BENEFICIATION OF WASTEWATERS FROM THE SOUTH AFRICAN CITRUS INDUSTRY – A FEASIBILITY STUDY

SG Burton • CR Garcin • JH Aucamp

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Water Research Commission

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Report to the
WATER RESEARCH COMMISSION

by

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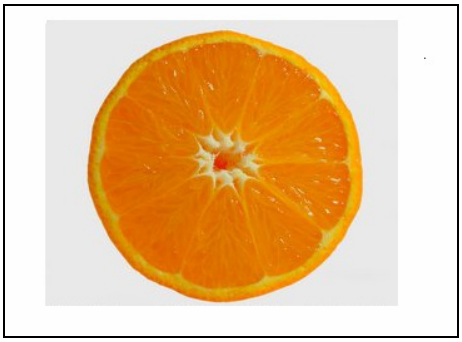
EXECUTIVE SUMMARY



In the citrus industry, the processing of fruit pulp to canned or packaged juice generates 1.5 ML wastewater per ton fruit containing, approximately 15% soluble solids and 30% pulp, and South Africa produces some 600 million kg citrus p.a. The waste streams from citrus processing are toxic to microorganisms and livestock due to the high concentration of organics including terpene-containing oils and flavanoids. They also contain significant amounts of carbohydrate, principally complex in nature and insoluble, and which add to the organic load of the wastewaters. Thus, the industry produces a significant volume of toxic wastewater which must be disposed of, adding chemical and biological oxygen demand burdens to treatment facilities, and depleting national water resources.

In this feasibility study, we proposed a solution of waste beneficiation whereby the contaminating organic oils and carbohydrates are removed from the citrus processing wastewater. These would then be used to generate selected products which add value in the industry.

These products were envisaged to be

(1) an oil extract suitable for addition to cosmetics etc.

(2) a carbohydrate-based material which could act as a carrier for the oils and which would constitute an antioxidant-dietary fibre food additive.

The benefits of the process were envisaged to include the following:

- Removal of these organics would yield cleaner, re-useable water.
- The organics present in the wastewater have significant potential for value-addition.
  - (1) The extracted oils represent a potentially high-value commercial product. The oils available from citrus processing (2 – 5 liters per tonne fruit) have high value ($500/L) in applications such as flavour, fragrance, nutraceutical and/or cosmetic components. In particular, the flavanoids are potent antioxidants.
  - (2) The complex carbohydrates are a potential source of dietary fibre which is known to have health benefits.
- This value addition would enhance the attractiveness of wastewater treatment and hence encourage water conservation and/or recycling.
- There would be potential for the process to be developed as a SMME-type enterprise positioned as a “satellite” business linked to large industry, thereby contributing to poverty alleviation.

The research objectives and approach

In an initial one-year feasibility study, the research programme involved the following objectives:

- To demonstrate the feasibility of the extraction of citrus oils from processing wastewater
- To demonstrate the feasibility of de-watering to obtain the complex carbohydrate fraction
- To generate two prototype products and conduct preliminary characterization
  - One would be an oil extract
  - The second would be a carbohydrate based-fibre product
- To obtain data to determine the potential benefit of such products
- To assess the potential marketability for the products
- To establish links with relevant South African industries.

The envisaged outcomes of the project would be:

- A feasibility study for a process beneficiating citrus industry wastewater
- A demonstration that a viable value-added product could be made
- Knowledge of the potential industry partners for wastewater supply and technology uptake
- Information regarding the potential market and value for the product
- Benefit to South African research and industry through collaboration with Australian researchers

Results of research

In the course of the project, supplies of samples of citrus processing wastewaters were required. These were sourced locally in the Western Cape, and they were found to be available in the summer season when the fruit crops were being processed. In the early stages of the project, the initial research was conducted on “synthetic” waste water solutions that were formulated in the laboratory, to enable the preliminary work to be conducted in time, since this approach had an advantage in that the synthetic water samples were simpler to work with in developing methods, and this allowed some very useful primary data to be obtained.

The findings of this preliminary feasibility study are summarised as follows:

- Citrus wastewaters were shown to contain useful amounts of antioxidants and complex carbohydrates which would be useful as extracts for use as dietary supplements.
- The wastewaters could be extracted using organic solvents to produce an antioxidant extract containing up to 65 mg phenolics per L wastewater extracted. This extract was found to have antioxidant activity comparable with other bioflavonoid products. However, the method was not selective and carotenoid fractions were extracted at the same time. While this is not necessarily a disadvantage in term of nutritional value, the carotenoids would alter taste and appearance of the product. Furthermore, the objective of extracting phenolic antioxidants would not be realised by this method without extensive further process development.
- The most effective method of extracting antioxidant phenolics was found to be the use of solid phase extraction on adsorbent resin. This method was found to be more selective than solvent extraction, and to provide a potential route to production of a clean antioxidant phenolic product.

- Complex carbohydrates were found to be extractable from citrus waste water, at a level of approximately 100 kg per ML wastewater. This fraction was found to be composed largely of insoluble dietary fibre which is potentially very valuable as a nutritional supplement. Isolation of the fibre was problematic in that removal of simple sugars from the fibre was difficult, but this separation is probably unnecessary since the simple sugars constituted only a small proportion of the extractable carbohydrate.
- The most useful approach found was to be spray-drying. Using aqueous or ethanolic extracts of fruit, prototype extracts were produced and spray-dried to yield an acceptable powder product which could be incorporated into foods as an antioxidant dietary fibre.
- While the value of an organic antioxidant product such as the resin-extracted product might be in the region of US$10 per kg and the value of the antioxidant powder suitable for addition to food products was estimated to be US$28 per kg, it was found not to be to assess the potential costs of producing the antioxidant fibre material without a more in-depth economic investigation. This work was not possible within the scope of the present project.

Recommendations

The project was a feasibility study intended to provide scope for later research. The following are recommendations for developing this research programme:

Further detailed characterization is required to confirm COD, BOD, organic and inorganic content, volumes and concentrations in different wastewaters from more industrial sources. Not all waste streams form citrus industries are likely to be suitable since some are cleaner than others, and preliminary purification would need to be minimized in a beneficiation process.

In addition, sourcing of the waste waters needs to be considered, since it was found that the processing only takes place during harvest periods, rather than all year. It is recommended that waste production should be monitored at some industry plants over the annual activity.

More detailed analysis of the nature of the antioxidant components in the extracts should be conducted. Identification of the particular phenolic components will be necessary for food supplement specification.

Further research will be required to develop and customize the extraction process and to further explore supported liquid membrane extraction methods, to optimize extraction of high value components.

The spray-drying technology used to produce the prototype products shows great promise and is recommended as a simple, clean and effective method to consider for future development.

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The Reference Group responsible for this project consisted of the following persons:

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Professor S Burton	University of Cape Town
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Dr J Aucamp	University of Cape Town

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CHAPTER 1
BACKGROUND AND INTRODUCTION

1.1 Project background

In the citrus industry, the processing of fruit pulp to canned or packaged juice generates 1.5 ML wastewater per ton fruit containing approximately 15% soluble solids and 30% pulp, and South Africa produces some 600 million kg citrus p.a. The waste streams are toxic to microorganisms and livestock due to the high concentration of organics including terpene-containing oils and flavanoids. They also contain significant amounts of carbohydrate, the majority being complex in nature and insoluble. Thus, the industry produces a significant volume of wastewater which must be disposed of, adding chemical and biological oxygen demand burdens to treatment facilities, and depleting national water resources.

In this feasibility study, waste beneficiation was proposed as a means whereby the contaminating organic oils and carbohydrates could be removed from the citrus processing wastewater. These would then be used to generate selected products which add value in the industry. These products were envisaged to be

(1) an oil extract suitable for addition to cosmetics etc. and/or

(2) a carbohydrate-based material which could act as a carrier for the oils and which would constitute an antioxidant-dietary fibre food additive.

1.2 Potential benefits of the proposed process

Removal of these organics would yield cleaner, re-useable water.

- The organics present in the wastewater have significant potential for value-addition.
  (1) The extracted oils represent a potentially high-value commercial product. The oils available from citrus processing (2 – 5 liters per tonne fruit) have high value ($500/L) in applications such as flavour, fragrance, nutraceutical and/or cosmetic components. In particular, the flavanoids are potent antioxidants.

(2) The complex carbohydrates are a potential source of dietary fibre which is known to have health benefits.

- This value addition will enhance the attractiveness of wastewater treatment and hence encourage water conservation and/or recycling.
- There is potential for the process to be developed as a SMME-type enterprise positioned as a "satellite" business linked to large industry, thereby contributing to poverty alleviation.

1.3 Brief overview of the citrus industry

Annually, approximately 1.6 million tonnes of citrus fruit are produced in South Africa. This consists of approximately 1.25 million tonnes of oranges, 175 thousand tonnes lemons and 200 thousand tonnes of grapefruit. Oranges produced are primarily from the Valencia, Delta's, Midnight and Navel cultivars. Approximately half of all the oranges are exported whilst the remaining produce is split near-equally between domestic fresh fruit consumption and processed products such as juices and jams [1].

The primary products of the citrus industry are fresh fruit, juice and jam. By-products are generally regarded as value-added products obtained by additional processing of the pulp, peel and effluent process water wastestreams. By-products obtained from the peel fraction include molasses, pectin, cold-pressed oils, and limonene [2 - 5]. Peels, when mixed with pulp and dried can also be used as low-grade cattle feed [6,7]. Peels, pulp and effluent wastewater are rich sources of citrus related phenolic compounds, which include phenolic acids, polymethoxylated flavones, flavanone and flavone glycosides and flavonoids [9].

Processing of oranges into juice results in 50% of the total weight of the orange being discarded as pulp and peel. During the juicing process a total of 1.5 ML wastewater is produced per tonne fruit, and contains approximately 15% soluble solids and 30% pulp [10,11]. Effluent citrus wastewater from processing plants is toxic to micro-organisms due to high concentrations of terpene oils and flavonoids. Additionally, it also contains sugars and cellulosic compounds that add to the chemical oxygen demand (COD) and biochemical oxygen demand (BOD) load of wastewater [12].

Both the citrus oils and bioflavonoids are groups of compounds used in the fragrance, cosmetic and nutraceutical industries that may be recovered as high value secondary products, thus enabling waste stream beneficiation [13,14,30]. Currently in South Africa, there is only one process which uses skins for chemical extraction of oils, while some peel is used as low-grade cattle feed near processing plants [15]. There is a new process under development by the CSIR in South Africa which will produce pectin from solid citrus waste, expected to be initiated in the next two years.

1.4 The composition of citrus fruit

Processing of oranges yields approximately 45% (w/w) fruit juice and 50% (w/w) peels and pulp. The remaining 5% is mainly made up of whole cells, essential oils and D-limonene.

Contained in the juice and pulp fractions are a variety of flavonoids and flavonoid glycosides unique to citrus fruit. The different citrus fruit (i.e. oranges, lime, grapefruit) also contain their own fruit-specific flavonoids. For example, naringin is only present in grapefruit, while eriocitrin occurs only in lemon juice. Narirutin and neohesperidin are most abundant in oranges and grapefruit and hesperidin, the most widely spread, is found in orange, grapefruit and lemon juice [16]. The concentrations of these compounds vary greatly depending on many factors including the type of fruit, cultivars and seasonal changes. Below is a list of the typical concentrations at which above-mentioned flavonoid glycosides occur in citrus fruit juices [17].

Table 1.1: Summary of concentrations for prominent citrus antioxidants. [17]

Flavonoid glycoside	Fruit	Concentration in juice (mg/100mL)
Hesperidin	Oranges/Grapefruit/Lemon	3-40
Narirutin	Oranges/Grapefruit	1-12
Naringen	Grapefruit	2-35
Neohesperidin	Oranges/Grapefruit	1-2
Eriocitrin	Lemon	20-25

Apart from the main flavanone glycosides, hesperidin and naringin, citrus peel also contains polymethoxylated flavones and numerous hydroxycinnamates all forming part of the total phenol content. Examples of hydroxycinnamic acids are ferulic, p-coumaric and sinapic acids which can occur at concentrations of 0.3-0.7 mg/mL in concentrated citrus peel molasses [2]. Polymethoxylated flavones occur at concentrations typically of less than 0.1 mg/mL in concentrated citrus peel molasses [2].

Citrus oils consist of mixtures of hydrocarbons of the terpene and sesquiterpene groups, oxygenated compounds and non-volatile residues. Terpenes do not contribute much to the flavour or fragrance of the oil; since they are mostly unsaturated compounds, they are unstable to heat and light, and rapidly oxidise in air. It is the oxygenated compounds that provide much of the characteristic flavour of citrus oil. The monoterpene hydrocarbons, D-limonene, myrcene, γ-terpene, linalool, α- and β-pinenes are usually the most abundant compounds in the oil. D-limonene makes up 40-50% of the monoterpene contents and is extensively used as a food additive to provide a citrus flavour, as a fragrance in perfumes, air fresheners, and personal care products. It can also serve as a natural replacement for petroleum-based solvents in paints and cleaning products and may also be used as an ingredient in pesticides and germicides [4,18]. Seeds are rich in limonoids (triterpenoids), but it is arguable whether the obtainable quantity is enough to make the extraction process feasible [8].

Food-grade pectins are used to improve gelling and texturising of foods, are functional in dietary fiber and a substance with a growing number of recognised pharmacological activities [3]. The structure of

pectin can be described as a complex anionic polysaccharide consisting mainly of blocks of (1-4)-linked homogalacturonan interrupted by one (1-2)-linked rhamnose unit. Pectins are characterised based on molecular size, viscosity and gelling properties [19]. The citrus peel contains approximately 50-60% pectin but normally only about 20% of the total pectin content is extracted successfully [3].

1.5 Extraction and recovery of flavonoids from citrus wastestreams

Several studies were found in literature where main flavonoid glycosides such as hesperidin and naringin were recovered from citrus waste streams [20,21], molasses [3] or from an aqueous extract of milled citrus [22,23]. The methods typically extract antioxidants with water followed by selective resin adsorption and elution allowing for removal of sugars as main contaminant. No process thus far has reported using modern readily transportable technologies such as supported liquid-membrane extraction (SLM) or continuous counter-current extraction methods and these unit operations will also be considered for possible process development.

1.6 Aims of the project

The main aims of the study were to investigate the feasibility of developing and implementing separation technologies that could lead to cleaner water effluent from the citrus industry and also yield high-value antioxidant and flavour/fragrance products from agro-industrial wastes, with inexpensive feed / fertiliser as secondary product.

In this one-year feasibility study, the objectives were to:

- Demonstrate the feasibility of the extraction of citrus oils from processing wastewater using modern separation technology (based on use of research previously conducted at UCT).
- Demonstrate the feasibility of de-watering to obtain the complex carbohydrate fraction. (This would be achieved through collaboration with researchers at the University of Sydney where specialist facilities are available).
- Generate two prototype products and conduct preliminary characterization
  - One would be an oil extract
  - The second would be a carbohydrate based-fibre product
- Obtain data to determine the potential benefit of such products and whether one of the two products is more viable than the other
- Formulate a preliminary assessment of the potential marketability for the products
- Establish links with relevant South African industries.

The outcomes were planned to be:

1. A feasibility study for a process beneficiating citrus industry wastewater
2. A demonstration that a viable value-added product could be made
3. Knowledge of the potential industry partners for wastewater supply and technology uptake
4. Information regarding the potential market and value for the product
5. Benefit to South African research and industry through collaboration with Australian researchers

Thus, the research plan was as follows:

Task 1: Extraction methods for and characterization of organic (oil) fraction

- Obtain wastewater samples from local citrus processing industries
- Characterise wastewater extracts in terms of chemical content and antioxidant capacity using HPLC, HPLC-MS, GC, GC-MS, mass analysis and standard antioxidant assays
- Quantify the extractable organics in the waste water samples on a mass basis
- Make first-order assessment of production (mass) and commercial (money) potential using estimated (known) industry fruit pulp processing (RSA and Oz/ international), corresponding waste (resource) quantities available, extracted oil yield (m/m from waste, from above), extraction cost (typical/estimated) and estimated unit value of final product.

Task 2: Dewatering methods for and characterization of carbohydrate fraction

- Use the spray-drying facility available to us at University of Sydney to dry samples of the waste to generate complex carbohydrate (dietary fibre) fractions. (1) using samples dewatered to varying degrees by centrifugation and filtration before drying
- (2) using samples before and after oil extraction
- Determine the composition of the resulting materials in terms of simple and complex carbohydrate content using chemical and HPLC analyses
- Determine the effect of residual oil content of the dried extracts of water samples on “dry-ability”
- Characterise the carbohydrate fraction samples with respect to solubility, antioxidant content and stability on storage
- Carry out first-order assessment of production (mass) and commercial (money) potential using estimated (known) industry fruit pulp processing (RSA and Oz/ international), corresponding waste (resource) quantities available, extracted oil yield (m/m from waste, from above), extraction cost (typical/estimated) and estimated unit value of final product.

Task 3: Market information

- Establish contact and familiarity with citrus juice producers
- Conduct a preliminary market survey (with aid of WRC services) on antioxidants and dietary fibre products
- Gather information on feasibility and scope of process development to commercialisation in South Africa

CHAPTER 2
Extraction and characterisation of antioxidants from citrus wastewaters

2.1 Introduction

Citrus wastewater was obtained from two orange juice producing companies in the Western Cape, once the fruit season was complete and juicing was in process (April 2006). These samples were used to determine typical composition and to ascertain whether there might be useful by-products that could be recovered from such waste streams. In addition, and to facilitate the more fundamental aspects of the study, extractions were also conducted using whole oranges and pulp produced from juicing these. Further, model phenolic compounds were obtained and "synthetic wastewaters" were formulated and used in analyses, in order to ascertain the potential efficiency of methods of extraction of the antioxidant components.

2.2 Materials and Methods

All analyses were performed in triplicate with the result expressed as the mean ± standard deviation. Purified, de-ionised water from a Millipore Elix 3 system was used for all analyses. All analyses were performed at 25 ± 2^{o}C. Chemicals and reagents were of analytical or HPLC grade as required, and were supplied by Merck or Fluka. Least-squares correlation coefficients (r^2) for all standard curves were greater than 0.99.

2.2.1 Wastewater samples

(Sample 1 was from Cape Fruit Processors in Paarl; Sample 2 was from Citrus Juices in Citrusdal)
Sample 1 was collected by an employee the first processing plant, from the combined total wastewater stream. It contained pulping process wastewater, cleaning and rinsing waters, and miscellaneous factory wastewater. This wastewater is discarded of directly into the municipal sewage system.

Sample 2 was collected directly from a wastewater tank in the second processing plant. This contained wastewater generated during the juicing process and was undiluted. After the wastewater

has been collected in the holding tank, it is discharged to a set dam where it is chemically treated to decrease the COD.

The companies were not at liberty to disclose the production processes for proprietary reasons, and therefore no estimations could be made as to quantities involved or to the specific processes involved in their generation. Both wastewaters were aqueous and contained only small quantities of visible oily fractions.

2.2.2 Citrus fruit materials

For preliminary testing, Valencia oranges were peeled to separate peel from the juice containing flesh. The flesh was partially homogenised to allow juice and pulp to be separated. The pulp was first filter-pressed to ensure most of the juice was removed after which it was dried at 37 °C for later use. The dried pulp was used as starting material for flavonoid extraction out of solid waste, although in practice wet waste will more likely be used. Using dried pulp allowed us to compare different solvents using the same 'preserved' citrus source. An aqueous alkaline extraction of 5% (w/v) dried pulp was obtained, neutralised and served as starting material for investigation of the recovery of flavonoids out of citrus wastewater.

2.2.3 Analytical methods

pH and conductivity were measured with appropriately calibrated instruments. Total acidity and alkalinity were determined according to APHA standard methods 2310 and 2320 respectively.

Total solids were determined by evaporating 100 ml of well-mixed wastewater samples in an oven at 80^{o}C, until constant weight was reached. The samples were evaporated in pre-weighed aluminium dishes, and were cooled to room temperature in a desiccator before weighing. Dissolved solids were determined in the same manner after a sample filtration (Millipore 0.45μm). Suspended solids were calculated as the difference between these two values.

A Merck reagent set (1.14555 HR) was used to determine chemical oxygen demand (COD), in conjunction with a 150^{o}C digestion block and a Nova Spectroquant photometer. Potassium hydrogen phthalate in distilled water was used as a standard, at concentrations of 425 and 850 mg.L^{-1} in distilled water, corresponding to COD values of 500 and 1000 mg.L^{-1} respectively. Distilled water was used as reagent blank.

2.2.3.1 <u>Reducing sugar analysis</u>

The DNS reaction mixture was prepared by weighing out 1 g of 3,5-dinitrosalicylic acid (DNS), 1.6 g NaOH and 30 g of Na/K-tartrate and making it up in 100 mL deionised H_2O [24]. Samples containing reducing sugars were prepared in water, ethanol, methanol, acetone or ethyl acetate to concentrations in the range of 200-1400 μg/mL when possible. 1 mL DNS reaction mixture and 1 mL sample were combined in a test tube. Another 8 mL deionised water was added, mixed well and heated for 5 min in a boiling water bath. Samples were left to cool to room temperature and absorbance measured at 546 nm using an Unicam Helios (Merck, Jhb).

A glucose stock solution of 1.6 mg/mL was prepared. Glucose solutions with final concentrations of 0, 0.4, 0.8, 1.2, 1.6 mg/mL were prepared by appropriate dilution. A standard curve was generated with absorbance readings in triplicate (Appendix, Figure A1). Samples were diluted with blank DNS solution (1 mL DNS reaction mixture + 9 mL deionised water) to ensure readings were in the linear range of the standard curve obtained.

2.2.3.2 Antioxidant capacity measurement

The radical scavenging potential of samples were measured with the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay as modified by Sanches-Moreno [25]. 0.1 mL of an antioxidant sample with water, methanol, ethanol, acetone or ethyl acetate as main solvent was added to 3.9 mL of a methanolic DPPH radical solution. The DPPH solution was always prepared to have an absorbance reading of 1.00-0.950 at 515 nm before addition and dilution with the sample. A time-dependent change in absorbance at 515 nm was measured until the reading stabilised. The DPPH radical concentration was determined using the calibration curve $A_{515nm} = 2935.68 \times [DPPH] - 2.18 \times 10^{-3}$. Results are displayed as the amount of radicals scavenged in mg/L and the time reactions required for establishing equilibrium. Not all samples tested reached equilibrium in a reasonable time and the assay was then usually terminated after 3 or 5 hr. Blank controls were included to determine the DPPH quenching drift observed over time.

2.2.3.3 HPLC analysis

Analyses were performed using a LaChrom Merck Hitachi liquid chromatography system constituting of a L-7400 UV-detector, L-7200 autosampler and L-7100 pump. Analysis was done with a reverse phase column (Waters Sperisorb® S5 ODS1 46x250 mm), an isocratic elution procedure with mobile phase containing $H_2O/CH_3CN/CH_3CO_2H$ (78:20:2, v/v/v) and flow rate of 0.7 $mL \cdot min^{-1}$ [21]. Extraction samples containing acetone, ethylacetate, ethanol, methanol or water were diluted 10 fold in the mobile phase and centrifuged, as a precautionary measure against precipitate formation, before being transferred to HPLC/GC vials for injection.

Hesperidin (>90% pure) from Fluka was used to obtain a linear calibration curve in the range of 0-45 mg/L (Appendix, Figure A2). Pure ferulic acid (Fluka) and *p*-coumaric acid (Sigm-Aldrich) were also used for attempted peak identification.

2.2.3.4 Total phenols assay

The estimation of total phenol content was coinducted using the Folin Ciocalteau (Sigma-Aldrich) reagent. This colorimetric assay was performed as reported by [26] although the original method was developed by [27]. 400 µL sample was added (without any dilution) to 400 µL Folin Ciocalteau in 4 mL cuvettes. After 3 minutes, 400 µL of 10% sodium carbonate (w/v) and 2.8 mL of distilled water were added and mixed well. The reaction was allowed to proceed for 90 min away from any light before spectrophotometric analysis at 765 nm. Distilled water was used, instead of the sample, to make up the blank control.

A standard curve was produced using gallic acid (Sigma-Aldrich) as typical plant phenol. A stock solution of 100 mg/L gallic acid was prepared in water and diluted to produce solutions with final concentrations of 100, 80, 60, 40, 20 and 0 mg/L. The assay was performed using the standards in triplicate, allowing for estimation of the total phenol content of extraction samples as gallic acid equivalents (GAE). The standard curve is presented in the Appendix, Figure A3.

2.2.4 Flavonoid recovery with solid-phase adsorption

Amberlite® XAD4 and XAD7HP (Supelco) were compared for their ability to adsorb the model flavonoid compound hesperidin from an aqueous solution (representing a synthetic wastewater sample). Both resins were washed repeatedly with deionised water until the pH has decreased to below 7.0. 0.5 g water soaked resin was incubated with 100 mL of a 20 $mg \cdot L^{-1}$ hesperidin solution. The hesperidin concentration was monitored at 288 nm using an absorbance extinction coefficient of 0.0293 $L \cdot mg^{-1} \cdot cm^{-1}$.

10 g dried pulp (prepared as described above) was extracted with 200 mL of an alkaline solution, prepared as 0.01 M NaOH; pH 11.92. Pectins were precipitated as calcium pectinate by adding KCl to the solution after which the solution was neutralised. The solution was clarified by filtration with Whatman No 1 filter paper and used as a flavonoid-rich solution for solid-phase flavonoid recovery experiments.

0.5 g Amberlite® XAD7HP was added to 100 mL flavonoid-rich solution and stirred at 300 rpm on a magnetic stirrer. The adsorption process was conducted overnight after which the resin was filtered of and flavonoids eluted using 100 mL ethanol. Samples of the flavonoid-rich solution was collected prior to and after the adsorption step as well as from the elution step for HPLC analysis.

2.2.5 Flavonoid extraction with food-grade organic solvents

Dried pulp was extracted with water, ethanol, methanol, acetone or ethyl acetate at a ratio of 5% (w/v). The solution was agitated on a magnetic stirrer at 300 rpm and the extraction process monitored at 320 nm until it reaches equilibrium. If the process has not reached equilibrium after 5 hr it was left overnight. Each organic solvent extraction was performed three consecutive times where fresh solvent was used for each step whilst retaining the pulp to ensure complete or maximal extraction. After the third organic solvent extraction a water extraction step was performed.

Dried pulp was also extracted three consecutive times with deionised water followed by three consecutive extractions with methanol. All extraction steps were stored individually and analysed to determine the antioxidant capacity, total phenol, hesperidin and reducing sugar content.

2.2.6 Extraction of industrial wastewaters

Low molecular weight phenolic compounds were extracted from the industry wastewater samples by conventional liquid/liquid extraction, using ethyl acetate as the non-miscible organic solvent. 100 mL

of sample was added to an equal volume of ethyl acetate in a separatory funnel, and gently shaken for several minutes. Phases were then allowed to separate, and the ethyl acetate was decanted and dried over anhydrous Na_2SO_4. The wastewater was extracted three times in this manner, and the combined ethyl acetate fractions were pooled. A Buchi R200 Rotavapor was used for *in vacuo* solvent removal at 45^{o}C, resulting in a sticky residue which was weighed to obtain total extract yield. The extract was subject to total phenols and HPLC analysis as described above.

2.3 RESULTS AND DISCUSSION

2.3.1 Analytical methods

Reducing sugar assay: The DNS assay is very useful for quantifying monomer carbohydrates such as glucose and fructose that can be used as a carbon-source for fermentation growth and that contribute greatly to the BOD. Complex sugars such as cellulose and starch, also useful carbon sources, are not detected by this method. However, the detection response is only linear in the range of 0.6-1.6 mg/mL glucose. Oxygen in the solution converts glucose to gluconic acid under alkaline conditions, desensitising the method at the lower concentrations of glucose (Appendix, Figure A1).

Total phenol assay: This is a very sensitive and selective method for detecting phenolic compounds. It does not distinguish between phenolic acids and flavonoids. This is desirable since not only contribute both these groups to environmental pollution, but their antioxidant capacity provides potential health benefits when recovered from the wastestream. The method detects polyphenols as gallic acid equivalents (GAE) with a linear response in the range of 0-100 mg/L gallic acid (Appendix, Figure A2).

Antioxidant capacity: The DPPH assay is commonly used for measuring the antioxidant capacity of pure compounds or mixtures of compounds such as plant extracts. DPP$^{\cdot}$ radicals absorb at 515 nm and when quenched or scavenged by an antioxidant, the solution is bleached. Figure 2.1 depicts a typical quenching/bleaching result of the radical scavenging process. The greater the reduction in absorbance, the greater the antioxidant capacity (ability to neutralise harmful oxygen radicals) of the sample. Table 1 summarises the antioxidant capacity of each extraction as mg/L DPPH quenched. Higher values indicate greater antioxidant capacity. The equilibrium time for water extracts samples and also some methanol extract samples were very long, exceeding 3 and 5 hr. When the equilibrium point was not determined the value is displayed with a 'greater as' (>) sign in Table 2.2.

HPLC analysis method: A typical separation of *p*-coumaric acid, ferulic acid and hesperidin is shown in Figure 2.2. Two well resolved peaks are present in both water and methanol extraction samples that still require identification. The peak at 12 min is likely to be narirutin, the other abundant flavonone glycoside in oranges while the peak at 6 min is likely to be a phenolic acid. Typical profiles of water and methanol extracts from dried citrus pulp are shown in Figure 2.2. Besides hesperidin, another two well resolved peaks are present as major components.

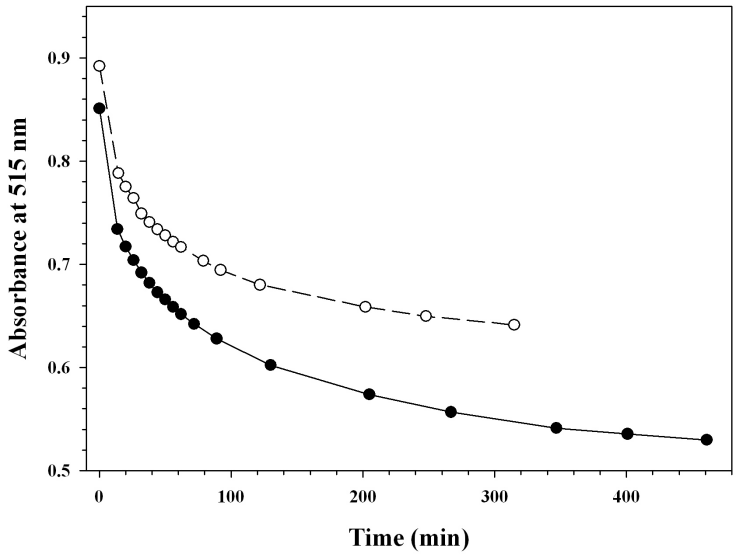

Figure 2.1: $DPPH^{\cdot}$ radical scavenging ability of the first (●) water and (○) methanol extract respectively. It is evident that the water extraction of orange pulp has a higher antioxidant capacity than the methanol extract.

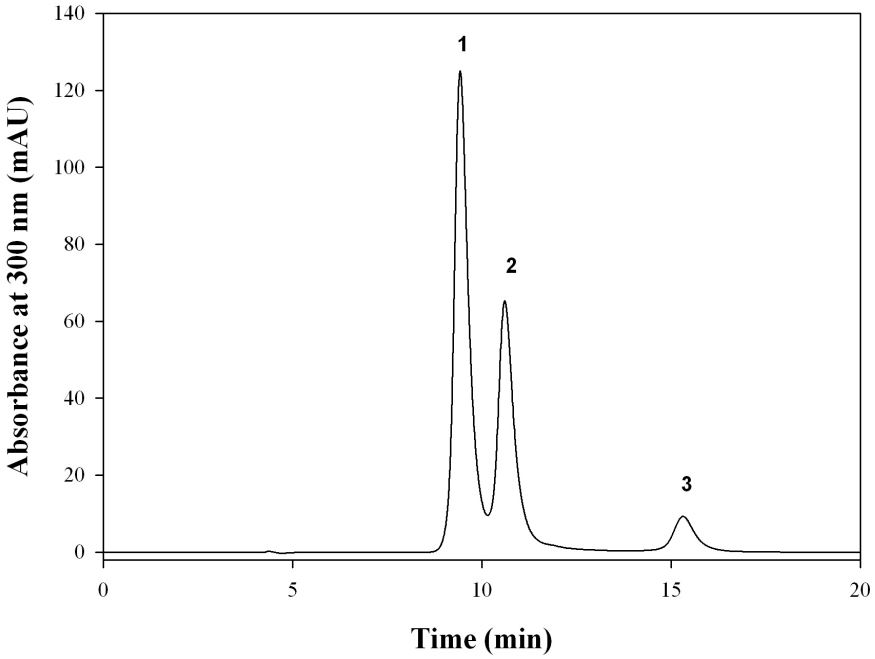

Figure 2.2: The HPLC method allowed separation of (1) *p*-coumaric acid, (2) ferulic acid and (3) hesperidin and would also resolve naringin and narirutin from hesperidin.

2.3.2 Recovery of antioxidants from synthetic wastewater

In the initial stages pf the project, where methods were being developed, solutions of model compounds were used as standards, since the wastewater samples were complex and contained very low (and of course, unknown) quantities of the compounds of interest. A solution of the flavonoid hesperidin was formulated and used as a synthetic wastewater to facilitate investigation of the extraction methods. Initially, solvent extraction was considered but it was found to be unselective, and was therefore more suitable for extracting both sugars and antioxidants. Also, the solvent used would need to be immiscible with water and hence non-polar, but our results (see Section 2.3.4) and the literature (see Chapter 1) indicated that the antioxidants present would be soluble in water and polar solvents. This approach was considered again in dealing with the industrial samples (see Section 2.3.5).

The use of adsorbent resins to extract flavonoid antioxidants was investigated as a more selective method. The rate of adsorption and the quantity hesperidin bound to the resin were determined for both resins XAD4 and XAD7HP. Results are depicted in Figure 2.3. The bound hesperidin (Q) was determined by estimating the hesperidin still remaining in solution (by HPLC) and subtracting that from the initial concentration. The adsorption process was followed for 5 hr. A flavonoid-rich solution was then prepared from oranges as described in the Materials and Methods section, and this was used to conduct an adsorption study with XAD7HP. The XAD7HP resin was selected since it out-performed XAD4 in the initial study. The batch adsorption, conducted in a beaker, was allowed to continue overnight with agitation at 300 rpm. Resin was separate from the flavonoid-rich solution and eluted with ethanol overnight by agitating in a beaker at 300 rpm. Not all flavonoids, including hesperidin, eluted in the first elution step. Four elution steps were required to remove all the flavonoids from the resin. HPLC analysis of the flavonoid-rich sample, filtrate after adsorption and elution step are shown in Figure 2.4.

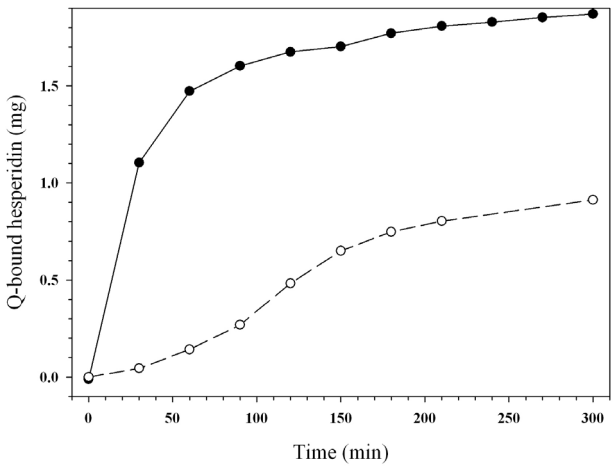

Figure 2.3: The adsorption of the model flavonoid hesperidin to two food-grade Amberlite® resins was tested. (○) XAD4 displayed slower adsorption kinetics and lower load capacity than (●) XAD7HP. The XAD7HP adsorbed nearly all the hesperidin in solution and was able to bind even more once placed in a fresh solution, giving it a load capacity of more than twice that of XAD4.

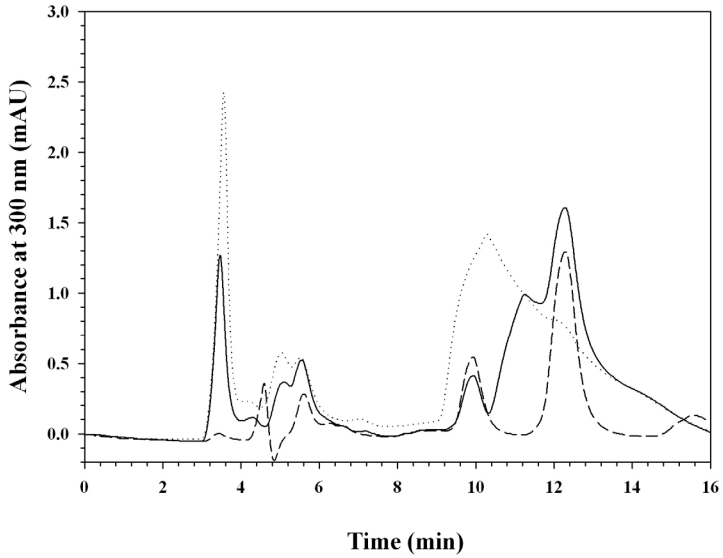

Figure 2.4: HPLC analysis profiles of samples from the Amberlite® XAD7HP batch adsorption process. (—) Represents the chromatogram of the flavonoid-rich solution prior to the solid-phase recovery study. (···) Represents the chromatogram of the flavonoid-rich solution retained after the adsorption process. The chromatogram represented by (– –) is from elution step where ethanol was used to remove flavonoids from the resin.

2.3.3 Extraction of flavonoids from orange pulp

In order to identify the flavonoids extractable from orange pulp and to obtain an estimation of the potential amounts of antioxidant flavonoids that might be extracted from citrus waste, dried pulp was extracted using aqueous and organic solvents. The extraction process was monitored at a wavelength of 320 nm, this wavelength being more specific for hesperidin and narirutin, and results are shown as extraction curves in Figure 2.5. The time an extraction step takes to reach equilibrium was deemed important in the context of designing a process, since it would affect productivity. Extractions were therefore allowed to reach equilibrium and with all organic solvents the experiments had to continue overnight due to the process being slow. The extraction liquid was filtered off and pulp samples re-extracted. Sample names as listed in Table 2.2 are informative of the extraction procedure; for instance MeOHEx(1) was the first extraction using methanol as solvent and MeOHEx(3) was the extraction liquid filtered off after the third consecutive extraction with methanol. Samples were subjected to all the analytical methods described earlier and the results summarised in Table 2.1. The results indicated that while water, and its mixtures with methanol and ethanol gave high concentration of the model flavonoid in the extracts, ethyl acetate gave only very low extraction. Figure 2. 6 shows the differences in elution profiles of samples extracted with water and methanol respectively. Identification of all the major peaks will provide valuable information for future process development.

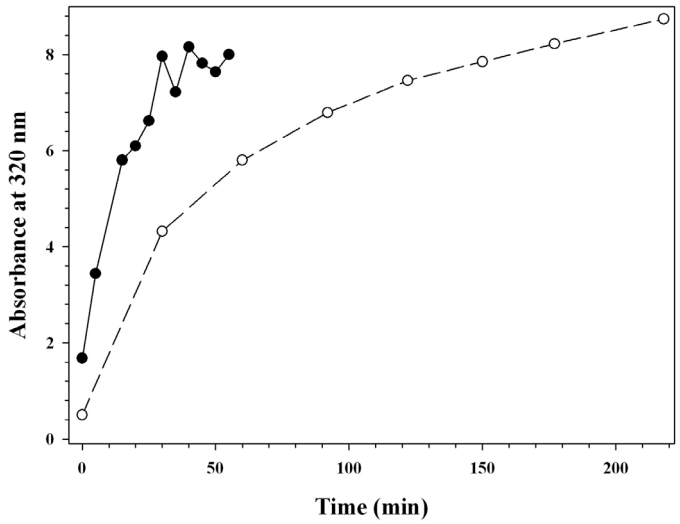

Figure 2.5: Extraction curves obtained using (●) water and (○) as extraction solvents, monitored with absorbance at 320 nm. In all instances organic solvent required a longer time to reach equilibrium than water.

Table 2.1: Summary of analysis data for extraction samples.

Sample name	DPPH Scavanged (mg/L)	Equilibrium Time (min)	Total polyphenol (GAE) (mg/L)	Total Hesperidin (mg/L)	Peak 6.15min Total area	Total reducing sugar as glucose (g/L)	Extraction time (min)
WaterEx(1)	0.11	>461	107.48	140.26	51223	19.99	60
WaterEx(2)	0.02	264	36.46	101.20	12372	2.76	60
WaterEx(3)	0.01	53	20.35	103.20	4572	0.83	60
MeOH1AftrWatrEx(3)	0.01	340	34.27	729.54	4560	1.09	Overnight
MeOH2AftrWatrEx(3)	0.02	338	28.70	1122.89	3032	1.23	Overnight
MeOH3AftrWatrEx(3)	0.03	336	37.91	2247.61	3955	0.73	Overnight
MeOHEx(1)	0.09	>195	108.01	2041.97	55913	21.33	Overnight
MeOHEx(2)	0.04	>197	54.12	2620.57	12125	1.30	Overnight
MeOHEx(3)	0.01	>313	19.60	751.87	3780	0.66	Overnight
Watr1AftrMeOHEx3	0.02	>315	26.13	82.73	3182	0.68	60
EtOHEx(1)	0.02	306	36.57	198.26	12732	5.93	Overnight
EtOHEx(2)	0.02	237	30.20	922.37	7550	3.32	180
EtOHEx(3)	0.01	93	15.75	499.73	3084	0.72	180
Watr1AftrEtOHEx3	0.07	178	84.57	171.46	35565	18.65	60
EthylAcEx(1)	0.00	48	7.88	0.00	0	0.63	Overnight
EthylAcEx(2)	0.00	53	6.92	0.00	0	0.69	Overnight
EthylAcEx(3)	0.00	33	5.53	0.00	0	0.67	Overnight
WatrEx1AftrEthylAcEx3	0.07	>169	102.71	378.69	50215	6.01	60
AcetoneEx(1)	0.00	455	21.37	181.71	0	2.78	280
AcetoneEx(2)	0.00	27	11.52	183.37	0	2.36	180
AcetoneEx(3)	0.04	174	41.06	789.67	0	14.55	240
WatrExAftrAcetone(3)	0.07	>502	69.37	204.78	20941	19.99	60
AlkalineEx(1)	0.06	>120	58.92	1818.08	26269	-	60
AlkalineEx(2)	0.05	40	62.37	783.70	12304	-	60

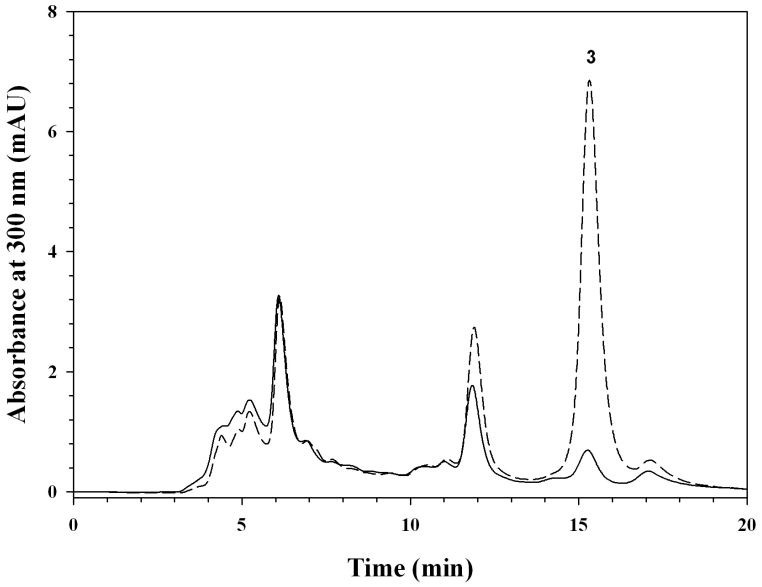

Figure 2.6: Typical HPLC chromatograms of (—) water and (– –) methanol extracts of orange pulp. Hesperidin (3) elutes at approximately 15.5 min. Other major peaks are observed at approximately 6 and 12 min respectively.

2.3.4 Characterisation of citrus industry waste waters

Samples of wastewater were obtained form local fruit juice processing companies, in late summer 2006. One sample, Sample 1, was a dilute mixed sample containing wash water as well as the water from the process. The other, Sample 2, was more concentrated and was obtained directly from the juicing plant. The analyses reflect this difference, particularly in terms of the organic content of the wastes. This is an important indication that the nature of the wastewater can vary and the waste stream used in the proposed process would need to be chosen carefully. Results of the primary analysis of the industrial wastewaters are shown in Table 2.1 . The UV-visible absorbance profiles of the samples were measured and these showed the presence of aromatic components with absorbance maxima typical of flavonoids (Figure 2.7). The wastewater samples were also analysed by HPLC in order to confirm the presence of aromatic (phenolic) components which correspond with the standard flavonoid compounds used in the study (Figure 2.8).

Table 2.1: Primary analysis of citrus wastewaters

Parameter		Sample 1	Sample 2
pH		3.8	11.4
Conductivity	($mS.cm^{-1}$)	0.67	3.71
Acidity (as $CaCO_3$)	($mg.L^{-1}$)	155 ± 3.2	-
Alkalinity (as $CaCO_3$)	($mg.L^{-1}$)	-	815 ± 7.4
COD	($mg(O_2).L^{-1}$)	5306 ± 35	3180 ± 9
Total phenols	($mg.L^{-1}$)	3.9 ± 0.2	63 ± 3
Reducing sugars	($g.L^{-1}$)	9.9 ± 0.5	6.1 ± 0.2

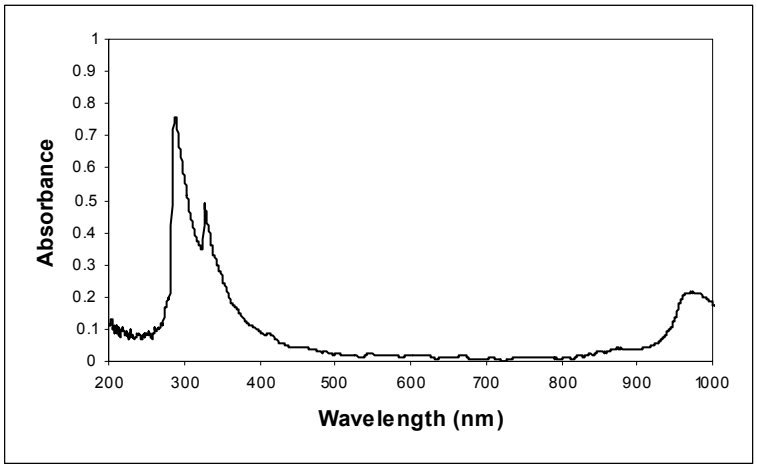

Figure 2.7a : UV-Visible spectrum of Sample 1

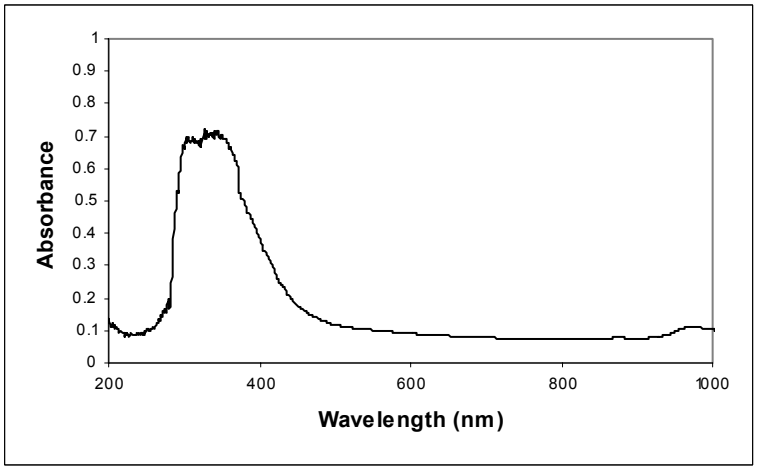

Figure 2.7b: UV-Visible spectrum of Sample 2

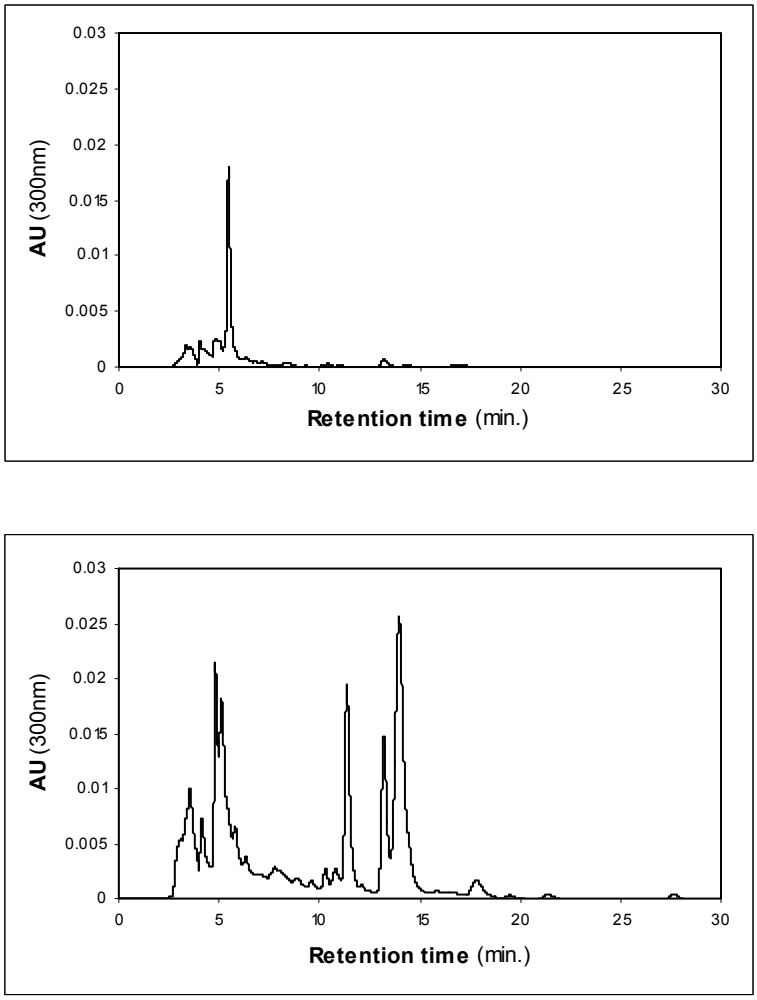

Figure 2.8: HPLC profiles of phenolic components in Samples 1 and 2

Sample 2 was extracted using ethyl acetate, which gave a bright yellow extract due to the presence of carotenoids as well as flavonoids. This gave a total of 174.9 mg extract obtained from 100 mL of wastewater, which represents a yield of 1.749 g extract per L of wastewater. This extract was re-suspended in 20 mL of methanol/water (50:50), and used for further analyses. The HPLC profile is shown below (Figure 2.9). Of the total amount of extract, for Sample 1 3.9 mg/L (0.2 %) was determined to be attributable to phenolic compounds by total phenol assay and for Sample 2 this quantity was 2.8 mg/L representing 1.6% of the extract.

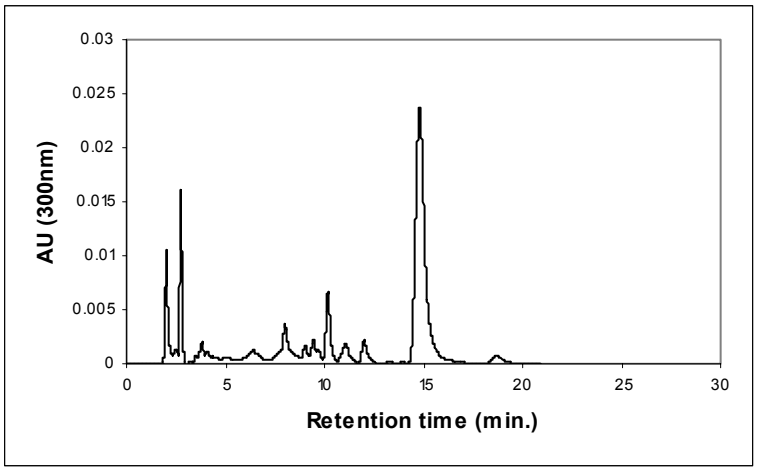

Figure 2.9: HPLC profile of ethyl acetate extract of wastewater Sample 2.

2.3.5 Discussion of recovery of antioxidants from wastewater

Three potential methods of extracting the antioxidants from citrus wastewaters were considered; these were precipitation, two-phase extraction using a supported liquid-membrane extraction system (SLM) or solid phase adsorption. Of these, selective precipitation would only be achievable for the low soluble compounds such as hesperidin, but not for the water-soluble antioxidants such as the phenolic acids. Also, simultaneous precipitation of all the antioxidants would be very difficult, bearing in mind the diverse nature of their chemical structures.

A SLM system ideally requires that two immiscible solvents be used. Ethyl acetate is one of few food-grade organic solvents that could be used and from the results in Table 2.2 it can be concluded that it is highly unselective for the antioxidants in citrus fruit. The use of non-food-grade solvents would push the process and validation cost of the product up since all traces of solvents unsuitable for human consumption must be removed. Another requirement for using a SLM system would be that the antioxidants such as hesperidin be soluble in the organic phase. Hesperidin, as reported in literature, does not dissolve well in most organic solvents with methanol performing the best [28]. Hesperidin dissolves readily in alkaline aqueous solution above pH 12 and seems to be reasonably stable under such conditions for short exposure times [21]. Acidification of alkaline solution would precipitate the bulk of the hesperidin out of solution.

The use of food-grade resins for recovery of hesperidin from wastewater has been reported in literature before [20,21,23]. In this study two reported resins were compared but the study may be extended to other resin types and manufactures with the aim of finding the most selective adsorption media. The results obtained indicated that the resin XAD7HP allows for faster adsorption kinetics and also has a larger load capacity than XAD4. All three major

compounds present in alkaline citrus pulp extract were shown to bind to XAD7HP, hesperidin being the most significant. Elution of adsorbed hesperidin can be achieved by using methanol or alkaline solution, rather than the ethanol used in our study. Although methanol is toxic and all traces would need to be removed later, for food products. The resin was non-selective for carbohydrates in the wastewater, which would allow the antioxidants to be recovered first before the carbohydrates are enriched/concentrated for later extraction and use.

The polyphenol and DPPH assays indicated that the water-extracted fraction from citrus pulp has both high polyphenol content and antioxidant capacity. HPLC analysis (Figure 2.6) was not conclusive but a slightly higher concentration of early eluting (4-6 min) aromatic compounds, likely to be phenolic acids, may contribute to the high antioxidant capacity. In addition, extraction of the industrial wastewaters showed a corresponding high concentration of phenolic components that are likely to be flavonoid antioxidants.

There was no clear correlation between the hesperidin concentration in extracts and their antioxidant capacity. This is to be expected since hesperidin is not the only antioxidant present in solution. Despite its high quantities it is not the most potent antioxidant in the extract. In most extraction samples where the antioxidant capacity tested high, the peak area of the major compound eluting at approximately 6 min is also at its highest. By combining hesperidin concentration and the peak area of the compound at 6 min a reasonable correlation with antioxidant capacity is obtained. The major peak at approximately 12 min behaves similar to hesperidin, being more soluble in methanol, ethanol and alkaline solution than in water. This behaviour and evidence from literature suggests that it might be narirutin, another flavonone glycoside present in oranges [21].

Although the alkaline solution extracted high concentrations of hesperidin, it also dissolves pectins that precipitate to form gel-like structures under acid conditions. In typically processes the pH is made alkaline and pectins simultaneously precipitated as calcium pectinates with addition of calcium hydroxide. Pectins are also high-value products used in foods that require gel-like textures.

Ethyl acetate was found to have low selectivity for antioxidants, hesperidin and sugars in the citrus pulp. However, it extracts some of the yellow pigments, carotenoids, which would alter the taste, colour and properties of an extract, but would add to the antioxidant activity.

2.4 Conclusions

Based on the results obtained it would be relatively straight-forward to obtain a low carbohydrate, high flavonoid extract that could be dried and commercialised as a citrus bio-flavonoid rich product.

- Several analytical procedures were implemented and are suitable for analysis of citrus waste streams. The HPLC method could be adjusted to elute and detect carotenoids present in the extracts.
- The best method for extracting the organic antioxidant fraction was found to be solid phase adsorption on resins. However, SLM technology still requires further assessment before it is excluded.
- Two food-grade resins were tested for their ability to recover flavonoids. From initial studies Amberlite XAD7HP worked well and the adsorption kinetics and characteristics now need to be established. Other food-grade resins may be tested for adsorption specificity.
- Several food-grade organic solvents were screened for their ability to extract flavonoids and sugars from citrus pulp. Water extracts dissolved the most reducing sugars and also gave the highest antioxidant capacity. Methanol dissolves the most hesperidin, but also high amounts of reducing sugars. Ethanol is a superior choice for dissolving hesperidin. An alkaline solution dissolved sugars, pectins and hesperidin very well.
- Designing of a suitable process will be aided greatly by deciding first what type of products will or can be manufactured. For example there is an option of producing high antioxidant capacity, low citrus flavonoid water extracts or low antioxidant high in citrus flavonoid content. The separation of sugars from water-soluble antioxidants is currently problematic and needs to be investigated further.

CHAPTER 3
Carbohydrate and antioxidant fibre products from citrus wastewaters

3.1 Introduction

Previous research has shown that high fibre functional food powder, “dietary fibre”, can be formulated from citrus fruit peels and this powder has been widely used by adding it into various foods in order to enhance their health benefits [32, 33]. The work reported in this chapter was conducted to investigate the feasibility of producing such products from extracts of citrus wastewater.

The complex carbohydrates in citrus exist largely in the white spongy and cellulosic tissue inside the peel. They provide a valuable source of dietary fibre, consisting of non-starch polysaccharides (NSP) which comes from the cell walls, forming structural support of the tissue. Non-starch polysaccharides include pectins, gums, hemicelluloses, cellulose, β-glucans and other polysaccharides. Dietary fibre can be broadly classified according to its solubility, namely soluble (SDF) and insoluble, (IDF), both of which are essential in the daily diet. Pectins, gums and certain hemicelluloses are soluble fibres, and citrus fruit are a good source of this soluble dietary fibre. The main advantage of dietary fibre from citrus fruits as compared to alternative sources of fibre such as cereals is its higher proportion of soluble dietary fibre, [34]. The health benefits of dietary fibre are recognised to include promotion of intestinal health, prevention of cardiovascular disease, and reduction in obesity and the effects of diabetes.

The objective of the present study was to characterise the dietary fibre available from citrus wastewaters, by determining the total carbohydrate, total reducing sugars, and antioxidants that might be associated with the complex carbohydrate (fibre), and to assess the potential value of these components in terms of health benefits and extractability. In order to do this, initially, “synthetic” wastewater samples were formulated in order to provide clean, defined samples for preliminary characterisation. Thus, the “pith” from orange peels was ground and washed to produce complex carbohydrate extracts representative of the complex carbohydrate component of citrus wastewaters. These extracts were then characterized in terms of the SDF and IDF content, and antioxidant activity. Following this, citrus extracts were produced using simple aqueous or organic extraction, and then spray dried (in collaboration with researchers at the University of Sydney) to produce combined antioxidant-plus-fibre extracts, in order to assess the potential for producing an antioxidant dietary fibre product.

3.2 Methods and materials

3.2.1 Fibre extraction

The peels from the Valencia cultivar oranges were used as the source of fibre for preliminary testing. The dietary fibre was first extracted according to the method of [35] and [36]. Orange peels were milled using the Waring blender. The selective removal of undesirable compounds (including simple sugars) and potentially pathogenic microorganisms from the fibre was achieved by washing each of the samples with 90^{o}C water. The volume of loosely packed fibre samples were estimated and they then were washed with a volume hot water equal to 2x equal volumes, after which fibre was dried by filter-pressing. The wash water of each of the sample size fractions was collected into the glass bottles as indicated in Figure 3.1 below. The labelled bottles were then stored in a 4^{o}C refrigerator in order to prevent growth of microorganisms. The water was collected for both the peels and the fibre product so that the mass balance on the sugars could be performed.

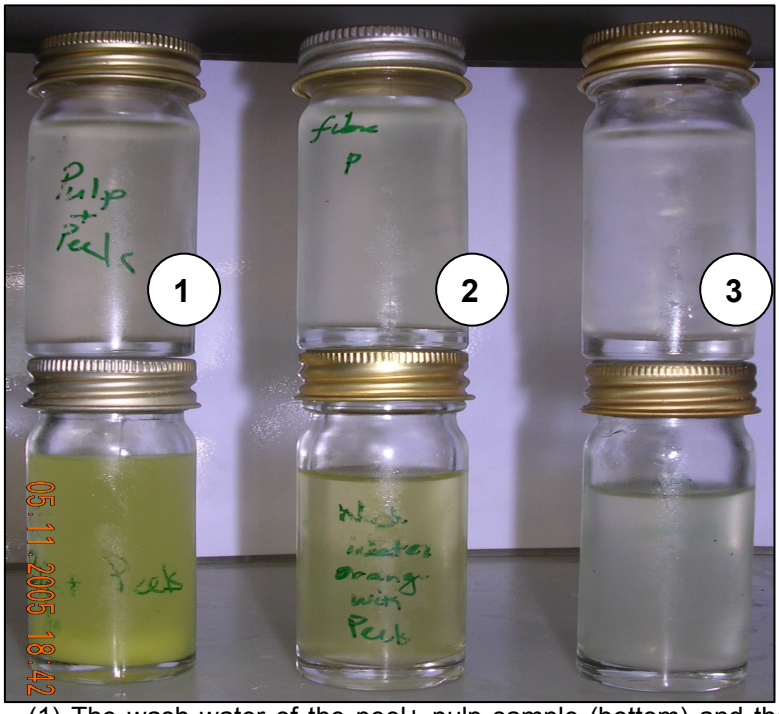

Figure 3.1: (1) The wash water of the peel+ pulp sample (bottom) and the fibre extract (top). (2) The wash water of the peels with flavedo (orange part) and its fibre (top and bottom). (3) The wash water of the pith and its fibre extract (bottom and top).

The residues were then put into a 75^{o}C oven for approximately 3 hours to dry. They were then stored in a 37^{o}C room to further dry over night. In order to extract sugars and flavonoids, 2 g samples of fibre were homogenised with 100 ml distilled water for 1 minute. The mixture was left over night for extraction to reach equilibrium, and then filtered.

All extracts were analysed for sugars, phenolic and antioxidant content using the methods described in Chapter 2.

Figure 3.2: The dried peels + pulp sample from a 75^{o}C oven.

3.2.2 Testing for Soluble and Insoluble Dietary fibre

This assay was used to determine the total dietary fibre content of the dried samples of the washed orange peels. It uses the combination of enzymic and gravimetric methods published in the 16th edition of the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC). The dried samples are gelatinized with heat stable □-amylase and then enzymatically digested with protease and amyloglucosidase to remove the protein and starch present in the sample. Ethanol is then added to precipitate the soluble dietary fibre while the residue (which is the insoluble DF) is dried and weight.

1 g samples of fibre were measured into 125ml conical flasks. This was followed by the addition of 50 ml of the pH 6.0 phosphate buffer in each flask. 0.1 ml of α-amylase was added in each flask, followed by gentle swirling of the flasks to allow mixing. The flasks were covered with aluminium foil and placed in a 95^{o}C water bath for 15 minutes with gently agitation for 5 minutes intervals. The solutions were then allowed to cool to room temperature before the pH was adjusted. The pH of the solutions was adjusted to 7.5± 0.2 by initially adding 10 ml of NaOH, followed by small amount of NaOH and HCl added as desired to reach the desired pH. Thereafter, 0.1ml of the 50mg/ml solution of protease was added (prepared immediately using 50mg of protease powder with 1ml of deionised water) to each of the flask. This was followed by covering the flasks with foil and placing them in a 60^{o}C water bath for 30 minutes with gentle swirling every 10 minutes. The solutions were allowed to cool. And the pH was adjusted to between 4.0 and 4.6. Following this, 0.1 ml of

amyloglucosidase was added to each flask, which were then covered with foils and placed into 60^{o}C water bath for further reaction.

The solutions were then filtered to separate the insoluble and soluble fibre fractions. The residue (insoluble) was placed into placed into a 75^{o}C oven until it was dry. Its mass was then measured the filtrate (which contained the soluble fibre) was mixed with 4 times its volume with 99% (v/v) of ethanol and left overnight to precipitate. After precipitation, the samples were filtered to recover the soluble fibre. Each was dried and then weight. Figure 3.3 below shows the soluble fibre after overnight precipitation.

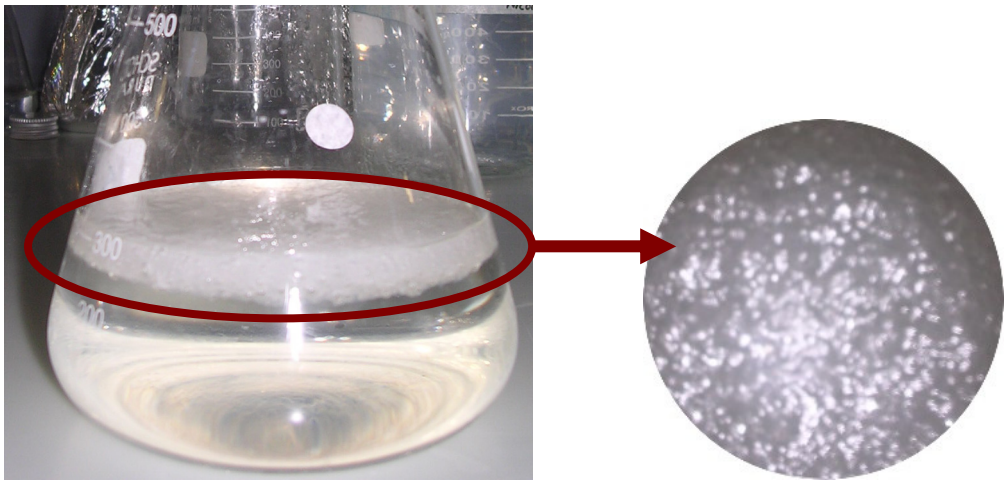

Figure 3.3: The side and top view of the soluble dietary fibre precipitate floating on ethanol.

3.2.3 Extraction of combined fibre-plus-flavonoid "antioxidant fibre" product

Samples of peel (20g) were extracted in 250 mL water at pH 2.5. 200 mL samples of the extracts were spray dried forming a powder. A Buchi-290 miniature spray dryer was then used to dry the extracts. This equipment operates co-currently with a two-fluid self-cleaning spray nozzle having a cap orifice of diameter 1.5 mm. The drying chamber is cylindrical and vertically orientated with a length and diameter of approximately 500 mm and 150 mm, respectively. The compressed air pressure (5 Bar), the liquid pump rate (1%, 0.18 mL min^{-1}), the main air (aspirator) flow rate (100%, 37.5 m^{3} hr^{-1}), and the nozzle rotameter reading (500 L hr^{-1}) were kept constant, while the inlet gas temperature was set at 60^{o}C. These powders were weighed, made up into solutions containing 10 mg/mL, and then assayed for total phenolics and antioxidant capacity as described in previous sections.

3.3 Results and discussions

3.3.1 Carbohydrate content of extracts

Wastewaters would be expected to contain both simple sugars and complex carbohydrate. The total sugars measurements were conducted to provide a bench mark for the amount of carbohydrate present in orange pulp after juicing, representing the maximum amount that might be extracted into the wastewater. Initially whole peels after juicing, and peels which had had the rind removed were used (Table 3.1) [The flavedo is the orange rind on the fruit and the albedo is the white part of the pith]. These results indicate that a large amount of carbohydrate can be washed off in the peels, particularly those retaining the flavedo, as would be the case with a juicing process. These carbohydrates could then be present in wastewaters, although they may later be diluted by added washing water.

Table 3.1: The Total carbohydrate content of different citrus materials

Total Sugars		
Raw material	g/g of raw material	g/g of fibre
white peel	0.273	0.153
peel+flaved	0.338	0.050
peel + pulp	0.354	0.099

3.3.2 The reducing sugars

Reducing sugars are soluble, simple sugars such as glucose which would not be desirable in fibre products since they have high calorific value and would make any dietary fibre product higher in energy content. It was thus necessary to assess the proportion of reducing sugars that would be present, in proportion to complex carbohydrate fractions. The % washed out shows the amount that was extractable using hot water. A large percentage of the reducing sugars, (19.5%) was washed off the whole peel, indicating that reducing sugars in the wastewater might well present a problem for production of a dried product. Not only would they add calorific content, but they also add to the stickiness of a dry fibre product.

Table 3.2 : Reducing Sugars for different citrus materials

Reducing Sugars			
Raw material	g/g of raw material	g/g of fibre	% washed out
white peel	0.049	0.044	10.8%
peel+flaved	0.050	0.041	19.5%
peel + pulp	0.104	0.086	16.6%

3.3.3 Dietary fibre composition

The peel clearly contains most of the fibre components of the citrus fruits, and of this, the majority was shown to be insoluble dietary fibre (approximately 0.7 g / g pulp). This is an advantage in terms of production of dietary fibre products, since a proportion of this would be present in the processing wastewaters. The soluble fibre was shown to be extractable using alkaline treatment, giving the yields shown in the table below, of about 0.2 g/g material. Based on these results, if juicing results in 50% of the fruit mass being removed, approximately 175kg of fibre would remain per tonne of fruit processed.

Table 3.3: Dietary fibre composition of citrus material

Raw material	TDF	IDF	SDF	% SDF	Ash
white peel	0.99	0.73	0.25	25.7%	0.014
peel+ flavedo	0.90	0.65	0.24	27.1%	0.122
peel + pulp	0.88	0.66	0.22	24.8%	0.135

3.3.4 Antioxidant capacity in wash water samples

Figure 3.4 below shows the comparison between the antioxidants capacity of different raw material. This comparison is measured in terms of the difference in the [DPPH$^{\bullet}$] scavenged. AS would be expected, the orange rind itself contained most of the antioxidant components, and the content in the wash extract shows that a significant portion would be present in washing waters.

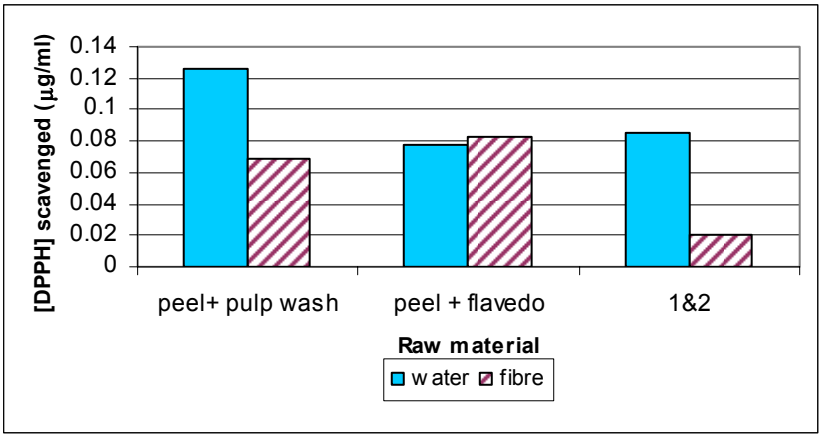

Figure 3.4: Comparison of the antioxidant capacity for different orange pulp materials

3.3.5 Production of a combined antioxidant fibre product

Based on the findings reported thus far, it was concluded that wastewaters would contain significant and useful amounts of dietary fibre and antioxidant organics. As an alternative approach to extraction of the antioxidant organics as described in Chapter 2, an extraction was considered in which the "synthetic wastewater" or selected industrial samples could simply be spray dried to give a combined fibre-plus-antioxidant product. The main problem envisaged here is that materials which are sticky can be extremely problematic to spray dry. However, collaboration with researchers at University of Sydney allowed us to produce a preliminary product which could be tested for its antioxidant properties.

Orange and grapefruit were tested in this experiment, and the procedure involved taking peels which had been juiced, and extracting them with hot water or ethanol, to give samples representative of wastewaters. The extracts were then spray dried (at the Department of Chemical Engineering, University of Sydney). The orange samples gave 0.1g per 10g peel extracted, and grapefruit samples gave 0.6 g per 10g extracted. This indicates that approximately 1% of the solids would be extracted into waste water streams. Solutions were made form these extracts to measure antioxidant activity. Table 3.4 shows the antioxidant activity and phenolics content of the powdered extracts.

These results show that spray-dried extracts were successfully produced and that they had very high antioxidant activity. This preliminary process thus has very significant potential for successful development. It is notable that the grapefruit samples gave higher results and may well represent a more favoured material to investigate in future.

Table 3.4: Total phenols and antioxidant activity for combined antioxidant fibre citrus extracts

Sample	Total phenols (mg / L gallic acid equivalents)	DPPH quenching (% decrease under standard conditions)
Grapefruit (water extract)	196	46
Grapefruit (ethanol extract)	66	45
Orange (water extract)	75	35
Orange (ethanol extract)	15	25

3.4 CONCLUSION

The results of the preliminary tests conducted suggest that 1% of the total fruit pulp could be expected to be present in the wastewater as complex carbohydrate, of which 70% would be insoluble dietary fibre and 25 % soluble dietary fibre. Simple sugars were also found to be present. It was clear that it would be possible to extract dietary fibre from citrus wastewaters, although it would not be simple to separate the simple reducing sugars. However, on a nutritional level, the amount of reducing sugars was considered to be low enough to be acceptable in a food product.

A novel antioxidant fibre powder product was produced by spray-drying water extracts of citrus pulp, showing that this technology could be used to obtain extracts. This technology can readily be used for dilute solutions such as wastewater streams. Further, the treatment did not destroy the antioxidant activity of the extracts. Thus, the spray-dried products were shown to be extremely promising candidates for further development.

CHAPTER 4
CONCLUSIONS AND RECOMMENDATIONS

4.1 Findings of the feasibility study

The feasibility study has shown that South African citrus processing waste waters are a source of antioxidants and complex carbohydrates that could be used for dietary fibre. The content of the waste waters was assessed and quantities of the useful components were estimated. The findings can be summarised as follows:

- If an organic, oil-based flavonoid-containing extract, a “bioflavonoid” nutraceutical product, is the desired product, the best method for extracting the organic antioxidant fraction was found to be solid phase adsorption on resins. However, SLM technology still requires further assessment before it is excluded.
- Complex carbohydrate in the form of soluble and insoluble dietary fibre could be extracted successfully. Thus, it would be possible to extract dietary fibre from citrus wastewaters, although this would not be simple to separate the simple reducing sugars. However, on a nutritional level, the amount of reducing sugars was considered to be low enough to be acceptable in a food product.
- Spray drying of water extracts was used to produce a powder with both antioxidant and fibre components in it. The spray-dried product was shown to be extremely promising candidates for further development
- Designing of a suitable process will be aided greatly by deciding first what type of products will or can be manufactured. For example there is an option of producing high antioxidant capacity, low citrus flavonoid water extracts or low antioxidant high in citrus flavonoid content. The separation of sugars from water-soluble antioxidants is currently problematic and needs to be investigated further.

This leads to the following important findings:

(1) The citrus processing wastewaters are highly likely to be contain *both* carbohydrates and phenolic antioxidant compounds

(2) To design a process for separating water-soluble sugars and antioxidants would be potentially problematic, as the phenolic compounds are not easily extracted using a non-water miscible solvent that is acceptable in food products.

(3) One (possibly expensive) possibility would be to also recover water-soluble antioxidants with a solid-phase adsorption step.

(4) A more economical approach would be to develop a spray-dried product that contains both the carbohydrate and the flavonoid components, together, as a potential food additive.

4.2 Industry contacts

The following industries were contacted and consulted.

Fruit Juices, a citrus juice producing plant located near Citrusdal. Plant also extracts oils from citrus but not with high efficiency. Waste products include pulp and peel containing amounts of oils as well as effluent water high in sugars.
Contact: Johan Zandberg (manager), 022-921-2831 (work)

CapeFruit Processes: Small processing plant that press and collect oil from oranges and lemons while intact. After oil removal, cut fruit open and collect juice. Waste products are pulp and peel containing minimal oil and effluent water high in sugars. Located on Wemmershoek road between Paarl and Franschhoek.
Contact: Tony Lamas; 021-867-0277 (work), 082 807 4410 (mobile), tony@riverside.co.za (e-mail)

SSB Transports: Company that transport pulp and peel from Citrusdal to storage silos in Ashton to be used as cattle feed. Citrus waste still contain 80% moisture and is often mixed with other feed. Located in Paarl.
Contact: Philip Watson 021 933 8035 (work), 082 8790 675 (mobile)

4.3 Preliminary market consideration

The following is a tentative estimate of potential market values for products obtainable from citrus wastewater.

The total South African citrus production is 1.6 million tonnes per year.
The total value of the crop is approximately R2 billion.
Of this, 25% is processed [0.4 million tonnes].
In the processing, some 1.5 ML water are produced per tonne [0.6million ML]

Our results indicate the presence of between 4 and 64 mg phenolics per L wastewater
Using 30 mg / L as an indicator value, and assuming that 1% of the national citrus processing wastewater stream was to be extracted, a total of 180 000 kg of antioxidant extract could be expected.

The current price of orange oil is approximately US$40 per gallon (approximately $8 / kg).
If our extract had a market price of only 10% that of pure orange oil, this suggests a potential market price for the extract of US$14 m p.a.

Fibre was extracted at a yield of 175 kg per tonne fruit and we might expect 10% of this to be present in wastewater. Alternatively, the current price of an equivalent antioxidant fibre

product derived from rice bran is currently available in the USA at $28 per kg (www.nutracea.com). Thus we might expect our antioxidant powder product to have a similar value.

4.4 Recommendations

The project was a feasibility study intended to provide scope for later research. The following are recommendations for developing this research programme:

Further detailed characterization is required to confirm COD, BOD, organic and inorganic content, volumes and concentrations in different wastewaters from more industrial sources. Not all waste streams from citrus industries are likely to be suitable since some are cleaner than others, and preliminary purification would need to be minimized in a beneficiation process.

In addition, sourcing of the waste waters needs to be considered, since it was found that the processing only takes place during harvest periods, rather than all year. It is recommended that waste production should be monitored at some industry plants over the annual activity.

More detailed analysis of the nature of the antioxidant components in the extracts should be conducted. Identification of the particular phenolic components will be necessary for food supplement specification.

Further research will be required to develop and customize the extraction process and to further explore supported liquid membrane extraction methods, to optimize extraction of high value components.

The spray-drying technology used to produce the prototype products shows great promise and is recommended as a simple, clean and effective method to pursue for future development.

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APPENDICES

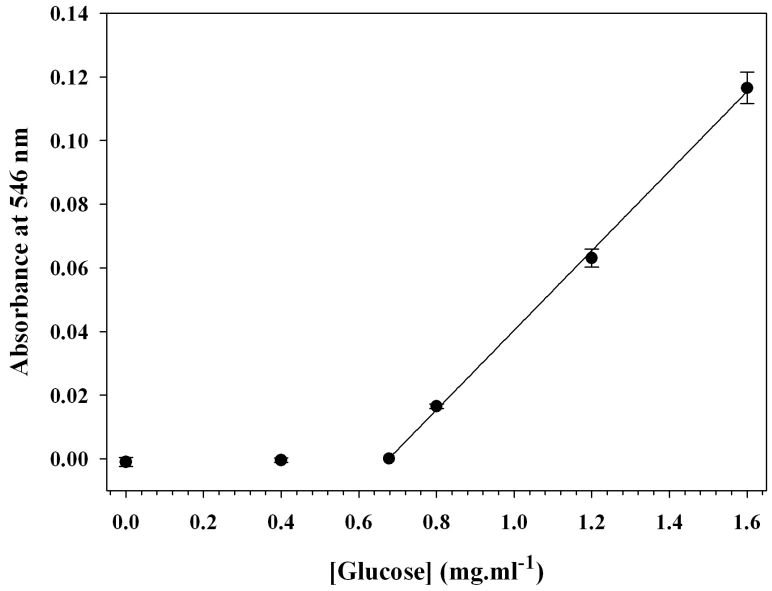

Figure A1: Glucose standard curve with DNS assay shows a linear response in the range of 0.7-1.6 mg/mL glucose. The lack of linearity in the lower range is the result of oxygen in the sample, destroying the glucose under alkaline conditions.

Linear equation: $A_{546nm} = 0.125*[Glucose] - 8.47*10^{-2}$; $r^2 = 0.995$

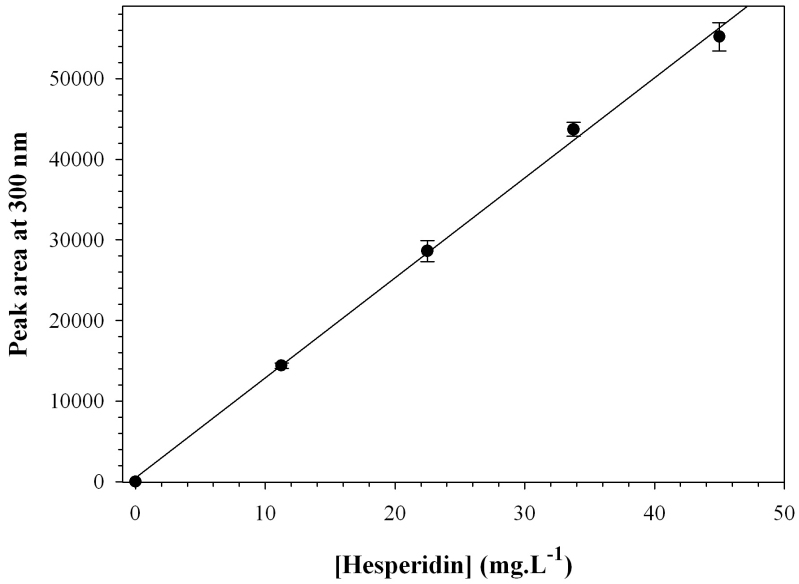

Figure A2: HPLC hesperidin standard curve shows a linear response in the range of 0-45 mg/L. The upper concentration is also close to the saturation point in a water solution.

Linear equation: Peak Area = 1241.77*[Hesperidin] + 435.4; $r^2 = 0.997$

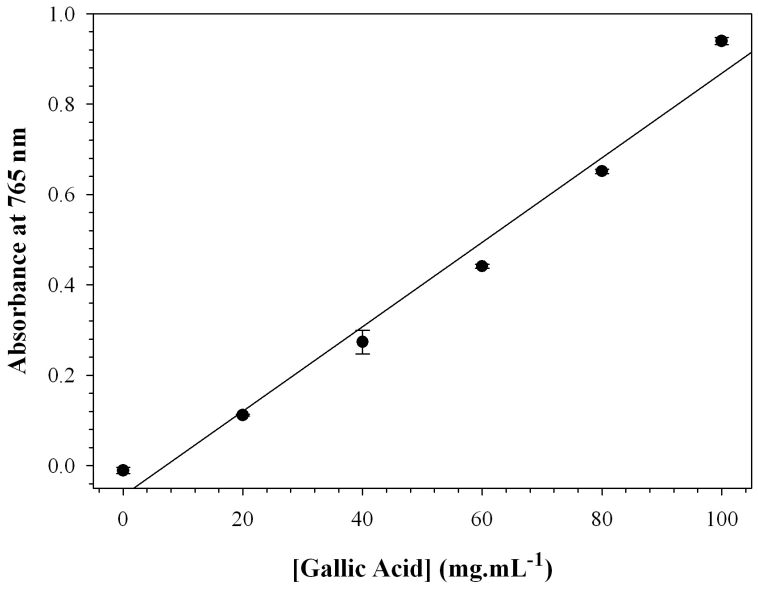

Figure A3: Gallic acid standard curve for total phenol determination shows a linear response in the range of 0-100 mg/L gallic acid. Gallic acid is a typical plant polyphenol that can be used for estimating total phenol content of plant extracts expressed as gallic acid equivalents (GAE).

Linear equation: $A_{765nm} = 9.34*10^{-3}*[\text{Gallic Acid}] - 6.61*10^{-2}$; $r^2 = 0.978$