

# Vitamin Extraction from Kakadu Plum

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## ABSTRACT

Kakadu plum (*Terminalia ferdinandiana*) is a native Australian fruit which is known to have very high concentrations of phytochemicals, including vitamin C concentrations of up to 100 times that contained in oranges. However, due to a combination of factors including the size, texture and flavour of the fruit as well as product and supply variability, it has not found widespread use in the food industry. For example, the fibrous nature of its flesh means the fruit is not easily juiced. The purpose of this study was to develop a small scale solid-liquid extraction of phytochemicals from Kakadu plum that could be used by the food industry to produce value added products, such as beverages.

The approach taken was to carry out laboratory extractions and analyse the extracts for Vitamin C, in order to produce data that can be used to model an extraction.

A mathematical model has been developed and model parameters have been found for the vitamin C extraction experiments that were carried out. This model and the calculated parameters have been used to model both equilibrium and non-equilibrium extractions.

## 1.0 INTRODUCTION

Although fruit and vegetables are known to provide specific benefits, the mechanism by which these benefits are provided, and the roles of individual phytochemicals are often unknown (Dillard and German, 2000). The food industry is now moving towards developing an understanding of specific types of phytochemicals and their properties (DeFelice, 1995).

A growing understanding of phytochemicals is leading to food products being marketed as “functional foods” because of their perceived health benefits. Functional foods are foods which are used for the health promoting properties that they provide (DeFelice, 1995).

Plants foods all have unique phytochemical compositions and therefore their own unique nutritional properties (DeFelice, 1995). These unique properties means that there are many fruits and vegetables which could serve a purpose in the functional food market (DeFelice, 1995). Many native Australian fruits are known to have high phytochemical concentrations and are likely to be used as functional foods in the future (Konczak et al., 2008). Native Australian fruits of interest include Cedar Bay cherry,

muntries, Kakadu plum, Burdekin plum, Davidson's plum, Illawarra plum, finger lime, riberry and brush cherry (Konczak et al., 2009).

### **1.1 Project Objectives**

The overall aim of this project is to develop a small scale continuous process for the extraction of phytochemicals from Kakadu plum. The extract produced by the process could potentially be used to produce functional foods.

### **1.2 Phytochemicals**

Possible benefits that phytochemicals may provide as part of a consumers diet include the degradation of toxic chemicals, acting as substrates in biological reactions, and enhancing absorption of other nutrients (Dillard and German, 2000). Health benefits associated with the intake of phytochemicals has included the prevention of diseases such as diabetes, cancer and heart disease (Craig, 1997).

Although there is significant evidence that phytochemicals can provide specific health benefits, the exact mechanisms are often unknown (Dillard and German, 2000). As understanding of phytochemicals grows, this understanding will play a part in the development of novel foods by the food industry.

#### **1.2.1 Kakadu Plum as a Functional Food**

Kakadu plums are a small fruit that grow on trees. They are approximately two centimetres long with a one centimetre diameter. There is a single seed in the middle of the fruit and the flesh is tough and fibrous.

Although its high phytochemical concentrations make Kakadu plum an ideal functional food, it is unpleasant to eat because of its rough texture and a displeasing taste (Netzel et al., 2007).

This research focused on the vitamin C content of Kakadu plum. Other important properties of Kakadu plum include phenolic content and antioxidant capacity. However, these were not investigated in this project.

#### *Vitamin C*

Kakadu plum has a vitamin C concentration of up to 100 times that of oranges (Netzel et al., 2007). Vitamin C is an essential vitamin. Also, vitamin C consumption above the essential level is recognised to provide additional health benefits and has been associated with a reduced incidence of some diseases (Vicente et al., 2009). Vitamin C is also an antioxidant and promotes the uptake of iron (Vicente et al., 2009).

#### *Phenolics*

Phenolic compounds can act as antioxidants and specific phenolics may also perform other biological functions (Netzel et al., 2007). Konczak et al found Kakadu plum to have six times the concentration of phenolic phytochemicals than blueberries (Konczak et al., 2010).

#### *Antioxidant Capacity*

Oxidative stress can cause lipid oxidation, protein and DNA damage (Vicente et al., 2009). This can contribute to diseases including cancer, diabetes and atherosclerosis

(Waris and Ahsan, 2006). Antioxidants slow down oxidation by neutralising reactive oxygen species and thus provide health benefits through reducing the damage that reactive oxygen species can cause (Vicente et al., 2009). Konczak et al found Kakadu plum to have four times the antioxidant capacity of blueberries (Konczak et al., 2010).

### **1.3 Potential Phytochemical Extraction Methods**

#### **1.3.1 Juicing**

Juicing is a method that can be used to extract phytochemicals from fruit. Juicing is the most common and well known method to extract the constituents of a fruit into a liquid (McLellan and Padilla-Zakour, 2004).

For most fruits, mechanical juicing is simple and very effective at producing an extract from the fruit (Harjo et al., 2004). However, the fibrous flesh of Kakadu plum makes juicing ineffective. In this project, when cutting up and then grinding Kakadu plum flesh with a mortar and pestle, a minimal amount of liquid was forced from the flesh. When apple and lemon flesh was ground in a mortar and pestle, for comparison, a large amount of juice quickly left the flesh.

Juicing was considered unsuitable because of the fibrous nature of Kakadu plum. Although there are potential methods of making the juicing process more efficient, it was decided that a better solution to this problem was to use solid-liquid extraction.

#### **1.3.2 Solid-Liquid Extraction**

Solid-liquid extraction involves bringing a solvent in contact with the solids to be extracted. This results in the desired components in the solids dissolving in the solvent, which can then be separated from the solids (Barbosa-Canovas and Ibarz, 2010).

There are a large range of solid-liquid extractors used in industry. Industrial extraction of phytochemicals is generally carried out in stirred tanks (Kassing et al., 2009). This type of extraction is generally carried out in a multiple stage counter-current batch process (Kassing et al., 2009). Continuous processes are used for extraction of phytochemicals; but this is generally limited to large scale extractions such as the oil-seed industry (Kassing et al., 2009).

## **2.0 EXPERIMENTAL METHODOLOGY**

The experimental work involved extracting the Kakadu plums with water and then testing the water extract for vitamin C.

## 2.1 Extraction Methodology

### *Extraction Procedure*

To prepare fruit samples for extraction, fruit stored at  $-20^{\circ}\text{C}$  were deseeded by cutting the flesh off the seed with a knife and then cut into 2x5mm pieces. They were then ground in a mortar and pestle and then thawed. Once the fruit had warmed to room temperature, it was ready to be extracted.

For each extraction, 1.5 g of Kakadu plum was used along with 30 mL of Milli-Q water. The fruit was then added to a 100 mL beaker. The beaker was then mixed using a magnetic stirrer bar (500 rpm). 30 mL of water was added to the beaker and the timer was started. The extraction was then carried out for the desired amount of time.

To remove the solid matter from the extract, the sample was filtered and the extract stored at room temperature.

## 2.2 Vitamin C Titration Procedure

The vitamin C titration procedure was carried out using a standard operation procedure from the University of Canterbury (University-of-Canterbury-Outreach, 2005).

### *Materials*

Potassium iodide, potassium iodate, starch solution, hydrochloric acid solution and ascorbic acid were obtained from Sigma Aldrich.

### *Solutions*

Required solutions were 0.6 mol/L potassium iodide, 0.002 mol/L potassium iodate, 1 mol/L hydrochloric acid and ascorbic acid standard solution (0.0000 to 0.0036 mol/L). These solutions were made by dissolving the chemicals in Milli-Q water.

### *Titration Procedure*

The sample to be titrated was prepared by pipetting 20 mL of kakadu plum extract diluted in Milli-Q water (or a standard) into the conical flask and then pipetting 5mL of potassium iodide solution, 1mL of hydrochloric acid solution and 1mL of starch solution. The amount the extract was diluted by depended on the concentration in the extract (the higher the concentration, the more the extract was diluted). This mixture was then titrated with the potassium iodate solution. The endpoint was reached when the reaction had a permanent trace of a dark black-blue colour.

## 3.0 EXPERIMENTAL RESULTS

### 3.1 Vitamin C Equilibrium in Water

Data was collected for the equilibrium separation of vitamin C between the fruit and the extraction solvent. This was carried out by extracting 1.5 g of solids four consecutive times in 30 mL of fresh extraction solvent for 15 min (which was sufficient time to ensure equilibrium). The replication of points (two for each extraction) is because each extract was assayed twice. The results are shown in Figure 1.

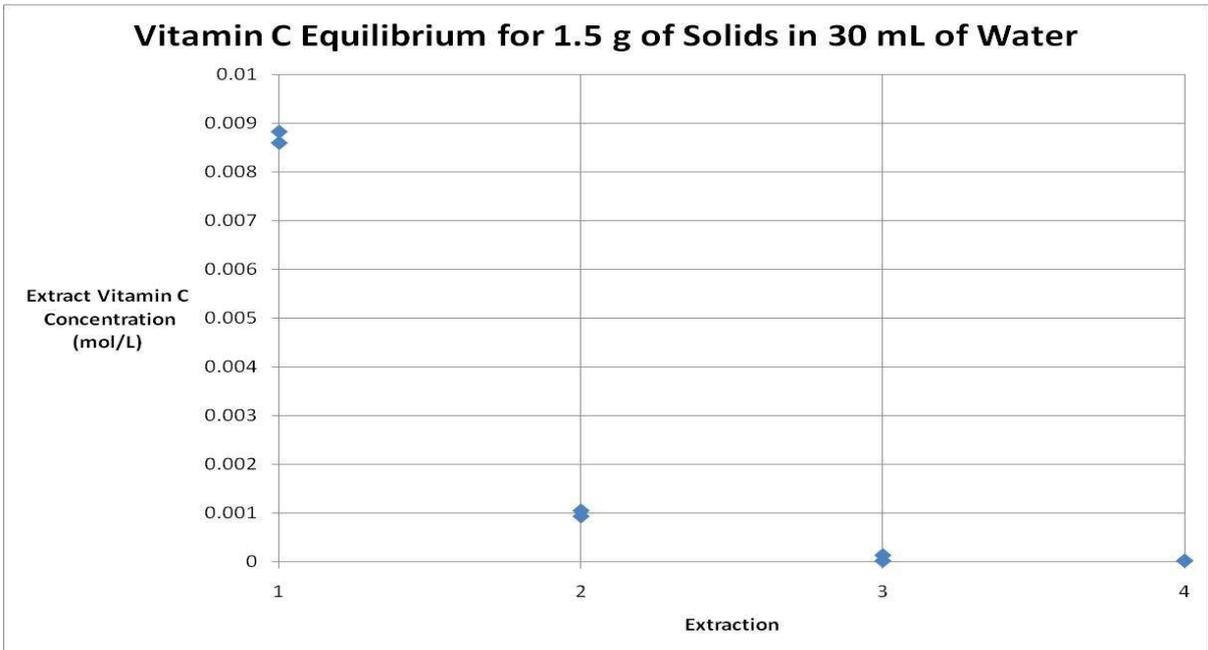


Fig. 1: Vitamin C concentration of extracts as four consecutive extractions are taken

### 3.2 Vitamin C Extraction Kinetics in Water

Experiments were also carried out to analyse the kinetics of the extraction of vitamin C in water. The extractions were carried out with 1.5 g of Kakadu plum (taken from the crushed fruit of three plums prepared each day) and 30 mL of water. The replication of points is because each extract was assayed twice. The results produced are shown in Figure 2.

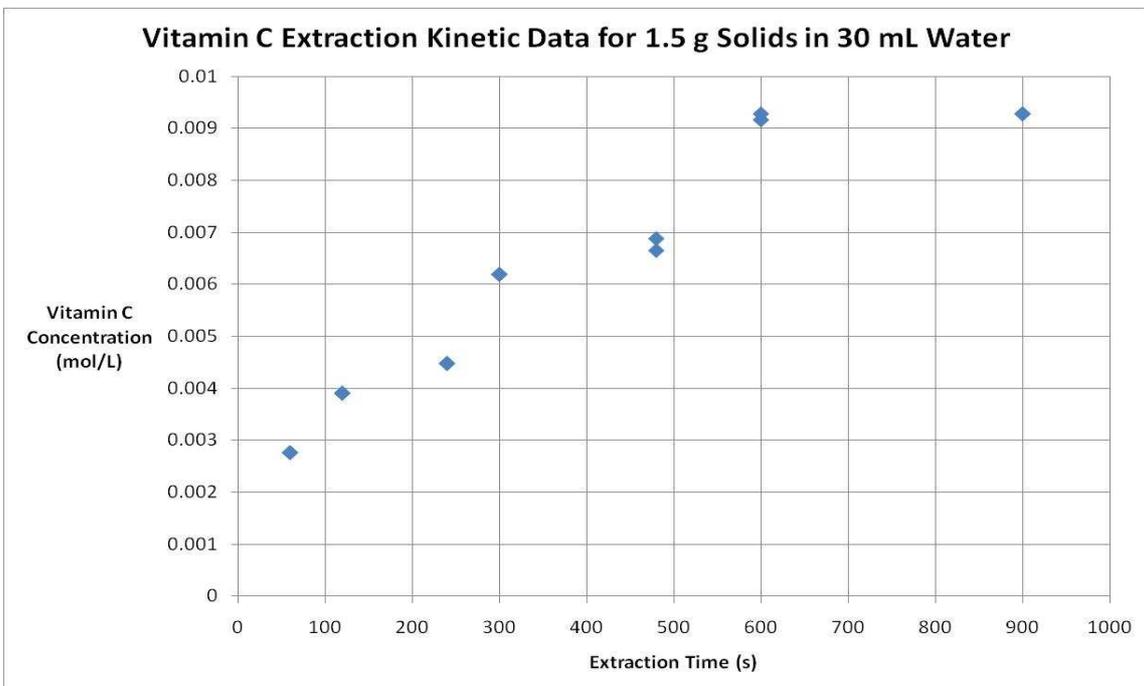


Fig. 2: Extract concentration versus time extracted for vitamin C extraction in water

## 4.0 MODELLING

### 4.1 Development of the Model

There are a wide range of models that could be used to model extraction, with different levels of complexity (Kassing et al., 2009). This project will initially use as simple a model as possible. The equation chosen is shown below (Miguel Aguilera, 2009):

$$N = K(c_s^* - c_s) \quad (1)$$

where  $N$  is the mass transfer rate,  $K$  is the overall mass transfer coefficient,  $c_s$  is the solvent concentration and  $c_s^*$  is the solvent concentration that would be in equilibrium with the solids (Miguel Aguilera, 2009).  $K$  will depend on a number of factors which will include the level to which the solids have been cut up and grinded. This equation has been used to derive an expression for the solvent concentration versus time,  $c_s$ , during an extraction:

$$c_s = c_s^*(1 - e^{-Kt}) + c_{s0}e^{-Kt} \quad (2)$$

For the equation for solvent concentration shown above to be useful, it is necessary to have an expression for  $c_s^*$ .  $c_s^*$  will be defined as:

$$E = c_p^*/c_s^* \quad (3)$$

where  $E$  is an equilibrium constant.  $E$  will depend on the ratio of the mass of solids to the volume of solvent. Therefore, for the model to be further developed, it would be necessary to find the dependence of  $E$  on the ratio of solids mass to solvent volume.

The above equations were used to derive the expression for  $c_s^*$  shown below:

$$c_s^* = \frac{n_s}{V_s} = \frac{n_{p0} + n_{s0}}{m_p E + V_s} \quad (4)$$

where  $n_s$  is the number of moles in the solvent,  $n_p$  is the number of moles in the solids,  $m_p$  is the mass of solids and  $V_s$  is the volume of solvent. Therefore,  $c_s$  can be given by:

$$c_s = \frac{n_{p0} + n_{s0}}{m_p E + V_s} (1 - e^{-Kt}) + c_{s0}e^{-Kt} \quad (5)$$

### 4.2 Calculated Model Parameters for Vitamin C Extraction

The derived model was fitted to the experimental data for vitamin C extraction. The value for the equilibrium constant,  $E$ , was calculated as 0.00233 L/g. It is important to note that this equilibrium constant is for a solid to solvent ratio of 1.5 g to 30 mL. The value for the overall mass transfer coefficient,  $K$ , was calculated as  $0.00366\text{s}^{-1}$ .

### 4.3 Modelling of Extraction Processes

The model derived above was used to model this extraction process, for the extraction conditions carried out in this project and then was applied to a two equilibrium stage process and a two non-equilibrium stage process. The two stage equilibrium process model calculated a final extract concentration of 0.00968mol/L, compared to a one stage equilibrium concentration of 0.00877mol/L. The two stage non-equilibrium model was

calculated for extraction times of 300s in each extractor and the final extract concentration was found to be 0.00810mol/L, compared to a single non-equilibrium stage of 600s giving an extract concentration of 0.00781mol/L.

## **5.0 DISCUSSION**

### **5.1 Experimental Results**

#### **5.1.1 Vitamin C Titration Results**

There was a large amount of variability shown in the vitamin C extraction experiments. Figure 2 shows that the individual extractions do not seem to follow a smooth curve. Because of the limited experimental results, any kinetic or equilibrium parameters obtained from the data are unlikely to be highly accurate representations of the underlying “average” kinetic parameters.

The reason for the small amount of experimental results is that a range of issues were encountered in importing the Kakadu plum samples into New Zealand.

A large source of variability in these experiments is likely to be the vitamin C content of the individual fruits. Although three fruits were used for each set of extractions, there is still likely to be great variability in the total vitamin C content of the mixtures produced. Different harvests of the fruit and regional differences will also have increased the variability. As a result of this, much more experiments need to be carried out for vitamin C extraction.

Experimental data was also only collected for a solid to solvent ratio of 1.5g to 30mL. To better understand the extraction, it is necessary to conduct extractions with various solid to solvent ratios.

#### **5.1.2 Antioxidant Capacity and Phenolic Content**

Antioxidant capacity and phenolic content were identified as important properties of Kakadu plum however the antioxidant capacity and phenolic content of extracts was not assayed for.

Assaying for these properties would provide a more complete understanding of Kakadu plum extraction. An appropriate assay for antioxidant capacity could be the TEAC assay or the ORAC assay (Zulueta et al., 2009). An appropriate assay for phenolic content could be the TPC assay (Kim et al., 2003).

### **5.2 Modelling**

#### **5.2.1 Calculated Model Parameters**

The model derived for the solvent concentration versus time was successfully used to fit equilibrium and kinetic parameters to vitamin C extraction with water. However, because of the limited number of experimental results used the estimated parameters will be inaccurate.

The equilibrium parameter calculated was also only applicable for solids to solvent ratios of 1.5g to 30mL. For the model to be useful, it will be necessary to develop a model that is applicable for all solids to solvent ratios. Therefore, the model will need to

be developed further by finding how the equilibrium constant,  $E$ , varies with the solids to solvent ratio and adding an expression for this into the model.

### 5.2.2 Modelling of Extraction Processes

The modelling carried out in the project showed that the kinetic parameters obtained from the extraction data can be used to model extraction processes. As expected, the models showed that two stages provide a better extraction than a single stage.

## 6.0 CONCLUSIONS

The experimental data collected on vitamin C extraction is only for one solid to solvent ratio and doesn't have enough data points to accurately show kinetics or equilibrium properties.

A model has been produced to model phytochemical extraction and this has been used to model simple extraction processes.

## 7.0 FUTURE WORK

Future work for this project will include continuing the vitamin C extraction experimental work and also beginning experimental work to look at the extraction of phenolic content and antioxidant capacity. Future experiments will be carried out over a range of solid to solvent ratios to see how this affects extraction.

A computer simulation of the models developed in this project will be produced. With the extra experimental data, this model could be used to help develop a small scale extraction of phytochemicals from Kakadu plum.

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## **9.0 BIOGRAPHY OF PRESENTER**

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