82.61	102.61	99.09	107.86
1.5	97.75	78.82	103.29	99.98	108.93
2	93.85	76.99	106.27	100.45	112.52
2.5	91.92	74.70	107.97	101.18	114.46
3	92.75	71.83	108.35	101.58	114.28
3.5	89.52	69.81	111.45	102.04	117.18
4	89.75	70.70	109.59	103.04	116.75
4.5	89.35	68.32	111.18	103.04	117.49
5	88.51	67.64	110.21	103.30	118.84
5.5	87.57	68.66	110.80	103.12	119.36
6	86.60	64.23	111.69	102.38	122.06
6.5	85.07	67.38	112.33	102.64	122.22
7	84.39	66.76	112.74	102.42	123.14
7.5	82.78	66.60	113.59	101.93	124.88
8	81.09	61.63	113.56	102.28	128.31
8.5	79.64	59.30	114.48	101.73	130.67

Sugar concentrations in mg g^{-1}

Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
9	26.476	6.513	11.829	16.117	39.065
9.5	26.759	6.788	11.893	15.397	39.164
10	26.086	6.983	11.957	15.331	39.642
10.5	25.994	6.273	11.926	15.427	40.381
11	25.653	6.623	11.920	15.338	40.466
11.5	24.938	6.720	12.042	15.434	40.866
12	24.564	6.857	12.027	15.469	41.083
12.5	24.453	6.463	12.037	15.518	41.529
13	23.924	6.675	12.076	15.584	41.741
13.5	23.680	6.531	12.205	15.508	42.076
14	23.740	6.334	12.120	15.518	42.288
14.5	23.337	6.331	12.153	15.666	42.514
15	22.635	6.389	12.389	15.666	42.921
15.5	22.343	6.409	12.448	15.739	43.061
16	22.030	6.731	12.301	15.787	43.151
16.5	21.810	6.746	12.350	15.862	43.232
17	21.567	6.614	12.440	15.921	43.457
17.5	21.046	6.776	12.504	15.946	43.728
18	21.653	6.118	12.429	15.909	43.891
18.5	21.576	6.230	12.507	16.047	43.640
19	21.005	6.090	12.696	16.251	43.959
19.5	20.957	6.032	12.722	16.262	44.027
20	20.437	6.401	12.710	16.279	44.172
20.5	20.005	6.427	12.898	16.270	44.400

Relative percentage change in sugar composition

Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
9	80.89	59.15	113.82	105.38	127.78
9.5	81.76	61.64	114.44	100.67	128.10
10	79.71	63.42	115.06	100.24	129.66
10.5	79.42	56.96	114.75	100.87	132.08
11	78.38	60.14	114.70	100.29	132.36
11.5	76.20	61.02	115.88	100.92	133.67
12	75.05	62.27	115.74	101.14	134.38
12.5	74.71	58.69	115.83	101.46	135.84
13	73.10	60.62	116.20	101.89	136.53
13.5	72.35	59.31	117.44	101.40	137.63
14	72.54	57.52	116.63	101.46	138.32
14.5	71.30	57.49	116.94	102.43	139.06
15	69.16	58.02	119.21	102.43	140.39
15.5	68.27	58.20	119.79	102.91	140.85
16	67.31	61.12	118.37	103.22	141.14
16.5	66.64	61.26	118.84	103.71	141.41
17	65.90	60.06	119.71	104.10	142.14
17.5	64.30	61.54	120.32	104.26	143.03
18	66.16	55.55	119.59	104.02	143.56
18.5	65.92	56.58	120.35	104.92	142.74
19	64.18	55.30	122.17	106.26	143.78
19.5	64.03	54.77	122.42	106.33	144.01
20	62.44	58.13	122.31	106.44	144.48
20.5	61.13	58.36	124.11	106.38	145.23

Sugar concentrations in mg g^{-1}

Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
21	20.140	6.468	12.902	16.275	44.216
21.5	19.765	6.485	13.004	16.370	44.377
22	19.642	6.189	13.185	16.409	44.576
22.5	19.348	5.706	13.321	16.629	44.997
23	19.082	5.511	13.477	16.604	45.326
23.5	18.196	5.656	13.241	16.657	46.250
24	18.054	5.675	13.345	16.729	46.197
24.5	17.932	5.716	13.423	16.879	46.049
25	17.846	5.538	13.656	16.933	46.027
25.5	17.738	5.193	13.801	16.952	46.316
26	17.453	5.117	13.796	17.104	46.529
26.5	17.386	5.422	13.861	17.005	46.327
27	17.401	5.493	13.710	17.004	46.392
27.5	16.398	5.981	13.666	17.078	46.877
28	16.287	5.596	13.802	17.331	46.984
28.5	16.856	5.194	13.713	16.829	47.407
29	16.744	5.139	13.687	16.845	47.585
29.5	16.654	5.001	13.749	16.855	47.740
30	17.030	4.902	13.737	16.977	47.353

Relative percentage change in sugar composition

Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
21	61.54	58.74	124.15	106.41	144.62
21.5	60.39	58.89	125.13	107.03	145.15
22	60.01	56.20	126.88	107.28	145.80
22.5	59.12	51.81	128.18	108.72	147.18
23	58.30	50.05	129.69	108.56	148.26
23.5	55.60	51.36	127.41	108.91	151.28
24	55.16	51.54	128.42	109.38	151.10
24.5	54.79	51.91	129.17	110.36	150.62
25	54.53	50.29	131.40	110.71	150.55
25.5	54.20	47.16	132.80	110.84	151.49
26	53.33	46.47	132.76	111.83	152.19
26.5	53.12	49.23	133.38	111.18	151.53
27	53.17	49.89	131.93	111.18	151.74
27.5	50.10	54.31	131.50	111.66	153.33
28	49.76	50.81	132.81	113.31	153.68
28.5	51.50	47.17	131.96	110.03	155.06
29	51.16	46.67	131.71	110.14	155.64
29.5	50.89	45.41	132.30	110.21	156.15
30	52.04	44.52	132.18	111.00	154.89

Minimal K_2CO_3, pH 4, heated at 15 °C

Sugar concentrations in mg g^{-1}					
Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
0	35.543	10.326	8.739	13.063	32.330
0.25	34.933	9.538	8.851	13.382	33.296
0.5	33.858	9.494	8.990	13.503	34.155
0.75	33.570	9.239	9.034	13.608	34.548
1	32.769	9.092	9.218	13.693	35.228
1.25	32.347	8.627	9.472	13.705	35.849
1.5	32.275	8.525	9.521	13.747	35.932
1.75	31.338	8.085	9.493	13.625	37.459
2	31.200	7.948	9.528	13.725	37.599
2.25	30.778	7.840	9.595	13.725	38.062
2.5	30.463	7.753	9.624	13.755	38.405
2.75	29.907	7.428	9.653	13.795	39.217
3	29.245	7.284	9.682	13.800	39.988
3.25	28.958	7.184	9.711	13.837	40.310
3.5	28.379	7.005	9.740	13.864	41.013
3.75	27.609	6.864	9.769	13.894	41.864
4	26.821	6.643	9.798	13.917	42.820
4.25	26.468	6.385	9.828	13.976	43.343

Relative percentage change in sugar composition					
Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
0	100.00	100.00	100.00	100.00	100.00
0.25	98.28	92.37	101.29	102.44	102.99
0.5	95.26	91.94	102.88	103.36	105.65
0.75	94.45	89.48	103.38	104.17	106.86
1	92.20	88.05	105.48	104.82	108.97
1.25	91.01	83.55	108.39	104.91	110.89
1.5	90.81	82.56	108.95	105.23	111.14
1.75	88.17	78.30	108.63	104.30	115.87
2	87.78	76.97	109.03	105.06	116.30
2.25	86.59	75.93	109.80	105.06	117.73
2.5	85.71	75.09	110.13	105.29	118.79
2.75	84.14	71.94	110.46	105.60	121.30
3	82.28	70.54	110.79	105.64	123.69
3.25	81.47	69.57	111.12	105.93	124.68
3.5	79.84	67.84	111.45	106.13	126.86
3.75	77.68	66.48	111.79	106.36	129.49
4	75.46	64.34	112.12	106.54	132.45
4.25	74.47	61.84	112.46	106.98	134.07

Sugar concentrations in mg g^{-1}

Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
4.5	25.869	6.184	9.857	14.003	44.087
4.75	24.909	5.836	9.887	14.027	45.340
5	24.653	5.542	9.916	14.043	45.845
5.25	24.706	5.330	9.946	14.072	45.946
5.5	24.800	5.218	10.176	14.125	45.681
5.75	24.838	5.184	9.706	14.225	46.047
6	25.122	5.025	9.936	14.125	45.792
6.25	24.169	4.953	10.066	14.325	46.487
6.5	24.507	4.828	10.096	14.332	46.236
6.75	24.928	4.287	10.127	14.352	46.305
7	23.940	4.349	10.157	14.372	47.182
7.25	24.692	4.393	10.187	14.472	46.255
7.5	24.054	4.663	10.218	14.572	46.493
7.75	23.371	4.363	10.249	14.604	47.414
8	22.525	4.746	10.279	14.633	47.816
8.25	22.533	4.238	10.310	14.675	48.243
8.5	21.802	4.390	10.341	14.882	48.584
8.75	21.675	4.427	10.372	14.795	48.731
9	21.453	4.395	10.403	14.739	49.010
9.25	22.050	4.284	10.535	14.756	48.375
9.5	21.765	4.482	10.466	14.802	48.484
9.75	21.519	4.488	10.497	14.834	48.661
10	21.525	4.528	10.329	14.872	48.745
10.25	20.186	4.329	10.560	14.892	50.032

Relative percentage change in sugar composition

Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
4.5	72.78	59.89	112.80	107.19	136.37
4.75	70.08	56.52	113.14	107.38	140.24
5	69.36	53.67	113.48	107.50	141.80
5.25	69.51	51.62	113.82	107.72	142.12
5.5	69.78	50.54	116.45	108.13	141.30
5.75	69.88	50.20	111.07	108.89	142.43
6	70.68	48.67	113.70	108.13	141.64
6.25	68.00	47.97	115.19	109.66	143.79
6.5	68.95	46.76	115.53	109.72	143.01
6.75	70.14	41.52	115.88	109.87	143.23
7	67.35	42.11	116.23	110.02	145.94
7.25	69.47	42.54	116.58	110.79	143.07
7.5	67.68	45.16	116.93	111.55	143.81
7.75	65.75	42.25	117.28	111.79	146.66
8	63.38	45.97	117.63	112.02	147.90
8.25	63.40	41.05	117.98	112.34	149.22
8.5	61.34	42.52	118.34	113.93	150.28
8.75	60.98	42.87	118.69	113.25	150.73
9	60.36	42.56	119.05	112.83	151.59
9.25	62.04	41.49	120.55	112.96	149.63
9.5	61.24	43.41	119.76	113.31	149.97
9.75	60.54	43.47	120.12	113.56	150.52
10	60.56	43.86	118.19	113.85	150.77
10.25	56.79	41.92	120.84	114.00	154.76

Sugar concentrations in mg g^{-1}

Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
10.5	20.858	4.375	10.592	14.902	49.272
10.75	20.400	4.593	10.624	14.910	49.473
11	20.843	4.483	10.656	14.920	49.098
11.25	20.996	4.649	10.588	14.945	48.822
11.5	19.610	4.740	10.720	14.968	49.963
11.75	19.911	4.660	10.752	14.980	49.697
12	18.676	4.483	10.784	15.004	51.053
12.25	18.019	4.749	10.816	15.028	51.387
12.5	18.531	4.389	10.849	15.094	51.138
12.75	17.489	4.403	10.881	15.105	52.122
13	16.621	4.329	10.914	15.110	53.026
13.25	16.420	4.547	10.847	15.137	53.048
13.5	16.542	4.631	10.980	15.165	52.683
13.75	15.912	4.285	10.813	15.208	53.782
14	15.833	4.392	11.046	15.216	53.512
14.25	15.740	4.593	11.079	15.255	53.333
14.5	15.601	4.328	11.112	15.285	53.674
14.75	15.451	4.483	11.145	15.473	53.448
15	15.395	4.525	11.179	15.373	53.529

Relative percentage change in sugar composition

Minutes	FOS	1-kestose	Sucrose	Glucose	Fructose
10.5	58.69	42.37	121.21	114.08	152.41
10.75	57.39	44.48	121.57	114.14	153.03
11	58.64	43.42	121.93	114.22	151.87
11.25	59.07	45.03	121.16	114.41	151.01
11.5	55.17	45.90	122.67	114.58	154.54
11.75	56.02	45.13	123.03	114.67	153.72
12	52.55	43.42	123.40	114.85	157.91
12.25	50.70	45.99	123.77	115.04	158.95
12.5	52.14	42.51	124.15	115.54	158.18
12.75	49.21	42.64	124.52	115.63	161.22
13	46.76	41.93	124.89	115.67	164.02
13.25	46.20	44.04	124.12	115.88	164.08
13.5	46.54	44.85	125.64	116.09	162.96
13.75	44.77	41.50	123.73	116.42	166.35
14	44.55	42.54	126.40	116.48	165.52
14.25	44.29	44.48	126.78	116.78	164.97
14.5	43.89	41.92	127.16	117.00	166.02
14.75	43.47	43.42	127.54	118.45	165.32
15	43.31	43.82	127.92	117.68	165.57

5.5 Blackcurrant juice bioactives results tables

Blackcurrant-yacon juice, excess K_2CO_3, pH 4.2, heated at 95 °C

Minutes	Anthocyanins cyn-3-glu mg L^{-1}	DPPH % I	FRAP µM	Polyphenols GAE mg mL^{-1}
0	1013.11	81.74	8416.66	330.67
0.5	989.61	81.80	10061.91	263.33
1	926.19	83.88	10725.16	306.67
1.5	867.65	87.47	12100.66	270.67
2	796.06	89.72	8649.66	263.33
2.5	785.60	83.40	8059.91	268.89
3	815.71	84.45	7848.16	364.22
3.5	823.26	86.39	8775.66	284.89
4	836.99	83.99	7358.16	269.78
4.5	843.31	84.78	9629.66	270.67
5	813.73	83.88	9040.12	308.22
5.5	836.00	83.02	10823.16	319.10
6	835.91	86.09	7709.91	233.78
6.5	833.34	82.86	10138.91	292.22
7	815.35	81.19	17147.66	299.33
7.5	859.45	79.89	10623.66	284.89
8	802.28	76.55	11358.66	320.89
8.5	831.04	79.89	6834.91	313.78
9	802.82	78.48	7370.41	241.78
9.5	793.53	69.66	13388.66	292.22
10	831.85	81.37	9725.91	292.22
10.5	773.60	89.33	10254.99	322.29
11	771.98	81.29	10574.66	263.33
11.5	762.96	82.09	8612.91	248.89
12	731.05	79.24	8873.66	284.89
12.5	731.04	63.96	10268.41	393.11
13	731.22	80.22	8200.49	284.89
13.5	749.71	70.49	9316.41	263.33
14	787.58	79.41	10166.91	356.89

Minutes	Anthocyanins cyn-3-glu mg L^{-1}	DPPH % I	FRAP µM	Polyphenols GAE mg mL^{-1}
14.5	757.28	79.09	11174.91	320.89
15	714.63	73.16	10697.16	365.11
15.5	728.43	75.76	8691.66	227.33
16	740.42	78.52	8468.82	270.67
16.5	720.49	74.94	8692.82	328.22
17	738.98	83.35	9790.66	349.78
17.5	744.66	68.87	9356.66	349.78
18	769.27	74.92	10544.91	313.78
18.5	658.72	78.09	8308.16	263.33
19	650.24	67.69	11651.49	356.89
19.5	658.72	77.71	8549.32	234.67
20	602.99	66.18	8143.66	212.89
20.5	588.39	86.81	7500.49	241.78
21	595.15	71.12	8355.99	212.89
21.5	576.21	67.74	10333.16	277.78
22	665.12	66.37	9211.94	283.11
22.5	591.09	71.02	12155.49	349.78
23	686.22	66.99	10502.32	356.89
23.5	656.74	68.94	10995.05	396.49
24	657.37	76.68	10032.81	343.00
24.5	660.98	63.36	11330.40	262.93
25	660.34	68.93	12695.66	241.84
25.5	637.17	71.84	11422.38	263.10
26	668.64	62.27	10198.50	205.42
26.5	693.89	71.49	10577.36	252.76
27	639.06	60.88	12464.61	222.89
27.5	665.30	79.87	11330.89	287.42
28	662.69	65.43	12359.24	253.00
28.5	620.04	62.32	8897.15	294.22
29	580.36	61.06	8369.40	283.78
29.5	569.99	65.34	8286.24	322.93
30	573.51	61.63	8290.40	231.82

Blackcurrant-yacon juice, minimum K_2CO_3, pH 4, heated at 95 °C

Minutes	Anthocyanins cyn-3-glu mg L^{-1}	DPPH % I	FRAP µM	Polyphenols GAE mg mL^{-1}
0	1013.11	81.74	8416.66	330.67
0.5	989.61	81.80	10061.91	263.33
1	926.19	83.88	10725.16	306.67
1.5	867.65	87.47	12100.66	270.67
2	796.06	89.72	8649.66	263.33
2.5	785.60	83.40	8059.91	268.89
3	815.71	84.45	7848.16	364.22
3.5	823.26	86.39	8775.66	284.89
4	836.99	83.99	7358.16	269.78
4.5	843.31	84.78	9629.66	270.67
5	813.73	83.88	9040.12	308.22
5.5	836.00	83.02	10823.16	319.10
6	835.91	86.09	7709.91	233.78
6.5	833.34	82.86	10138.91	292.22
7	815.35	81.19	17147.66	299.33
7.5	859.45	79.89	10623.66	284.89
8	802.28	76.55	11358.66	320.89
8.5	831.04	79.89	6834.91	313.78
9	802.82	78.48	7370.41	241.78
9.5	793.53	69.66	13388.66	292.22
10	831.85	81.37	9725.91	292.22
10.5	773.60	89.33	10254.99	322.29
11	771.98	81.29	10574.66	263.33
11.5	762.96	82.09	8612.91	248.89
12	731.05	79.24	8873.66	284.89
12.5	731.04	63.96	10268.41	393.11
13	731.22	80.22	8200.49	284.89
13.5	749.71	70.49	9316.41	263.33
14	787.58	79.41	10166.91	356.89

Minutes	Anthocyanins cyn-3-glu mg L^{-1}	DPPH % I	FRAP µM	Polyphenols GAE mg mL^{-1}
14.5	806.07	80.22	10982.59	356.33
15	840.42	70.91	11593.98	298.00
15.5	839.79	81.36	10890.46	362.67
16	767.11	76.86	10958.48	319.33
16.5	692.63	78.43	9330.98	362.67
17	654.57	78.41	11647.37	352.00
17.5	689.65	77.65	10467.65	360.00
18	639.96	81.35	11473.43	406.00
18.5	657.55	68.59	11204.76	395.00
19	695.51	74.70	9670.26	319.33
19.5	637.44	80.87	11926.37	298.00
20	636.99	74.80	9971.65	425.67
20.5	666.39	73.65	11728.32	384.33
21	729.06	80.37	12100.32	341.00
21.5	628.33	85.02	10837.10	352.00
22	628.33	73.40	10341.10	362.67
22.5	637.08	78.09	13098.83	391.44
23	631.22	77.88	9967.43	397.73
23.5	628.33	82.21	9254.49	401.33
24	622.47	73.31	8634.46	355.67
24.5	604.98	74.40	9704.71	394.33
25	660.43	75.06	11290.87	362.67
25.5	616.61	80.29	11912.84	416.67
26	619.50	79.16	9832.15	347.67
26.5	603.26	83.56	11663.44	416.67
27	654.66	83.13	9623.14	368.00
27.5	599.12	89.76	10070.44	349.33
28	660.43	78.12	7772.88	386.33
28.5	647.96	84.93	7617.37	366.00
29	625.45	76.82	8477.01	319.33
29.5	639.96	88.06	8193.58	331.40
30	621.23	93.97	9024.43	364.67

Blackcurrant juice, no yacon or K_2CO_3, pH 2.8, heated at 95 °C

Minutes	Anthocyanins cyn-3-glu mg L^{-1}	DPPH % I	FRAP µM	Polyphenols GAE mg mL^{-1}
0	1189.21	85.57	14453.90	416.67
0.5	1163.75	86.78	13209.11	317.67
1	1123.60	84.48	12022.78	420.60
1.5	955.62	85.77	15180.18	436.61
2	985.64	83.58	15216.91	308.89
2.5	1132.37	86.96	10592.45	524.44
3	1039.11	88.37	12393.78	285.26
3.5	1045.68	89.27	9614.11	371.11
4	1006.05	88.38	13664.78	386.89
4.5	942.63	84.29	10994.65	321.59
5	930.15	83.98	12327.38	344.44
5.5	968.78	85.73	12788.51	285.26
6	838.57	88.39	13927.25	371.11
6.5	946.34	86.38	13191.51	386.89
7	817.32	85.98	14595.71	321.59
7.5	719.67	84.40	11002.91	317.67
8	802.61	86.46	15845.11	314.89
8.5	764.02	85.38	17967.58	321.59
9	816.65	84.45	19306.78	197.11
9.5	940.10	82.57	15918.11	232.67
10	838.07	89.15	10260.98	452.22
10.5	771.95	91.60	15543.38	358.33
11	928.97	86.77	12960.05	371.11
11.5	869.60	84.99	14206.25	361.22
12	786.12	85.68	15289.65	201.11
12.5	825.08	87.57	13635.85	128.89
13	849.87	86.77	13392.25	356.67
13.5	773.30	87.79	15272.65	393.67
14	798.94	89.57	14679.51	385.11

Minutes	Anthocyanins cyn-3-glu mg L^{-1}	DPPH % I	FRAP µM	Polyphenols GAE mg mL^{-1}
14.5	884.95	88.50	14179.11	416.67
15	747.16	87.97	13912.78	272.22
15.5	952.58	83.56	12068.55	253.78
16	565.85	94.60	13150.18	164.44
16.5	615.94	85.97	13197.18	243.81
17	621.51	86.10	13976.85	308.89
17.5	760.48	85.82	12561.18	272.22
18	816.65	87.96	12768.11	297.26
18.5	837.56	81.48	13445.71	215.89
19	823.22	85.90	12604.31	238.59
19.5	805.35	83.97	14311.65	253.81
20	713.60	86.00	13965.98	164.44
20.5	781.56	83.23	14073.98	220.48
21	691.50	82.12	14520.38	220.48
21.5	690.83	96.25	13004.52	222.21
22	758.46	83.47	12709.32	326.89
22.5	644.28	87.45	15718.60	344.44
23	827.10	88.55	13960.92	243.81
23.5	779.88	84.04	11905.39	242.70
24	731.81	82.96	11361.35	243.81
24.5	758.29	85.68	11645.65	328.11
25	857.80	87.32	13549.05	416.67
25.5	724.56	85.30	14295.40	253.81
26	668.90	84.98	11798.58	292.67
26.5	777.18	83.59	13996.13	273.00
27.5	851.56	85.68	11847.77	226.00
27.5	818.16	86.37	12084.53	222.69
28	794.38	91.39	10327.45	205.26
28.5	780.55	85.79	9840.85	326.89
29	667.89	85.39	10172.41	227.11
29.5	641.24	82.96	9832.29	263.33
30	656.08	87.62	10829.31	203.33

Blackcurrant-yacon juice, no K_2CO_3, pH 2.8, heated at 95 °C

Minutes	Anthocyanins cyn-3-glu mg L^{-1}	DPPH % I	FRAP µM	Polyphenols GAE mg mL^{-1}
0	1361.24	87.77	10945.30	236.67
0.5	1267.64	80.48	9986.84	380.33
1	1279.28	81.40	9317.09	317.67
1.5	1356.86	87.15	11385.14	246.89
2	1299.18	76.00	11412.69	285.26
2.5	1144.86	78.88	7944.34	236.67
3	1132.37	77.75	9295.34	420.60
3.5	1178.08	78.05	7210.59	358.33
4	1062.33	74.57	10248.59	380.11
4.5	1167.52	80.67	8245.99	356.67
5	1077.56	81.03	9245.54	371.11
5.5	1191.41	82.43	9591.39	393.67
6	1214.01	83.20	10445.44	385.11
6.5	1139.29	80.03	9593.64	436.61
7	1122.76	79.39	10946.79	317.67
7.5	967.43	77.44	8252.19	416.67
8	1035.40	76.94	11883.84	386.89
8.5	1085.82	80.94	13475.69	361.22
9	1127.82	76.83	14480.09	277.70
9.5	1010.10	74.73	11938.59	314.89
10	890.86	75.37	7995.74	308.89
10.5	1007.40	74.29	11657.54	277.10
11	932.51	75.30	9720.04	321.59
11.5	1012.63	76.29	11654.69	297.26
12	1025.45	80.03	11467.24	197.11
12.5	856.11	74.58	10226.89	201.11
13	949.38	74.29	12044.19	232.67
13.5	895.07	69.56	11454.49	215.89
14	933.02	73.86	11009.64	274.89

Minutes	Anthocyanins cyn-3-glu mg L^{-1}	DPPH % I	FRAP µM	Polyphenols GAE mg mL^{-1}
14.5	820.69	80.29	13634.34	221.50
15	814.45	73.49	10434.59	272.22
15.5	927.79	71.04	10051.41	238.59
16	919.19	81.39	9862.64	253.78
16.5	948.20	82.74	11897.89	220.48
17	858.47	78.90	10482.64	227.22
17.5	847.17	77.19	9420.89	164.44
18	806.02	79.60	9876.09	153.16
18.5	771.28	80.30	10084.29	243.78
19	712.99	80.48	9953.24	253.81
19.5	745.13	83.19	10733.74	243.81
20	789.83	77.59	10474.49	308.89
20.5	834.69	75.49	12555.49	292.67
21	788.99	79.29	10890.29	273.00
21.5	807.37	77.67	8753.39	220.48
22	836.38	84.38	9531.99	226.00
22.5	673.29	80.39	11788.95	198.26
23	818.50	82.33	12470.69	220.48
23.5	713.60	79.29	8929.04	222.69
24	769.76	81.39	9521.01	242.70
24.5	626.57	79.63	8734.24	222.21
25	692.34	77.39	9161.79	205.26
25.5	646.64	78.38	10721.55	277.32
26	641.24	76.94	8848.94	326.89
26.5	612.06	80.68	10497.10	227.32
27.5	649.00	82.85	8885.83	208.26
27.5	533.30	83.37	9063.40	227.11
28	637.19	81.38	8745.59	221.47
28.5	518.63	84.39	7380.64	243.81
29	589.63	83.35	7629.31	328.11
29.5	546.45	82.49	9374.22	220.48
30	667.05	87.77	8121.99	263.33