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The effects of drying conditions and carrier selection on the production of an antioxidant rich powder

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A thesis submitted to the Faculty of Engineering,
The University of Sydney in fulfilment of the requirements for the Degree of Master of Philosophy

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Declaration

Apart from the assistance mentioned in the acknowledgements, the studies contained within this thesis were planned and executed by the author, and have not been previously submitted for any degree to a University or Institution.

Roshan Premarajah

23rd February 2014
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First and foremost, I would like to express my most sincere thanks to Professor Timothy Langrish. I really appreciate the opportunity you have given me to do undertake postgraduate studies. Tim, your support and patience over the past 6 years, have been second to none. Together with Tim, I would like to thank the students and academic staff of the School of Chemical and Biomolecular Engineering, University of Sydney, and in particular the spray drying group including Md Intiaz-Ul Islam, Don Chiou, Jawad Ahmad, Perry Johnson, Tino Kausmann and Nima Yazdanpanah.

I would like to thank my family including Risha Premarajah, Ratnarajah Premarajah, Iris Leong and Kevin Plowman for their ongoing love and support.

Finally, my deepest appreciation to my wife, Tamara Premarajah, for all the emotional support and for keeping me sane over the past 9 years. We have shared many special memories and I look forward to achieving more of our dreams into the future.
Summary

The beneficial impacts of consuming an antioxidant-rich diet have been discussed widely in the literature and includes their anti-carcinogenic effects. Antioxidant-rich, bioactive liquids can be created through extraction from antioxidant rich plants. A liquid is not ideal for today’s fast moving consumer society as it may need to be refrigerated and may not have a long shelf life. A bioactive powder is much more convenient as it can be stored at room temperature for extended periods which also enables cheaper transportation (ie. sea cargo rather than air mail) around the globe. The main aim of this study was to develop a method that would allow a bioactive extract to be dried in a repeatable and reproducible manner, such that it could be stored as a free flowing and non-sorbing powder.

Typical of many fruit juices and extracts, the bioactive extract used in this study is a sticky liquid. Due to the stickiness of this material drying is particularly difficult without the use of a drying aid. Traditionally, high molecular weight materials, such as maltodextrin and gum arabic are used as a drying aid due to their higher glass-transition temperatures. Higher glass-transition materials can be dried into more crystalline, free-flowing, low hygroscopicity powders.

Carriers are usually undesirable materials and hence are used in the lowest percentages feasible. However, maltodextrin has been used as a carrier in relatively high proportions (above 50%) for fruit juices, due to its specific wall properties and ability to interact with the dried materials to reduce stickiness. The resulting product is relatively free flowing and easy
to store. In this study, two desirable material were used as carriers: natural fibre and whey protein isolate (WPI). Fibre is known to improve gut health, and whey protein is used as a dietary supplement to increase muscle mass. The beneficial nature of these two carrier materials reduces the need to minimise these components in the bioactive powder.

In this study, multiple drying methods and conditions were studied and the efficacy of two carriers was considered. Drying methods utilised included oven, vacuum, freeze, and spray drying and the carriers studied were a natural sugar cane fibre and whey protein isolate. The antioxidant extract that was studied was an aqueous extract of the Rosella plant (*Hibiscus sabdarifa* L.). This is a commercially, and hence consistent, extract that is a rich purple in colour due to the high concentration of anthocyanins.

The drying methods were assessed to identify which one should be investigated further. Oven, vacuum and freeze drying were found to take a relatively long time and had a negative effect on the conservation of antioxidant capacity of the extract. Spray drying was found to be the most suitable drying method, with yields of up to 70%, when drying the extract-natural fibre mixture, and up to 98%, when drying the extract-WPI mixture. An increase in the initial antioxidant content was discovered when spray drying with both carriers, which may be due to Maillard reactions occurring throughout the drying process. This increase in antioxidants when spray drying extract with a carrier has not been compared to extract dried on its own as there was not enough powder collected without a carrier.
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List of Abbreviations

AAPH – 2,2’-azobis(2-amidinopropane) dihydrochloride
AAPH₂ – 2,2’-azobis (2-methylpropionamide) dihydrochloride
ABAP – 2,2’-azobis (2-amidinopropane) dihydrochloride
ABTS – 2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
AOX – Antioxidant capacity
BHA – Butyl hydroxyl anisole
BHT – Butyl hydroxyl toluence
B-PE – B-phycoerythrin
CAA – cellular antioxidant activity
CLSM – confocal laser scanning microscopy
DCHF – 2’,7’-dichlorofluorescin
DCHF-DA – 2’,7’-Dichlorofluorescin diacetate
DPPH – 2,2-diphenyl-1-picrylhydrazyl
FCR – Folin-Ciocalteu reagent
FL – fluorescin
FRAP – Ferric ion reducing antioxidant power
FRASC – Ferric reducing/antioxidant power and ascorbic acid concentration
HBSS – Hanks’ Balanced Salt Solution
HPLC – High pressure liquid chromatography
ORAC – Oxygen radical absorbance capacity
PGSS – Particles from gas saturated solutions
ROS – Reactive Oxygen Species
TEAC – Trolox equivalent antioxidant capacity
Tₜ – Glass transition temperature
TPTZ – 2,4,6-tripyridyl-s-triazine
TRAP – total radical-trapping parameter
Chapter 1. Introduction

Free radicals and other reactive species present in the body can have a large effect on, and have been suggested to have some role in the development of, many degenerative diseases, such as heart disease, cancer and Parkinson’s disease. These chronic diseases have been linked with by oxidative stress on body tissues (Halliwell and Gutteridge, 1999; Wolfe and Liu, 2007; Wong et al., 2006), which is the imbalance between reactive oxygen species (ROS) and antioxidant defence. This imbalance can result from either an increase in ROS or a breakdown in the level of antioxidant defence, and therefore the balance of antioxidants and ROS in the body is crucial. An increase in ROS within the body can arise from common sources, such as smoking, exposure to heat and UV light. The antioxidants used to protect against ROS can come from internal sources, namely the antioxidants produced within the human body, such as glutathione, or external sources, such as antioxidants obtained from fruits and vegetables. The use of traditional synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), has been reduced in recent times since these chemicals are suspected to be carcinogenic and to cause liver damage (Chen et al., 1992; Clevelence et al., 1997; Grice, 1988; Kubola and Siriamornpun, 2008; Senevirathne et al., 2006). Therefore, there is a significant market demand to replace these synthetic antioxidants with natural antioxidants derived from plant material.

In this study, a commercially available Rosella extract (Hibiscus sabdariffa L.), which is high in antioxidants, has been chosen as the sample material. The main antioxidants in the Rosella extract are anthocyanins (Gradinaru et al., 2003), which are the largest group of natural pigments of plants and are responsible for the rich dark colourings of fruits such as cherries.
and berries and vegetables, such as eggplant. Drying the bioactive extract would facilitate increased shelf life and reduced microbial degradation due to the reduction in the moisture content. Drying would also increase the attractiveness to industry since there would be significantly reduced transportation costs, and it may simplify the process of adding an antioxidant-rich powder to currently-available consumer food products.

Several different drying techniques are possible, including tray drying; drum drying; freeze drying; and spray drying. When drying sensitive materials, several factors need to be considered, such as the drying time and the harshness of drying conditions. Tray drying could be considered the oldest form of drying and could be as simple as leaving a tray of sample in the sun. More realistically, in an industrial application, a solution may be spread thinly on trays and placed into an oven where heated air is circulated (Mccabe et al., 1993). This process may be expensive and lengthy due to the labour involved in loading and unloading the trays from the oven and the relatively small surface area across which evaporation of the moisture can occur.

Drum drying is similar to tray drying but the surface area of the drying interface is increased because the solution to be dried is continuously being spread across the outer surface of a rotating, internally heated drum (Genskow et al., 2008). Freeze drying involves the removal of water or another solvent from a frozen product by sublimation. Sublimation is the passing from a solid (frozen state) directly to a gaseous state bypassing the liquid phase. Freeze drying can be considered to be a three-step process consisting of: prefreezing; primary drying; and secondary drying. The amount of manual labour involved in tray drying and the multi-step nature of freeze-drying make both techniques significantly expensive and time consuming. Alternatively, spray drying is a one-step process which involves atomising the liquid feed, to increase the surface area, and then mixing this fine mist with a drying gas (typically heated air). The dry particles are then separated from the gas stream, usually through the use of a cyclone.

Spray dryers are used in a variety of industries, where a product is to be directly transformed from a feed solution or suspension into a solid particulate state in a single-step continuous
Single-step processes are attractive in food and pharmaceutical industries, which require minimal handling to allow high product quality. Spray-drying methods are also desirable due to short contact times and relatively low particle temperatures compared with gas temperatures (Rodriguez-Hernandez et al., 2005), which enables some food properties, such as flavour, colour and nutrients to be retained in high percentages (Kuts and Samsonyuk, 1989).

Desobry et al. (1997) compared spray, drum and freeze drying in relation to encapsulation and preservation of β-carotene. The researchers focused their attention on two features of the powders, including the degradation during the drying process and the physical properties of the resulting powder, which made the antioxidant either less or more susceptible to oxidative degradation. During freeze drying, the drying occurs through sublimation of the frozen water, and hence there should be no water phase reactions and little oxidation due to the vacuum.

Spray drying, while offering the largest surface area from which evaporation can occur, also has the greatest surface area across which oxidation can occur. The short contact time, typically of 2-3 seconds in small-scale dryers (Arpagaus and Schwartzbach, 2008), mitigates this. The size and shape of the obtained powders had a large effect on the preservation of the β-carotene. The spray-dried powder was small and spherical in shape, which provided a large surface area for oxidation to occur. The drum-dried and freeze-dried material were ground after drying and this grinding stage resulted in larger particles compared with spray drying, which offered more protection to the β-carotene. Even though the freeze-dried powder had a similar size to drum-dried products, it was more susceptible to oxidative degradation since there were large pores extending into the interior of the particles, which would allow easier diffusion. Therefore, the outcome of the study conducted by Desobry and colleagues (1997) was that drum drying was the most favourable way to dry encapsulated β-carotene. This suggests that freeze drying is not the best way to protect the antioxidant nature of the liquid extract. However, these data suggest that another study should be undertaken in which the drum-dried and freeze-dried particles should be ground to the same size as the spray-dried particles to give a more accurate assessment of the effect of the drying method on the level of preservation for the oxygen-sensitive product.
Another important aspect of this work (with the bioactive extract) is to protect the antioxidant nature of the liquid extract. Encapsulation has been found to: protect sensitive food components; ensure against nutritional loss; utilise otherwise sensitive ingredients; incorporate unusual or time-release mechanisms into the formulation; mask or preserve flavours and aromas; and transform liquids into easily handled solid ingredients (Desai and Park, 2005). In this work, encapsulation has been used to exploit the protection of sensitive materials and to produce an easily-handled powder. Gradinaru et al. (2003) established that the encapsulation of anthocyanins with pullulan protects the bioactive anthocyanins from degradation by reducing the reaction kinetics. It has also been found that spray drying α- and β-carotenes with dextrose-equivalent hydrolyzates as encapsulants increases the shelf-life 72-220 fold (Wagner and Warthesen, 1995). Chiou and Langrish (2007) found that the encapsulation of the Hibiscus sabdariffa L. inside natural fruit fibres results in the production of a free-flowing powder.

This thesis has been structured to give a summary of the current knowledge on drying of antioxidant rich fruits, vegetables and liquids. It then outlines the empirical data derived in this research project, which started by assessing different drying methods. Spray drying was identified as the most suitable drying method for the natural fibre-bioactive liquid, and then further experimentation was done to optimise the spray drying yields and to identify the best drying conditions for the retention of polyphenolic components. Finally, another carrier, whey protein isolate, was investigated to reveal the yields and antioxidant retention that could be achieved through the use of a different carrier.
Chapter 2. Review of the Literature

INTRODUCTION

The waste streams of many food-processing industries have an adverse environmental impact due to the presence of residual phenols from the plant material used. These residual phenols lead to increased biochemical and chemical oxygen demand, with detrimental effects on the flora and fauna of discharge zones. Also, in solid residues for obtaining fertilisers, relatively high levels of phenolic compounds are a problem because of their inhibition of germination properties (Negro et al., 2003). These same polyphenols have advantageous effects for human health, such as the inhibition of oxidation for low-density lipoproteins (Frankel et al., 1993; Frankel et al., 1995; Lila, 2004; Mazza, 1998; Meyer et al., 1997), thereby decreasing heart disease risks (Williams and Elliot, 1997). They also have anti-inflammatory activity and anti-carcinogenic properties (Maeda-Yamamoto et al., 1999; Miyake et al., 1999). Therefore, it is beneficial to extract the polyphenols from the waste streams of food processing industries and to re-implement them into processed foods, in either liquid or solid forms. Spray drying has the potential to create these solid forms.

Fang and Bhandari (2012) demonstrated the difficulty in spray drying a sugary liquid when they recovered no powder when spray drying bay berry juice on its own. The work conducted by Chiu and Langrish (2007) showed a method to produce a novel and natural nutraceutical. In their work they found that a bioactive extract could be encapsulated in a natural fruit fibre and hence counteract the hygroscopic nature of the powdered extract. This study has continued that work with the drying of an extract and fibre slurry. The bioactive/fibre slurry has been dried with a variety of methods to identify an appropriate drying method. The drying method has been assessed on the basis of the amount of antioxidant degradation that has occurred throughout the process. The time of drying and temperature of drying are proposed to be the most important factors (Saeed et al., 2008), but other factors such as particle size and fibre to extract ratio (amongst others) must also be considered.
ANTIOXIDANTS

The consumption of antioxidants has been known to be beneficial for a long time (Pandey and Rizvi, 2009; Shahidi, 1997). It is also well known that the conventional processing of foods degrades, and in some cases destroys, the antioxidative properties of the foods that are consumed. Consumers are also becoming increasingly concerned about the use of traditional synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), based on concerns that these compounds may be carcinogenic (Chen et al., 1992; Clevidence et al., 1997). Therefore, significant research has focused on the extraction and utilisation of antioxidants from natural sources, such as plants.

The importance of antioxidant consumption is emphasised in a phenomena known as the French paradox. It is known that people in certain districts of France have a diet with significantly high levels of saturated fats and relatively high plasma cholesterol levels, but they have a low heart disease mortality rate (Renaud and De Lorgeril, 1992). This phenomena is believed to be due to the fact that in these same districts of France, there is a high consumption of red wine and hence an associated high level of phenolic/antioxidant intake (Meyer et al., 1997), which results in the inhibition of oxidation for low-density lipoproteins in the blood (Lila, 2004; Mazza, 1998).

There are antioxidants from many different sources, but the current study has focussed on those derived from plant material, with a particular emphasis on those contained in lemons, sugar cane and hibiscus. Antioxidants are found in plants to protect them against tissue injuries as the tissue oxidises and combines with proteins and other components. In addition, phenolic compounds in plants may serve as defence systems against animals eating them, and they may protect against oxidation when the plant is cut. Also, by-products of photosynthesis may produce high levels of oxygen, free radicals and reactive oxygen species (ROS). Therefore, there are many antioxidant compounds that plants utilise to survive (Shahidi, 1997). These compounds include: phenolic acids; flavanoids; isoflavones; gallate esters (hydrolysable tannins); lignans; coumarines; stilbenes; flavanones; and oligomeric proanthrocyanidins (Shahidi 1997). The best-known natural antioxidants that have been
proven to be beneficial to human health are: tocopherols; vitamin C; and carotenoids (Packer, 1996; Shahidi, 1997).

**Antioxidants Present in Common Dietary Sources**

Table 1 lists a few commonly used plants and their total polyphenol content, as measured by different researchers. Many of these plant materials are used in the preparation of a variety of ethnic foods and/or beverages. The most relevant material to this study is Rosella (*Hibiscus sabdariffa* L.), since this is an extract that has been extensively analysed.

Table 2 shows a list of common waste products and their polyphenolic contents. These polyphenols may be extracted and used as natural antioxidants in place of synthetic antioxidants.
<table>
<thead>
<tr>
<th>Plant</th>
<th>Total polyphenol content</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betel, <em>Piper betel</em></td>
<td>18 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Wong et al., 2006</td>
</tr>
<tr>
<td>Carob pods, <em>Ceratonia siliqua L.</em></td>
<td>19.2 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Kumazawa et al., 2002</td>
</tr>
<tr>
<td>Cekur manis, <em>Sauropus androgynus</em></td>
<td>21 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Wong et al., 2006</td>
</tr>
<tr>
<td>Curry tree, <em>Muraya keonigii</em></td>
<td>26 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Wong et al., 2006</td>
</tr>
<tr>
<td>Ginger leaves, <em>Alpinia</em> sp.</td>
<td>3.92-19.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Chan et al., 2008</td>
</tr>
<tr>
<td>Ginger leaves, <em>Boesenbergia rotunda</em></td>
<td>2.6 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Chan et al., 2008</td>
</tr>
<tr>
<td>Ginger leaves, <em>Curcuma</em> sp.</td>
<td>2.30-5.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Chan et al., 2008</td>
</tr>
<tr>
<td>Ginger leaves, <em>Elettariaopsis</em> sp.</td>
<td>3.03-4.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Chan et al., 2008</td>
</tr>
<tr>
<td>Ginger leaves, <em>Etlingera</em> sp.</td>
<td>11.10-23.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Chan et al., 2008</td>
</tr>
<tr>
<td>Ginger leaves, <em>Hedychium coronarium</em></td>
<td>8.20 ± 0.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Chan et al., 2008</td>
</tr>
<tr>
<td>Ginger leaves, <em>Kaempferia</em> sp.</td>
<td>1.12-1.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Chan et al., 2008</td>
</tr>
<tr>
<td>Ginger leaves, <em>Scaphochlamys kunstleri</em></td>
<td>2.03 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Chan et al., 2008</td>
</tr>
<tr>
<td>Ginger leaves, <em>Zingiber</em> sp.</td>
<td>2.91 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Chan et al., 2008</td>
</tr>
<tr>
<td>Horse radish tree, <em>Moringa pteriosperma</em></td>
<td>14 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Wong et al., 2006</td>
</tr>
<tr>
<td>Laksa, <em>Polygonium hydropiper</em></td>
<td>14 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Wong et al., 2006</td>
</tr>
<tr>
<td>Local celery, <em>Apium graveolens L.</em></td>
<td>6 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Wong et al., 2006</td>
</tr>
<tr>
<td>Petai, <em>Parkia speciosa</em></td>
<td>32 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Wong et al., 2006</td>
</tr>
<tr>
<td>Rosella, <em>Hibiscus sabdariffa</em></td>
<td>12 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Wong et al., 2006</td>
</tr>
</tbody>
</table>

<sup>a</sup> mg gallic acid equivalents/100 g dry sample
<sup>b</sup> mg gallic acid equivalents/100 g fresh sample
Table 2: Total polyphenol content of selected waste products.

<table>
<thead>
<tr>
<th>By-product</th>
<th>Total polyphenol content</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bitter gourd leaf</td>
<td>474 ± 0.71(^b)</td>
<td>Kubola and Siriamornpun, 2008</td>
</tr>
<tr>
<td>Bitter gourd stem</td>
<td>259 ± 1.2(^b)</td>
<td>Kubola and Siriamornpun, 2008</td>
</tr>
<tr>
<td>Grapefruit peel</td>
<td>155 ± 10.3(^a)</td>
<td>Gorinstein et al., 2001</td>
</tr>
<tr>
<td>Hazelnut skin</td>
<td>206.1(^c)</td>
<td>Contini et al, 2008</td>
</tr>
<tr>
<td>Hazelnut waste shell</td>
<td>72(^c)</td>
<td>Contini et al, 2008</td>
</tr>
<tr>
<td>Lemon peel</td>
<td>190 ± 10.6(^a)</td>
<td>Gorinstein et al., 2001</td>
</tr>
<tr>
<td>Mango peel</td>
<td>57.24(^b)</td>
<td>Ribeiro et al., 2008</td>
</tr>
<tr>
<td>Mango seed kernel</td>
<td>82.54(^b)</td>
<td>Ribeiro et al., 2008</td>
</tr>
<tr>
<td>Orange peel</td>
<td>179 ± 10.5(^a)</td>
<td>Gorinstein et al., 2001</td>
</tr>
</tbody>
</table>

\(^a\) mg chlorogenic acid/100g fresh material  
\(^b\) mg gallic acid equivalents/g dry sample  
\(^c\) mg gallic acid equivalents/g roasted sample

Table 3 shows a selection of wines and their polyphenolic contents. It shows that there are more polyphenols in red wines than in white wines and berry wines. This fits with the perception in society that a glass of red wine has a beneficial effect on the human body. It also supports the “French paradox”. The wide variations in results are due to the regional differences and/or the quality of the wines. Table 4 shows the polyphenolic content for a selection of varieties of beer. Similarly, tables Table 5 and Table 6 show the polyphenolic content of a selection of teas and fruit juices respectively. This shows the wide variety of places that polyphenols are found in the common diet.
Table 3: Total polyphenolic content of selected natural (not concentrated) fruit juices and wines.

<table>
<thead>
<tr>
<th>Wine</th>
<th>Total polyphenol content</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple</td>
<td>160-470&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Heinonen et al., 1998</td>
</tr>
<tr>
<td>Black currant and crowberry</td>
<td>1020&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Heinonen et al., 1998</td>
</tr>
<tr>
<td>Black currant and bilberry</td>
<td>1010-1060&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Heinonen et al., 1998</td>
</tr>
<tr>
<td>Black and red currant</td>
<td>515-1270&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Heinonen et al., 1998</td>
</tr>
<tr>
<td>Black currant</td>
<td>520-1820&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Heinonen et al., 1998</td>
</tr>
<tr>
<td>Black and red currant and strawberry</td>
<td>720&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Heinonen et al., 1998</td>
</tr>
<tr>
<td>Black currant and strawberry</td>
<td>655-950&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Heinonen et al., 1998</td>
</tr>
<tr>
<td>Cowberry and birch sap</td>
<td>775&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Heinonen et al., 1998</td>
</tr>
<tr>
<td>Cranberry</td>
<td>680&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Heinonen et al., 1998</td>
</tr>
<tr>
<td>Green and white currant</td>
<td>250-270&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Heinonen et al., 1998</td>
</tr>
<tr>
<td>Red currant</td>
<td>440-495&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Heinonen et al., 1998</td>
</tr>
<tr>
<td>Rowanberry and apple</td>
<td>690&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Heinonen et al., 1998</td>
</tr>
<tr>
<td>Red wine</td>
<td>750-4500&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Kiralp and Toppare (2006)</td>
</tr>
<tr>
<td>Red wine</td>
<td>1390-1600&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Heinonen et al., 1998</td>
</tr>
<tr>
<td>Red wine</td>
<td>3500 ± 100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Lugasi and Hovari, 2008</td>
</tr>
<tr>
<td>Red wine</td>
<td>1730 ± 583&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2003</td>
</tr>
<tr>
<td>White wine</td>
<td>265&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Heinonen et al, 1998</td>
</tr>
<tr>
<td>White wine</td>
<td>392 ± 157&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Lugasi and Hovari, 2003</td>
</tr>
</tbody>
</table>

<sup>a</sup> mg gallic acid equivalents/L
<sup>b</sup> mg catechin equivalent/L

Table 4: Total polyphenolic content of a selection of beers.

<table>
<thead>
<tr>
<th>Beer</th>
<th>Total polyphenol content (mg catechin equivalent/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lager beers</td>
<td>376 ± 73</td>
<td>Lugasi and Hovari, 2003</td>
</tr>
<tr>
<td>Dark beers</td>
<td>473 ± 114</td>
<td>Lugasi and Hovari, 2003</td>
</tr>
</tbody>
</table>
Table 5: Total polyphenolic content of a selection of teas and herbal infusions.

<table>
<thead>
<tr>
<th>Tea</th>
<th>Total polyphenol content</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greek Mountain tea,</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sideritis syriaca</em></td>
<td>88 ± 0.42</td>
<td>Atoui et al., 2005</td>
</tr>
<tr>
<td>Mint, <em>Mentha piperita</em></td>
<td>106 ± 0.18</td>
<td>Atoui et al., 2005</td>
</tr>
<tr>
<td>Chamomile,</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Matricaria recutita</em></td>
<td>106 ± 0.37</td>
<td>Atoui et al., 2005</td>
</tr>
<tr>
<td>Dictamnus, <em>Origanum dictamnus</em></td>
<td>109 ± 3.20</td>
<td>Atoui et al., 2005</td>
</tr>
<tr>
<td>Eucalyptus,</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eucalyptus globules</em></td>
<td>113 ± 1.33</td>
<td>Atoui et al., 2005</td>
</tr>
<tr>
<td>Sage, <em>Salvia fruticosa</em></td>
<td>124 ± 1.57</td>
<td>Atoui et al., 2005</td>
</tr>
<tr>
<td>Linden, <em>Tilia</em> sp.</td>
<td>184 ± 1.72</td>
<td>Atoui et al., 2005</td>
</tr>
<tr>
<td>Black Ceylon tea,</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Camellia sinensis</em></td>
<td>847 ± 8.89</td>
<td>Atoui et al., 2005</td>
</tr>
<tr>
<td>Chinese green tea,</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Camellia sinensis</em></td>
<td>1216 ± 32.0</td>
<td>Atoui et al., 2005</td>
</tr>
<tr>
<td>Iced green tea</td>
<td>0.8 ± 0.1\textsuperscript{b}</td>
<td>Seeram et al., 2008</td>
</tr>
<tr>
<td>Iced black tea</td>
<td>0.4 ± 0.0\textsuperscript{b}</td>
<td>Seeram et al., 2008</td>
</tr>
<tr>
<td>Iced white tea</td>
<td>0.9 ± 0.0\textsuperscript{b}</td>
<td>Seeram et al., 2008</td>
</tr>
</tbody>
</table>

\textsuperscript{a} mg gallic acid equivalents/cup where 1 cup =240mL

\textsuperscript{b} mg gallic acid equivalents/mL

Table 6: Total polyphenol content of selected common fruit juices.

<table>
<thead>
<tr>
<th>Juice</th>
<th>Total polyphenol content</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pomegranate juice</td>
<td>3.8 ± 0.2\textsuperscript{a}</td>
<td>Seeram et al., 2008</td>
</tr>
<tr>
<td>Concord grape juice</td>
<td>2.6 ± 0.4\textsuperscript{a}</td>
<td>Seeram et al., 2008</td>
</tr>
<tr>
<td>Blueberry juice</td>
<td>2.3 ± 0.4\textsuperscript{a}</td>
<td>Seeram et al., 2008</td>
</tr>
<tr>
<td>Black cherry juice</td>
<td>2.1 ± 0.1\textsuperscript{a}</td>
<td>Seeram et al., 2008</td>
</tr>
<tr>
<td>Acai juice</td>
<td>2 ± 0.1\textsuperscript{a}</td>
<td>Seeram et al., 2008</td>
</tr>
<tr>
<td>Cranberry juice</td>
<td>1.7 ± 0.2\textsuperscript{a}</td>
<td>Seeram et al., 2008</td>
</tr>
<tr>
<td>Orange juice</td>
<td>0.7 ± 0.1\textsuperscript{a}</td>
<td>Seeram et al., 2008</td>
</tr>
<tr>
<td>Apple juice</td>
<td>0.4 ± 0.1\textsuperscript{a}</td>
<td>Seeram et al., 2008</td>
</tr>
<tr>
<td>Sugar cane juice</td>
<td>160\textsuperscript{b}</td>
<td>Duarte-Almeida et al., 2006</td>
</tr>
</tbody>
</table>

\textsuperscript{a} mg gallic acid equivalents/mL

\textsuperscript{b} mg catechin equivalents/L
Table 7: Total polyphenolic content of selected fruits.

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Total polyphenol content</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple</td>
<td>71.36-124.2(^{a})</td>
<td>Khanizadeh et al., 2008</td>
</tr>
<tr>
<td>Lemon</td>
<td>164 ± 10.3(^{a})</td>
<td>Gorinstein et al., 2001</td>
</tr>
<tr>
<td>Orange</td>
<td>154 ± 10.2(^{a})</td>
<td>Gorinstein et al., 2001</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>135 ± 10.1(^{a})</td>
<td>Gorinstein et al., 2001</td>
</tr>
<tr>
<td>Bitter gourd, <em>M. charntia L.</em></td>
<td>324 ± 1.63(^{b})</td>
<td>Kubola and Siriamompun, 2008</td>
</tr>
<tr>
<td>(green fruit)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bitter gourd, <em>M. charntia L.</em></td>
<td>224 ± 0.86(^{b})</td>
<td>Kubola and Siriamompun, 2008</td>
</tr>
<tr>
<td>(ripe fruit)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highbush blueberry, <em>Vaccinium corymbosum L.</em></td>
<td>33(^{b})</td>
<td>Castrejon et al., 2008</td>
</tr>
</tbody>
</table>

**Grapes**

<table>
<thead>
<tr>
<th>Grape</th>
<th>Total polyphenol content</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grape</td>
<td>119.963(^{a})</td>
<td>Jacob et al., 2008</td>
</tr>
<tr>
<td>Black magic grapes</td>
<td>169.27(^{a})</td>
<td>Harkensee et al., 2006</td>
</tr>
<tr>
<td></td>
<td>1258.89(^{c})</td>
<td>Harkensee et al., 2006</td>
</tr>
<tr>
<td>Palieri grape</td>
<td>152.43(^{a})</td>
<td>Harkensee et al., 2006</td>
</tr>
<tr>
<td></td>
<td>1139.80(^{c})</td>
<td>Harkensee et al., 2006</td>
</tr>
<tr>
<td>Victori grape</td>
<td>49.33(^{a})</td>
<td>Harkensee et al., 2006</td>
</tr>
<tr>
<td></td>
<td>374.26(^{c})</td>
<td>Harkensee et al., 2006</td>
</tr>
</tbody>
</table>

\(^{a}\) mg gallic acid equivalents/100g fresh sample  
\(^{b}\) mg gallic acid equivalents/g dry sample  
\(^{c}\) mg gallic acid equivalents/100 g dry sample

**Antioxidants Present in Lemon Peels**

The chemical components of citrus fruits are important in the human diet and for human health. It is known that they are effective in decreasing erythrocyte aggregation and blood coagulation in vitro (Baldi *et al.*, 1995) and, for some of them, the anticarcinogenic activity in vitro and in vivo are well known (Baldi *et al.*, 1995; Verma, 1992).

The lemon peel bioflavanoids are characterised by the presence of four groups of flavonoids (Baldi *et al.*, 1995), and these flavonoids are named in Table 8.
Table 8: The four groups of flavonoids that characterise lemon peel bioflavonoids.

<table>
<thead>
<tr>
<th>Flavones-O-glycosides</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luteolin-7-rutinoside</td>
<td>Gottlieb et al., 1975</td>
</tr>
<tr>
<td>Diosmetin-7-rutinoside (diosmin)</td>
<td>Gottlieb et al., 1975</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flavones-C-glycosides</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>6,8-di-C-glucosyl-luteolin</td>
<td>Park et al., 1983</td>
</tr>
<tr>
<td>6,8-di-C-glucosylapigenin</td>
<td>Park et al., 1983</td>
</tr>
<tr>
<td>6,8-di-C-glycosyl-chrisoeril</td>
<td>Park et al., 1983</td>
</tr>
<tr>
<td>6,8-di-C-glucosyl-diosmetin</td>
<td>Park et al., 1983</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flavanols</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutin (quercetin-3-O-rutinoside)</td>
<td></td>
</tr>
<tr>
<td>3,5,6,4'-tetrahydroxy-6,8,3'-trimethoxy-flavone (limocitrol)</td>
<td></td>
</tr>
<tr>
<td>3,5,7,4'-tetrahydroxy-8,3'-dimethoxy-flavone (limocitrin)</td>
<td></td>
</tr>
<tr>
<td>3,5,7,3'-tetrahydroxy-6,8,4'-trimethoxyflavone (isolimocitrol)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flavanones</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hesperitin-7-rutinoside (hesperidin)</td>
<td></td>
</tr>
<tr>
<td>Naringenin-7-rutinoside</td>
<td></td>
</tr>
<tr>
<td>Eriodictyol-7-rutinoside (eriocitrin)</td>
<td></td>
</tr>
</tbody>
</table>

There are several other polyphenols known to be present in lemon peel, including: phenolic acids and phenyl propanoids (coumarins, phenyl propanoid glycosides) (Macheix et al., 1990). Numerous phenolic compounds were identified through a study done by Baldi et al. (1995), and these compounds are shown in Table 9.
Table 9: Phenolic compounds identified in lemon peels by Baldi et al. (1995).

<table>
<thead>
<tr>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Limonoids</strong></td>
</tr>
<tr>
<td>Nomilinic acid</td>
</tr>
<tr>
<td>Limonin</td>
</tr>
<tr>
<td>Limonoic acid</td>
</tr>
<tr>
<td>Limonin 17-β-D-glucoside</td>
</tr>
<tr>
<td><strong>Phenyl propanoids</strong></td>
</tr>
<tr>
<td>Coumarin</td>
</tr>
<tr>
<td>Isoimperatorin</td>
</tr>
<tr>
<td>Bergamottin</td>
</tr>
<tr>
<td>Bergaptol</td>
</tr>
<tr>
<td>Limettin derivative a</td>
</tr>
<tr>
<td>Limettin derivative b</td>
</tr>
<tr>
<td><strong>Phenyl propanoids glycosides</strong></td>
</tr>
<tr>
<td>Citrusin A</td>
</tr>
<tr>
<td><strong>Flavanoids</strong></td>
</tr>
<tr>
<td>Flavones-C-glucosides</td>
</tr>
<tr>
<td>6,8-di-C-glucopyranosyl-luteolin</td>
</tr>
<tr>
<td>6,8-di-C-glucopyranosyl-apigenin</td>
</tr>
<tr>
<td>Flavanols</td>
</tr>
<tr>
<td>Limocitrol</td>
</tr>
<tr>
<td>Isolimocitrol</td>
</tr>
<tr>
<td>Limocitrin</td>
</tr>
<tr>
<td>Rutin</td>
</tr>
<tr>
<td>Flavones-O-glucosides</td>
</tr>
<tr>
<td>Diosmetin-7-rutinoside</td>
</tr>
<tr>
<td>Flavanones</td>
</tr>
<tr>
<td>Hesperitin-7-rutinoside (hesperidin)</td>
</tr>
<tr>
<td>Eriodictyol-7-rutinoside (eriocitrin)</td>
</tr>
<tr>
<td>Naringenin-7-rutinoside</td>
</tr>
</tbody>
</table>

**Antioxidants Present in Sugar Cane**

There has been limited research into the polyphenolic composition of sugar cane, and therefore Duarte-Almeida et al. (2006) tried to identify the phenolic compounds present in sugar cane juice. Using the Folin-Ciocalteu assay to determine the total phenolic content, it
was found that the phenolic content of the sugar cane juice was relatively high at 160 mgCAE/L. These researchers used high-performance liquid chromatography (HPLC)-diode array detection (DAD) to determine the phenolic compounds from the juice. The sugar cane juice was found to contain flavanoids (apigenin, luteolin and tricin derivatives), and phenolic acids (mainly caffeic, sinapic and isomers of chlorogenic acid).

**Antioxidants Present in Green Tea**

In this study, there must be a positive control to verify that the antioxidant levels found are comparable with other researchers. Therefore, a commonly tested solution is used, namely green tea. Table 10 lists the level of total polyphenols that several researchers have found to exist in green tea.

<table>
<thead>
<tr>
<th>Tea type</th>
<th>Total polyphenol content *</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green tea</td>
<td>65.8-106.2</td>
<td>Khokhar and Magnusdottir (2002)</td>
</tr>
<tr>
<td>117.3</td>
<td>Samman <em>et al.</em> (2001)</td>
<td></td>
</tr>
<tr>
<td>61-200</td>
<td>Schulz <em>et al.</em> (1999)</td>
<td></td>
</tr>
<tr>
<td>205.2</td>
<td>Gramza <em>et al.</em> (2006)</td>
<td></td>
</tr>
</tbody>
</table>

* mg gallic acid equivalents/g dry matter

**Antioxidants Present in *Hibiscus Sabdariffa* L.**

Rosella (*Hibiscus sabdariffa* L.) is a very darkly coloured flower. The darkness of the flower is due to the presence of anthocyanins, the biggest group of water-soluble natural pigments (Gradinaru *et al.*, 2003). Anthocyanins are believed to enhance a plant’s resistance to insect attack (Strack and Wray, 1993). The main anthocyanidins in rosella are delphinidin-3-sambubioside or delphinidin-3-xylosylglucoside or hibiscin and cyanidin-3-sambubioside or cyaniding-3-xylosylglucoside or gossypicyanin, as shown in Figure 1 and Figure 2.
Antioxidants Present in Fibre

It has been shown that the by-products of fruits and greens may be useful in the food industry as high dietary-fibre ingredients (Grigelmo-Miguel and Martín-Belloso, 1999). These by-products often have a relatively high residual polyphenolic content (Ajila et al., 2008). Since the fibre itself has a polyphemolic content and is not “blank” this must be considered throughout this work so that the antioxidant content of the extract is not overestimated in the extract-fibre product.
EXTRACTION

Laboratory Scale

The method of extraction of the polyphenols from waste streams has a significant effect on their applications and antioxidant properties. There are a variety of methods, employed by current researchers, to extract the phenolic contents of plants and vegetables, including maceration and homogenisation using a variety of solvents at different temperatures. In many cases, these extraction processes are preceded by drying and milling stages to ensure that a dry powder is initially attained.

Parejo et al. (2002) carried out a study to compare the radical scavenging activity of six distilled and nondistilled Mediterranean herbs and aromatic plants, and therefore needed to extract the polyphenols from the plant material. They went about this by drying the plant material in the open air (in the field) and then crushing the material. The plant material was again dried in the lab in an oven at 40°C and powdered in a mill. The extraction process that was followed is shown in Figure 3.
An amount of plant material was extracted with methanol by maceration for 24h (stirring for 4h). This solution was then filtered, the methanol was evaporated and the extract redissolved in water, kept at 4°C for 12h, and filtered again, thus obtaining the crude extract (CE1). This CE1 was then partitioned with hexane (200mL repeatedly until the organic solvent was colourless), thus obtaining both the hexane fraction (HxF) and “clean” or “defatted” crude extract (CE2). The CE2 was then successively partitioned with dichloromethane and ethyl acetate (as for the hexane partition), thus obtaining the dichloromethane (DCF), ethyl acetate (EAF), and aqueous (WF) fractions.
Another study carried out by Jayaprakasha et al. (2008) involved the freeze drying of slices of rio red (Citrus paradise MAcf.) grapefruit and sour orange (Citrus aurantium L.). These freeze-dried samples were then powdered using a mixer. The lyophilized citrus fruit powders were extracted using a Soxhlet type extractor with 500mL of hexane, ethyl acetate (EtOAc), acetone, methanol (MeOH) and MeOH:water (80:20) for 8h each separately. All the extracts were cooled, filtered, and concentrated to remove 95% of the solvent and freeze dried separately. These researchers have stated that the different extraction solvents will lead to the extraction of different polyphenols. Hexane will extract non-polar compounds such as carotenoids, and EtOAc will extract polar limonoids and flavanoid aglycones and glucosides. The acetone, MeOH and MeOH:water (80:20) will extract medium polar and polar compounds, such as aglycones and glucosides of flavanoids, limonoids, ascorbic acid and sugars, depending on their polarity.

Hayouni et al. (2007) implemented a similar technique but used different solvents, and also compared the effect of having a fatty material or a defatted material. The study carried out by Hayouni et al. (2007) involved the extraction of phenols from fruits: Tunisian kermes oak (Quercus coccifera L.) and phoenician juniper (Juniperus phoenicea L.) The first extraction procedure involved the finely-ground powder undergoing a Soxhlet extraction with hexane for six hours at 65˚C to remove the fatty materials. The defatted powder was divided into five fractions. Each fraction was separately re-extracted in a Soxhlet apparatus with 250mL of one of the following solvents: absolute chloroform; absolute acetone; mixture 1 (acetone:water:acetic acid – 90:9.5:0.5); mixture 2 (ethyl acetate:methanol:water – 60:30:10); and water. The water extraction involved the powder being infused with 100mL of freshly boiled distilled water for 10 minutes. The infusion was then filtered through Whatman No. 1 paper and rapidly cooled under tap water. The second extraction procedure was carried out in one step. The same solvents were used but without the initial Soxhlet extraction (6h at 65˚C in hexane) and therefore the extract was not defatted. The extract was filtered through a 0.45μm filter paper.

Hayouni et al. (2007) showed that the second method (the one-step extraction) was significantly superior to the first method. Unexpectedly, the efficiency of the solvents used differed between the two methods. When using method 1 the order of the extracts was:
acetone > mixture 1 > mixture 2 > infusion > chloroform, but when method 2 was used the order of extracts was: mixture 1 > mixture 2 > chloroform > acetone > infusion. These results were the same when Q. coccifera fruits were used, except that when method 2 was used, mixture 2 gave a higher yield than mixture 1. The technique of extraction, as well as the extracting solvent, significantly affected extraction yield, total polyphenol and biological activities (antioxidant and antibacterial) of several extracts from J. phoenicea L. and Q. coccifera L. fruits. The results from this study indicate that selective extraction from natural sources, by appropriate solvents and suitable methods, is important for obtaining fractions with high antioxidant and antibacterial activities.

Another variation of the extraction process was carried out by Pellegrini et al. (2007), in which they homogenised spinach with acetone containing 2,6-di-tert-butyl-4-methylphenol (BHT – a commonly used synthetic antioxidant), while keeping the sample refrigerated by means of an ice bath, followed by sonication for 30 seconds. The homogeniser was rinsed with acetate, and the homogenate was centrifuged. The pulp residue was re-extracted by the addition of acetone four times. All fractions were pooled and diluted to 100mL with acetone.

The same technique was used to extract tomato. The only difference was that the initial extraction solvent was tetrahydrofuran (THF stabilised with BHT) rather than acetone stabilised with BHT. The rest of the process was identical to the one described above.

The methods described above were used to extract of the antioxidants from an intact plant and/or part of a plant. In this study, an aqueous extract was the starting point so this author determined that this extraction had already taken place and hence none of these methods were used.

MEASUREMENT OF ANTIOXIDANT POTENTIAL

Chemical Antioxidant Assays

There are numerous assays to identify the antioxidant capacity of a fruit extract. The choice of the test method will affect the significance and relevance of the evaluation (Frankel and
Meyer, 2000). There are a number of factors that influence the choice of antioxidant assay, such as the presence of lipophilic or hydrophilic antioxidants, any molecules of the extract that will interfere with the tests and reduce the accuracy of the method, and the method of quantification for antioxidant activity. Due to antioxidants acting in different ways, there are various aspects of the antioxidants that are detected and measured by different assay methods.

**Folin-Ciocalteu Reagent Assay (Determination of Total Phenolics)**

The Folin-Ciocalteu reagent (FCR) is an assay designed to measure the total phenolic content of a solution. The FCR fundamentally measures the sample’s reducing capacity (Huang et al., 2005). The concept behind the assay is the reaction of the sample with the FCR and the measurement of the absorption of light at a long wavelength of 765nm. There are many benefits of using the FCR assay, including:

1) the commercial availability of the reagent;
2) the long wavelength of the chromophore minimises possible interference from the sample matrix, which is often coloured; and
3) it is a commonly accepted assay, is routinely practiced in research laboratories throughout the world and hence there is a large body of comparative data (Huang et al., 2005).

The above properties of the FCR assay are all beneficial, but there are also some particular disadvantages of this assay. Firstly, the FCR is only capable of measuring non-lipophilic antioxidants, since the assay is carried out in aqueous solutions (Huang et al., 2005). Secondly, the method appears to be somewhat non specific, because there are many ways in which the antioxidants react, and a correct measure of natural polyphenols requires extensive pre-treatment of the samples to eliminate the reducing interferences (Stevanato et al., 2004), which can be a problem. Thirdly, the number of interferences is significant (Singleton et al., 1999). The interferences fall into three categories: inhibitory; additive; and enhancing or augmenting. As the names suggest the interferences will give falsely low, high or a mixture of both readings.
**Trolox Equivalent Antioxidant Capacity Assay (Ability to Scavenge Radicals Generated)**

The Trolox Equivalent Antioxidant Capacity (TEAC) assay is based on the generation of the ABTS \([2,2'\text{-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)}]\) radical cation and a subsequent spectrophotometric method to determine the antioxidant activity of pure substances (Salah et al., 1995), aqueous mixtures and beverages (Miller et al., 1996; Rice-Evans et al., 1996).

The TEAC assay was first proposed by Miller et al. in (1993), but it was subsequently improved upon by Ré et al. in (1999). The difference between the two methods is the way in which the ABTS is generated. The original ABTS assay was based on the activation of metmyoglobin with hydrogen peroxide in the presence of ABTS to produce the radical cation, in the presence or absence of antioxidants. This technique was criticised on the basis that the faster reacting antioxidants might also contribute to the reduction of the ferryl myoglobin radical (Ré et al., 1999). The improved technique for the generation of ABTS involves the pre-formation of the radical cation prior to the addition of the antioxidant.

The improved method is a more suitable assay for the following reasons:

1. the chemistry involves the direct generation of the ABTS radical monocation with no involvement of an intermediate radical;
2. it is a decolourisation assay, and thus the radical cation is formed prior to addition of the antioxidant sample, rather than the generation of the radical taking place continually in the presence of the antioxidant; and
3. it is applicable to both aqueous and lipophilic testing regimes (Ré et al., 1999).

One disadvantage of the TEAC assay is the fact that there is no integrated method to measure the differing reaction kinetics between samples, since the TEAC assay is an end-point assay (Huang et al., 2005). The TEAC assay has been criticised due to the differing reaction times used in the experiment. Multiple researchers (Samaniego Sánchez et al., 2007; Wang et al., 2004) have mentioned that at least 30 minutes was required for the reaction to take place, when only few to several minutes were used in previous methods. This shorter time frame has
resulted in the underestimation of the antioxidant capacity. There are also suggestions that the TEAC assay is not suitable for complex solutions or solutions that contain antioxidants of very different kinds (Zulueta et al., 2009).

**Ferric Ion Reducing Antioxidant Power Assay**

The Ferric Ion Reducing Antioxidant Power (FRAP) assay is very similar to the TEAC assay (described above), but the oxidant is ferric salt [Fe(III)(TPTZ)$_2$Cl$_3$], where TPTZ is 2,4,6-tripyridyl-s-triazine rather than ABTS. The other difference between the FRAP and TEAC assays is that the FRAP assay is carried out under acidic (pH 3.6) conditions (Huang et al., 2005), while the TEAC assay is not pH sensitive. Similarly, it is an end-point assay, with the final measurement taken at four minutes.

There are several difficulties with this assay, and these involve:

1) Fe(III)(TPTZ)$_2$ and other Fe(III) species exist in solution simultaneously. This situation leads to interference reactions, since many metal chelators, which are in many food extracts, could form complexes that are also capable of reacting with antioxidants (Huang et al., 2005). Therefore, the measured antioxidant capacity may be artificially higher than that of the sample.

2) Absorption may not be complete at the final measurement time of four minutes and may continue for several hours, as shown by Pulido et al. (2000). These authors have shown that there are many samples that show these lengthy absorption times, which include a: caffeic acid, tannic acid, ferulic acid, ascorbic acid, and quercetin.

Although the FRAP assay has these disadvantages, its advantages include speed, low cost and robustness compared with many other assays. This robustness involves the fact that other inhibition assays, to date (such as the chemiluminescent assay, the TEAC assay and the TRAP assay), are indirect methods that measure the ability of antioxidants to inhibit the oxidative effects of reactive species purposefully generated in the reaction mixture. In inhibition assays, antioxidant action induces a lag phase; exhaustion of antioxidant power is denoted by a change in signal, such as rate of oxygen utilisation, fluorescence or chemiluminescence (Benzie and Strain, 1999). Measurement of these signals requires specialised equipment and
the tests can be time-consuming, technically demanding and may lack sensitivity (Benzie and Strain, 1999; Schofield and Braganza, 1996). The FRAP assay has, in its favour, the lack of the need for highly specialised equipment or skills, or a critical control of timing and reaction conditions (Benzie and Strain, 1999).

A further development from the FRAP assay is the modification to give the ferric reducing/antioxidant power and ascorbic acid concentration (FRASC) assay. The FRASC assay gives three indices of antioxidant status: the total reducing (antioxidant) power; the absolute concentration of ascorbic acid; and the relative concentration of ascorbic acid to the total antioxidant power of the sample (Benzie and Strain, 1999).

**DPPH Assay (Free Radical Scavenging Activity)**

This assay is used to determine the hydrogen donating or free radical scavenging ability of a sample through the use of the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH') (Atoui et al., 2005; Koleva et al., 2002; Parejo et al., 2002). The method utilises a spectrophotometer to measure the absorbance for the mixture of the sample and either methanolic (Atoui et al., 2005; Parejo et al., 2002) or ethanolic (Koleva et al., 2002) solutions of DPPH'. There is also a slight difference in the wavelength at which to measure the mixture, since both Koleva et al. (2002) and Parejo et al. (2002) have carried out the measurements at 517 nm, whereas Atoui et al. (2005) have taken measurements at 515 nm. These measurements are used to calculate the concentration of an antioxidant needed to decrease the initial DPPH concentration by 50% (EC$_{50}$) (Sánchez-Moreno et al., 1998) and the antiradical efficiency (AE = 1/EC$_{50}$) or antiradical power (ARP). The lower the EC$_{50}$ or the higher the AE, the higher is the antioxidant activity (Atoui et al., 2005; Brand-Williams et al., 1995).

**Nitro-Blue Tetrazolium Reduction Method (Superoxide Anion Scavenging Activity)**

The nitro-blue tetrazolium (NBT) reduction method is performed to assess the superoxide anion scavenging activity. In this method, O$_2^•^-$ reduces the yellow dye (NBT$^{2+}$) to produce the blue formazan, which is measured spectrophotometrically at 560 nm. Antioxidants are able to
inhibit the purple NBT formation (Chu et al., 2000; Cos et al., 1998; Parejo et al., 2003). The results are presented in terms of percentage inhibition of the NBT reduction with respect to the reaction mixture without sample (buffer only) and are calculated by the following equation:

\[
\%\text{inhibition} = \left( \frac{C_{\text{ABS}} - CB_{\text{ABS}}}{C_{\text{ABS}} - CB_{\text{ABS}}} - \frac{S_{\text{ABS}} - SB_{\text{ABS}}}{S_{\text{ABS}} - SB_{\text{ABS}}} \right) \times 100
\]  

(1)

Where:
- \(C_{\text{ABS}}\) = absorbance of the control
- \(CB_{\text{ABS}}\) = absorbance of the blank control
- \(S_{\text{ABS}}\) = absorbance of the sample
- \(SB_{\text{ABS}}\) = absorbance of the blank sample

\(\beta\)-Carotene Bleaching Method (Antioxidant Activity)

In this method, antioxidant activity is measured by the ability of a compound to minimize the coupled oxidation of linoleic acid and \(\beta\)-carotene in an emulsified aqueous system, which loses its orange colour when reacting with oxidising radicals (Parejo et al., 2003). In a study carried out by Parejo et al. (2003), 2,2'-azobis (2-methylpropionamide) dihydrochloride (AAPH\(\_2\)) was used as a water-soluble radical azo-initiator, which decomposes itself at a temperature-controlled rate, 32°C, yielding molecular nitrogen and two carbon radicals (R\(\cdot\)). Then the R\(\cdot\) reacts rapidly with molecular oxygen to produce ROO\(\cdot\). The reaction is carried out in situ in the cuvette: 1990μL of the aqueous emulsion (\(\beta\)-carotene/linoleic acid and saturated with oxygen) are equilibrated at 32°C for 6 minutes. The oxidising reaction is started by adding 10μL of AAPH\(\_2\) (0.9M). Ten minutes after vortexing (mixing) the mixture, 100μL of plant extract dissolved in DMSO at a concentration of 250μg/mL (DMSO for the control) is added, and the mixture is vortexed again. The absorbance is measured at 470nm in a spectrophotometer until a plateau in the absorbance is reached (90 minutes). BHA (250μg/mL) is used as a reference synthetic antioxidant, and a Tween 20 solution is used as a blank. The antioxidant activity (AOA) is given by the following equation:

\[
\%\text{AOA} = \left( 1 - \frac{S_{\text{ABSat0min}} - S_{\text{ABSat90min}}}{C_{\text{ABSat0min}} - C_{\text{ABSat90min}}} \right) \times 100
\]  

(2)

where: \(S_{\text{ABS}}\) = absorbance of the sample
The Oxygen Radical Absorbance Capacity (ORAC) assay was first developed by Glazer’s laboratory (Delange and Glazer, 1989) and later improved by Cao et al. (1993). The improved method is unique in that the reaction is driven to completion, and therefore the area under the reaction kinetic curve is proportional to the total ORAC of the sample (Cao et al., 1993). This assay was initially designed to measure antioxidant capacities in human serum (Cao et al., 1993) but has also been used by different laboratories to measure complex matrices, such as tea (Cao et al., 1996), fruits (Wang et al., 1996) and vegetables (Cao et al., 1996). It differs from the assays described so far since it is a fluorescence assay, so a fluorescence spectrophotometer is required to take fluorescence measurements at appropriate time intervals.

For this assay, 2,2’-azobis(2-amidinopropane) dihydrochloride (AAPH) is used as the peroxyl radical generator to start the reaction. Once the AAPH is added, the reaction mixture is incubated at 37°C. The antioxidants in the reaction mixture will act to slow the decay of the fluorescence of the fluorescent material that also exists in the reaction. Fluorescence is measured every five minutes at the emission wavelength of 565nm, using an excitation wavelength of 540nm, until no more fluorescence is detected.

There is some variation in the literature regarding what fluorescent probe should be used. The original assay, as developed by Glazer’s laboratory, utilised B-Phycoerythrin (B-PE) as the fluorescent probe. This probe has been used throughout the literature but has come under considerable scrutiny, especially from Ou and co-workers (Ou et al., 2001). Ou and co-workers demonstrated that fluorescin (FL) is a superior fluorescent probe when compared with B-PE when conducting the ORAC assay. This is further supported by a report published by the U.S. Department of Agriculture (2007) in which the ORAC values of 277 foods are listed, and the standard method adopted is that of Prior et al. (Prior et al., 2003) in which FL is used, rather than B-PE, as the fluorescent probe.
There are several reasons why B-PE is a less than ideal compound for use as a fluorescent probe. Firstly, batches of commercially available B-PE have been found to possess different fluorescence intensities and reactivities to peroxyl radicals. Ou et al. (2001) has attributed this to the isolation process that was used to extract the phycoerythrin from *Porphyridium cruentum* (a unicellular species of red algae). They also found that B-PE interacted with polyphenols, particularly proanthocyanidins (Prior et al., 2005), through nonspecific protein binding, and this resulted in falsely low ORAC readings. Another disadvantage of B-PE is instability, which results in the fluorescence of B-PE dropping substantially over a short period of time in the absence of AAPH. Finally, due to the fact that B-PE is a protein isolated from *P. cruentum*, B-PE is very costly. As an alternative, FL is inexpensive, does not interact with other compounds and is stable and hence not prone to photobleaching (Ou et al., 2001). Industry has accepted the method to the point that some nutraceutical manufacturers are beginning to include ORAC values on labels (Bank and Schauss, 2004; Wright, 2004). This acceptance of ORAC as an accurate and reproducible assay has been further confirmed through its selection as the reference method in a publication from the U.S. Department of Agriculture (2007).

*Multimethod Approach*

Some authors, such as Frankel and Meyer (2000), have pointed out the deficiency of using a one-dimensional method to evaluate multifunctional antioxidants. This is also reflected in the disparity of literature in regards to the ranking of particular antioxidants. For example, Cao and Prior (1998) have shown a weak correlation between results from the TEAC, FRAP and ORAC assays. Finally, given the fact that antioxidants are multifunctional, the assessment must also reflect this. There should be sufficient testing to allow an overall, and more accurate, portrayal of the antioxidant activity of a sample, compared with a single testing method, so a multimethod approach has been suggested by a variety of authors, including Parejo *et al.* (2002), Koleva *et al.* (2002) and Seeram *et al.*, (2008).
Biological Methods of Determining Antioxidant Capacity

Rancimat Method

Pure lard can be used as a substrate to evaluate the antioxidant activities of fruit extracts, and a study by Baldi et al. (1995) has already used this method to measure the antioxidant activities of lemon peel extracts. The study done by Baldi et al. (1995) involved pure lard, without any additives, being mixed with the extracts and placed in a Rancimat at a constant temperature (100°C) and with a steady air supply (20mL/min). The results of these experiments are in the form of an induction time. Longer induction times suggest stronger antioxidant activities.

Spontaneous Lipoperoxidation of Rat Brain Homogenates

The method used by Duarte-Almeida (2006), as set out by Ohkawa et al. (1979), involves the use of the brain of a rat to measure antioxidant activity. Male Wistar rats are anesthetised with ethyl ether, decapitated and the brain carefully removed from the skull, washed with ice-cold 0.9% NaCl solution, weighed and homogenised at 4°C in 50mM phosphate buffer (pH 7.4), in a ratio of 1g of wet tissue to 9mL of buffer.

The measurement of thiobarbituric reactive substances (TBARS) is performed through incubation of the brain homogenates with the same volume (different concentration) of extract. The absorbance of the resultant mixture is read at 532nm. The antioxidant activity is expressed as the percentage of inhibition of TBARS formation compared with the control undergoing maximum lipid peroxidation at the assay conditions.

Cellular Antioxidant Activity Assay

This is a relatively new assay that is attempting to bridge the gap between the relatively widely used chemical assays (as described in Section 1.4.1) and human clinical trials which are sometimes considered to be expensive, time consuming and not suitable for initial
antioxidant screening of foods and dietary supplements (Liu and Finley, 2005). The chemical assays have come into question about their ability to predict in vivo activity. Some chemical assays are performed at nonphysiological pH and temperature, and none of them take into account the bioavailability, uptake and metabolism of the antioxidant compounds (Liu and Finley, 2005; Wolfe and Liu, 2007). The protocols often do not include the appropriate biological substrates to be protected, relevant types of oxidants encountered, or the partitioning of compounds between the water and lipid phases and the influence of interfacial behaviour (Frankel and Meyer, 2000; Wolfe and Liu, 2007). Biological systems are much more complex than the simple chemical mixtures employed by most assays, and antioxidant compounds may operate through multiple mechanisms (Liu and Finley, 2005). Cell culture models provide an approach that is cost-effective, relatively fast, and address some issues of uptake, distribution and metabolism.

The cellular antioxidant activity (CAA) assay as described by Wolfe, Liu and their laboratory involves the use of a 2',7'-dichlorofluorescin (DCHF) probe in cultured human HepG2 liver cancer cells, which fluoresces when oxidized by peroxyl radical to 2',7'-dichlorofluorescein (Wolfe et al., 2008). Briefly the method is to seed the HepG2 cells at a density of 6x10^4/well on a 96-well microplate in 100μL of growth medium/well. Twenty-four hours after seeding, the growth medium is removed and the wells are washed with PBS. Triplicate wells are treated for 1h with 100μL of pure phytochemical compounds or fruit extracts plus 25μM 2',7'-Dichlorofluorescin diacetate (DCFH-DA). When a PBS wash is utilised, wells are washed with 100μL of PBS. Then 600μM 2,2'-azobis (2-amidinopropane) dihydrochloride (ABAP) is applied to the cells in 100μL of Hanks’ Balanced Salt Solution (HBSS), and the 96-well microplate is placed into a fluorescence microplate reader at 37ºC. Emission at 538nm is measured with an excitation at 485nm every 5min for 1h. Each plate should include triplicate control and blank wells; control wells containing cells treated with DCFH-DA and oxidant; blank wells containing cells treated with dye and HBSS without oxidant.

Even at the same research laboratory, the method has varied slightly from paper to paper. Wolfe et al. (2008) used the method as described above, but Wolfe and Liu (2008) slightly modified the method. This may indicate that the method is still at the developmental stage and
the researchers are still refining the method. It would be beneficial to monitor the situation, and later determine which method turns out to be the most repeatable and reproducible.

**Chromatographic Method of Measuring Antioxidant Composition**

*High-Performance Liquid Chromatography (HPLC)*

HPLC is a fast and reliable method to determine the compounds present in a sample. It has been used in many studies to determine the composition of many fruit and vegetable extracts for assessing their potential use as a source of natural antioxidants (Agócs et al., 2007; Amakura et al., 2000; Baldi et al., 1995; Dastmalchi et al., 2008; Duarte-Almeida et al., 2006; Sivakumar et al., 2005). It is used to separate and analyse compounds through the mass transfer of analytes between stationary and mobile phases (Ho and Stuart, 2003; Meyer, 1999; Poole and Poole, 1991; Skoog et al., 2000). HPLC involves a liquid mobile phase to separate the components of a mixture. The components are first dissolved in a solvent and then forced to flow (via the mobile phase) through a column (stationary phase) under high pressure (Ho and Stuart, 2003). The analyte motion through the column is hindered by specific physical or chemical interactions with the stationary phase. The time taken for a specific compound to exit the column is the residence time of that compound. The retention time under a particular set of conditions is considered to be unique to each compound.

Figure 4 shows a typical HPLC set-up. The main thing that changes in the arrangement is the detector used. The most common detectors include: ultra-violet; fluorescence; chemiluminescence; electrochemical; and mass spectroscopy.
Schwarz et al. (2001) have stated that the concentration of individual antioxidants in plant extracts determined by HPLC is the preferred way to provide standardised information. They have also stated that the antioxidant pattern is usually rather complex, thus making the prediction of a mixture’s potency, based on compositional data, difficult. Therefore, assays to test the antioxidant activity must be run in parallel.

Other researchers that utilise HPLC to determine the concentration of individual antioxidants include Samman et al. (2001), Duarte-Almeida et al. (2006) and Meterc et al. (2008). Samman et al. (2001) and Duarte-Almeida (2006) used HPLC to determine the phenolic content of green tea and rosemary extracts. Meterc et al. (2008) used HPLC to determine the concentration of catechins and caffeine in their green tea extract.

Considerations and limitations of this method include:

1) The need to identify the specific antioxidant chemicals first and provide pure reference standards.
2) The situation may be that individual chemical concentrations may not reflect the overall antioxidant behaviour.

**Titration Methods of Measuring Antioxidant Ability**

Titrative methods can utilise an oxidising reagent to oxidise an antioxidant and then promote a colour change in the solution being tested. For example, ascorbic acid can be oxidised by 2,6-dichlorophenol-indophenol (DCPIP), as prescribed by the Association of Official Analytical Chemists (AOAC) in their ‘Official Methods of Analysis’ (AOAC, 1990). The oxidation of ascorbic acid by DCPIP is shown in Figure 5.

![Figure 5: Oxidation of ascorbic acid by DCPIP (Hughes, 1983).](image)

Other possible reagents include iodine solution with a starch indicator, and a method involving more stable (over time) iodate and iodine solutions has also been proposed (University of Canterbury, 2007). The majority of studies, which utilise titrative methods to analyse Vitamin C content (Goula and Adamopoulos, 2006; Kebede *et al.*, 1996; Nindo *et al.*, 2003), however, prefer to use DCPIP as the titration reagent.

There are a number of potential problems when using titrative methods to determine the Vitamin C content of a sample. The above methods are not selective (i.e. DCPIP does not
solely oxidise AA if there are other oxidisable materials present), and interference can occur for a number of physical and chemical reasons (Hughes, 1983). Also, the titration method is based on the oxidation of ascorbic acid to dehydroascorbic acid. However, vitamin C is defined as the combination of both ascorbic and dehydroascorbic acids. Therefore, while titrative methods may be used to measure ascorbic acid levels, they may not be suitable to measure total vitamin C levels unless dehydroascorbic acid is reduced to ascorbic acid prior to titration (Park, 2001).

**Potential Degradation Behaviour**

Ascorbic acid has been studied as an index of the nutritional quality of foods because of its labile nature compared with other nutrients in foods (Rojas and Gerschenson, 2001). Goula and Adamopolous (2006) state that in general, rapid drying retains a greater amount of ascorbic acid than slow drying, meaning that the vitamin C content of vegetable tissue is greatly reduced during slow sun-drying while dehydration by spray drying and freeze drying reduces these losses. This statement is supported by the work of Xu and Langrish (2009), in which they measured the vitamin C retention of oven-dried samples. They found that the lowest degradation occurred when the highest drying temperature was used, since the moisture was rapidly removed and hence the degradation rates were considerably lowered. It would be considered that higher temperatures would lead to greater amounts of thermal degradation if the moisture content stayed constant and was not related to the degradation rate. However, the moisture content is related to the degradation rate, where the degradation rate decreases as the moisture content decreases. The moisture content also decreases during drying, by definition. Hence, both the temperature and the moisture content must be considered at the same time. Here high temperatures do not necessarily lead to high amounts of degradation, because high temperatures may mean a rapid reduction in moisture content that actually reduces the amount of degradation due to the low moisture content more than the amount of degradation is increased due to the high temperatures.

A paper that explores the dual effects of moisture content and temperature on the thermal degradation kinetics is that of Goula and Adamopoulos (2006) where they have simulated the
degradation of vitamin C during drying. Firstly, they have stated that the degradation kinetics are first-order and have defined the degradation equation as:

\[
\frac{C}{C_0} = \exp\left(-\frac{t}{\tau}\right) \text{ Equation 1}
\]

With:

\[
k = 278.6624 \cdot \exp(0.0660 \cdot X) \cdot \exp\left(-\frac{5548}{\tau}\right) (\text{min}^{-1}), \quad \text{For } X \leq 65 (R^2 = 0.993)
\]

\[
k = 7440.089 \cdot \exp(-0.0115 \cdot X) \cdot \exp\left(-\frac{4718}{\tau}\right) (\text{min}^{-1}), \quad \text{For } X \geq 65 (R^2 = 0.993)
\]

The term \(\exp(0.0660 \cdot X)\) means that decreasing \(X\) decreases the reaction rate (through the effect on \(k\)), while the term \(\exp\left(-\frac{5548}{\tau}\right)\) means that increasing \(T\) increases the reaction rate (again through the effect on \(k\)).

**Drying of Extract**

There are a number of factors that should be considered when deciding what type of dryer should be used. Firstly, it is essential that the dryer is able to produce the desired product at an appropriate rate. Then factors, such as thermal sensitivity of the materials and fragility of the solids formed, must be considered, as well as the reliability, safety and economic viability of the operation.

**Tray Drying**

Tray drying, in its various forms, may be considered to be the oldest form of drying. This form of drying could be as simple as leaving a solution out in the sun to dry. More elaborate forms of tray drying may involve spreading the solution out to form a thin layer and placing it in an oven at an elevated temperature to assist the drying process. A typical batch tray dryer may consist of support racks that carry a number of shallow trays, perhaps 200cm² and 5 to 15cm deep, which are loaded with material to be dried (Mccabe et al., 1993). Heated air can be circulated by a fan, and moist air can be continuously vented through an exhaust duct. When the drying process is completed, the racks can be manually taken out of the dryer. This
process can be expensive to operate due to the labor required to load and unload the dryer. This drying process may be useful when there is a low production rate (McCabe et al., 1993).

**Freeze Drying**

Freeze drying is a batch process that is usually implemented for thermally-sensitive materials. Traditionally, it has most commonly been used in the food and pharmaceutical industries (Labconco Corporation, 2004). Freeze drying involves the removal of water or another solvent from a frozen product by a process called sublimation. Sublimation involves a material passing from a solid (frozen state) directly to a gaseous state, bypassing the liquid phase. Freeze drying can be considered a three-step process, consisting of: prefreezing; primary drying; and secondary drying.

The sample to be dried must be suitably frozen to allow sublimation to occur, hence prefreezing is an important step in the process. The method of prefreezing and the final temperature of the final product, can affect the ability to successfully freeze dry the material (Labconco Corporation, 2004). The cooling rate may determine the way in which the ice crystals form. Faster freezing rates give rise to small ice crystals that result in faster sublimation during the primary drying phase, but slower secondary drying (Craig et al., 1999; Roy and Gupta, 2004). Removal of water by freezing increases the solute concentration and hence the viscosity. At some point, a saturation value is reached and no further increase in concentration/viscosity is possible. At this stage, glass transition [involving the parameter T_g’ (glass-transition temperature associated with the maximum freeze concentration)] takes place (Roy and Gupta, 2004). The significance of glass transition will be explained in the following paragraphs. Another factor that is important and must be addressed during prefreezing is that of the area of the frozen sample over which the water vapour from the sublimation process can leave. Clearly, the water vapour must pass through the dry layer at the top of the sample. A thin layer of sample is optimal when primary drying is occurring. One method of increasing the surface area is to “shell freeze” the sample. Shell freezing is achieved through rotating the vessel in a low temperature bath, causing the liquid product to freeze in a thin layer on the inside surface of the vessel (Labconco Corporation, 2004).
Primary drying involves the sublimation of the ice away from the sample. The temperature is critically important. The temperature should be low enough to ensure that no melting occurs but should be high enough to allow fast drying times. It has been reported that a temperature increase of only 1˚C results in a 13% reduction in drying time (Craig et al., 1999; Roy and Gupta, 2004), but it also been shown by Hatley (1992) that carrying out primary drying above the glass-transition temperature produces freeze-dried samples of inferior quality. Therefore, the best temperature to carry out primary drying is at the glass-transition temperature, in order to achieve the fastest drying rate that is consistent with good product quality.

Secondary drying occurs when all the frozen water has been removed through sublimation. This stage of freeze drying is the removal of “unfrozen” water from the freeze concentrate (Craig et al., 1999). Secondary drying is usually assisted by increasing the shelf temperature, usually to 25-60˚C, and therefore it is very important that all ice has been removed through sublimation in order to prevent melt-back. The rate of drying is relatively fast down to approximately 2% moisture content, after which it becomes significantly slower as the diffusion of water molecules through the sample becomes more difficult. This slowing of the drying rate is mainly due to the reduced porosity of the sample and not the sample thickness (Craig et al., 1999; Roy and Gupta, 2004).
A review article by Abascal et al. (2005) challenges the assumptions that freeze drying preserves the medicinal qualities of plants well and is superior to other preservation methods. They suggest that freeze drying may not be the optimum method for preserving volatile-rich plants for clinical research or patient use. Many of the results from a variety of studies that involve different plant materials were contradictory. Freeze drying appeared to reduce carotenoid preservation but may nonetheless be preferable to air drying and oven drying where carotenoid extraction is not an issue. The retention of polyphenols through various drying techniques also appears to be varied, but it appears that freeze drying is a good method for preserving large molecular-weight condensed tannins. The authors conclude that there is an unwarranted and unexamined assumption in botanical research that freeze drying preserves the constituents of plants, and there is evidence that these assumptions may be erroneous. In freeze drying, materials are exposed to low absolute temperatures for long times. This processing approach does not necessarily give less degradation than at higher absolute temperatures for shorter times, as with spray drying.

**Spray Drying**

Spray dryers are used in a variety of industries, where a product is to be directly transformed from a feed solution or suspension into a solid particulate state in a single-step continuous process. Single-step processes are attractive in the food and pharmaceutical industries, where it is desired to minimize the overall degree of handling. Spray-drying methods are also desirable due to short contact times and relatively low particle temperatures compared with gas temperatures (Rodriguez-Hernandez et al., 2005), which enables some food properties, such as flavour, colour and nutrients to be retained at high percentages (Kuts and Samsonyuk, 1989).

The spray drying process consists of four main stages (Masters, 1976):

1) Atomisation of feed liquid into a spray;
2) Spray-gas contacting (mixing and flowing);
3) Drying of spray (moisture evaporation); and
4) Separation of dried product from the gas.
The atomisation of the feed liquid allows very fine droplets to be formed and hence allows a greater contact area between the feed liquid and the gas. This increased surface area gives rapid drying. The design of the nozzle and/or air flow rate of atomising gas determines the size of the droplets and the direction in which the droplets leave the nozzle. Therefore, the nozzle can have a significant impact on the shape, size and moisture content of the solid particles. Spray drying is a process that produces dry solids, which often exist in an amorphous state (Vega and Roos, 2006).

There are a number of methods by which the gas can be introduced into the chamber, and these methods affect aspects such as the level of mixing between the gas and the fluid. Methods of improving mixing can include increasing the swirl of the inlet gas. There are several factors that must be considered when selecting the configuration to be used in a spray dryer. Such factors include the product sensitivity to high temperatures. Countercurrent dryers give the highest thermal efficiencies, although they result in the highest product temperatures. This limits their use to products that are not affected by such high temperatures. Co-current dryers suffer from relatively low efficiencies, though they have the advantage of low product temperatures unless back-mixing occurs (Coulson et al., 1991), which makes them more acceptable for thermally-sensitive products, such as bioactive compounds.
The drying of the spray of droplets, through the evaporation of moisture, occurs as soon as the gas and liquid come into contact. The temperature-time history is affected by the inlet gas and particle temperatures, the inlet gas humidity, the inlet solids composition, the gas and liquid flow rates and the drying kinetics of the particles.

The separation of the gas and solid particulate product is usually achieved through the use of a cyclone. The sizes of the particles are important when considering the efficiency of the cyclone. If the particles are relatively small, the low centrifugal forces will result in poor separation. For this reason, bag filters are typically used after the cyclones in spray dryers.

Another important factor, which is of increasing interest, is the actual drying gas used. There are certain situations in which air may not be an appropriate drying agent. For example, when
drying a flammable or oxygen sensitive material, the use of air may cause undue risk from fire or degradation, respectively. In such situations, it may be more appropriate to use an inert gas, such as carbon dioxide or nitrogen.

*Designs of spray dryers*

There are many different types of industrial spray dryers available. There are also both different types of atomizers and different types of drying chamber. There are ten main nozzle concepts and four main chamber types. The choices of the atomizer type and drying chamber are usually based on the viscosity and the product sensitivity to the feed temperature, respectively.

<table>
<thead>
<tr>
<th>Atomizer</th>
<th>Pressure</th>
<th>Rotary</th>
<th>Two-fluid</th>
<th>Spinning-cup plus gas blast</th>
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<tr>
<td><strong>Drying Chamber</strong></td>
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<td>Co-current</td>
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<td>Pastes</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

*Atomizers*

Atomization is usually driven by liquid pressure, centrifugal movement or gas-liquid shear. Regardless of what sort of energy is used to drive the atomizer, the aim is essentially to break down the unstable threads of liquid into rows of drops (Coulson *et al.*, 1991).
In a pressure atomizer, otherwise known as a nozzle, liquid is forced under pressure through an orifice, and the form of the resulting liquid flow sheet can be controlled by varying the direction of flow through the orifice. Pressure nozzles are somewhat inflexible due to the fact that large ranges of flow rates require very large variations in differential pressure (Coulson et al., 1991).

Rotary atomizers are commonly used in industrial-scale dryers due to being very versatile. A rotary atomizer has the ability to handle a wide range of feed rates and liquids with a wide range of properties. The rotating element can take many forms, but bowls, vaned discs and slotted wheels are commonly used (Coulson et al., 1991).

A two-fluid nozzle has been used in this project. The liquid and gas enter in the same direction in an annular configuration, with the liquid forming the central jet and the gas forming the annulus of the inlet flow. A two-fluid nozzle is the most versatile, as can be seen from Table 11, with both low and high viscosity solutions, as well as slurries and pastes, being able to be handled by this type of atomizer.

*The effect of spray drying conditions*

The traditional input variables of a spray dryer are the drying air temperature, input liquid flow rate, the volumetric flow rate of drying air and the nozzle shear rate of the liquid stream. These four variables have an impact on the size, residence time and drying temperature of droplets in the dryer. Logically, these variables all have an effect on the drying efficiency of the dryer. The smaller the droplets, the greater the surface area to volume ratio becomes which provides a larger surface area for the moisture to be evaporated. The slower the droplets come into the dryer, the longer they will be exposed to the drying gas and hence will increase the time for the evaporation to take place. The higher the temperature of the drying gas, the greater the evaporative coefficient in the dryer and hence the faster the droplets can be dried.
Particles from Gas Saturated Solutions

A new and novel method to dry natural extracts has been tried by Meterc et al. (2008) in Germany. Their technique is based on a high-pressure spray technique called “particles from gas saturated solutions (PGSS)” and has been described as a “high-pressure process for the gentle drying of natural extracts”. It is especially relevant to this current study, because their work also involves an investigation of the degradation of polyphenolic compounds.

A schematic diagram of the PGSS drying plant is shown in Figure 8. As can be seen in the diagram, high-pressure carbon dioxide is passed through a heat exchanger and heated before entering the static mixer, where it contacts the solution to be dried. This solution to be dried is also pre-heated. Figure 9 shows that the static mixer contacts the solution and the supercritical carbon dioxide intensively at high pressures and elevated temperatures. During the mixing, supercritical carbon dioxide is partly dissolved in the solution, and the mixture is sprayed by a single path nozzle into a spray tower. Driven by the expansion of the gas, fine droplets are formed, and the heated gas evaporates the solvent, which leaves together with CO$_2$. The powder obtained is collected at the bottom of the spray tower (Meterc et al., 2008).

![Figure 8: Schematic flow diagram of PGSS dryer (Meterc et al., 2008).](image-url)
The PGSS drying process has not been thoroughly studied, but the preliminary study by Meterc et al. (2008) has shown promise when drying green tea extracts. The promise lies in the fact that the drying process is carried out at low temperature (30-60ºC) and in an inert, oxygen-free atmosphere. The variations in the spray temperature, ranging from 33 to 65ºC, have no significant effect on the amount of total polyphenols, but higher temperature results in lower water residue in the sample. A more thorough investigation should be carried out to determine the differences in retention of catechins and polyphenols for this drying method compared with other drying methods, such as spray drying, freeze drying and vacuum drying.

**POTENTIAL MAILLARD PRODUCT FORMATION THROUGH HEATING**

It is well known that a browning reaction can occur during food processing due to heating. This browning may be due to heating effect causing a reaction between reducing sugars and amino acids in the food (Wang et al., 2011). Manzocco’s review (Manzocco et al., 2001) of non-enzymatic browning and antioxidant capacity in processed foods has highlighted a number of different research papers that have shown that oxidised polyphenols can exhibit a higher antioxidant activity than non-oxidised polyphenols. The food systems studied include pear and white grape juices (Spanos and Wrolstad, 1992), tea extracts (Manzocco et al., 1998), and commercial cognacs (Da Porto et al., 2000). Melanoidins are thought to be
important in preventing oxidative damage and diseases through their ability to scavenge a variety of Reactive Oxygen Species (ROS) (Wang et al., 2011).

**PRESERVATION OF BETα-CAROTENE (AN ANTIOXIDANT) BY DIFFERENT DRYING METHODS**

Desobry et al. (1997) explored spray, drum and freeze drying in relation to encapsulation and preservation of β-carotene. Their focus was on two features of the powders, including the degradation during the drying process and the physical properties of the resulting powder, which made the antioxidant either less or more susceptible to oxidative degradation. During freeze drying, drying occurs through sublimation of the frozen water, and hence there should be no liquid water-phase reactions and little oxidation due to the vacuum. During drum drying, the water boils off at 100°C, and the powder temperature then begins to rise above that temperature. The high temperature may cause the maltodextrin matrix to collapse, accelerating degradation, but the short residence time of 45 seconds mitigates this degradation.

Spray drying, while offering the largest surface area from which evaporation can occur, also has the greatest surface area across which oxidation can occur. The short contact time of 2-3 seconds mitigates this oxidation and degradation effect. The size and shape of the obtained powders had a large effect on the preservation of the β-carotene in Desobry et al. (1997). The spray-dried powder was small and spherical in shape, which provides a large surface area for oxidation to occur. The drum-dried and freeze-dried materials were ground after drying. This grinding stage resulted in larger particles compared with spray drying, and the larger particles offered more protection to the β-carotene. Even though the freeze-dried powder had a similar size to drum-dried products, it was more susceptible to oxidative degradation since there were large pores extending into the interior of the particles, which would allow easier diffusion of oxygen into the freeze-dried material. Therefore, the outcome of the study conducted by Desobry et al. (1997) was that drum drying was the most favourable way to dry encapsulated β-carotene. This suggests that freeze drying is not the best way to protect the antioxidant nature of the liquid extract. However, these data suggest that another study should be
undertaken in which the drum-dried and freeze-dried particles should be ground to the same size as the spray-dried particles to give a more accurate assessment of the effect of the drying method on the level of preservation for oxygen-sensitive products.

CARRIERS

Carriers are typically selected for their encapsulation properties, whether by creating a membrane wall around the product core (Shu et al., 2006), reduction of stickiness (Bhandari et al., 1993) or via material absorption (Chiou and Langrish, 2007). The spray dryer can be used to encapsulate a material when the “active” material is entrapped within a protective matrix (Ré, 1998). This encapsulation procedure may be used for many reasons:

- converting liquids and sticky materials to free-flowing powders, for easier handling (dry handling) and greater yields;
- protecting a product from the surrounding environment;
- protecting the environment from a hazardous or toxic product;
- decreasing the evaporation or transfer rate of volatile core material to the outside environment;
- masking the undesired properties of the active component; and
- controlling the release rate of the core material under desired conditions (Ré, 1998).

Ré (1998) mentions several features of microencapsulation that are generally relevant to the food industry, including the following aspects that are particularly relevant to the current study: protecting sensitive food components from other food ingredients during storage; protecting against nutritional loss and even adding nutritive materials to foods after processing; and providing an attractive appearance for displaying and merchandising food products (having more flavour and being more nutritious to better meet the needs of today’s consumers). For example, there is currently bread (Tip Top UP Hyfibe + Calcium) on the Australian market that is sold and marketed as being high in calcium and fibre.

Traditionally, encapsulants have included: carbohydrates, such as maltodextrin, starches and corn syrup solids; gums, such as acacia gum, known commonly as gum arabic; and proteins, such as milk protein and gelatin. These encapsulants have been chosen due to specific wall
properties (properties of the particle wall) that meet specific criteria (mechanical strength, compatibility with the food product, appropriate thermal or dissolution release, appropriate particle size) (Brazel, 1999; Gharsallaoui et al., 2007).

In this study, the focus has been furthering the work started by Chiou and Langrish (2007), in which fruit fibres were used to encapsulate the bioactive material and hence form a novel nutraceutical. The appeal of using fruit fibres is that they are natural materials, they are important in maintaining a healthy digestive system, and they exist as a waste product from a variety of industries, including the citrus processing sector. While it will be beneficial for consumption, it will also lessen the need for waste treatment from industry and will lead to the creation of a value-added waste product.

**VISUALISING THE PRODUCT**

**Microscopy**

*Scanning Electron Microscopy*

There is potential application for Scanning Electron Microscopy (SEM) to be applied to better understand the encapsulated bioactive compounds. SEM offers both a way to look at the topographical nature of the compound, specifically its shape and external wall properties, and the differing densities in the product can also be visualised. The Backscattered Electron Images (BEI) produced by the technique allow areas of differing densities in the sample to be studied. This may be beneficial when analysing the fibre for its internal channels. It may also lead to a clearer image of how the fibre and bioactive extract interact. Secondary Electron Images (SEI) give a clearer image of the outside of the particle and hence may show any deposition of bioactive extract on the outside of the fibre. SEM also offers the possibility of cutting the individual particles with laser beams, which may offer value in that the exact cross-sectional composition of the novel nutraceutical may be assessed.
Confocal Laser Scanning Microscopy

Confocal laser scanning microscopy (CLSM) is a fluorescence analysis technique that involves the scanning of one or more focused beams of light across the sample. These optical sections are combined to create an image of the sample. CLSM can also combine these optical sections into a three-dimensional reconstruction of the image. Therefore, CLSM gives a non-destructive visualisation of the inner structures of objects, provided the material is sufficiently translucent.

It has been found that CLSM allows the unambiguous identification of encapsulated products (Lamprecht et al., 2000). Through Lamprecht’s work, it was shown that staining an oil phase with a fluorescent marker allowed the quick and relatively easy identification of encapsulated oil rather than other droplet-like structures. Chiou and Langrish (2007) also identified CLSM as an appropriate method when identifying bioactive components of a bioactive fibre mixture, where bioactive compounds showed specific fluorescence behaviour.

AIM AND SCOPE

Extracts to be Studied

This work has primarily analysed the commercially available Rosella (*Hibiscus sabdariffa* L.) extract. This has been supplemented with other bioactive extracts, such as sugar cane juice and Kakadu plum extract. The integration of other bioactive liquids into this work has allowed the universality of the results to be assessed. The needs of commercial products mean that this work must be applicable to the sometimes variable nature of consumer preferences.

Extraction of Powder

The resultant powder that is obtained from the drying process has been extracted with water. This has been chosen due to the potential applications of the powder. As a food product, it
must be analysed in a manner similar to its end use. This has given an indication of the in vivo benefit that the powder may have. Also, due to the aqueous extraction, the extract contains only water soluble components. The data from the extraction and then analysed for antioxidant capacity has been normalised through calculating the total solids in the extracted solution and has been compared on that basis.

**Measurement of Antioxidant Potential**

As discussed, the antioxidant potential is a fairly sophisticated concept due to the multifaceted nature of antioxidants and antioxidant-rich foods. This is a concept that the food industry and, in particular, the nutraceutical industry has been forced to address. A multimethod analysis of the powders has been found to be appropriate to get a comprehensive view of the product. This study will therefore utilise several chemical assays. The chemical assays chosen have been Folin and Ciocalteu’s assay to determine total phenolics (FCR) and the Oxygen Reducing Antioxidant Capacity (ORAC). FCR has been chosen due to its robust nature and its proven reproducibility. It has been used as a standard phenolics test in the viticulture industry for many years. The ORAC assay has been adopted by many manufacturers as the assay of choice, with some going as far as publishing ORAC values on the nutritional information panel of their products. This interest in ORAC has also been helped by the U.S. Department of Agriculture adopting it as the reference method for a report published in 2007, as well as establishing an ORAC database for foods/food ingredients. The ORAC assay may have been adopted as the method of choice, since to some extent the assay replicates what is occurring in the body as it is undertaken at 37°C in a buffer solution.

**Drying Method**

The primary drying method of choice in this work has been spray drying due to the very low residence times involved and the continuous, single-step nature of the product, which is ideal for food applications to minimise handling, and hence contamination and losses. Tray and vacuum drying are considered relatively expensive (Peters *et al.*, 1920; Sinnott, 1983) due to the high labour costs as they are batch processes. Further to high labour costs, freeze drying
has high energy costs due to the low operating temperatures and long processing times. It could be said that spray drying is the most economical form of drying being studied here, but the spray drying work has been supplemented with tray drying, vacuum drying and freeze drying.

**Carriers**

Natural fruit fibres have been the primary choice of carriers. This will allow the continuation of the work proposed by Chiou and Langrish (2007) to give a more natural product with both components (fibre and extract) having a nutritional benefit. In this work, the fruit fibre used has been assessed for its antioxidant benefit so as to not overestimate the antioxidant benefit/retention of the extract. The antioxidant capacity of the input fibre and the bioactive extract have been measured and added together. The reduction in the antioxidant effect of each have been assessed through the drying process. The physical properties of the fibre carrier have also been assessed before and after drying to ensure there is no breakdown, such as the rupturing of cell walls.
Chapter 3. Materials and Methods

Bioactive extract
Throughout this work, the extract used was a commercially available Rosella extract that was provided by Vic Cherikoff Foods Pty. Ltd. The Rosella extract is an aqueous-based extract of the *Hibiscus sabdariffa* L. plant. This Rosella extract is dark purple in colour, which is characteristic of an extract obtained from a plant that is high in anthocyanins.

Fibre and extract mixture
The ingredients used to make the slurry mixture used in the spray-drying process consisted of fine milled sugar cane pith fibre (<30μm, KFSU Pty. Ltd.) and Rosella extract (*Hibiscus sabdariffa* L., Vic Cherikoff Food Services Pty. Ltd.). The slurry mixture was prepared using equal parts of fibre and extract based on the weight of the total dissolved solids, respectively. The combined extract was diluted with water until the mixture had a solids concentration of 10%. The slurry mixture was agitated, throughout the spray-drying process, with a magnetic stirrer to ensure that a homogenous solution was fed into the dryer. Sugar cane juice was obtained from a local provider located at Haymarket, Sydney. The slurry was prepared in the same way as that of the Rosella and fibre.

Oven drying
The ovens used throughout this study were both electric, digitally controlled, fan forced ovens. One was made by Labec (160L) and the other by Thermoline Scientific (150L). They were used interchangeably as they both generate very similar conditions when they have consistent settings.

Vacuum drying
The vacuum dryer was a Qualtex vacuum (~80L) dryer from Andrew Thom Ltd. It was connected to the school’s central vacuum system. The dryer had a heavy steel door that was locked in by a screw mechanism and had a stopper valve to seal the system and to allow the vacuum to be released when required.
Freeze drying
The freeze dryer used was a Labconco Vac-Stop tray dryer (~45L). The machine operated at -50°C at a pressure of 0.067atm. The samples were frozen in a convention -15°C freezer as a small layer (approximately 5mm) in a plastic specimen jar and a petri dish resulting in an exposed surface area of approximately 2cm² and 6cm² respectively.

Spray drying
The spray dryer used throughout this study was a Buchi B-290 laboratory scale spray dryer as seen in Figure 10. This is a co-current dryer with a two-fluid self-cleaning nozzle. The drying chamber of the spray dryer is a 48 cm long, 15.5 cm diameter vertically orientated cylinder, with a conical base.

Figure 10: Buchi B-290 spray dryer (Buchi, 2010)

Oxygen Reducing Antioxidant Capacity Assay
The ORAC method used here was based on the one developed by Prior and colleagues (Prior et al., 2003), since it has been chosen by the U.S. Department of Agriculture (U.S. Department of Agriculture (USDA) et al., 2007) as the reference method when assessing other analytical techniques. The method used in this study involved mixing 20μL of appropriately diluted extract with 200μL of a fluorescein solution (FL) and 37.5μL of a 2,2’-azobis (2-methylpropionamidine) dihydrochloride solution (AAPH). The fluorescein solution was made in three steps. The FL stock #1 solution was made by diluting 22.5mg of fluorescein (sodium salt) in 50mL of 0.075M phosphate buffer solution (pH 7.0) (PBS). FL stock #2 was made by diluting 50μL of stock #1 in 10mL of PBS. The working FL was made
by diluting 320μL of FL stock #2 in 20mL of PBS. The AAPH solution was made by dissolving AAPH in PBS at a concentration of 17.2mg/mL.
Chapter 4. The effect of different drying methods on the antioxidant retention of a bioactive solution

4.1 OVERVIEW
The aim of this set of experiments was to determine the effect of different drying methods (oven drying, freeze drying, vacuum drying and spray drying) on the antioxidant retention of a bioactive solution (*Hibiscus sabdariffa* L.). The identification of the most effective drying method will be the basis of further work in this project to determine the best drying conditions. The dried products were tested for their antioxidant retention using the Oxygen Reducing Antioxidant Capacity assay (ORAC). There were relatively similar levels of degradation amongst the drying methods, with more degradation occurring when the material was oven dried at higher temperatures and for longer times. The highest retention rates of antioxidants occurred with vacuum drying for 23 hours (98%), freeze drying for 40 hours (97%) and spray drying with an inert gas (98%) (in this case, nitrogen). These results suggest that a high-throughput drying method should be used for preserving the antioxidant levels in this material due to the much faster drying times with similar levels of degradation. The outcome of these findings was that spray drying was selected as the best form of drying for a bioactive solution.

4.2 INTRODUCTION
This study compares different ways of drying the extract including: freeze drying; spray drying; oven drying; and vacuum drying. This chapter compares the drying methods regarding the differences in degradation of the extract, which is based on antioxidant retention after drying. There are various factors that need to be addressed when comparing drying methods and these are considered to be important in this case: drying time/rate; drying
temperature; and exposure to oxygen as summarised in Table 12. Freeze drying involves the sublimation of the solid ice straight to a gas. It has the unique advantage that there should be few or no liquid water-based reactions occurring. It is a relatively slow process (typically 48 hr drying time), carried out at low temperatures (~50°C in this case) and in a vacuum (no exposure to oxygen). Spray drying has a very short contact time (1-2 s) between the material to be dried and the drying gas (usually heated air), carried out at moderate outlet temperatures (~60°C in this case), and there is the option of exposure to oxygen or not by the choice of drying gas. Oven drying is also a relatively slow process, with moderate to high temperatures (50-80°C), and exposure to atmospheric air and hence oxygen. Vacuum drying is also a relatively slow process, with low temperatures (38°C), and no exposure to oxygen.

<table>
<thead>
<tr>
<th>Drying Method</th>
<th>Drying Time</th>
<th>Drying Temperature (°C)</th>
<th>Exposure to Oxygen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze drying</td>
<td>48 hr</td>
<td>-50</td>
<td>very low</td>
</tr>
<tr>
<td>Spray drying</td>
<td>1-2 s</td>
<td>60 (outlet temp.)</td>
<td>low or very high</td>
</tr>
<tr>
<td>Oven drying</td>
<td>0.25-23 hr</td>
<td>50-80</td>
<td>high</td>
</tr>
<tr>
<td>Vacuum drying</td>
<td>23 hr</td>
<td>38</td>
<td>very low</td>
</tr>
</tbody>
</table>

The drying time is a relatively simple concept, since the longer the material takes to be dried, the longer it may be undergoing microbial degradation. It has been stated by Borompichaichartkul and colleagues (2009) that once the moisture content is reduced to 1.2-1.6% (d.b.), there is higher stability against lipid oxidation. It is also a well-established concept that chemical reactions are slower as the water activity decreases (Georgetti et al., 2008; Goula and Adamopoulos, 2006). The drying temperature is a very important factor when discussing degradation reactions. It is commonly known that heat speeds up most reactions. Normally, it would be safe to assume that the hotter the material is that undergoes drying, the faster the degradation occurs, but the hotter the material, the faster it dries and the more quickly the moisture content is reduced. Therefore the balance between high temperatures and fast drying times is not clear, so the best drying method has not been established yet. However, this may not be the case with some degradative enzymes, such as polyphenol oxidase, may be inactivated due to higher temperatures (Lim and Murtijaya, 2007;
Obied et al., 2008; Suvarnakuta et al., 2011). The exposure to oxygen is a straightforward concept when discussing the drying of antioxidants.

4.3 MATERIALS AND METHODS

Bioactive extract
The extract used was the Rosella extract as described in the materials and methods section.

Spray drying
The Buchi B-290 spray dryer was used. The settings were as follows: aspirator rate 100% (0.0127 kg/s); inlet drying air temperature of 110°C; pump rate of 5% (1.9ml/min); atomisation air rotameter 35 (to the bottom of the gauge ball) (~455L/h); and the nozzle cleaner set to 9 (51 strikes/min). The rosella extract was diluted to a 10% solids concentration, before it was spray dried.

Spray drying with inert gas loop
A Buchi B-290 spray dryer was connected in series with a Buchi B-295 inert loop. The inert loop reduces the oxygen content of the drying gas. The inert loop reduces the atmospheric oxygen content of air (~21%) down to a relatively low level of oxygen (~0.3%). The key attribute that is being explored is whether or not the oxygen content of the drying gas has a significant effect on the retention of antioxidants in the bioactive extract.

Spray drying rosella/fibre slurry
The rosella/fibre slurry was prepared based on the total dissolved solids (TDS) of the rosella solution. The TDS of the rosella was determined by mass measurements before and after oven drying of the solution at 100°C for 24 hours, with the weighing being performed on a Mettler analytical balance (model AB204S, ±0.0001g). When preparing the slurry, the amount of solids in the rosella was matched with fibre, and the total solids in the solution was fixed at 10%wt/wt through the addition of water. The slurry was kept well mixed throughout the spray-drying process through the use of a magnetic stirrer.
Oven drying
Two similarly-sized fan-forced ovens were used during these experiments. One was made by Labec and the other by Thermoline Scientific. The experiments were conducted by spreading a thin layer (1-2mm) of the bioactive extract on aluminium foil and placing this foil on a tray in the oven. When the bioactive extract had been subjected to the pre-determined drying conditions (ie. drying temperature and length of drying) it was removed from the oven, scraped off the aluminium foil and placed in an air-tight bag in the freezer before the ORAC testing was performed.

Freeze drying
Freeze drying was performed in a Labconco Vac-Stop tray dryer operated at a temperature of -50°C and a pressure of 0.067atm. The different conditions explored in the freeze dryer were simply the surface area across which the mass transfer of sublimation could occur. The initial test involved a plastic bottle that had a cross-sectional area of 15.6cm², and the second test involved a Petri dish with a significantly larger cross-sectional area of 60.2cm². Both freeze-drying experiments dried the same amount of sample and were conducted over 40 hours, with the dried material being placed in an air-tight bag in the freezer before ORAC testing was done.

Vacuum drying
Vacuum drying was conducted in a Qualtex vacuum dryer from Andrew Thom Ltd. The bioactive solution was placed on aluminium foil, placed on baking trays and then placed in the vacuum oven. Again, after drying, the sample was removed from the aluminium foil and placed in air-tight bags in the freezer before ORAC testing.

Oxygen Reducing Antioxidant Capacity Assay
The ORAC method used here has been described in the Materials and Methods section.
4.4 RESULTS

Table 13: Drying results including oven drying (OD), freeze drying (FD), spray drying (SD) and vacuum drying (VD).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time (hr)</th>
<th>Antioxidant retention (%)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>4</td>
<td>89</td>
<td>OD</td>
</tr>
<tr>
<td>80</td>
<td>5</td>
<td>87</td>
<td>OD</td>
</tr>
<tr>
<td>80</td>
<td>6</td>
<td>89</td>
<td>OD</td>
</tr>
<tr>
<td>80</td>
<td>7</td>
<td>86</td>
<td>OD</td>
</tr>
<tr>
<td>80</td>
<td>10</td>
<td>83</td>
<td>OD</td>
</tr>
<tr>
<td>50</td>
<td>23</td>
<td>92</td>
<td>OD</td>
</tr>
<tr>
<td>120</td>
<td>1</td>
<td>47</td>
<td>OD</td>
</tr>
<tr>
<td>120</td>
<td>16</td>
<td>37</td>
<td>OD</td>
</tr>
<tr>
<td>160</td>
<td>0.25</td>
<td>51</td>
<td>OD</td>
</tr>
<tr>
<td>-50</td>
<td>48</td>
<td>97</td>
<td>FD SA-60.2cm² - petri dish</td>
</tr>
<tr>
<td>-50</td>
<td>48</td>
<td>88</td>
<td>FD SA-15.6cm² - plastic bottle</td>
</tr>
<tr>
<td>Inlet: 110, Outlet: 62</td>
<td>0.0003-0.0006</td>
<td>92</td>
<td>SD without fibre</td>
</tr>
<tr>
<td>Inlet: 110, Outlet: 62</td>
<td>0.0003-0.0006</td>
<td>78</td>
<td>SD with fibre</td>
</tr>
<tr>
<td>Inlet: 110, Outlet: 62</td>
<td>0.0003-0.0006</td>
<td>98</td>
<td>SD without fibre and with inert loop</td>
</tr>
<tr>
<td>38.2</td>
<td>23</td>
<td>98</td>
<td>VD</td>
</tr>
</tbody>
</table>
When analysing the results shown in Table 13 and Figure 11, it can be seen that, when considering oven drying, it appears that the drying temperature does have a significant effect on the amount of antioxidants that are retained by the sample, while drying time does not have so significant an impact. This may be due to the high rate of drying at the initial stages of drying, and then the relatively low rates of degradation when the moisture content of the sample is reduced. The work of Goula and Adamopolous (2006) implies strongly that the degradation rate of antioxidants decreases very rapidly at low moisture contents. Therefore, once a sample is dried, little further degradation may be expected to occur, so the impact of additional drying time, further than that required to dry the sample, may be expected to be small, explaining the modest influence of drying time on the levels of antioxidants.

The freeze-drying process had a small effect on the retention of antioxidants. However, an interesting point here is that the vessel in which the sample was dried had a significant impact, possibly due to the difference in drying area per unit mass. The thinner layer of sample offered by the Petri dish allows a greater area over which the mass-transfer process can take place. Therefore, the greater surface area, allowing a faster rate of mass transfer via sublimation, resulted in lower levels of degradation which again supports the theory that faster drying rates will give better products when considering antioxidant activity.
For spray drying, the results indicate that it is better to spray dry the bioactive extract without fibre as a carrier. This may suggest that the oxidation reaction is a heterogeneous one and that the fibre provides sites for degradation to occur. It can also be seen from Table 2 that spray drying with a minimal amount of oxygen lowers the amounts of oxidation that the sample undergoes, since without oxygen, the oxidation process cannot occur, and so with low oxygen levels, oxidation is minimised.

Table 13 shows extremely low levels of degradation in vacuum drying. Again, in an environment without oxygen, low levels of oxidation, and hence degradation, are expected. Also, the relatively low temperatures in vacuum drying (compared with oven and spray drying) also help to explain the low levels of degradation for the relatively short periods of time when the sample is exposed to oxygen (i.e., the time from when the sample is placed into the vacuum oven until vacuum conditions are reached).

Figure 12: Antioxidant retention as a function of drying temperature (vacuum, freeze and oven drying) and outlet drying temperature for spray drying.
When a comparison is conducted simply between antioxidant retention and drying temperature at these lower temperatures, as can be seen in Figure 12, it can be noted that there is very little degradation below the drying temperature of 60°C. This may be due to the inactivation of oxidative enzymes as shown in the work of Choi and colleagues (Choi et al., 2006) in their study on the effect of heat treatment on Shiitake mushrooms, in which they found that when Shiitake mushrooms were heat treated at 100-121°C for 15-30 minutes, the free flavanoid content was significantly (p<0.05) increased compared with the untreated Shiitake mushrooms. The connection between the two heat treatments is that both resulted in a measurable increase in the polyphenolic content. More work needs to be done to gain a full understanding of what is occurring but it can be seen that heat treatment of a solid mushroom, albeit at higher temperatures and for a longer time, is shown to result in a higher antioxidant content similarly to heat treating an aqueous extract, where it can be assumed the polyphenols are already more available.

The strong temperature effect that appears in Figure 12 is likely to be significant, because it would normally be expected that degradation would be a function of the moisture content (and implicitly the drying method).

4.5 DISCUSSION

Other researchers have suggested an increase in antioxidant activity after drying (Chang et al., 2006; Choi et al., 2006). This study has shown this not to be the case with this material and these drying methods. It is suggested here that any partially oxidised polyphenols did not have a higher antioxidant activity, as supported by Manzocco and colleagues (Manzocco et al., 2000), and perhaps that the formation of novel compounds with higher antioxidant activities did not occur as suggested by Choi (Choi et al., 2006). However, it is also possible that the polyphenols were all fully oxidised during the drying processes performed here.

Upon analysis of the results and considerations of the many factors which influence an industrially-viable drying process, it appears that spray drying is the most appropriate form of drying. Firstly, the drying time involved in a spray dryer is small, compared with the times that are required by the other drying methods. The quality of the powders is similar, with the
exception of a few drying conditions such as the samples that underwent high-temperature oven drying and spray drying with fibres. Therefore, the highest-throughput drying method should be selected, which is spray drying, as studied in the following chapter.
Chapter 5. The effect of spray drying conditions on yields for bioactive extracts

The experiments described herein have been published as: Premarajah, R. & Langrish, T.A.G. (2009) The Effect of Spray Drying Conditions on Yields of Bioactive Extracts, Chemeca 2009, Perth, Australia.

5.1 OVERVIEW
This chapter has established trends in the yields when varying the inlet, and hence the outlet, temperatures of the inlet drying air when spray drying bioactive solutions. The inlet and outlet temperatures of the spray drying process were found to have a significant effect on the yield of the product. The best yield found from this study was found at an inlet temperature of 110°C with a 1.9mL/min feed rate and an outlet temperature of 64 °C, giving a yield of 53% of total materials sprayed. The results suggest that the same principles apply to the spray drying of bioactive materials as to the spray drying of pure sugars.

5.2 INTRODUCTION
The aim was to exploit the benefits of encapsulation whilst ensuring the constituents of the powder are completely natural. Therefore, the extract has been spray dried using sugar cane fibre as an encapsulant (Chiou and Langrish, 2007) to minimise the hygroscopic nature and extend the shelf life of the powder. Another advantage of using fibre as an encapsulant is that it is known to maintain a beneficial flora and fauna in the gut to promote health. Again, it is important to remember the difficulty of spray drying sugar rich substances such as the work done by Fang and Bhandari (2012) where they recovered no powder when bayberry juice was spray dried alone. Fang and Bhandari (2012) found that the addition of a small amount of protein (1%) and a large amount of maltodextrin (>30%) was effective in recovering powder (>50%).
5.3 MATERIALS AND METHODS

Fibre and extract mixture

The ingredients used to make the slurry mixture used in the spray-drying process consisted of fine milled sugar cane pith fibre (<30μm, KFSU Pty. Ltd.) and Rosella extract (*Hibiscus sabdariffa* L., Vic Cherikoff Food Services Pty. Ltd.). The slurry mixture was prepared using equal parts of fibre and extract based on weight and weight of total dissolved solids, respectively. The combined extract was diluted with water until the mixture had a solids concentration of 10%. The slurry mixture was agitated, throughout the spray drying process, with a magnetic stirrer to ensure a homogenous solution was fed into the dryer. Sugar cane juice was obtained from a local provider located at Haymarket, Sydney. The slurry was prepared in the same way as that of the Rosella and fibre.

Drying conditions

The slurry mixture was fed into a Buchi B-290 mini spray dryer. The settings were as follows: aspirator rate 100% (0.0127 kg/s); drying air temperature ranging from 70 to 200ºC; pump rates of 5 and 25% (1.9-9.3 ml/min); atomisation air rotameter 35 (to the bottom of the gauge ball) (~455 L/h); and the nozzle cleaner set to 9 (51 strikes/min). These operating conditions resulted in outlet temperatures in the range of 45 to 124ºC.

5.4 RESULTS

The empirical data (as shown in Appendices) obtained was expressed in a graphical form in Figure 13 and Figure 14. A quadratic trend line was also added to represent the relationship between the plotted points. For example, the quadratic equation of the 1.9 mL/min curve of Figure 13 was calculated using MS Excel as: 

\[-0.0041x^2 + 0.6903x + 22.826\]  

with an R² value of 0.8749. It should be noted that the seemingly low yields are typical of this scale of spray dryer. One of the reasons for the much lower product yields from small dryer is wall deposition. Wall deposition is when a wet droplet exits the nozzle and directly impacts with
the wall where it dries. Other researchers, that use small scale dryers, such as Bhandari et al (1997) have previously chosen 50% recovery “as a measure of spray drying performance as it is easily measured with reproducible results.” They have dried similarly sticky materials such as sucrose with yields only as high as 19.4%. Jayasundera et al (2011a) have discussed yields as high as 76% with a small scale (Buchi B90) spray dryer when drying sugar rich foods with protein coatings. By contrast, the spray drying of milk in industrial scale spray dryers is rarely performed if the yields are less than 99.5%. There is clearly a large difference between the yields that can be reasonably expected from small scale and industrial scale equipment.

The reasons for this large difference in yields is rarely discussed in the literature, but a key finding is reported in the work of Hanus and Langrish (2007). They found that wet particles (ie. sticky particles) were hitting the walls of the small dryer directly from the atomiser, prior to drying. This wet particle deposition is thought to be much less significant in industrial scale dryers, and hence there are much greater yields in larger dryers.

Figure 13: Yield of rosella-fibre powder as a function of inlet gas temperature.
A further set of experiments was conducted to further explore the trends found with the yields of the Rosella-fibre powders. The sugar cane-fibre results, as compared with the Rosella-fibre results, are shown in Figure 15.
Figure 15: Yield of sugar cane-fibre powder compared with Rosella-fibre powder, both spray dried at 1.9mL/min pump rate.

5.5 DISCUSSION

The results have been plotted as both a function of inlet and outlet temperatures. The inlet temperature is an independent, input value while the outlet temperature is more representative of the particle temperature throughout the dryer. The inlet temperature is a user setting while the outlet temperature is a measured attribute of what is happening in the dryer taking into consideration the daily fluctuations of the drying conditions such as the relative humidity of the drying air. As seen in Figure 16, the temperature of the drying particles does not reach the inlet air temperature. As the solution enters the dryer, the energy is transferred from the hot inlet gas to the cooler inlet feed solution. As the water starts to evaporate, the particles are cooled under the evaporative cooling effect. As the solution gets dryer and dried particles form there is less of an evaporative cooling effect and the powder particles and the surrounding gas reach an equilibrium temperature before the gas and powder exit the dryer.
Figure 16: Schematic representation of temperature behaviour along a concurrent dryer.

Figure 13 shows the relationship between the inlet temperature of the spray dryer and the yield produced. There is a peak in the yield at certain inlet temperatures. There is a difference in this peak yield temperature for the spray dryer operated at the two pump flow rates. The physical difference between these two pump rates is the humidity of the air in the dryer. With a lower pump rate, the humidity is lower and the amount of evaporative cooling of the inlet air is less, which explains why the inlet temperature with the higher pump rate must be significantly higher to observe the same outlet gas temperature. To show this mathematically, a mass and energy balance must be done over the dryer as previously done by Langrish (2009).

Energy input
Both the hot air and the liquid cause energy to enter the dryer, and the energy flow rate is the product of the enthalpy of each stream and the mass flow rate of that stream. For the hot air, the enthalpy is given by:

$H_e = C_{pa}(T_e - T_{ref}) + \nu A + C_{pv}(T_a - T_{ref})$  \[ \text{Equation 2} \]
where $C_{pa}$ = specific heat capacity of dry air $\approx 1$ kJ kg$^{-1}$ K$^{-1}$, $T_a$ = air temperature, °C, $T_{ref}$ = reference temperature, 0°C, $Y$ = air humidity, kg water (kg dry air)$^{-1}$ = latent heat of vaporisation $\approx 2500$ kJ kg$^{-1}$ K$^{-1}$, $C_{pv}$ = specific heat capacity of pure water vapour $\approx 1.8$ kJ kg$^{-1}$ K$^{-1}$.

The enthalpy of liquid water (same pattern for solids in water) is given by the equation:

$$H_l = C_{pl}(T_l - T_{ref})$$

where $C_{pl}$ = specific heat capacity of liquid water $\approx 4.2$ kJ kg$^{-1}$ K$^{-1}$, $T_l$ = water temperature, °C.

Picking one spray drying experiment of this experimental matrix, we have the following conditions in a laboratory-scale spray dryer: 0.000003166 kg s$^{-1}$ liquid, 90% water, 10% hibiscus solids (assume specific heat capacity 1.5 kJ kg$^{-1}$ K$^{-1}$) at 25°C enters with 0.0126 kg s$^{-1}$ air having a temperature of 90°C and a humidity of 0.01 kg water/kg dry air. The total energy flow rate into the dryer is the sum of the energy flow rates from the hot air and from the liquid feed. The enthalpy of air is given by the equation:

$$H_a = C_{pa}(T_a - T_{ref}) + Y[\lambda + C_{pv}(T_a - T_{ref})]$$

$$= 116.62 \text{ kJ kg}^{-1} (\text{dry air})$$

The dry air flow rate is equal to 0.0126 kg s$^{-1}$ x 1 kg dry air/1.01 kg total air (if the humidity is 0.01 kg water/kg dry air) = 0.01248 kg s$^{-1}$. The energy flow rate entering the dryer with the air is then 116.62 kJ kg$^{-1}$ x 0.01248 kg s$^{-1}$ = 1.455 kW. The enthalpy entering with the liquid (water) stream is given by

$$H_l = C_{pl}(T_l - T_{ref})$$

$$= 105 \text{ kJ kg}^{-1}$$

The flow rate of water is 0.9 x 0.000003166 kg s$^{-1}$ = 0.000002849 kg s$^{-1}$, so the energy flow rate entering with the water is 105 kJ kg$^{-1}$ x 0.000002849 kg s$^{-1}$ = 0.000299 kW. The enthalpy entering with the hibiscus solids is given by

$$H_s = C_{pa}(T_a - T_{ref})$$

$$= 1.5 \text{ kJ kg}^{-1} K^{-1}(25^\circ C - 0^\circ C)$$
= 37.5 kJ kg\(^{-1}\)

The flow rate of hibiscus solids is 0.1 \times 0.000003166 kg s\(^{-1}\) = 3.166 \times 10\(^{-7}\) kg s\(^{-1}\), so the energy flow rate entering with the hibiscus solids is 37.5 kJ kg\(^{-1}\) \times 0.000003166 kg s\(^{-1}\) = 0.00001185 kW. Hence the total energy flow rate entering the dryer is 1.455 + 0.000299 + 0.00001185 = 1.4553 kW.

Energy output

Neglecting the energy losses from the dryer, which are small, energy leaves the dryer mainly through the cooler, moister air, but also to a lesser extent through the solids (which contain some moisture). The assumption is made that the outlet solids are close to being in equilibrium with the outlet gas, so it follows from this assumption that the temperature of the gas and the solids is likely to be very similar (the same), and the outlet moisture content of the solids is likely to be equal to the equilibrium moisture content of solids in contact with the outlet gas. If there is a fixed temperature difference between the outlet solids and the outlet gas, then this difference can be readily included in the calculations. The gas stream takes up all the moisture that is evaporated from the solids. Hence the outlet moisture content of the solids may be related to the outlet humidity of the gas by a mass balance.

Continuing the previous example, the unknown variables are the outlet solids moisture content (X\(o\)), the outlet solids temperature (T\(Po\)), the outlet gas temperature (T\(Go\)) and the outlet gas humidity (Y\(o\)). These may be determined from mass and energy balances for the dryer, together with the assumptions outlined above.

The inlet solids moisture content is known (X\(i\) = 0.9 kg water/0.1 kg solids = 9 kg kg\(^{-1}\)), as is the inlet gas humidity (Y\(i\) = 0.01 kg water (kg dry gas)\(^{-1}\)). The dry solids flow rate in (F) is the hibiscus solids flow rate of 0.000003166 kg s\(^{-1}\), while the dry gas flow rate (G) is 0.01248 kg s\(^{-1}\). Hence the mass balance over the dryer gives the following equation:

\[
Y_o = Y_i + \frac{\dot{F}}{\dot{G}} (X_i - X_o) \quad \text{Equation 4}
\]

\[
= 0.01 + \frac{0.000003166}{0.01248} (9 - X_o)
\]

\[
= 0.01 + \frac{9 - X_o}{3941.9}
\]
Previously, the total energy flow rate entering the dryer was found to be 1.4553 kW, and there is energy leaving the dryer in both the air and the solids. For the air, the enthalpy is

\[ H_{Ca} = C_{pa}(T_{Ca} - T_{ref}) + Y_o[\lambda + C_{pa}(T_{Ca} - T_{ref})] \]

\[ = 1 \, \text{kJkg}^{-1}K^{-1}(T_{Ca} - 0^\circ\text{C}) + Y_o[2500\text{kJkg}^{-1}K^{-1} + 1.8\text{kJkg}^{-1}(T_{Ca} - 0^\circ\text{C})] \]

\[ = T_{Ca} + Y_o[2500 + 1.8T_{Ca}] \, \text{kJkg}^{-1} \quad \text{Equation 5} \]

In the solids, the enthalpy is

\[ H_{Sc} = C_{ps}(T_{Sc} - T_{ref}) + X_oC_{ps}(T_{Sc} - T_{ref}) \]

\[ = 1.5\text{kJkg}^{-1}K^{-1}(T_{Sc} - 0^\circ\text{C}) + X_o4.2\text{kJkg}^{-1}K^{-1}(T_{Sc} - 0^\circ\text{C}) \]

\[ = 1.5T_{Sc} + X_o4.2T_{Sc} \, \text{kJkg}^{-1} \quad \text{Equation 6} \]

An advantage of using a dry solids basis is that the dry gas (0.01248 kg s\(^{-1}\)) and dry solids (0.0000003166 kg s\(^{-1}\)) flow rates are identical at the inlet and the outlet of the dryer if there is little leakage, so from

\[ T_{Ca} + Y_o[2500 + 1.8T_{Ca}] \, \text{kJkg}^{-1} \quad \text{Equation 5} \]

and, we have:

\[ 1.4553 = 0.0000003166[1.5T_{Sc} + X_o4.2T_{Sc}] + 0.01246[T_{Ca} + Y_o(2500 + 1.8T_{Ca})] \quad \text{Equation 7} \]

The actual vapour pressure (\(p_v\)) divided by the saturation vapour pressure is the relative humidity (\(\psi\)). The relative humidity of the outlet gas needs to be calculated first, from the gas temperature (\(T_{Go}\)) and the gas humidity (\(Y_o\))

\[ \psi = \frac{p_v}{p_{v\text{sat}}} \quad \text{Equation 8} \]

The maximum vapour pressure at the outlet gas temperature (\(T_{Go}\)) is the saturation vapour pressure (\(p_{v\text{sat}}\)), which may be calculated using the Antoine equation, one version of which is:

\[ p_{v\text{sat}}(p_o) = 133.3\exp\left(18.3036 - \frac{3016.44}{T_o + 229.82}\right) \quad \text{Equation 9} \]
The outlet gas humidity ($Y_o$) is related to the actual vapour pressure ($p_v$) by the following equation (Keey, 1978; Strumillo and Kudra, 1986):

$$Y = 0.622 \frac{p_v}{p_{sat} - p_v} \quad \text{Equation 10}$$

to calculate the actual vapour pressure ($p_v$) from the gas humidity ($Y_o$) gives:

$$p_v = \frac{0.622 p_{sat}}{1 + Y_o / 0.622} \quad \text{Equation 10}$$

The relative humidity ($\psi$) can therefore be calculated from the gas temperature ($T_{Go}$) and the gas humidity ($Y_o$) using $\psi = \frac{p_v}{p_{sat}}$ Equation 8.

$$p_{sat}(T_o) = 133.3 \exp \left( 18.3036 - \frac{351.64}{173.15 + T_o} \right) \quad \text{Equation 9 and}$$

$$p_v = \frac{0.622 p_{sat}}{1 + Y_o / 0.622} \quad \text{Equation 10}.$$ Together with the gas and solids temperatures ($T_{So} = T_{Go}$), this relative humidity may be used to estimate the equilibrium moisture content ($X_{emc}$), which is a first approximation to the outlet moisture content ($X_o$). For different materials, the equilibrium moisture content may be estimated by different equations. A sorption isotherm for lactose can be used instead of a hibiscus isotherm as Chiou and Langriss (2007) have shown that the crystallization process is similar and hence the sorption isotherm can also be considered similar. Using the sorption isotherm for skim milk powder in (Kockel et al, 2002):

$$X_e = X_{emc} = 0.1499 \exp \left[ -2.306 \times 10^{-3} (T_{so} + 273.15) \cdot \ln \left( \frac{1}{\psi} \right) \right] \quad \text{Equation 11}$$

Overall, the equations to be solved are:

$$Y_o = 0.01 + \frac{Y_o - Y_o}{2 + 8} \quad \text{Equation 12}$$

$$X_e = 0.1499 \exp \left[ -2.306 \times 10^{-3} (T_{so} + 273.15) \cdot \ln \left( \frac{1}{\psi} \right) \right] \quad \text{Equation 13}$$

$$\psi = \frac{p_v}{p_{sat}} \quad \text{Equation 14}$$

$$p_{sat} = 133.3 \exp \left( 18.3036 - \frac{351.64}{173.15 + T_o} \right) \quad \text{Equation 15}$$

$$p_v = \frac{0.622 p_{sat}}{1 + Y_o / 0.622} \quad \text{Equation 16}$$

$$= 1.4453$$

Through iteration, we arrive at a final value for $X_o$ of 0.01 kg kg$^{-1}$, and an outlet gas and solids temperature of 63˚C.
The other difference between the two pump rates as seen in both Figure 13 and Figure 14 is that the yield with the higher pump rate is significantly lower. This difference in yield can be explained through the larger droplets produced at the higher pump rate, with all other factors constant. In this case, a two fluid nozzle (gas and liquid) nozzle has been used. Since the gas flow rate has been kept constant with the liquid flow rate being increased, the relative velocity of the gas to the liquid has been reduced and hence there is less shear and larger droplets. These bigger droplets have more momentum as they leave the nozzle and, as such, there is a greater chance that they will hit the wall and dry on the walls, which adds to the losses due to wall deposition as discussed by Hanus and Langrish (2007). Another effect of the larger droplets is the slower rate of drying due to the lower surface area to volume ratio. The particle is likely to be wetter and hence more sticky. This results in increased particle deposition after leaving the drying chamber, such as in the cyclone. This was noticed visually by the cyclone having a thicker layer of powder, as noticed by a darker colour, when the higher pump rate was used.

Considering Figure 13 and Figure 14, it can be seen that figures of both the yield as a function of inlet temperature and outlet temperature at both flow rates show very clearly the peak-in-yield trends. However, when scrutinising Figure 14 more closely, it can be seen that, when considering yield as a function of outlet temperature, the peak-in-yield curves appear to converge with the peaks being very close between 60 and 65˚C. This convergence of peaks suggests that the outlet gas temperature may be more representative of the conditions in the spray dryer.

The initial low yield at low inlet gas temperatures can be explained by the inlet gas, due to the low temperature, not drying the incoming droplets sufficiently. Therefore, there is enough residual moisture content in the powder to make stickiness a problem and hence give a low yield. Through increasing the temperature of the inlet gas, the resulting powder is drier and hence less sticky, giving a higher yield. In particular, the lower moisture content results in an increase in the powder’s glass-transition temperature, as predicted by the Gordon and Taylor equation (Gordon and Taylor, 1952). Therefore the overall difference between the particle and glass-transition temperatures decreases, and the resulting powder is less sticky, which creates a greater yield. Initially the glass-transition temperature (T_g) increases at a greater rate than
the particle temperature (T), which causes the stickiness to decrease and hence the yield to increase. The point at which the temperature of the powder increases at a faster rate than the glass-transition temperature is when the yield starts to decrease. When the overall difference between the particle and glass-transition temperatures starts to increase so too does the stickiness of the powder and hence the yield decreases. This peak in yield trend is in agreement with the results found by Bhandari et al. (1997) and Intiaz-Ul-Islam and Langrish (2008).

The peak-in-yield trend in bioactives was followed by spray drying sugar cane juice. The decrease in yield above the temperature at which the peak yield was obtained was followed for this material. However, the gradual increase in yield up to the peak yield was not confirmed. As seen in Figure 15, there is a sharp jump in yield, over a very small range of outlet temperatures. Over a range of only 3°C there is an increase in the yield of the powder by 2.5-3 times. This researcher strongly suggests that this behaviour is not a one-off aberration as it was found to be repeatible in repeat runs. There is no full explanation of this behaviour, but it is suggested through the work of Hanus and Langrish (2007) that there may be a critical dryness at which the particles in the drying chamber will bounce off the walls rather than stick to the walls. Therefore, it is suggested that this critical dryness is approached at an outlet temperature of 60°C and has been achieved at an outlet temperature of 63°C. It is also possible that the lower air inlet temperatures gave significantly more amorphous and sticky products (Chiou et al., 2008), explaining the much lower yields than for the higher air inlet temperatures, which gave higher yields.

It can be seen that there is a strong relationship between the outlet gas temperature and the yield as suggested previously by Bhandari et al. (1997). This may indicate that the outlet temperature should be an increased focus when analysing spray-drying data. When comparing the results found by Bhandari (1997) and the results found in this study, it can be noted that the outlet temperatures for both materials are similar, at 65-70°C, for the peak yields. This suggests that the glass-transition temperatures of sucrose and of the compounds in this bioactive extract are very similar.
5.6 CONCLUSIONS

This study has found that these two bioactive extracts display the same type of temperature-yield behaviour that has been observed before with a sugar, sucrose. A new finding is that the yield increased more sharply as the air temperature was increased for sugar cane juice than has been previously reported for other spray-dried sugars and bioactive materials. This may be due to the existence of a critical dryness, as suggested in the discussion, but it would be very interesting to see a study conducted to further our understanding of this behaviour. Having studied the yields from spray drying, the antioxidant retention (as briefly reviewed in Chapter 4) will be investigated in the next chapter.
Chapter 6. The effect of spray drying conditions on the antioxidant retention for a bioactive extract

6.1 OVERVIEW
The aim of this study was to determine the effect of drying air temperature (70-150ºC) on the antioxidant retention of a bioactive solution (Hibiscus sabdariffa L.) that was dried in a Buchi B-290 spray dryer. The resulting powder was tested for its antioxidant retention using the Oxygen Reducing Antioxidant Capacity assay (ORAC). This study found that the varying drying temperatures did not significantly affect the antioxidant retention of the sample. These results suggest that the focus should be on obtaining more product (higher yield) rather than to minimise the degradation by changing the operating conditions in this spray-drying situation.

6.2 INTRODUCTION
Spray drying may be the most suitable to dry this antioxidant extract since there are very short contact times (typically 1-2 seconds) and relatively low particle temperatures compared with gas temperatures (Rodriguez-Hernandez et al., 2005), which enables some food properties, such as flavour, colour and nutrients to be retained in high percentages (Kuts and Samsonyuk, 1989). Spray dryers are used in a variety of industries, where a product is to be directly transformed from a feed solution or suspension into a solid particulate state in a single-step continuous process. Single-step processes are attractive in food and pharmaceutical industries, which require minimal handling to allow high product quality. The difficulty faced in this work is the stickiness of the extract due to it containing low molecular weight sugars which have proven difficult to spray dry in the past (Bhandari et al., 1993).

With the background of drying techniques and the benefits of encapsulation as outlined in chapter 2 (pp51-53), the aim of this study was to: a) minimise the degradation of antioxidants in the liquid extract and b) to obtain the greatest amount of powder from the spray dryer. To achieve these aims the effect of inlet air temperature was explored.
6.3 MATERIALS AND METHODS

Fibre and extract mixture

The fibre and Rosella extract slurry was formed as per the description in the materials and methods section.

Drying conditions

The slurry mixture was fed into a Buchi B-290 mini spray dryer. The settings were as follows: aspirator rate 100% (0.0127 kg/s); drying air temperature ranging from 70 to 150°C; pump rates of 5% (1.9ml/min); atomisation air rotameter 35 (to the bottom of the gauge ball) (~455L/h); and the nozzle cleaner set to 9 (51 strikes/min). These operating conditions resulted in outlet temperatures in the range of 41 to 82°C.

Extraction of Samples

Two extraction techniques were employed throughout this study. The first was a very simple water extraction method, wherein a spray-dried sample (1g) was extracted in a 70mL screw cap jar with 20mL of water. To ensure the powder was well wetted, the jar was gently shaken and then the solution remained at room temperature for 1 hour. After the hour, the supernatant was removed, appropriately diluted and analysed for antioxidant capacity via the Oxygen Reducing Antioxidant Capacity (ORAC) assay (Prior et al., 2003, U.S. Department of Agriculture (USDA) et al., 2007). The differences in solution concentrations were taken into account by obtaining the total dissolved solids (TDS) of the supernatant and comparing the samples in terms of TDS.

The second technique was a slightly modified version of one previously used by Prior and colleagues (2003). In short, the spray dried powder was mixed with a solution of acetone, water and acetic acid (70:29.5:0.5). The mixture was vortexed for 30 seconds and then sonicated for 5 minutes, with the tube being inverted once during the sonication step to ensure the powder was still adequately suspended. After the sonication, the tube was left at room temperature for 10 minutes with occasional shaking. The tube was then centrifuged at 3500rpm for 15 minutes before the supernatant was removed and transferred to a 25mL volumetric flask, where the solution was made up to 25mL through the addition of water.
Oxygen Reducing Antioxidant Capacity Assay
The ORAC method used here has been described in the Materials and Methods section.

Calculations
The final ORACFL values were calculated by using a regression equation (\(Y = aX + b\)) between the Trolox concentration (X) and the net area under the FL decay curve (Y). Linear regression was used in the range of 6.25-50\(\mu\)M Trolox. Data have been expressed as micromoles of Trolox equivalents (TE) per 100 gram of sample (\(\mu\)molTE/100g). The area under the curve (AUC) was calculated using the rectangle rule, and the net AUC was calculated by subtracting the AUC of the blank by that of the sample.

6.4 RESULTS
Figure 17 shows the relationship between the antioxidant content of the rosella fibre mixture as a function of the outlet temperature of the spray dryer. This figure shows how the resulting powder compared with the initial level (100%), as a function of outlet temperature. The graph shows a minimum value of 28% and a maximum value of 51%. The expression of the results as a function of outlet temperature has been selected since the outlet air temperature is more representative of the conditions the particles undergo through the spray drying process (Bhandari et al., 1997, Premarajah and Langrish, 2009).
It is also possible that the inlet gas temperature may be a more important parameter than the outlet gas temperature, if the bulk of the degradation occurs near to the inlet of the dryer. When the percentage of initial antioxidants is plotted against the inlet temperature in Figure 18 it can be seen that there is no better correlation. This apparent lack of a correlation between the inlet or the outlet temperature may indicate that the degradation is not occurring more strongly at the inlet of the dryer and that the degradation occurs throughout the spray dryer.
Figure 18: Percentage of initial antioxidants as a function of inlet temperature.

Figure 19: Antioxidant capacity (μmol TE/100g) as a function of outlet temperature.
Figure 19 shows the antioxidant content of the rosella fibre powder. The initial rosella extract is not included because the extract is initially in liquid form, and both of the extraction techniques require a powder sample. An untreated powder sample cannot be obtained directly from the liquid extract since it cannot be stated, with any level of certainty, that the drying process chosen to produce it, perhaps freeze drying, would have no effect on the level of antioxidants in the solution. However, an ORAC test of the raw extracts showed an initial reading of 24000 μmol Trolox Equivalents (TE)/100g. The graph shows a mean of 12600 with a minimum of 9000 and a maximum of 16200μmol Trolox Equivalents (TE)/100g.

Figure 17, Figure 18, Figure 19 and Figure 20 show no significant effect of either inlet or outlet temperature on the antioxidant content of the spray-dried rosella-fibre powder. Statistical analysis performed in Excel has shown a very low R² value of 0.01 and that with 95% confidence the data has a gradient between -0.1 and 0.1. This demonstrates a lack of correlation within the data set, and hence it can be said that there is no statistically significant trend.
Figure 21: Yield as a function of outlet temperature.

Figure 21 shows the yields produced from the spray dryer as a function of outlet air drying temperature. It shows a slight peak-in-yield trend. Similar peak-in-yield trends have been found in previous studies for biological materials such as fruit juices, sucrose and a similar rosella-fibre mixture (Bhandari et al., 1993, Imtiaz-Ul-Islam and Langrish, 2009). As discussed in Chapter 4, it can be seen that the peak yield is close to the outlet gas temperature of 60°C, further confirming the strong relationship between outlet gas temperature and peak in yield for a given material.

6.5 DISCUSSION

When analysing the results, it may (at first) appear that there is a significant effect of temperature, since there is approximately 40% variation in the results seen in Figure 19. However, if it is considered that an Arrhenius based temperature effect is expected (Erenturk et al., 2005), with the rate of (degradation) reactions doubling with every 10°C temperature rise, then over the 40°C change in outlet temperature that is experienced in this experimental
matrix, it would be expected that a 24 factor change in antioxidant content would occur. Even with a short contact time, the temperature should affect antioxidant retention. Hence, when the Arrhenius-based temperature effect is considered, the 40% variation in antioxidant content is not significant when a much larger change might have been expected. In addition, the variation in the antioxidant content is not systematic, as demonstrated by the low $R^2$ value for the correlation between antioxidant content and outlet temperature and the 95% confidence interval for the slope of the relationship between the two parameters (-0.1,0.1), which suggests that no significant correlation exists between them. Therefore, these experimental results suggest that the effect of temperature on the antioxidant content during spray drying is not significant. This is most likely to be due to the relationship between the rate of removal of moisture and the exposure of the thermally sensitive antioxidant with respect to the rate and amount of antioxidant degradation. In particular, increasing the temperature increases the degradation rate, but it also decreases the moisture content more quickly, which decreases the degradation rate, so the overall effect is not expected to be very strong or significant, as seen in this study.

The effect of outlet air drying temperatures on the yield has been less distinct than expected. A peak-in-yield trend is usually expected when drying materials containing low molecular weight sugars (Bhandari et al., 1993, Imtiaz-Ul-Islam and Langrish, 2009, Premarajah and Langrish, 2009). The peak in yield trend over this range of temperatures is explained by the following physical processes as previously discussed in chapter 5. At the lower temperatures, the droplets are not dried sufficiently, and the particles leave the dryer fairly wet and hence sticky. As the temperature increases, the particles leave the dryer with a lower moisture content and hence are less sticky, resulting in a higher yield. In particular, the lower moisture content results in an increase in the powder’s glass-transition temperature, as predicted by the Gordon and Taylor equation. Therefore, the overall difference between the particle and glass-transition temperature decreases, and the resulting powder is less sticky, which results in a greater yield of product. Initially, the glass-transition temperature (Tg) increases at a greater rate than the particle temperature (T), which causes the stickiness to decrease and hence the yield to increase. The point at which the temperature of the powder increases at a faster rate than the glass-transition temperature is when the yield starts to decrease. When the overall difference between the particle and glass-transition temperatures starts to increase, so too does
the stickiness of the powder and hence the yield decreases. The lack of a distinct peak-in-yield trend in this study emphasises the importance of verifying the composition of the sample solution, particularly when it is a natural product, as is the case here. The data shows no significant effect of either the inlet or outlet temperature on the antioxidant retention of the dried samples. This is evidence that the degradation occurs throughout the dryer and is not stronger at the inlet or outlet.

The standard error in the ORAC results (Figure 19) is 17% of the mean, which is reasonable for these biological samples. The variance within the data sets may be due to inhomogenous sampling of powders. The samples for each extraction technique are 1g each from an approximate 10g output from the spray dryer. Due to the small output from the dryers this is an appropriate sample size for the analytical testing. Further variance may be derived from inconsistencies in the slurry solution and perhaps even biological variability within the sample.

These results suggest that this Rosella antioxidant-rich extract can be dried in a spray dryer with the same levels of degradation across this temperature range and hence the focus should be on obtaining the maximum yields from the spray-drying process. Therefore, the technology is available to industry to create an antioxidant rich powder to be used as a nutraceutical or as an additive to add value to food products.

6.6 CONCLUSIONS

The results obtained from this study suggest that there is no significant effect of spray drying temperature on the antioxidant content of this rosella fibre mixture. This lack of an effect is most likely due to the low exposure times and relatively low temperatures. The rapid rate of drying also reduces the overall degradation as the particles become less mobile and hence less susceptible to damage.
Chapter 7. The effect of using Whey Protein Isolate as a carrier for a bioactive extract

7.1 OVERVIEW AND INTRODUCTION

Spray drying of sticky materials, such as food extracts containing low molecular weight sugars, can be difficult as they tend to be sticky (Adhikari et al., 2003). This stickiness can be overcome by adding additives to the materials. Common additives include maltodextrin, gum Arabic and proteins. This researcher has previously used natural fibre as an additive and it was successful to some extent (Premarajah and Langrish, 2009). The additives are usually used to reduce wall deposition of the material on the walls of the spray dryer and hence to increase the yields of the drying process. These additives often have high glass transition temperatures ($T_g$) and are added to materials with sticky materials that have a low glass-transition temperature and so the glass transition temperature of the bulk material is increased.

Unlike the previously mentioned carriers that were used to change the physical properties of the bulk solution, whey protein isolate has been used due to its surface-active nature. Faldt and Bergenstahl (1994) and Wang and Langrish (2010) have both found that adding very small amounts, as low as 1%, of protein gave upwards of 40% protein on the surface of the particles. Adhikhari and colleagues (2007) have found that WPI molecules are strong surfactants and preferentially migrate to the droplet-air interface, which explains the greater concentration of protein at the particle surface rather than in the bulk material. Another theory is that casein diffuses away from the surfaces of the droplets more slowly than lactose and other sugars and hence accumulates at the surface (Meerdink and Van't Riet, 1995). Whatever the mechanism of the accumulation of casein at the surface of the particles, it has been demonstrated empirically through the work of Wang and Langrish (2010) and this study has sought to exploit this encapsulating property of casein with a different sugary material.
7.2 MATERIALS AND METHODS

Whey protein isolate and extract mixture
The spray-dried solution consisted of Whey Protein Isolate (WPI) (Fitlife WPI, Castle Hill NSW) and Rosella extract (*Hibiscus sabdariffa* L., Vic Cherikoff Food Services Pty. Ltd). The solution was prepared by adding WPI at a concentration of 1% based on the total dissolved solids of the Rosella extract. The combined solution was diluted with water to a solids concentration of 10%. The combined mixture was stirred for at least 30 minutes before spray drying to ensure that the WPI was dissolved in the solution. The solution was also stirred during the entire spray-drying process to ensure that a homogenous solution was fed into the dryer.

Spray-drying experiments
The spray-drying experiments were carried out using a Buchi B-290 advanced spray dryer (Buchi, Switzerland). The settings were as follows: aspirator rate 100% (0.0127 kg/s); drying air temperature ranging from 90 to 150°C; pump rate of 5% (1.9 ml/min); atomisation air rotameter 35 (to the bottom of the gauge ball) (~455 L/h); and the nozzle cleaner set to 9 (51 strikes/min). The operating conditions resulted in outlet temperatures in the range of 54 to 88°C.

Oxygen reducing antioxidant capacity assay
The ORAC method used here is described in the materials and methods section.
7.3 RESULTS AND DISCUSSION

Figure 22 shows a peak-in-yield trend which has been seen in many other studies involving spray drying of substances containing low molecular weight sugars (Bhandari et al., 1997; Imtiaz-Ul-Islam and Langrish, 2009; Premarajah and Langrish, 2009). The interesting point to note here is both the relatively high yields that are rarely seen in spray dryers of this small scale and the sharp drop in yield at around 92°C. This rapid decrease in yield has been seen in other studies such as Premarajah and Langrish (2009), when sugar cane juice was spray dried. This behaviour is not fully understood but it has been suggested through the work of Hanus and Langrish (2007) that there may be a critical dryness at which the particles in the drying chamber will bounce off the walls rather than stick to the walls.

The peak in yield trend has been previously discussed in chapter 6. The initial lower yield is explained by the drying gas temperature being lower than optimal and therefore the powders still have some residual moisture that causes stickiness of the powder hence lowering the yield. As the outlet gas temperature increases the drying gas is drying the particles more thoroughly and hence stickiness is less of a problem. A more thorough explanation involves
the behaviour of the glass-transition temperature ($T_g$) as the droplet and subsequent particle dries. According to the Gordon and Taylor equation, stickiness is strongly correlated with the difference in the particle temperature and the glass-transition temperature ($T_g$). The glass transition temperature is affected by the moisture content of the particle such that, as the moisture content of the particle decreases, there is an increase in the glass-transition temperature. Therefore, in this case, on the left side of the peak, the glass-transition temperature ($T_g$) increases at a greater rate than the particle temperature ($T$), which causes the stickiness to decrease and hence the yield to increase. On the right side of the peak the particle is relatively dry and hence the particle temperature ($T$) increases at a greater rate than the glass-transition temperature ($T_g$), which increases $T-T_g$ and hence stickiness increases and hence the yield drops.

Figure 23: Increase in antioxidants as a function of outlet temperature.

Figure 23 shows an increasing of antioxidant activity as a function of outlet gas temperature from the dryer. There are many explanations that may account for this increase in antioxidant activity. As discussed in Chapter 2, the formation of Maillard Reaction Products may be the reason for an increase in antioxidant activity after food processing. Manzocco’s review
(Manzocco et al., 2001) of non-enzymatic browning and antioxidant capacity in processed foods has highlighted a number of different research papers that have shown that oxidised polyphenols can exhibit a higher antioxidant activity than non-oxidised polyphenols. The food systems studied include pear and white grape juices (Spanos and Wrolstad, 1992), tea extracts (Manzocco et al., 1998), and commercial cognacs (Da Porto et al., 2000). The reason that these partially oxidised polyphenols have a higher antioxidant content may be due to the formation of novel compounds that have a high antioxidant activity, which was seen in the work of Choi and colleagues (Choi et al., 2006) in their study of Shiitake mushrooms. Choi’s paper also suggested that the lack of antioxidant degradation may be due to the heat treatment deactivating the endogenous oxidative enzymes contained in the mushrooms. This is supported by the work of Dewanto and colleagues (Dewanto et al., 2002) that also showed that heat may have prevented enzymatic oxidation and that heat treatment improved the nutritional value of tomatoes.

A table from (Nicoli et al., 1999) has summarised the possible effects of food processing as:
- No effect
- Loss of naturally occurring antioxidants
- Improvement of antioxidant properties of naturally occurring compounds
- Formation of novel compounds having antioxidant activity (ie. Maillard reaction products)
- Formation of novel compounds having pro-oxidant activity (ie. Maillard reaction products)
- Interactions among different compounds (eg. Lipids and natural antioxidants, lipids and Maillard reaction products)

The increase in antioxidants may appear to be increasing or may in fact appear to be decreasing if the single high point at 90°C (from Figure 23) is removed. However, if it is considered that an Arrhenius based temperature effect is expected (as discussed in Chapter 6), then again the effect is insignificant. This is supported by the work of Ahmad and Langrish (2012) in which they state that the evolution of Maillard reactions at lower temperatures (135°C) is modest. This does not mean that Maillard products are not forming, as there are in fact
low molecular weight sugars experiencing high temperatures, albeit for a short amount of
time, but does support the relatively low evolution of Maillard products.
The similarly low exposure time and relatively low temperatures support the findings in both
the usage of fibre or WPI as a carrier that there is a relatively low impact on the antioxidant
generation/degradation of the samples. One reason that there is a consistent increase in AOX
when considering WPI as a carrier and a consistent decrease when using fibre as a carrier
may be due to where in the particles that the antioxidant rich extract is being deposited and
hence how much protection is being offered. WPI may be acting as an encapsulant with the
extract tending to be contained within the thin WPI coating whereas the fibre may be acting
more as a drying agent with significantly more extract deposited on the surface of the fibre.
This phenomena may be further explored through electron microscopy to explore where the
extract is being deposited in relation to the carrier. These electron micrographs may also
expose the integrity of the encapsulation provided by the WPI and fibre.

The relatively low effect on the AOX suggests that yield and additive concentration should
have a much more significant impact on the choice of a drying aid. When comparing fibre
and WPI as drying aids, WPI would appear to be much better, as much lower quantities are
required and much better product yields may be achieved under the same spray drying
conditions.
This research compared oven, freeze, tray, vacuum and spray drying. The first finding was that spray drying was identified as the most promising approach to drying a natural, antioxidant rich food extract as there was minimal handling, continuous operation and a desirable output composition. The yields derived from small-scale dryers were considered to be satisfactory (~55%) when using natural sugar cane fibre as a carrier. The yields were found to display a peak in yield trend with the peak corresponding to an outlet temperature of approximately 60°C. The feed flowrate into the spray dryer was found to have a significant impact on the yield, with the higher flowrate resulting in lower yields due to higher wall deposition. When discussing the peak in yield trend, an interesting finding was that the sugar cane juice-fibre slurry resulted in an extremely sharp jump in yield. This sharp jump was not a one-off aberration and was found to be repeatable. This sharp increase in yield should be investigated further and a similar trend may be found with the drying of other sugar-rich solutions. This will further increase our understanding of peak in yield behaviour and may lead to the effective drying of materials previously thought to be too difficult.

The main technique used to determine the antioxidant capacity of each sample was the Oxygen Reducing Antioxidant Capacity (ORAC) assay as it is a reliable high throughput method. This assay was used successfully and showed that the satisfactory yields were coupled with a suitable amount of retention of antioxidant potential. The antioxidant retention was not significantly affected by drying temperature and this is thought to be due to the low exposure times and relatively low temperatures. Rapid rate of drying also reduces antioxidant degradation as the particles become less mobile and hence less susceptible to damage. It
would be interesting to see if this author’s prediction of drying in an industrial scale dryer will, in fact, result in a similar retention of antioxidant capacity of the samples with a marked increase in yields.

The final experiments of this study have shown extremely promising evidence that whey protein isolate can be used as an encapsulant for sticky materials. The extremely high yields (>95%) that have been experienced in small scale dryers is unusual and a deeper understanding of how the whey protein isolate is aiding this would be an extremely great step forward for the drying industry. Whey protein isolate is already targeted towards the bodybuilding and health markets so the coupling with an antioxidant extract may allow a differentiation in the WPI market.

The finding that the antioxidant capacity is actually increased throughout the drying process should be further investigated from a food technology and dietary point of view to ensure that any possible Maillard by-products are safe for consumption and to ensure that they are not carcinogenic as BHA and BHT are thought to be.
References


Polyphenols Laboratories As (2008a) Cyanidin 3-Sambubioside. Sandnes, Norway, CorePublish CMS.

Polyphenols Laboratories As (2008b) Delphinidin 3-Sambubioside. Sandnes, Norway, CorePublish CMS.


Xu, Z. & Langrish, T.A.G. (Year) Thermal Degradation of Vitamin C During the Oven-Drying ProcessConference, NameConference, Perth, Australia,

# Appendices

## FIGURE 12

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## FIGURE 13

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