Horticulture Innovation Australia

Final Report

Freeze Dried Finger Lime Pearls for Food Service and Retail

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Sunfresh Marketing Co-op

Project Number: CT11003

CT11003

This project has been funded by Horticulture Innovation Australia Limited with co-investment from Sunfresh Marketing Co-op and funds from the Australian Government.

ISBN 0 7341 3245 X

Published and distributed by:
Horticulture Innovation Australia Limited
Level 8, 1 Chifley Square
Sydney NSW 2000
Tel: (02) 8295 2300

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MEDIA SUMMARY

Sunfresh Avocado Marketing Co-op has identified an opportunity to develop a unique range of native finger lime products. The unique lime crystals that make up the fruit pulp, "pop" in the mouth when consumed fresh or freeze dried, releasing a fresh burst of lime flavour.

This project consisted of two stages; each investigating a different processing technology platform. Stage 1 of the project involved the development of a process for the freeze drying of finger lime pearls, to produce a shelf stable food ingredient for export. The second stage of the project involved the development of pretreatment and processing protocols for High Pressure Processing (HPP) of chilled finger limes with extended shelf life without the addition of preservatives, for food service and retail markets.

Stage 1 of the project involved development of a process for producing high quality, shelf stable freeze dried finger lime products. The freeze drying process that was developed produced crystal-like finger lime products that retained their color, flavour and bioactivity. This freeze dried product is suited to the food service market as it delivers the finger lime flavour and color characteristics, while remaining shelf stable.

Stage 2 of the project involved development of a fresh-like ready-to-use HPP finger lime products, suited to the export retail market sector. The high pressure process maintains the finger lime color, flavour and bioactivity with extended chilled shelf life. Development of a process for the HPP of finger limes has produced a high quality product that has shelf stability of up to 3 months chilled, without the requirement for the addition of preservatives. Being able to produce a fresh ready-to-use finger lime product range with extended shelf life, will allow Sunfresh to penetrate Asian export markets.

The development of a range of unique finger lime products will allow Sunfresh and Finger Lime growers to develop new export and domestic markets in the retail and food service sectors, providing a range of product variants and market positioning opportunities.

TECHNICAL SUMMARY

The objective of this project was to develop processes for producing unique value added finger lime products with extended shelf life to enable export into premium retail and food service markets in Asia.

Sunfresh, with the assistance of CSIRO have developed processes to produce i) freeze dried finger lime pearls for the food service market and ii) high pressure processed ready-to-use finger lime pearls for the retail and food service sectors.

The outcomes of this project will be beneficial to the fledgling finger lime industry and not just Sunfresh Marketing Co-op. The majority of fruit produced is currently either sold fresh at local markets or exported as whole frozen fruit.

Presently most commercial plantings of finger limes are confined to northern NSW and southern Queensland, with a few plantings dotted along the coast as far south as Sydney. Most domestic fruit is sold in the central fruit and vegetable markets in Brisbane, Sydney and Melbourne. Small quantities of fruit are also supplied direct to retailers and restaurants, particularly in northern NSW and south-eastern Queensland where most of the fruit is currently grown. Around 50% of Australian finger lime production is currently exported to markets in Europe and Asia. In 2008/09 total production from Australia was estimated to be around 10 tonnes.

Sunfresh has a member base of over 160 growers of tropical fruit such as avocado and limes across Queensland, Northern New South Wales and Western Australia. Many of these growers also have an orchard of native finger limes so Sunfresh have a well-established supplier network. Following market penetration by Sunfresh in South East Asia, the wider Australian industry will have access to adopt the learnings from this project. Success will provide opportunities for other finger limes growers to leverage off the market awareness of finger limes and their uses in premium Asian markets.

INTRODUCTION

Currently in Australia, finger limes are usually sold as fresh or frozen whole fruit. Sunfresh have identified an opportunity to capture the growing interest of Restaurant Chefs and food service in the finger lime fruit by producing a range of freeze dried and fresh finger lime products. This range would consist of different varieties of fruit, utilising ‘pearls’ or skin slices that are produced through freeze drying or high pressure processing.

The Finger lime (Citrus australasica F.Muell) is a rare rainforest tree originally from the Eastern coast of Australia. At present most commercial plantings of finger limes are in northern NSW and southern Queensland with a few plantings along the coast as far south as Sydney. Finger lime cultivars are produced commercially and protected with Plant Breeder Rights.

Figure 1. Finger Lime varieties vary in size and color.

The fruit from these native trees has been used as a food source by the Australian indigenous people for thousands of generations. The finger lime fruit has a range of stunning skin and pulp colours; the flesh is caviar like in appearance with a unique lime flavour. Because the fruit is so similar in appearance to fish eggs it is often referred to as ‘lime caviar’, ‘caviar lime’ or ‘citrus caviar’. Sunfresh plan to market their product as ‘Finger Lime Pearls’.

Figure 2. Native finger lime tree.

At present most interest and demand for finger limes is from food service and the restaurant trade. The promotion of finger limes as a commodity has been undertaken by Sunfresh at national and international food fairs where it received exposure from promotion by celebrity chefs and has appeared in travel and food magazines.

Currently both domestic and export market prices are very good largely because volumes are still small and demand is outweighing supply. Returns to growers for export quality fresh fruit are between $40-60/kg and $25-40/kg on the domestic fresh fruit market. The potential for freeze dried Finger lime is exemplified by one customer from Sweden, an import company specialising in teas and spices seeking an initial order of 250kg of dried fruit cubes and strips for an identified market opportunity. This one customer has indicated follow up orders of this size will be required.

Dried finger lime has a range of uses that surpasses the capacity of the fresh market to absorb all that is grown. When promoted in either a Chain store or at farmers markets the response is similar with demand increasing with awareness. Freeze dried product provides an appropriate shelf life to allow penetration into countries that currently have phytosanitary restrictions on fresh product.

Existing companies supplying finger lime fruit are Australian Finger Lime Company, Finger Limeing Good Pty Ltd, Wild Finger Lime and Sunfresh. These companies currently only sell whole fresh fruit or frozen semi processed fruit for domestic food service as well as for export, so there are no competitors in the market place at this time.

Sunfresh have identified an opportunity to export this unique finger lime pearl product to consumers through retailers in premium Asian markets. Sunfresh, as a co-operative operation has the experience and capability to draw the finger lime growers together to supply export markets with centrally processed fruit. As Sunfresh begins to achieve market penetration, other growers, food production or processing companies will also be able to leverage of Sunfresh’s export activities to also enter the market. Sunfresh will maintain its competitive advantage as market leader through a continuous innovation and product improvement strategy.

Development of freeze dried and high pressure processed finger limes enables Sunfresh to increase crop utilisation through processing fruit that is outside the quality specifications for the fresh market, while still providing a premium versatile and unique product that maintains its quality attributes with extended shelf life.

Freeze drying, uses both a vacuum and a freezing process to remove water from perishable foods. The result is a product that can be stored at room temperature for years without spoilage, or packed in limited storage spaces and reconstituted with water later. A freeze dried product is ideal for export as it negates the use of cold chain distribution and the need to transport water contained within a food product. Freeze drying is an ideal drying technology for finger limes as the process has the potential to have minimal effect on the products color, flavour and aroma, the key attributes differentiating this fruit.

High pressure processing (HPP) is a way to process foods to destroy microbial contaminants without using heat. It has the potential to produce high quality, fresh, nutritious, ready-to-eat foods without using chemical preservatives. HPP involves subjecting food to 300 to 700 MPa of pressure. For comparison, atmospheric pressure is normally around 0.1 MPa and the pressure at deepest point in the ocean is around 110 MPa. The high pressure used in HPP kills most micro-organisms, by damaging cell components such as cell membranes.HPP products have the greatest potential to achieve consumer acceptance and market penetration because of their fresh eating quality an extended shelf life.

Sunfresh Marketing Co-op is a Primary Industry Co-operative that was established in July 1995 as grower/packer organisation to market avocados and other sub-tropical fruit for growers in the South east of Queensland. The Cooperative negotiates and completes the sale and supply, by variety, size, quality and quantity to the buyer on behalf of its member growers. For over ten years the company has demonstrated its commitment to develop and grow exports by establishing strong international partnerships in Singapore, Thailand and Hong Kong exporting to these countries.

The research challenges that Sunfresh overcame were the development of pretreatment and processing protocols to produce a freeze dried product and a ready-to-use retail HPP product. This involved investigating the effect of varying operating parameters of the processing technologies and developing processing protocols that provide effective processes for freeze drying and HPP processing.

The research program investigated;

- Ideal fruit variety and optimum quality suitable for processing,
- Pre-treatment requirements,
- Processing parameters,
- Packaging requirements,
- Product performance and functionality,
- Shelf life and
- Consumer and market validation.

METHODOLOGY

Sunfresh worked with Dr Mala Gamage and the team from CSIRO Animal, Food and Health Sciences to develop pretreatments and protocols for processing both skin and flesh of finger limes using freeze drying technology and high pressure processing. CSIRO also assisted with the development of appropriate packaging material and formats for the finished product along with preliminary evaluation of shelf life, product performance, and quality attributes such as taste, texture and appearance; of both freeze dried and high pressure processed products.

The project was conducted in two distinct stages: Stage 1 freeze drying and Stage 2 high pressure processing.

Stage 1: Freeze Drying.

The methodology for development of the freeze drying process is detailed below.

Objective 1: Development of a laboratory scale process for freeze drying finger limes.

1. Literature and patent review

   CSIRO undertook a literature and patent review to obtain background information on pre-treatments and formulations for freeze drying of citrus products to develop and select protocols for trials. This review will determine if Sunfresh has freedom to operate with this opportunity. A review of the bioactives within finger lime was also be undertaken.

2. Proof of concept

   Proof of concept trials with three products were undertaken by CSIRO's Dr Mala Gamage. This activity was to identify preparation requirements including pre-treatment of fruit and peel, formulation of products for product stability and increasing surface area.

3. Trial sample quality evaluation

   CSIRO and Sunfresh will undertake preliminary evaluation of the trial samples to assess shelf life, rehydration performance, taste, texture, moisture content and appearance of freeze dried products.

   Preliminary investigation of packaging requirements.

   A report detailing the recommended pre-treatments, formulations, processing protocols and packaging requirements was produced. The report also included the findings from the sample evaluation of quality and functionality.

Objective 2: Development of pilot scale freeze drying process

4. Pilot scale fruit freeze drying trials and economic assessment.

   Sunfresh worked with CSIRO to develop the preparation and freeze drying process at pilot scale. This will enable an assessment of the commercial requirements for the process.

   During this project phase process parameter data allowed for equipment sizing and process layout design, as inputs to development of cost models. Sunfresh will undertaking an assessment of cost structures and return on investment to ensure the opportunity creates value for the business.

As the project proceeded, Sunfresh Marketing Co-op had an opportunity to undertaken a preliminary investigation of High Pressure Processing of the Finger Lime vesicles that make up the fruit pulp. This investigation was conducted with CSIRO Food & Nutritional Science at Werribee. The resulting product from this preliminary investigation had outstanding quality, in particular the products appearance, taste and colour.

Sunfresh had also recognised that a 'fresh' or minimally processed finger lime pearl product would have greater appeal to the market and offer a wider range of applications in meal formats, than a freeze dried product. The majority of interest and demand for finger limes from Sunfresh has been from the restaurant trade. Currently both domestic and export market prices for fresh fruit are very good largely because volumes are still small and demand is outweighing supply.

While freeze dried finger lime products potentially have a greater range of uses and market opportunities than fresh product, its utilisation will be restricted due to the 'none fresh' product outcome. Consumers are willing to pay a premium for fresh, in particular in Export markets. A high pressure processed product has the greatest potential to achieve consumer acceptance and market penetration because of the fresh eating quality together with an extended shelf life. Existing companies supplying finger lime fruit such as Australian Finger Lime Company, Finger Limeing Good Pty Ltd, Wild Finger Lime and Sunfresh would be able to achieve greater crop utilisation and market acceptance because of the competitive advantages achievable with the HPP technology.

With support from Horticulture Australia through a project variation, Sunfresh Marketing Co-op continued to work with Dr Mala Gamage and the team from CSIRO to undertake a research and development program to determine the process and protocols for HPP processed finger lime pearl products for the retail and food service market. HPP finger limes provide the greatest opportunity for market penetration due to greater customer acceptance of a minimally processed product with fresh fruit characteristics. HPP finger lime product will be chilled rather than frozen, providing improved market positioning opportunities.

Stage 2: High Pressure Processing.

The methodology for development of the high pressure processing treatment is detailed below;

Objective 1: Preliminary evaluation of High Pressure Processing (HPP) Finger limes.

> CSIRO conducted preliminary HPP trials to determine HACCP procedures and the required pre-treatment and processing protocols for high pressure processing (HPP) of finger lime pearl products. CSIRO also undertook preliminary evaluation of shelf life, taste, texture and appearance of this ready-to-use product. Undertake modelling of HPP processing of finger lime pearls to determine commercial feasibility.

Objective 2: Economic modelling of HPP process.

> Sunfresh together with CSIRO undertook the economic modelling of the HPP technology to evaluate the financial viability of the process and to provide data to guide the future selection of equipment.

Objective 3: Development of pilot scale HPP process.

> CSIRO undertook pilot scale HPP trials to determine the optimum processing protocols and packaging for fresh or minimally processed finger lime pearl products. This research involved conducting microbiological challenge testing along with standard shelf life testing and product quality assessment.

Objective 4: Product validation.

> This research objective involved the measurement and validation of product quality outcomes during trial 'runs' through the cold chain distribution systems. Results from the evaluation of prototype product performance through the cold chain provides feedback to guide changes in processing procedures and parameters at manufacture.
>
> Modifications to the process would also be made based upon initial consumer feedback from this validation work. Product validation is the final R&D step involving process modifications to ensure finished product withstands distribution conditions and meets consumer acceptance in-market.

RESULTS AND DISCUSSION

Stage 1: Freeze Drying Process.

CSIRO Animal, Food and Health Sciences have successfully completed this research project which involved the development of procedures, pre-treatments and formulations for freeze drying finger lime fruit and peel. Some preliminary evaluation of trial samples has been undertaken by both Sunfresh and CSIRO, to assess rehydration performance, taste, texture, moisture content and appearance of products. The freeze drying process that has been developed has produced a dried finger lime product that maintains its key attribute of flavour and is shelf stable.

Literature Review

The initial activity conducted in this project was a literature review of the finger lime fruit, in particular its bioactives and finger lime processing techniques. The literature search (patents and scientific publications) was conducted using ISI web of Knowledge and Food Science and Technology abstracts. The Literature Review conducted by CSIRO has been included at the end of this report as Attachment B: Freeze drying and bio-actives of finger limes (Citrus australasica F.Muell; Rutaceae). 28 December 2011.

The bioactives of finger limes have been widely studied and reported in literature. According to the literature, finger limes contains both hydrophilic and lipophilic bio actives. Total phenolics of the hydrophilic fraction are lower in finger limes compared to blue berries (cv. Biloxi). Gallic acid, chlorogenic acid and cyaniding 3-glucosed are among bioactives of the hydrophilic fraction while vitamin E and Lutein were the predominant bioactives in the lipophilic fraction. Finger limes are rich in ascorbic and citric acids. The antioxidant activity of the hydrophilic fraction (ORAC_H) is lower than that of the blue berries but the antioxidant activity of the lipophilic fraction (ORAC_L) is comparable to blue berries and avocado. The ORAC_H contribution towards the total antioxidant activity of finger lime is higher (> 3 folds) than the ORAC_L contribution. Finger limes are considered as a high value product due to their seasonal production, perishable nature and the current market demand.

Freeze Drying Process

Although the literature review and patent search did not identify specific patents or research articles on freeze drying of finger limes, research publications and patents on the pre-treatments and freeze drying of citrus products were collated and summarised to assist with the experimental design. Based on the findings of the literature review, hot water blanching and ultrasound were selected by CSIRO's Dr Mala Gamage as pre-treatments for evaluation during the project. These treatments were selected specifically in an attempt to reduce the oil content in the peel in order to reduce the bitterness of the finished freeze dried products.

Finger limes have a very short production season and a preservation technology is needed to prolong the availability of finger limes throughout the year. Finger limes have a very exotic colour, flavour and aroma and the preservation of these qualities are essential for extending the shelf life. Freeze drying has been identified as a potential method of preservation by the Sunfresh Marketing Co-Operative based on the shelf stable nature of freeze dried products since it is generally used for dehydration of heat sensitive high value plant materials. Hence, this study was conducted to evaluate the potential of using freeze drying as preservation method for finger limes.

CSIRO and Sunfresh determined a process for the freeze drying of finger limes based on the literature and preliminary exploratory investigations. The proposed process is a blanching treatment at 90°C for 5 min and slicing of the blanched limes to obtain freeze dried products with moisture contents at or below 4% and water activity at or below 0.2. The schematic representation of the proposed freeze drying processing procedure is illustrated in Figure 3 Proposed freeze drying process.

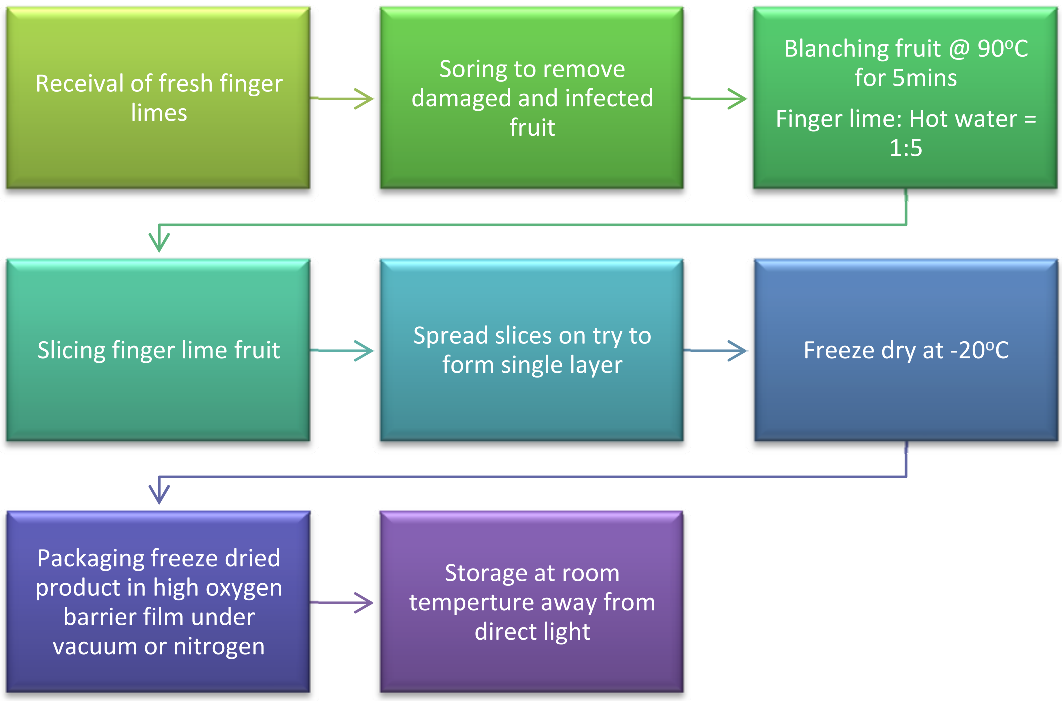


Figure 3: Proposed freeze drying process

Pre-treatment

Blanching (70°, 80° and 90°C for 30 sec – 7 min) and Blanching + ultrasound (40 kHz + 400 kHz for 3 min) pre-treatments were evaluated using fresh and frozen limes. Blanching and ultrasound pre-treatments were used in an attempt to remove oil from the finger lime peel. Pre-treated limes were freeze dried at a commercial freeze drying facility as whole finger limes or slices. Dehydrated limes were analysed for moisture content, water activity, rehydration ratio and oil content. Demonstration samples were also provided to Sunfresh for further long-term evaluation.

The dehydration time for pre-treated whole finger limes was ~71 hours and the freeze dried products were unevenly dried and effects of pre-treatments were not visible. Slicing of pre-treated finger limes enhanced the dehydration process and the dehydration time ranged between 47 – 51 hours. The sliced products were evenly dehydrated and the effects of pre-treatments were visible.

With the increase of the blanching temperature and blanching time the moisture content and the water activity of the freeze dried samples decreased to or below the critical levels (of 4% and 0.2 respectively) that determines the product stability during storage. Freeze dried products with a final moisture content of 3.3 - 4% and a water activity of 0.18 - 0.2 were obtained by blanching finger limes at 90°C for 5 - 7 min. The final moisture content and the water activity of the corresponding untreated samples were in the range of 8.0 to 8.9% respectively.

Blanching + Ultrasound pre-treatments were not effective in reducing the moisture content and water activity of freeze dried products compared to the blanching treatment alone.

Rehydration

Rehydration ratio of the freeze dried limes was not influenced by the blanching pre-treatments. The rehydration ratio of freeze dried products made from fresh finger limes (5.2 - 5.5 g/g) were higher than that of the products made from frozen raw material (3.5 g/g). However, this needs to be confirmed by further commercial trials. Freeze dried finger limes (made out of fresh raw material) rehydrated to a moisture content of 76% after 24 hours of rehydration and this was close to the initial moisture content of fresh limes which was 81%.

During dehydration soluble material was released from the freeze dried finger lime slices and this acidified and increased the total soluble solid content of the rehydration liquid. The colour of the rehydration liquid was also changed due to the solubilisation of pigments such as anthocyanins.

The rehydrated freeze dried products possessed the characteristic aroma and colour of finger limes. The colour of the finger limes peel changed from green to red after subjecting to blanching. The colour of the finger lime pulp was also intensified by the blanching treatment in most of the experiments.
Oil content of the freeze dried finger limes was variable and showed no relationship to the applied blanching conditions or to the final moisture content of the samples. In this study the bitterness of the final products was not assessed and correlated with the oil content of the final product, however, it is important to evaluate bitterness as a quality parameter to assess and correlate with the oil content in future studies.

Both fresh and frozen limes could be used as raw material for the production of freeze dried finger limes. It is recommended that if the freeze drying process is to be commercialised then further commercial trials should be conducted to confirm suitable blanching conditions for frozen finger limes.

Pre-treatment of finger limes at 90°C for 5 mins was essential for the reduction of moisture content and water activity of the freeze dried material. The application of ultrasound treatment after blanching showed no added benefit to the freeze drying process of finger lime. The slicing of blanched finger limes enhanced the removal of moisture during freeze drying and reduced the drying time by ~ 20 hours and enabled the production of freeze dried material with a low moisture level and water activity.

Sunfresh and CSIRO investigated blanching and ultrasound as pre-treatments. Blanching and/ or ultrasound pre-treatments were not effective in reducing the oil content of the dehydrated material. The oil content of freeze dried material showed no relationship with the final moisture content or the water activity. Blanching pre-treatments improved the colour of freeze dried material by changing the peel colour from green to red. The pulp colour was also intensified by the Blanching treatment.

Both fresh and frozen limes could be used as raw material for the production of freeze dried finger limes.

Figure 4a: Results of freeze dried finger lime slices

Figure 4b: Results of freeze dried finger lime slices.

Generally, freeze dried products with low water activities (0.2 – 0.4) could be shelf stable for 12 – 24 months when stored at low oxygen levels (in packaging with high oxygen barrier properties under vacuum). Freeze drying could be considered as a good method of preserving finger limes, if the cost of freeze drying is not a limiting factor.

Stage 2: High Pressure Processing (HPP)

Sunfresh reviewed the export market opportunity for finger limes and came to the conclusion that a fresh ready-to-use retail product format was more likely to achieve greater consumer acceptance and so achieve greater market penetration. A ‘fresh’ or minimally processed finger lime pearl product will have greater appeal to the consumer than a freeze dried product. The use of HPP technology allows Sunfresh to achieve a ready-to-use finger lime pearl products with optimum fresh like eating quality while maximising shelf life. This type of finger lime product is new and novel and has not been produced anywhere in the world. Sunfresh Marketing Co-op undertook preliminary investigation of High Pressure Processing (HPP) technology; which resulted in a product with outstanding quality attributes, in particular appearance, taste and colour. The change in research direction to high pressure processing would deliver a more commercially viable product and provide market sustainability through the competitive advantage offered by the technology.

Figure 5: CSIRO's High Pressure Processing pilot plant.

HPP Process

CSIRO and Sunfresh developed a process for the HPP processing of finger limes based on the literature and preliminary exploratory investigations. The process involved subjecting the finger lime fruit to a chlorine dip to destroy any fungi and microbiological contamination, prior to extraction of the pearls from the skin. Finger limes naturally contain high levels of oil within the skin of the fruit. This oil component of the fruit is bitter in taste and quickly undergoes oxidative rancidity. Extraction of pearls and pulp has to be undertaken in such a way as to ensure minimal oil is included with the pulp.

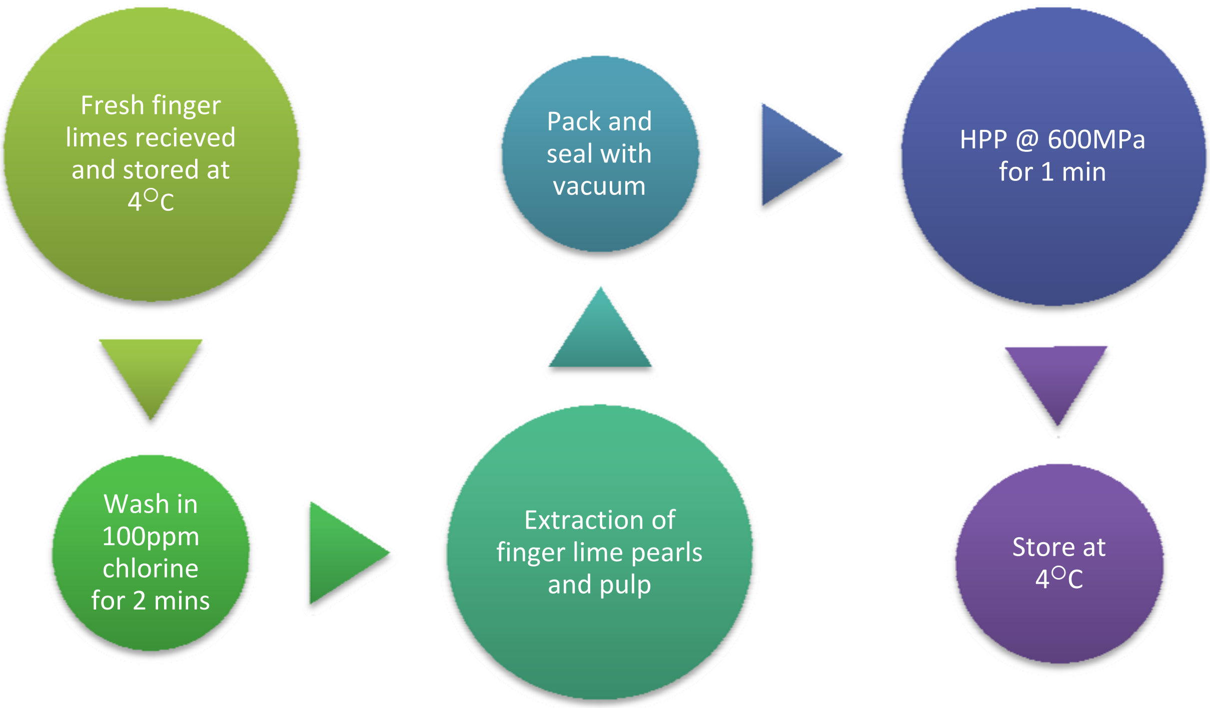


Figure 6: High pressure processing procedure.

Figure 7. CSIRO Pilot Plant facilities.

Figure 7 shows the pilot Plant facilities and the HPP Unit used throughout the project and Figure 8 shows the work in preparing samples for the HPP trials.

Figure 8. Preparing finger limes for high pressure processing trials.

HPP Research Outcomes

- HPP processing did not affect the integrity of juice vesicles on day zero and juice vesicles remain intact after storage at 4 to 8C for 10 days. HPP finger lime pulp can be stored at 4°C for 90 days (3 months) with minimal changes to the sensory quality and microbial counts.
- High pressure processing can be used for shelf life extension of finger lime pulp/pearls. The storage of HPP treated finger lime pearls at 4°C is essential for maintaining the product quality over a period of 3 months.

- Packaging of juice vesicles or the pulp in juice was not as appealing when compared to the pulp packed with no juice.
- The colour of red finger lime pulp was slightly intensified by the HPP treatment while the colour of green finger lime pulp turned olive green after HPP.
- The microbiological challenge tests demonstrated that the HPP treatment of finger lime pearls/pulp at 600 MPa for 1 to 5 minutes @ 4°C was able reduce the microbial counts of the products below detection limits. The microbiological challenge testing has proven that HPP treatment is very effective in inactivating pathogenic (*E. coli, Lactobacillus plantarum)* and spoilage (*Saccharomyces cerevisiae)* organism counts by 5 log cfu/g.
- Storage at 8°C affected the quality parameters after 30 days of storage. However, the microbial counts remained unchanged at 8°C storage temperature.
- Colour degradation at 4°C storage could be minimised by applying full vacuum (- 0.9 bar) to the product at the stage of packaging.

Figure 9. High pressure processed finger lime pearls samples.

Economic modelling

CSIRO undertook preliminary modelling of HPP operating and capital costs. This was based on estimated HPP operational throughput scenarios provided by Sunfresh Marketing Co-op along with other confidential proprietary production and operating information that CSIRO has access to.

Production variables included capital costs, interest rate for capital costs, depreciation period of capital costs, labour costs, days of production per annum, process temperature, process pressure, vessel size, package size, product density, power demand and efficiency factors of pumps etc, costs for electrical energy, water, steam, and maintenance. CSIRO provided HPP specific information such as operating conditions including cycle time, compression times, process temperature and pressure, filling efficiency of the vessel, water and electrical energy consumption, steam consumption, product viscosity and product density, etc.

CSIRO evaluated the dependencies and impact of those variables on the process costs and thereby developed specific cost models for HPP on one specific HPP product format. The financial viability evaluation also included a sensitivity analysis to identify possibilities to further minimize the estimated processing costs and/or estimated packaging efficiencies.

Sunfresh Marketing Co-op have been able to evaluate the operating and capital cost estimations provided by CSIRO to determine the potential financial investment viability (ie. ROI and IRR) for high pressure processing of finger limes.

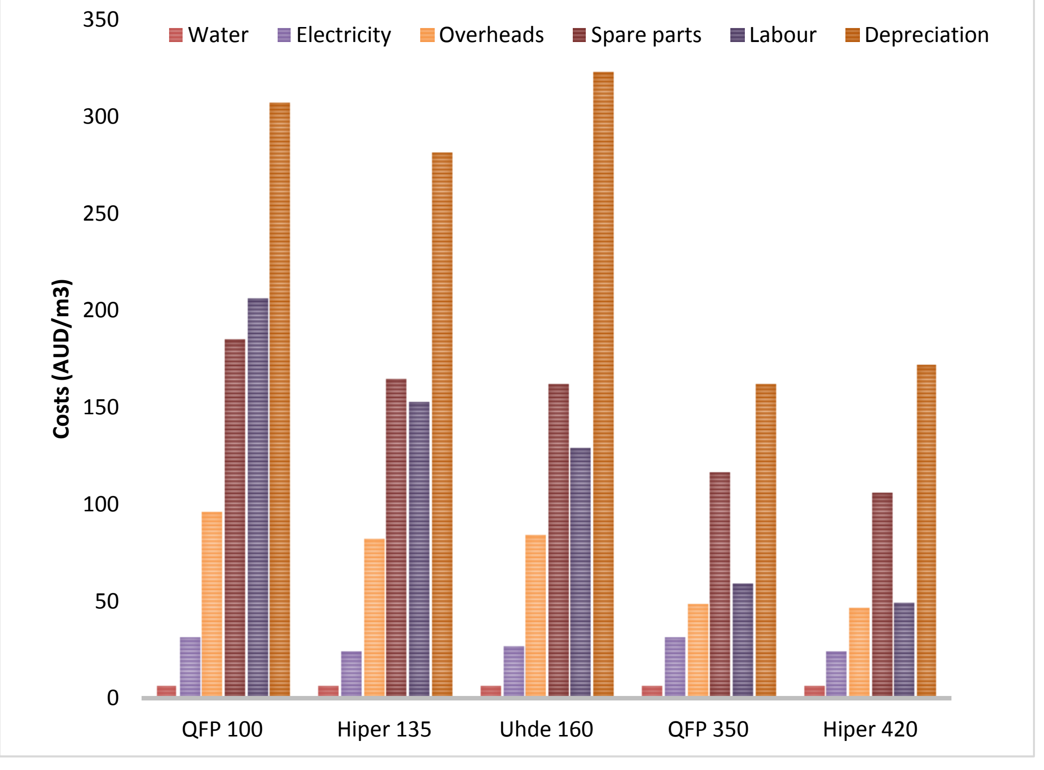


Figure 10: Operating cost comparison of HPP machines.

Attachment A: HPP Cost Calculations, provides a breakdown of the variables used in the financial modelling along with a detailed summary of the modelling results for each HPP Brand and Model that Sunfresh could purchase or lease.

The results of the financial modelling indicated that the purchase of a high pressure processing unit was not economical viable unless the capital equipment could be fully utilised. Given that the finger lime growing season is short (~ 4months) and there is limited production at this stage due to the infancy of the industry and its market, the allocation of capital towards a HPP unit cannot be justified unless other products can be processed and market opportunities developed.

Sunfresh have decided to investigate the option of undertaking contract high pressure processing with a third party and will develop potential value chain and business models required to capitalise on the opportunity for ready-to-use fresh finger lime pearls for retail markets in Asia. Currently there are a number of HPP units in operation in Australia that provide toll processing services; Longfresh (Sydney), Pressure Fresh Australia (Bundaberg), Simpson Farms (Childers), Preshafood Limited (Melbourne) and Moira Mac's (Bendigo).

Market Validation

Sunfresh worked with Ken Melia from FoMo (Fear of Missing Out) to undertake market validation of both freeze dried and high pressure processed finger lime products. Due to the short growing season for finger lime combined with limited supply availability it wasn't possible to undertake commercial production trials without waiting a further 5 months for the fruiting season to commence. So in order to undertake market validation work it was decided that Sunfresh would work with FoMo to simulate the cold chain in order to evaluate and validate the product stability and acceptability.

Preliminary market validation of freeze dried product was achieved by simulating the distribution conditions, which in the case of freeze dried product was achieved by storing vacuum packaged freeze dried finger lime pearls and skin products at room temperature for 12 months. Freeze dried product was sampled at 6 months and then again at 12 months to assess consumer acceptability of the product's quality attributes, colour, flavour and rehydration. The freeze dried finger lime product maintained all of its key quality attributes throughout the storage period, demonstrating that the process produces a 'robust' product.

Research samples of vacuum packaged, high pressure processed finger lime pearls were held under chilled conditions for up to 6 months, to simulate the cold chain to overseas markets. Again samples were periodically opened to evaluate the quality parameters of aroma, flavour colour, texture, mouth feel and microbiological stability. All the samples of chilled HPP finger lime products maintained acceptable levels of all quality attributes.

HPP finger lime product can be stored at 4°C for 90 days with minimal change in the products sensory attributes and microbial counts, while storage at 8°C produced some deterioration in the products quality parameters of colour, aroma and texture; but the product is still acceptable.

Ken Melia undertook some preliminary consumer validation by presenting the product to a sample group of consumers and facilitated discussions with them to understand their perceptions and expectations of a fresh ready-to-use retail finger lime product (HPP product). The results from this market validation work will be used to finalise the processing and packaging protocols to ensure when commercial trials are undertaken that the finished product will meet consumer expectations through repeat purchases. This ensures the greatest chance of commercialisation success.

CONCLUSIONS & RECOMMENDATIONS

This project has successfully developed commercially and economically viable procedures and processes for both freeze drying and high pressure processing of finger limes for domestic and export markets. The freeze dried products that have been developed are shelf stable, while the HPP product is a chilled product. Each product format provides a different competitive advantages. Freeze dried product doesn't require a cold chain distribution system to deliver it to the market, while on the other hand high pressure processed product although requiring a cold chain distribution system, has superior visual and organoleptic attributes.

Sunfresh's Board has decided that high pressure processing technology provides the greatest opportunity for Sunfresh's value adding strategy to deliver a range of finger lime products through greater consumer convenience, appeal, fresh like product and extended shelf life without the addition of preservatives.

Sunfresh have determined that until other unique product opportunities are identified, that HPP processing can provide a competitive advantage, then toll processing should be the commercialisation strategy for the finger lime product range. To ensure financial viability of the process and to maximise profitability of a range of ready-to-use fresh finger lime pearl products, it is essential to ensure maximum productivity through mechanisation of the process where possible. Any mechanisation of the extraction of pearls from the skin will need to ensure minimal inclusion of contaminants, such as seed and oil from the skin. Sunfresh will now undertake some research and development of a process for mechanical assistance in the removal of pearls from the finger lime.

Sunfresh will continue to develop a finger lime grower network for those producers wishing to become involved in value adding for export and retail, in addition to the fresh market. This will ensure supply continuity for premium export markets in Asia.

Figure 8: Oysters with added finger lime pearls.

APPENDIX A: FINANCIAL MODELLING

Currency	AUD	
Exchange rates	www.x-rates.com	
USD:EUR	1.298500	
USD:AUD	1.05058	
USD:JPY	0.012142	
General		
Electric power	0.174544	AUD/kWh
Water	1.9134	AUD/m³
Factory overheads	13	%*
Depreciation period	10	a
Interest rate per period	10	%/a

* of total cost per year

Production		
Process pressure	600	MPa
Starting temperature	20	°C
Days of production / a	290	d/a
Hours / d	16	h/d
Cycle time	8.0	min
Pressure come up time	3.0	min
Filling estimated	40	%
Package size	0.5	kg
Product density	1.10	kg/L

Manning	*No.*	*Costs / a*	
Engineer(s) per shift	0.1	70000	AUD/a
Worker(s) per shift	2	45000	AUD/a
Hours per shift	8		h

Manufacturer		**Hiperbaric**						**Avure Technologies**						**Multivac/Uhde**			**Kobelco**	
Unit Models	*Units*	**Hiper 55**	**Hiper 120**	**Hiper 135**	**Hiper 300**	**Hiper 300T**	**Hiper 420**	**QFP 35L**	**QFP 100L**	**QFP 215L**	**QFP 320L**	**QFP 350L**	**QFP 687L**	**HPHT 50L**	**160L**	**350L**	**CIP 130T**	**CIP 30L**
Capital costs	AUD	786,841	1,102,842	1,355,643	2,052,740	3,807,586	2,569,085	547,982	1,095,964	1,622,796	1,701,629	2,017,920	2,018,881	1,009,938	1,842,283	2,520,421	2,777,527	3,326,860
Size	L	55	120	135	300	600	420	0	100	215	320	350	687	50	160	350	260	30
Vessel inner diameter	mm	200	200	300	300	300	380	230	308	390	475	386	540	200	380	380	450	400
Weight	t	23	35	45	70	140	85	9	17.5	37	30	46	46	17	45	70	?	?
Production																		
Number of cycles / day	1/d	120	120	120	120	240	120	120	120	120	n/a	120	n/a	120	120	120	n/a	120
Number of cycles / year	1/a	34,800	34,800	34,800	34,800	69,600	34,800	34,800	34,800	34,800	n/a	34,800	n/a	34,800	34,800	34,800	n/a	34,800
Hourly production	L/h	123.75	270	303.75	675	1350	945	78.75	225	483.75	n/a	787.5	n/a	112.5	360	787.5	n/a	67.5
Hourly production	1/h	248	540	608	1,350	2,700	1,890	158	450	968	n/a	1,575	n/a	225	720	1,575	n/a	135
Yearly production	m³/a	574	1,253	1,409	3,132	6,264	4,385	365	1,044	2,245	n/a	3,654	n/a	522	1,670	3,654	n/a	313
Packs per year	1000/a	1,148	2,506	2,819	6,264	12,528	8,770	731	2,088	4,489	n/a	7,308	n/a	1,044	3,341	7,308	n/a	626
Yearly operating costs																		
Energy	AUD/a	12,535	27,350	30,769	68,375	273,500	95,725	10,370	29,629	63,703	n/a	103,702	n/a	12,535	40,113	87,748	n/a	6,838
Water	AUD/a	2,942	6,419	7,221	16,047	32,094	22,466	1,872	5,349	11,500	n/a	18,722	n/a	2,675	8,558	18,722	n/a	1,398
Spare parts	AUD/a	121,800	139,200	208,800	313,200	626,400	417,600	104,400	174,000	208,800	n/a	382,800	n/a	104,400	243,600	348,000	n/a	69,600
Manning	AUD/a	194,000	194,000	194,000	194,000	194,000	194,000	194,000	194,000	194,000	n/a	194,000	n/a	194,000	194,000	194,000	n/a	194,000
Depreciation charge	AUD/a	207,567	290,927	357,615	541,508	1,004,432	677,718	144,556	289,113	428,090	n/a	532,322	n/a	266,419	485,990	664,881	n/a	877,617
Miscellaneous costs	AUD/a	70,050	85,526	103,793	147,307	276,955	182,976	59,176	89,972	117,792	n/a	160,101	n/a	75,404	126,394	170,736	n/a	149,429
Total	AUD/a	**608,894**	**743,422**	**902,198**	**1,280,437**	**2,407,381**	**1,590,485**	**514,375**	**782,063**	**1,023,885**	**n/a**	**1,391,647**	**n/a**	**655,433**	**1,098,656**	**1,484,086**	**n/a**	**1,298,882**
Treatment Costs																		
Cost/Pack	AUD/pack	0.5302	0.2967	0.3201	0.2044	0.1922	0.1814	0.7039	0.3746	0.2281	n/a	0.1904	n/a	0.6278	0.3289	0.2031	n/a	2.0736
Cost/Cycle	AUD/cycle	17.50	21.36	25.93	36.79	34.59	45.70	14.78	22.47	29.42	n/a	39.99	n/a	18.83	31.57	42.65	n/a	37.32
Cost/m³	AUD/m³	1060.42	593.41	640.13	408.82	384.32	362.73	1407.70	749.10	456.15	n/a	380.86	n/a	1255.62	657.72	406.15	n/a	4147.13
Cost/t	AUD/t	**964.02**	**539.46**	**581.94**	**371.66**	**349.38**	**329.75**	**1279.73**	**681.00**	**414.69**	**n/a**	**346.23**	**n/a**	**1141.47**	**597.93**	**369.23**	**n/a**	**3770.12**

QFP 320L only operational up to 400 MPa
QFP 687L only operational up to 320 MPa
CIP 130T only operational up to 400 MPa

Financial Model Variables

Hiperbaric 135 (Model only)

Days of production / a	290	d		
Hours / d	16	h/d		
Process pressure	600	MPa		
Starting temperature	20	°C		
Vessel size	135	L		
Number of vessels	1	U		
Cycle time	8	min	0.133333	h
Compression time	3	min	0.05	h
Filling degree	40	%		
Package size	0.5	kg		
Product density	1.1	kg/L		
Power (compression) required	101.310912	kW		

Capacity per cycle	0.054	m³/Cycle
Cycles / a	34800	1/a
Capacity / h	0.405	m³/h
Packs / h	810	units/h
Production / a	1879.2	m³/a
Packs / a	3758.4	1000/a
Cycles / d	120	1/d

Demand		
Electric power	93.8064	kWh/m³ product
Water	1.72140509	m³/m³ product

Funding costs				
Exchange rate	EUR:AUD	0.791138		
System (incl installation)	1251362.36	AUD	990000	EUR
No. extra pumps required	1			
Extra pump	94800.1788	AUD	75000	EUR
Accessories	9480.01788	AUD		EUR
Sum	1355642.56	AUD		

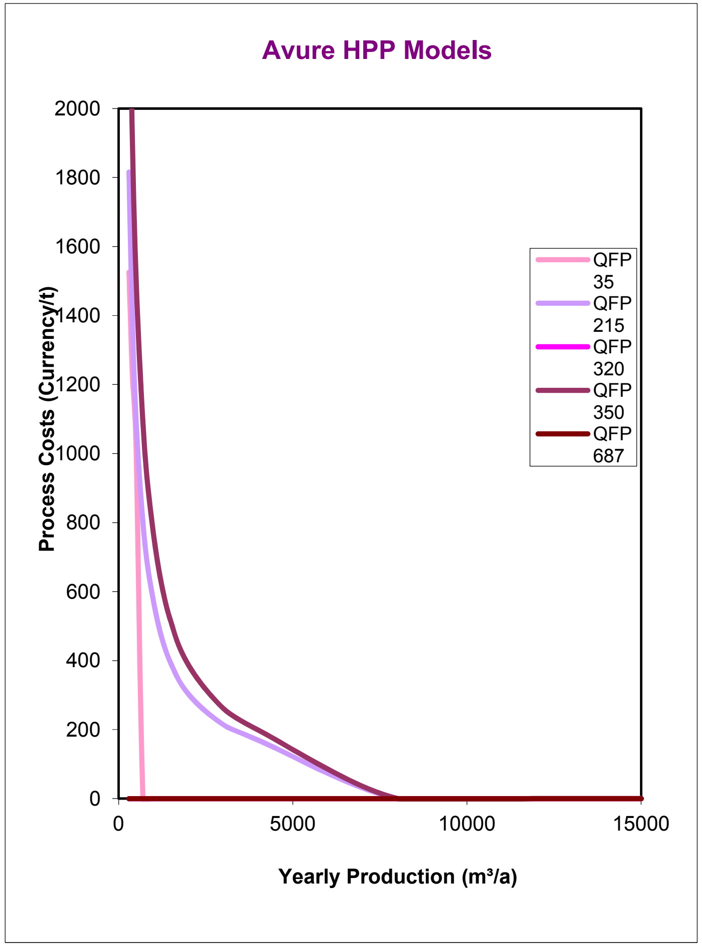
General expenses		
Electric power	0.174544	AUD/kWh
Water	1.9134	AUD/m³
Maintenance (spare parts)	2.50	AUD/cycle Factory
overheads	13	% of total cost per year
Depreciation period	10	a Interest rate per
period	10	%

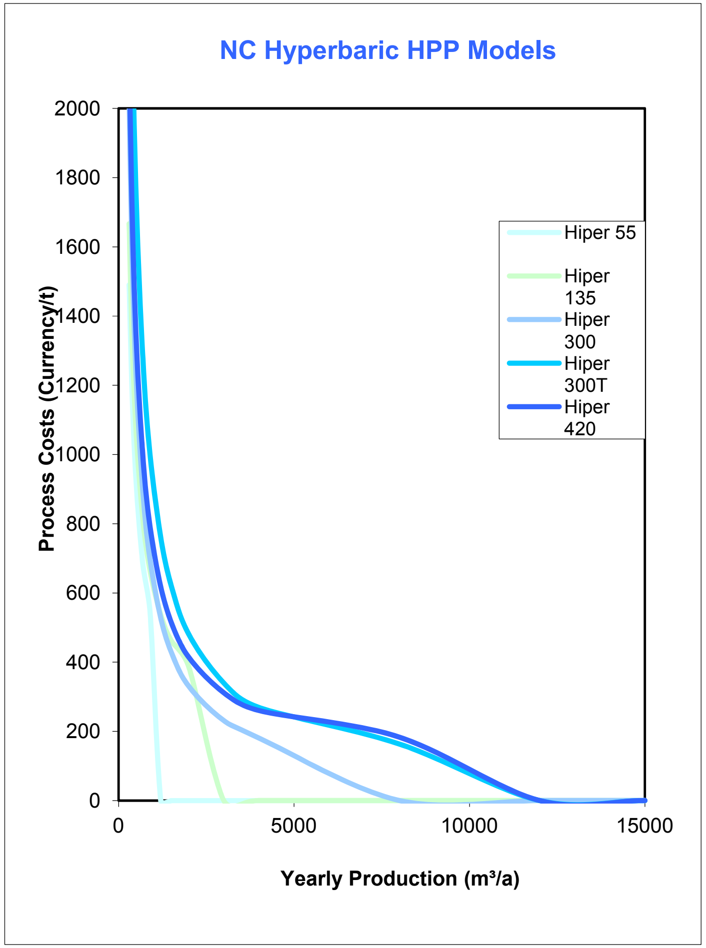
Manning					
Engineer(s)	0.1	per shift	Costs:	70000	AUD/a
Worker(s)	2	per shift	Costs:	45000	AUD/a
Number of shifts	2				
Overall number of workers	4.2				
Manning costs	194000	AUD/a			

Running Costs / a		
Electric power	30769	AUD/a
Water	6190	AUD/a
Spare parts	74289	AUD/a
Manning costs	194000	AUD/a
Depreciation charge	220625	AUD/a
Miscellaneous costs	68363	AUD/a
Sum	594236	AUD/a

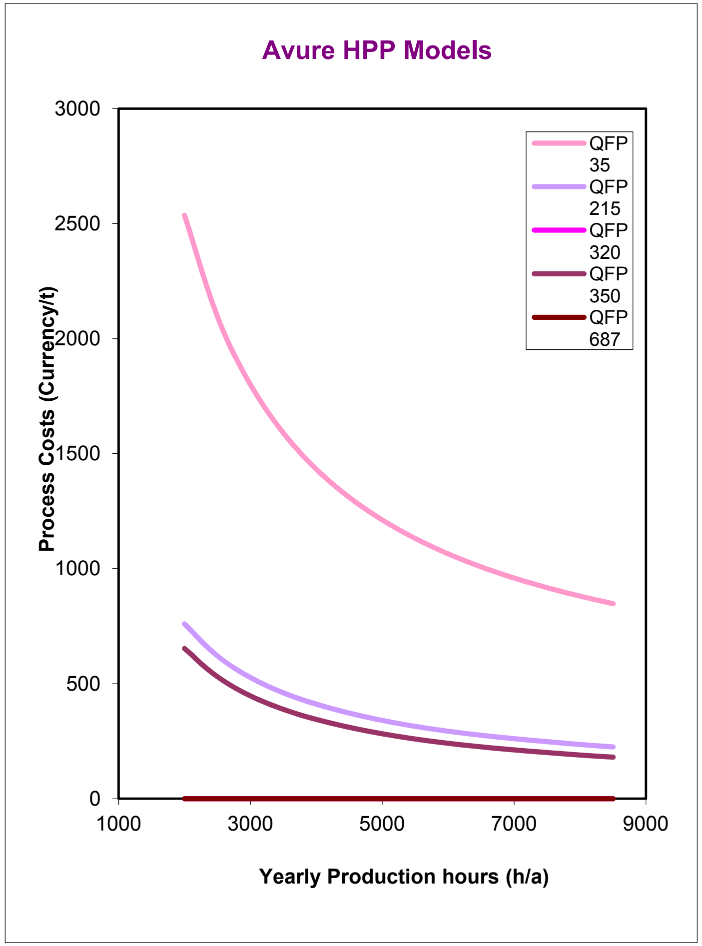
Cost per m³ product:	**316.22**	**AUD/m³**
Cost per tonne product:	**287.47**	**AUD/t Cost**
per cycle:	**17.076**	**AUD/cycle**
Cost per package:	**0.158**	**AUD/pack**

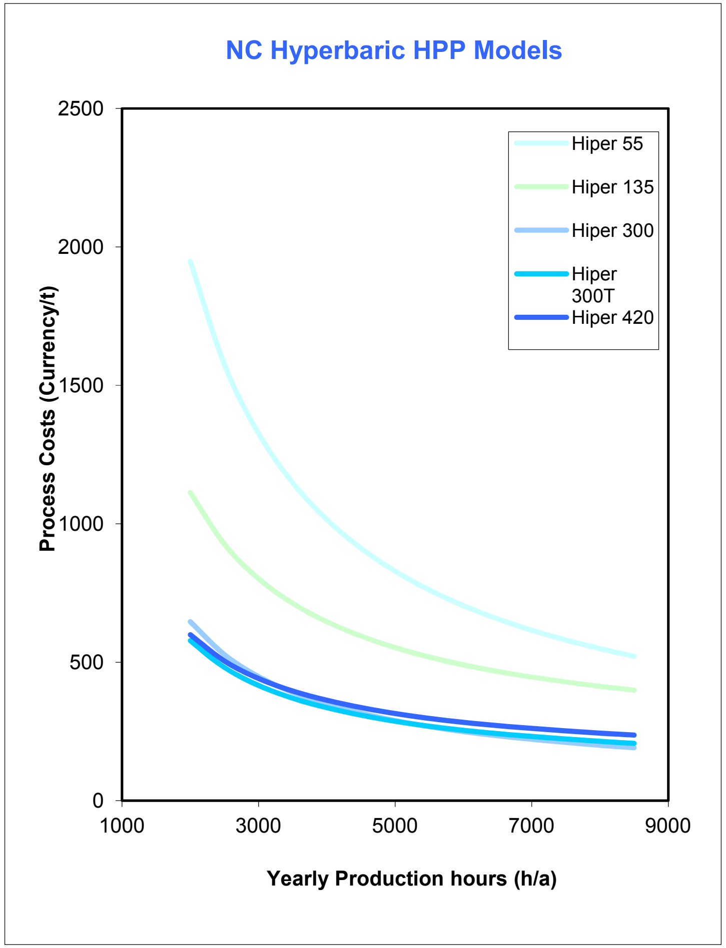
Yearly Production vs Process Costs





Year production hours vs Process Costs





ATTACHMENT B: HAZARD ANALYSIS AND FOOD SAFETY PLAN

High pressure processed finger lime pulp/pearls

PRODUCT DESCRIPTION

PRODUCT:	HPP finger lime pulp/pearls
DESCRIPTION:	Finger lime pulp treated with High Pressure at 600 MPa for 3 min at 4°C starting temperature.
INTENDED USE: **Human consumption or not**	The manufactured samples will be used for sensory testing – April – Aug 2013 Consumer feedback study – Feb 2014
TARGET POPULATION: **General Consumption Internal Taste Panel/ External Taste Panel** **Sensitive Population: Infant/Aged**	Trial product is for client and CAFHS staff – April – Aug 2013 General consumers - Feb 2014
GMO	None
ALLERGENS	Not aware
PACKAGING:	Amcor retort pouches
STORAGE CONDITIONS & DISTRIBUTION:	Storage at 4°C
INGREDIENTS USED	This recipe will include: - Fresh finger lime pulp/pearls
CHEMICAL AND NUTRITIONAL LABELLING:	Not applicable for trial
MICROBIOLOGICAL CRITERIA:	Compliance with CAFHS FRAT team specifications
INTENDED SHELF LIFE:	10 days at 4°C

For Human Consumption Ready to Eat product, in event of pathogen detection, it is the responsibility of the project leader to retrieve all product processed for proper disposal.

Authorised by: ____________________________ Date: ________________

Ref.No.:_______________

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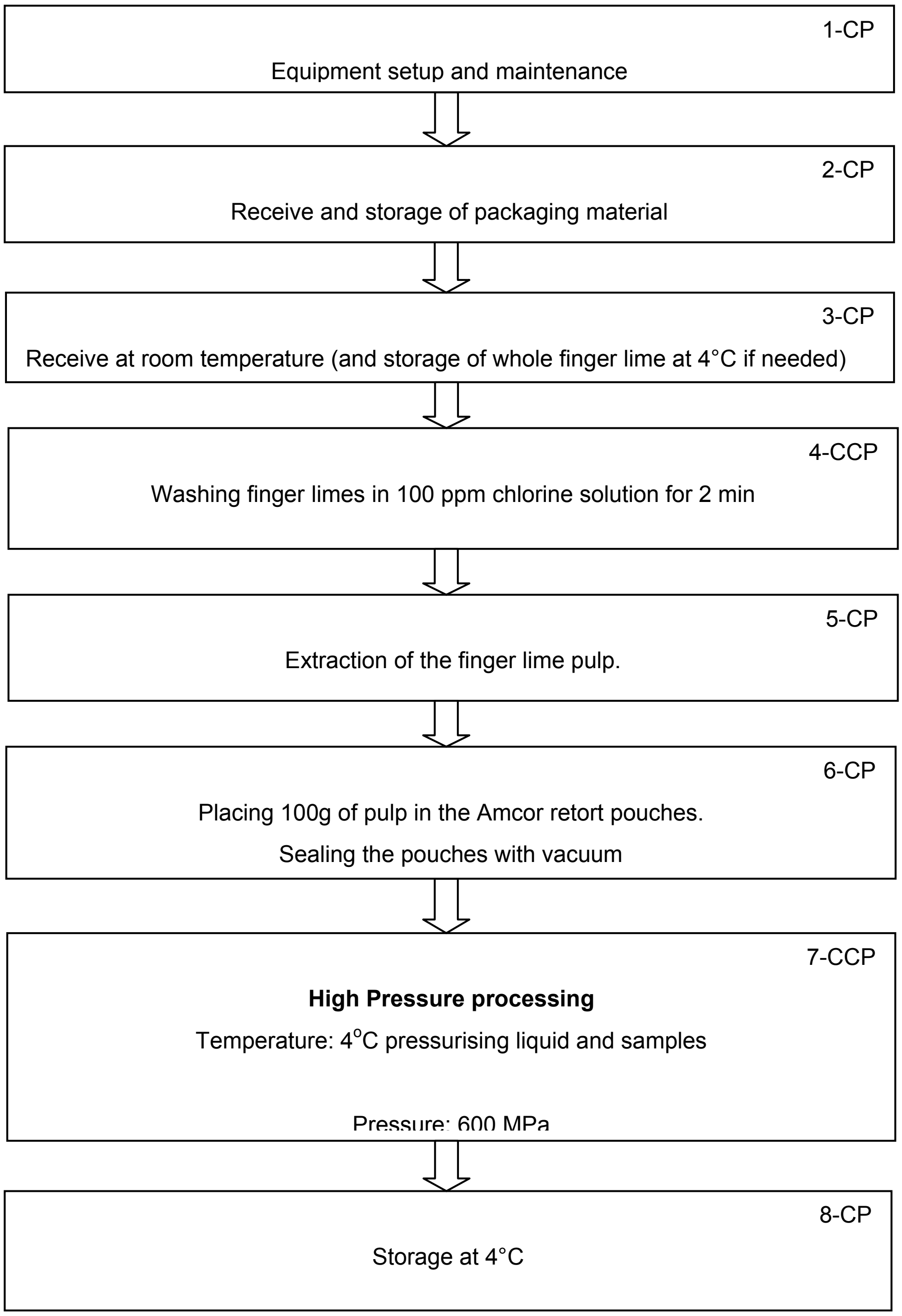
HPP Finger Lime – Process Diagram



Form 22

HACCP FOOD SAFETY PLAN

Product Description: **Trial production of high pressure treated finger lime pulp / pearls**

Issue Date: **13 – 14 March 2013 and Feb 2014**

Supersedes: **New**

Appendix:

TYPE OF HAZARD RISK (Likelihood) RESULT (Severity)

M – Microbiological	A – **High, likely to happen**	**1 – Critical, automatically results in unsafe product**
C – Chemical	B – Medium, could happen	2 – Serious, probably result in unsafe product
P – Physical	C – Low, not likely to happen	3 – Major, may result in unsafe product with potentially serious consequences
		4 – Minor, may result in unsafe product with no serious consequence
		5 – None, will not result in unsafe product

							Monitoring				
Step	**CP /CCP**	**Process/ Operation**	**Potential Hazard**	**Type of Hazard**	**Risk/ Result**	**Control Measure**	**Critical Limit**	**Inspection Frequency**	**Person Responsible**	**Record**	**Corrective Action**
1	CP	Equipment Setup / Maintenance	Contamination if equipment is not set up correctly or well maintained	P	C4	Correct setup and maintenance as per equipment SOP	Correct set up upand operation. Maintain in good order	Before the start of the trial	CSIRO Project Staff	Trial record data	Repair or replace equipment if not functioning correctly
2	CP	Receival and storage of packaging material	Contamination Damaged packaging	M C P	C4	Obtain from certified food-grade suppliers Store in clean & dry place Visual check on package integrity Absence of pest activity	Certificate of analysis conforms to specifications	Every delivery of packaging material materials	CSIRO Project Staff	Certificate of analysis Inward Goods Receipt- Log Sheet #1	Reject products

							Monitoring				
Step	**CP /CCP**	**Process/ Operation**	**Potential Hazard**	**Type of Hazard**	**Risk/ Result**	**Control Measure**	**Critical Limit**	**Inspection Frequency**	**Person Responsible**	**Record**	**Corrective Action**
3	CP	Receival and storage of finger limes	Contamination	M	C4	Obtain from certified suppliers. Finger lime is received and stored at less than 4°C	Certificate of analysis conforms to specifications Temp. < 4°C	Every delivery of finger lime	CSIRO Project Staff	Certificate of analysis. Record receival temperature on Log Sheet#1	Reject product
			Chemical contamination with insecticides and fungicides	C	C4	Obtain from certified finger lime supplier	Certificate of analysis conforms to specifications and levels are below the MRL's as specified in FSANZ Food Standards Code	Every delivery of finger lime	CSIRO Project Staff	Certificate of analysis	Reject product
4	CCP	Washing, Cleaning and Sanitisting finger lime	Microbiological contamination hazards	M	C3	Wash water temperature slightly warmer (5-10°C) than the produce to prevent water being sucked inside the fruit	Slightly warmer (5-10°C) than the lime finger	Every new batch washed	Project leader	Processing record sheet	Change to clean water when water is showing too much food debris
						Sanitise the washed finger lime with food grade 100 ppm (free) chlorine (sodium hypochloride is a choice) for 5 minutes	100 ppm (free) chlorine	Every new batch cleaned	Project leader	Processing record sheet	Re-sanitise

							Monitoring				
Step	CP /CCP	Process/ Operation	Potential Hazard	Type of Hazard	Risk/ Result	Control Measure	Critical Limit	Inspection Frequency	Person Responsible	Record	Corrective Action
5	CP	Preparation of finger lime pulp. Cleanliness of knives and cutting boards , plastic containers and measuring utensils	Contamination Chemical residues Foreign matter	M C P	C4	Equipment cleaning according to SOP & visual inspection before use ATP Swab	Cleaning completed Visually clean Swab count <100 RLU	Prior to usage	CSIRO Project Staff	Cleaning SOP Equipment Cleaning- Log Sheet #2	Use equipment only after being sanitized Repeat procedures if the swab counts > 100 RLU
6	CP	Placing finger lime pulp in Amcor pouches and sealing. Cleanliness of plastic containers and measuring utensils	Contamination Chemical residues Foreign matter	M C P	C4	Equipment cleaning according to SOP & visual inspection before use ATP Swab	Cleaning completed Visually clean Swab count <100 RLU	Prior to usage	CSIRO Project Staff	Cleaning SOP Equipment Cleaning- Log Sheet #2	Use equipment only after being sanitized Repeat procedures if the swab counts > 100 RLU

7	CCP	High Pressure treatment	Microbial pathogens not inactivated as intended	M	C1	Time/Pressure/Temp of HPP treatment	600 MPa for 3 min	Every HPP run	FPC certified HPP operator	HPP log sheet	Re- HPP if the run is not correct
8	CP	Cold storage	Contamination Chemical residues Foreign matter	M C P	C4	Equipment cleaning according to SOP & visual inspection before use ATP Swab	Cleaning completed Visually clean Swab count <100 RLU	Prior to usage	CSIRO Project Staff	Cleaning SOP Equipment Cleaning-Log Sheet #2	Use equipments only after being sanitized Repeat procedures if the swab counts > 100 RLU
9	CP	Microbiological testing and clearance	Microbial growth and spoilage	M	C4	Micro level testing as confirmation that the product meets specifications and if satisfactory product is released	5 samples from each batch tested for *Salmonella* (ND in 25 g), *L. monocytogenes* (ND in 25 g), *E. coli* (<3 MPN/g).	5 samples from each batch	Project leader	Micro level certification	If micro results are positive re-sample and re-test. If re-test samples are positive destroy all product from production run

INWARD GOODS RECEIPT

Log Sheet #1

Project:

Product Description	Supplier / Manufacturer	Batch no	Amount	Certification of Analysis / Specifications	Product was intact & suitable for use Temperature upon receival conformed to specification	Signed

EQUIPMENT CLEANING

Log Sheet #2

Project:

Equipments	Detergent	Cleaning regime	ATP Swab Count (100 RLU max.)	Visual inspection satisfactory	Signed

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OUTWARD GOODS RECORD

Log Sheet #3

Project:

Products	Packaging	Amount	Transport & storage conditions	Temperature during dispatch	Micro results satisfactory	Signed

ATTACHMENT C: LITERATURE REVIEW

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Freeze drying and bio-actives of finger limes

(*Citrus australasica* F.Muell; *Rutaceae)*

28 December 2011

Dr Mala Gamage

Sunfresh Marketing Co-op Limited.

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Contents

List of Tables

Executive Summary

Finger limes (*Citrus australasica* F.Muell; Rutaceae**)** are native to Australia and they have become very popular among chefs in recent years. Finger lime production is seasonal and it is necessary to develop a preservation technique to extend the shelf life to meet the demand during the off season. Freeze drying is considered as a suitable method of preservation, since it is generally used for dehydration of heat sensitive high value plant materials to preserve their natural properties and bioactives.

A literature and a patent search were conducted using ISI web of Knowledge and Food Science and Technology abstracts. Specific patents or research articles on freeze drying of finger limes were not retrieved. Literature on important bioactives of finger limes was available and was collated in this review. Research articles and patents on the pre-treatments and freeze drying of citrus products were summarised to assist with the experimental design. The literature search was further extended to incorporate the quality parameters of freeze dried foods to assist with quality assessment of the test products.

Finger limes contains both hydrophilic and lipophilic bioactives. In the hydrphylic bioactive fraction contained low levels of total phenolics compared to blue berries (cv. Biloxi). Finger limes were rich in ascorbic and citric acids. Gallic acid, chlorogenic acid and cyaniding 3-glucosed were among the predominant hydrophilic bioactives. Vitamin E and lutein were predominant in the lipophilic bioactive fraction.

The antioxidant activity of the hydrophilic fraction (ORAC_H) of finger limes was lower than that of the blue berries but the antioxidant activity of the lipophilic fraction (ORAC_L) was comparable to blue berries and avocado. The ORAC_H contribution towards the total antioxidant activity of finger lime was 3 folds higher than the ORAC_L contribution. The nutritional impact of finger lime may depend on the amount of finger lime caviar consumed at a given time. Generally finger limes are used in small quantities in salads, cocktails, desserts and as a garnish with seafood,

Reported pre-treatments of freeze dried citrus products included the application of calcium ions, heat and ultrasound. From the collated quality testing parameters, suitable tests will be identified to evaluate the effects of pre-treatments.

1 Introduction

Finger limes (*Citrus australasica* F.Muell; *Rutaceae)* are native to Australia and are well known for their caviar like juice vesicles, taste, flavour and the unique shape. They have a tart lemony lime taste and are used with seafood, salads, cocktails and desserts. . Finger limes are commercially grown in Australia and USA (Sfgate.com 2011). In recent years finger limes have become very popular among chefs

Citrus products contain many bio-actives. They mainly include antioxidants and micro elements (Konczak and Roulle 2011). The health promoting properties of processed fruit and vegetables are strongly related to their processing record such as storage, preparation and processing which have significant effects on the content of bioactive compounds (Hunter 2002).

Shelf life extension of citrus fruits is achieved through cold storage (<10°C) but this only allows an extension of shelf life by 6-20 weeks (Wills, Graham *et al.,* 2007). Citrus is preserved in the form of juice, frozen juice concentrates, pectin, peel, seed oil and squash (Salunkhe and Kadam 1995).

Dehydration is commonly used to improve the stability of food, since water activity is reduced and thereby reduce the microbiological activity and minimize the physical and chemical changes during storage (Mayor and Sereno 2004). Conventional drying processes could considerably change product characteristics. The dehydration of finger limes using conventional drying process is reported to result in a poor quality end product (personal communication with Sunfresh Marketing Co- Limited.).

Freeze drying is generally used for preserving heat sensitive high value plant materials. This is achieved through the removal of ice through sublimation and bound water molecules through desorption. Since finger lime has a good niche market and has a high value it is important to evaluate the use of freeze drying as a preservation technique. For these applications it is essential to preserve its natural properties and preserve the bioactives. Preserving finger lime by freeze drying will fulfil both requirements.

This literature review aims to identify the important bioactives in finger limes and suitable pre treatments, freeze drying conditions for finger limes. To assist with the future experimental stage, the literature search was extended to incorporate the quality parameters of freeze dried food.

2. Literature and patent Search stratergy

Literature search was conducted using Food Science and Technology Abstracts and Web of Knowledge data bases. Search terms used were finger lime, *Microcitrus australiasica*, freeze drying, freeze-drying, freeze dry*, freeze-dry*, limes, citru*, fruit, vegetable*, pre treatments and "pre-treatment for freeze drying". These terms were used in topic and title fields using boolean operator "AND". Time span ranged from 1969 – 2011.

Patent search was conducted on Derwent Innovation Index using search terms finger lime, *Microcitrus australiasica*, freeze drying, freeze-drying, freeze dry*, freeze-dry*, limes, citru*, fruit, vegetable*, pre treatments and "pre-treatment for freeze drying". Time span ranged from 1963 – 2011. Since very few patents were retrieved following codes were also used.

International Patent Classification code: A23B-007/02

Derwent Class Code: D13 (Other foodstuffs and treatment)

Derwent Manual Codes: D03-A04; D03-H01L

3 Finger lime

Finger lime (*Citrus australasica* F. Muell; *Rutaceae*) originated from the rainforests of southern Queensland and northern New South Wales. Fruits are oblong and are about 6–7 cm long and are 1 cm in diameter. Fruits are of different colours such as green, yellow, pink, red and purple. The globular juice vesicles of finger lime resemble caviar and are becoming popular as a gourmet food. The fruit is used for making juice, marmalade, pickle and dried products. Dried finger lime is used as a flavouring spice (Konczak *et al.,* 2010). Selected trees from the wild have been used to propagate commercially grown cultivars. Finger limes are cultivated at commercial scale in Australia and in the United States of America.

4.0 Bioactives

4.1 Bioactives of citrus

Fruits in general contain many bioactive compounds and micronutrients which have antioxidant properties. Bioactive compounds scavenge free radicals and are essential in the prevention of oxidative stress at the cellular level. Consumption of fruits is encouraged by medical and government authorities in order to improve health and well being of people.

Citrus fruits are well known for their vitamin C content, but are also good sources of vitamin A, thiamine, riboflavin, niacin, folic acid, and dietary fibre (pectin). Nutritional profiles of some citrus fruit and fresh juices are available at *http://www.enotes.com/food-encyclopedia/citrus-fruit*.

Citrus products contain many bio-actives such as flavanoids, limanoids, and caratinoids. They possess a variety of bioactive properties such as free radical scavenging, anti-lipo-peroxidation and metal ion chelation (Mahinda *et al.,* 2010); (Chen *et al.,* 2011).

Citrus antioxidants belong to lipid soluble and water soluble groups. Generally, the commonly consumed fruits are considered as poor sources of lipophilic antioxidants (0.3% - 3.1%) and as a result contribute less towards the total antioxidant activity of fruits (Konczak and Roulle 2011).

4.2 Bio actives of finger limes

Konczak *et al.* (2010) quantified the hydrophilic bioactives and the antioxidant capacity of the hydrophilic extracts obtained from commercially grown native Australian fruits including finger limes. This research group has also evaluated the lipophilic bioactive fraction and the mineral composition of commercially grown finger limes and other native Australian fruits (Konczak and Roulle 2011) .

4.2.1 Hydrophilic bio-actives

The hydrophilic bioactives of finger limes included the phenolic compounds, organic acids and vitamin C.

Total phenolics

Table 1 – Total phenolic content of hydrophilic fraction of antioxidants in finger limes grown in Australia.

Finger lime type	DW/FW ratio	Total phenolics (FC; µmol GAE/g FW)**	Source
Green	0.2090	6.8 ± 0.4	Konczak *et al.,* 2010)
Pink[1]	0.1880	9.2 ± 0.5	Konczak *et al*,. 2010
Red	ND	8.6 ± 0.59	Netzel *et al*., 2007
Yellow	ND	10.9 ± 0.48	Netzel *et al*., 2007

The data are means ± SD of 3 determinations. DW- Dry weight; FW- Fresh weight; **Results of total phenolic compounds (FC) corrected for vitamin C. FC – Folin- Ciocalteu assay. ND- Not detected.

The total phenolic content of pink and green finger lime ranged from 6.6 to 9.2 µmol GAE/g FW (Table 1) and was similar to red (8.6µmol GAE/g FW) and yellow (10.9µmol GAE/g FW) varieties evaluated in a separate study (Netzel *et al.,* 2007). Total phenolic content of blue berries (26.5 ± 0.6

μmol GAE/g FW) was higher than that of the finger limes. The most common fruits, such as cranberries (42.2 μmol GAE/g FW), blackberries (38.8 μmol GAE/g FW), apple (12.3–20.4 μmol GAE/g FW) and orange (19.8 μmol GAE/g FW) contained higher levels of total phenolics (USDA 2007).

Table 2– The phenolic compounds identified in the hydrophilic fraction of antioxidants of finger limes grown in Australia (Konczak *et al.,* 2010).

Finger lime type	Phenolic compounds*			
	280 nm (μmol GA E/gFW)	326 nm (μmol CHA E/gFW)	370 nm (μmol RE/gFW)	520 nm (μmol 3GE/gFW)
Green	8.7 ±0.6	1.4 ±0.03	–	–
Pink	10.6 ± 2.7	0.45 ± 0.01	–	0.06 ± 0.01

The data are means ± SD of 3 determinations; FW- Fresh weight; * HPLC quantification of phenolic compounds was calculated as: GA -μmol gallic acid E/g FW for compounds detected at 280 nm, CHA -μmol chlorogenic acid E/g FW for compounds detected at 326 nm, RE-μmol rutin E/g FW for compounds detected at 370 nm and C3G-μmol cyanidin 3-glucoside E/g FW for anthocyanins (520 nm).

The green and pink finger limes contained gallic acid, chlorogenic acid (Table 2). Rutin was not present in pink or green finger limes. Anthocyanins were identified in pink (Konczak *et al*., 2010) and red finger lime (Netzel *et al.,* 2007). Cyanidin 3-glucoside and Peonidin 3-glucside were the main anthocyanins present in red finger lime (Netzel *et al.*, 2007) while Cyanidin 3-glucoside was predominant in pink finger limes.

Antioxidant capacity of hydrophilic fraction:

Table 3– The antioxidant capacity in hydrophilic fraction of antioxidants of finger limes grown in Australia (Konczak *et al.,* 2010).

Finger lime type	FRAP (μmol Fe^{+2}/gFW)	ORAC-H (μmol TEq/gFW)
Green	12.6 ± 0.5	45.9 ± 6.6
Pink	23.2 ± 0.8	65.1 ± 12.8

The data are means ± SD of 3 determinations; FW- Fresh weight; FRAP -ferric reducing antioxidant power assay. ORAC-H - Oxygen radical absorbance capacity for hydrophilic compounds assay.

FRAP and ORAC assay values of green finger lime were lower than that of pink finger lime (Table 2). The antioxidant activity (FRAP) of green and pink finger lime was 4 and 2 folds lower than that of blueberry cv. Biloxi, respectively (Konczak *et al.,* 2010). Based on TEAC assay, antioxidant activity of red and yellow finger limes were 3 folds lower than blueberry cv. Biloxi (Netzel *et al.,* 2007). According to Konczak *et al*., (2010) the total reducing capacity (FRAP assay) of the red finger lime fruit was 24.6 ± 0.3 µmol Fe+2/gFW, which was very similar to the total reducing capacity of pink finger lime (23.2 ± 0.8 µmol Fe+2/gFW).

Vitamin C and organic acids:

Table 4 – Total phenolic content of hydrophilic fraction of antioxidants in finger limes grown in Australia.

Finger lime type	Ascorbic acid mg/g FW	Citric acid mg/g FW	Malic acid mg/g FW	Oxalic acid mg/g FW
Green	0.26 ± 0.01	46.8 ± 0.47	ND	0.08 ± 0.02
Pink	0.91 ± 0.02	58.8 ± 1.70	ND	0.20 ± 0.03

The data are means ± SD of 3 determinations. FW- Fresh weight; ND- Not detected.

The pink finger limes contained more vitamin C than the green variety (Table 4). Australian citrus fruits, finger limes and Australian desert lime, were found to be inferior to blueberry in antioxidant capacity; however, they were a good source of vitamin C.

Citric acid was present in all Australian native citrus fruits. Among them, pink finger lime and green finger lime had a higher citric acid content compared to lemon aspen and Australian desert lime. Citric acid was predominant in pink finger limes, green finger limes and lemon aspen while malic acid was predominant in the Australian desert lime. Malic acid was not detected in finger limes. Finger limes also contained oxalic acid.

4.2.2 Lipophilic bioactives

Table 5– Lipophilic phytochemical in Australian grown finger limes (Konczak and Roulle 2011).

FL	Vitamin E (mg/100g FW)	Vitamin E components - Tocopherols (mg/100g FW)			Lutein (mg/100g FW)
		Alpha	Gama	Delta	
Green	0.521±0.033	0.517±0.033	0.004±0.0004	ND	0.401±0.027
Pink	2.360±0.235	2.335±0.233	0.025±0.002	ND	0.139±0.011

Results are presented as means ± standard deviation (*n* = 3); ND, not detected; T-traces (peak area less than 3% of the total peak area).

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The main lipophilic phytochemicals detected in finger limes were of vitamin E (α- tocopherol, γ-tocopherol and δ-tocopherol) and lutein(Table 4). The levels of vitamin E components in Australian citruses varied from 2.4 to 0.5 mg/100 g FW, with the highest levels in pink finger lime. Vitamin E is one of the most important lipid-soluble antioxidant, found in Australian bush fruits and it protects human cells against lipid peroxidation. According to Konzak and Roulle (2011) α-Tocopherol was the main component of vitamin E mixture of green and (99.2%), Pink finger lime (98.9%) (Table 5). The α-Tocopherol content of pink limes was four folds higher than that in green limes. The values reported for edible portion of lemon and lime were 0.15 mg/100 g FW and 0.22 mg/100 g FW respectively. The α-Tocopherol content of lemon peel was 0.25 mg/100 g FW (USDA 2010).

Lutein is a pigment that improves visual function and symptoms in atrophic age-related macular degeneration (ARMD) (Richer *et al*., 2004). Australian green and pink finger limes (peels included) contained 40 and 14 times higher levels of lutein compared to lemon and grapefruit juice respectively (approximately 0.010 mg/100 g FW; USDA 2010). In addition to vitamin E and lutein traces of chlorophyll a and b were reported in finger limes Konzak and Roulle (2011).

Antioxidant capacity of lipophilic fraction

Lipophilic fractions of Australian citruses possess high levels of oxygen radical scavenging capacity (20.5–26.7%; Table 2). However, a low positive correlation (R2 = 0.3637) was observed between the level of lipophilic compounds and the ORAC_L activity of commercially grown Australian natives by Konczak and Roule (2011).

Table 6 – Oxygen radical absorbance capacity in the lipophilic fraction of Australian grown finger limes (Konczak and Roulle 2011).

FL	ORAC_T (μM TEq/g FW)	ORC_L (μM TEq/g FW)	ORAC_L (%)	ORAC_H * (%)
Green	57.81	11.86 ±1.25	20.51	79.49
Pink	88.82	23.73± 0.75	26.72	73.28

Results are presented as means ± standard deviation (n = 3).

ORAC-T: total oxygen radical absorbance capacity; μM TEq/g; FW: micromole Trolox equivalent/g fresh weight; ORAC-L: oxygen radical absorbance capacity – lipophilic compounds; ORAC-H: oxygen radical absorbance capacity – hydrophilic compounds; * For ORAC- H values refer to: Food Chemistry (2010), 123, 1048–1054.

The ORAC_T values of finger limes (57.8 – 88.8 μM TEq/g FW) were in the same range as blue berries (65.5 μM TEq/g FW; USDA 2007). The contribution of lipophilic fraction towards the ORAC-T values of pink and green finger limes were 20.51 and 26.73 % respectively (Table 6) and were comparable to avocado (28.6%; Wu *et al*., 2004). The ORAC_H contribution towards total antioxidant activity was > 3 folds higher than the ORAC_L contribution.

4.2.3 Mineral composition

The iron (Fe) content of Australian native fruits was between 1.24 (Davidson's plums) to 7.29 (Green finger lime; mg/ 100g DW; Table 7). The Fe content of the pink finger limes was in the middle range. The highest Fe content was found in lemon aspen (13.25 mg/ 100g DW).

Finger limes (0.715 – 1.13 mg/ 100g DW) together with Riberry (1.135 mg/ 100g DW), and lemon aspen (0.834 mg/ 100g DW) contained the high levels of copper (Cu) among the native Australian crops studied by (Konczak and Roulle 2011). These values were approximately twice the level of Cu observed in European fruits and vegetables with some exception such as European squash (0.9 mg/100 g DW), celery root (1.2 mg/100 g DW) and artichoke (1.0 mg/100 g DW) (Ekholm *et al*., 2007).

The manganese (Mn) content of Australian native fruits was between 22.75 (mg/ 100g DW) for riberry and 0.400 (mg/ 100g DW) for pink finger limes. The Mn content of the finger limes was in the lower range. The zinc (Zn) content of native Australian fruits ranged from 4.24 (Quandong) to 0.426 mg/ 100g DW (Davidson's plums). Finger limes contained 0.780 – 0.848 mg/ 100g DW.

Australian citrus fruits contained the highest level of Calcium (Ca) (330 mg/100g DW) among the native Australian crops studied by (Konczak and Roulle 2011).

Quandong contained the highest magnesium (Mg) level (217.9 mg/100g DW) and the Australian desert limes contained the lowest level (4.5 mg/100g DW) reported for Australian native fruit. Content of Mg in finger limes were closer to the higher levels in this range. The Mg level varys in fruit and vegetables depending on the country of origin (see (Konczak and Roulle 2011).

Quandong contained highest level of potassium (K) per 100 g dry weight than any other crop evaluated in this study. The K in other fruits ranged between 1242.6 mg/ 100g DW of pink finger lime to 1905.5 mg/ 100g DW in Kakadu plum. Green finger lime also had K values closer to the lower end of the scale.

Phosphorous (P) Content was at the highest range (166.9 – 141.7 mg/ 100g DW) in finger limes. The lowest P levels were found in Kakadu plums (52.45 mg/ 100g DW).

Table 6 – The mineral element composition (mg/100 g DW) of native Australian finger limes (Konczak and Roulle 2011).

Mineral	Green FL	Pink FL	Physiological function
Fe	7.290	3.670	Haemoglobin – oxygen transport
Cu	0.715	1.31	
Mn	0.450	0.400	
Zn	0.848	0.780	Co- factors of enzymes responsible for DNA repair system (Fenech, 2005).
Ca	352.7	334.1	Co- factors of enzymes responsible for DNA repair system (Fenech 2005)
Mg	139.5	111.1	Co- factors of enzymes responsible for DNA repair system (Fenech, 2005).
K	1459.6	1242.6	Required at levels > 100mg/day (Özcan 2004)
P	166.9	141.7	
Se	<0.001	<0.001	
Mo	0.0104	0.0083	
Ni	0.0349	0.0563	Need in minute quantity, high levels can be toxic
Cd	0.005	0.004	
Pb	0.004	0.004	Toxic properties
Al	0.405	0.644	
Co	0.002	0.003	Need in minute quantity, high levels can be toxic
Na	11.1	8.7	

Results are based on three independent evaluations (*n* = 3).

Selenium (Se) content of all native Australian fruits was less than 0.001 mg/ 100g DW.

Molybdenum (Mo) content was highest in Quadong (0.0556 mg/100g DW) and was the lowest in Australian desert lime (0.0077 mg/100g Dw) while the finger limes contained Mo at lower levels.

The levels of Nickel (Ni) in green and pink finger lime were 0.349 and0.0563 (mg/100 g DW) respectively. In apples and raspberry consumed in Finland the level of Ni varied from 0.007 to 0.088 mg/100 g DW (Ekholm *et al*., 2007). This indicates that, the Ni levels in finger limes were within the same range as in fruits consumed in Finland.

Cadmium (Cd) levels ranged between 0.004 mg/100g DW for pink finger lime and 0.04 mg/100g DW for lemon aspen. Green finger lime had 0.005 mg/100g DW).

Lead (Pb) is known to have toxicological effects. Both green and pink finger limes contained 0.004 mg/100 g DW of lead. The levels of lead found in fruit and vegetables vary depending on the country of origin. Lower levels of Pb were reported for fruit and vegetables consumed in Finland (.0.002 -0.016 mg/ 100 g DW) compared to that of Australia native fruit. Konczak and Roulle (2011) recalculated the Pb levels of finger limes to µg/100 g FW to compare with the Pb levels reported for in Mexican fruits. The levels of Pb in Australian native green and pink finger lime were 0.846 and were 0.827 µg/100 g respectively. In Mexican fruits the Pb levels ranged from 0 to 243 µg/100 g FW. However the majority of Mexican fruit contained 20 to 50 µg/100 g FW of Pb (Sanchez-Castillo *et al.,* 1998). According to (Konczak and Roulle 2011) the levels of Pb in Australian fruits were higher than that reported for fruits in Finland but lower than the Pb levels reported in Mexican fruits.

Aluminium (Al) in Australian fruits was comparable to and slightly higher than fruits consumed in Finland. The level of Al in Davidson's plum (22.8 mg/ 100 g DW) was higher than in other fruits grown in Australia and higher than in European fruits, but only half of that in vegetables capsicum and paprika produced in Turkey (Ozcan, 2004). Al levels in finger limes were at the lowest levels detected in Australian natives (0.405 – 0.644 mg/100g DW).

Australian finger limes contained 0.002 to 0.003 mg/100 g DW of cobalt (Co). These levels were comparable to the Co levels reported by (Ekholm, Reinivuo *et al.,* 2007) for fruits consumed in Finland (0.002 to 0.011 mg/100 g DW) (Konczak and Roulle 2011).

Quandong contained the highest level of sodium (Na). Na levels of other tested Australian fruits ranged between 47.1 mg/ 100g DW riberry in to 2.2 mg/ 100g DW in Australian desert lime. The sodium content of finger limes ranged from 8.7 – 11.1 mg/ 100g DW.((Konczak and Roulle 2011).

The nutritional impact of finger lime may depend on the amount of finger lime caviar consumed by an individual. Generally finger lime is used with another food such as seafood, salads, cocktails and desserts.

5.0 Citrus processing

Citrus is generally processed in to juice, concentrates, and frozen concentrates. Citrus fruits are dehydrated rarely. Dehydration is used in the preservation of citrus species that have medicinal properties (Chen *et al.,* 2011). However, in recent years the interest on freeze dried citrus products has been developing as indicated by the number of publications and patents on this area of study.

5.1 Dehydration:

Dehydration is one of the oldest food preservation methods. Dehydrated fruits can be obtained by various conventional methods, most commonly air drying. (Krokida and Maroulis 2000; Moraga,Talens *et al.* 2011). Conventional drying methods are based on the application of heat to remove moisture from food material. Drying involves simultaneous heat and mass transfer and can result significant changes in the chemical composition, structure, and physical properties of food material. Removal of water and application of heat cause stresses in the cellular structure of food material leading to formation of pores and shrinkage. These structural changes depend on variation in moisture transport mechanisms and external pressure. Therefore, the drying method and conditions applied significantly affect the product characteristics such as porosity, shrinkage and bulk density (Rahman 2003). The colour, flavour, texture and nutritional value of products are affected by the high temperatures and long drying times associated with conventional hot air drying. (Lin *et al*., 1998; Krokida and Maroulis, 2000; (Moraga, Talens *et al,* 2011).

5.2 Freeze drying (FD)

During freeze drying ice is removed from a frozen material through the process of sublimation and the removal of bound water molecules through the process of desorption. During sublimation ice changes directly to water vapour without proceeding through the liquid (water) phase. To assist this phase transition heat energy is applied to the frozen product under vacuum at a pressure and temperature below the triple point of water (4.58 torr; 0.0098°C;(J 2010). FD process is controlled by different parameters, such as drying time, pressure, sample thicknesses, chamber temperature, sample temperatures and relative humidity (Menlik *et al.,* 2010).

FD results in high-quality dehydrated products due to the absence of liquid water and the low temperatures used during the drying process. The presence of solid state water during freeze-drying protects the primary structure and minimizes changes in the shape of the product, with minimal reduction of volume (Ratti 2001). FD contributes to the preservation of bioactive, vitamins, original flavour and aroma of the dried products (George and Datta 2002). Therefore, freeze-drying appears as a promising technique for dehydration of thermal-sensitive materials (Marques *et al.,* 2009) and high value foods such as: (a) seasonal and perishable commodities, (b) baby foods, (c) nutraceutical foods; (d) foods with distinguished organoleptic properties (herbs or coffee); and (e) foods with special end use(outdoor activities, military rations or instant meals).

5.3 Types of freeze drying

5.3.1 Conventional freeze drying

During controlled freeze drying (FD) temperature is maintained low to preserve the appearance and characteristics of the dried product. FD results in high quality products with a porous structure, minimum shrinkage, good rehydration capacity and products with minimal changes to the colour, taste, aroma and nutrients.

Although FD is more expensive than other dehydration techniques, it yields products such as freeze-dried apple and banana snacks which are available in retail shop outlets (Moraga, Talens *et al.,* 2011). Freeze drying is reported to cause loss of flavour compounds that are volatile at temperatures below triple point. (Lin *et al.,* 1998).

In conventional FD conduction or radiant heating is used to heat the shelves that hold the materials. The poor heat transfer in conventional FD leads to long drying time, related high energy consumption and high capital costs (Hammami and Rene, 1997: Ratti, 2001).

5.3.2 Microwave freeze drying

The use of microwave heating in place of traditional conduction or radiant heating in the FD process has proven to result better heat and mass transfer rate. Microwave freeze drying

(MFD has been used successfully for drying beef, skim milk, cabbage, sea cucumber , vegetable soup , potato, apple and banana (Wang *et al.,* 2011).

Adding salt and sugar significantly increased the MFD drying rate due to increase in the absorption of microwave. A recent study on effect of osmotic dehydration combined with MFD showed that osmotic treatment is effective in accelerating MFD drying rate (Wang *et al*., 2010b). These studies were focused on finding the optimum processing parameters to achieve shorter drying time, higher final product quality, and better energy efficiency.

Huang *et al*., (2011) evaluated the effects of MFD, FD, microwave vacuum drying (MVD) and vacuum drying (VD) on texture, colour, rehydration, sensory, microstructure and other quality parameters of re-structured potato chips. MFD chips were preferred by consumers and had the best quality compared to FD, MVD and VD products. Incorporation of microwave drying reduced the drying time and improved the quality of dried products.

Table 7– List of patents and summary of information obtained from the preliminary patent search conducted using Derwent innovations index

Patent No/ Year/ Title	Commodity type / advantage	Novelty	Impact on this project
JP2009017822-A ; JP4368911-B2 / 2009 / Manufacture of powder from fruit for e.g. cosmetic involves squeezing fruit juice from citrus fruit, liquefaction processing of squeezed fruit residue using a grinder and freeze-drying the residue.	Fruit: Uniform grinding was performed and the resulting powder was easily dissolved in water. Whole fruit fruit was utilized. Oxidation or heat denaturation of fruit components was prevented and a good quality product was obtained.	Powder from fruit for e.g. cosmetic is manufactured by squeezing fruit juice from citrus fruit, liquefaction processing of squeezed fruit residue using a grinder and freeze-drying the residue.	Japanese patent may have minimal effect on this project
KR2008096273-A; KR900810-B1/ 2009 / Preparing powder of Korean citrus peel by separating and splitting peels of washed fruits, reacting mixture by mixing split peels with water, adding enzyme treatment agent having e.g. cellulose and pulverizing after freeze-drying	Korean citrus peel: The method enabled high yield of active ingredients and improved flavonoid aglycone compound concentration. The powder of Korean citrus peel obtained by this method had excellent electron donating ability, nitrite scavenging activity, angiotensin-converting enzyme inhibitory activity, anticancer activity and antioxidant activity and provides improved health.	Method involves washing Korean citrus fruits, separating and splitting peels of the washed fruits, reacting the mixture by mixing split peels with water in a ratio of 1:3 for 15-20 hours, adding enzyme treatment agent having cellulose, beta-glucanase, and xylanase as main ingredients and pulverizing after freeze-drying, where a enzyme treatment density of Korean citrus peel is 1.1-1.8% of total volume of compounds.	Korean patent. May be of interest to the proposed project if commercial enzymes are used to treat peel before FD. Need further clarification. Table 7 continued….

Patent No/ Year/ Title	Commodity type / advantage	Novelty	Impact on this project

JP2007077139-A/ 2007/ Composition for use in health food/beverage products and supplement for lowering blood glucose level and preventing arteriosclerosis, contains freeze dried and pulverized fruit skin, pulp lees and seeds of Citrus sudachi	Citrus sudachi: Natural raw material was utilized effectively and composition having hypoglycemic effect is produced. The composition does not produce side effects, highly safe to use and increases survival rate.	Composition contains freeze dried and pulverized fruit skin, fruit pulp and seeds of Sudachi (Citrus sudachi). Preferred Process: product is placed in an oven at 40-60degreesC for 24-26 hours. Freeze drying process is performed in an oven at -40 to -25degreesC.	May be of interest as a pre-treatment. However, shorter heat treatment times will be evaluated in the proposed project.
KR2005122158-A/2005/ Freeze-dried uncooked food composition containing chlorophyll, carbohydrates, vitamins and minerals in large quantities	Cereals, vegetables, seaweeds and the like:	A method of preparing a freeze dried uncooked food composition by freeze-drying. The composition contains chlorophyll, carbohydrates, vitamins and minerals in large quantities.	Korean patent may have minimal effect on this project
JP60070030-A; JP91020220-B / 1985/ Freeze-dried lemon prepn. - by removing turpentine oil, dipping in aq. soln. of saccharide and freeze drying	Lemon: Method gives freeze-dried lemons which retain their shape when cut into round slices and has a good odour and taste, no bitterness, and have high keeping quality.	Raw lemon slices are dipped and treated in hot water for 30 sec - 4 minutes to remove the turpentine oil. Slices are then dipped in aq. soln. of 1-10% saccharide containing sodium ascorbate and then freeze-dried. The saccharides are sorbitol, glucose, mannose, fructose and cane sugar.	Japanese patent may be of interest to the proposed project. Need further evaluation. Table 7 continued….

Patent No/ Year/ Title	Commodity type / advantage	Novelty	Impact on this project
RD222005-A/ 1982/ Prepn. of flavouring product - by desorption from substrate to form frost mixed with water soluble solids prior to freeze drying	coffee, tea, cocoa, oranges, grapes, lemons, cherries, strawberries, potatoes or peanuts: A stable, water soluble, freeze-dried food or beverage prod. is prepd. from aroma- and flavour-bearing substrates Flavour and aroma losses are minimised.	Initially a stable, concentrated, aromatic, dry prod. is prepd. from the substrate in a desorption process in which wet steam is passed through a zone containing the substrate. This may be applied either in a pulsed manner, or slowly and continuously avoiding flooding of the zone.	May need to capture the flavour lost during freeze drying. May be of interest to the proposed project.

5.4 Freeze drying of citrus products

One publication was found on freeze drying of peel and endocarp of Kaffir lime a citrus used for traditional medicine in Thailand (Chaisawadi, Thongbutr *et al.,* 2008).Few patents were observed on freeze drying of citrus (JP60070030-A; JP91020220-B; JP2007077139-A) and citrus peel products (KR2008096273-A; KR900810-B1) but a specific patent on the freeze drying of finger lime was not found in the patent literature. One patent described blanching as a pre- treatment before freeze drying of lime to remove citrus oil from the peel to control bitterness in the final product (JP60070030-A; JP91020220-B).

Please note that although the Derwent Innovations Index begins its coverage in 1963 it does not uniformly cover all patent jurisdictions from that date, and therefore the patent listing provided in this report cannot be used as a guarantee of the freedom to operate. The table 7 shows country/jurisdiction coverage of patent families in the Derwent Innovations Index. The quality of the patent abstracts of some of the non-English language patents is also questionable, in term of comprehensiveness and in their use and spelling of vital names and terminology. Many of the foreign language patents also have insufficient detail in the abstract to understand why some of the International Patent Classisification Codes were allocated.

While the Derwent database is one of the most comprehensive databases available, if financial or legal decisions are to be made in this area, it is recommended that the services of a patent attorney be sought to confirm the freedom to operate.

5.5 Pre treatments

Quality of dehydrated products depends on the drying condition as well as on the pre treatments. Pre treatments are applied to inactivate enzymes that can lead to unacceptable colour and flavour changes (Negi and Roy 2000; Rawson 2011).

5.5.1 Blanching

Blanching is used as a pre treatment for air dried products and helps in enzyme inactivation, reduction of drying time, improving the final texture and rehydration properties. However, some studies showed adverse effects of blanching as discussed in following examples. Wang *et al.* (2010) reported that blanching pre treatment caused starch gelatinisation and swelling of cells in potato slices prior to FD. During freezing blanched potato was more damaged than the un-blanched samples and showed more micro-structural changes at pre frozen and sublimation stages. However, blanching was effective in improving rehydration capacity and to minimise shape changes of the freeze dried potato samples.

Blanching is also reported to result thermal degradation of the product and reduction in the content of bioactive compounds (Hansen *et al.,* 2003); (Kidmose *et al.,* 2004). To overcome these adverse effects of blanching, research has been conducted on identifying alternate pre treatment.

5.5.2 Ultrasound

Ultrasound is identified as a possible pre treatment to replace blanching. Rawson *et al*. (2011) evaluated the use of ultrasound (20k Hz; amplitude 24.4, 42.7 and 61.0 µm; 3 and 10 min) and blanching (80°C for 3 min) as pre-treatments for air dried and freeze dried carrots. The bioactive retention of the ultrasound treated FD product was similar to the untreated FD product. Ultrasound treatment significantly improved the retention of bioactive compounds polyacetylenes and carotenoids in hot air dried carrots. FD carrots were superior in quality compared to the air dried samples. This study indicated that ultrasound pre-treatment has a high potential as an alternative to conventional blanching treatment (Rawson, 2011).

5.5.3 Partial drying

To minimise the cost of FD the use of partial hot air drying has been evaluated by few research groups. Donsi *et al*. (1998) used partial forced convection drying at 50°C at a relative humidity of 15% to a predetermined moisture level (0.6 – 0.8%) for potato, apple, carrot and courgettes. The structure of the plant material was intact and was not collapsed after the partial air drying. The quality of partial hot air dried and FD samples was comparable to that of fully FD products. By using the combined process considerable level of energy and time saving was achieved.

Banana slices pre-dried with sequential infrared radiation, dried slowly during freeze-drying compared to the samples with no pre-drying treatment. The textural changes that occurred during the pre-drying step were considered to be responsible for the decreased drying rate during freeze drying (Pan *et al.,* 2008) .

5.5.4 Application of calcium ions

Wang *et al.* (2010) immersed potato slices in 0.5% $CaCl_2$ solution for 10 min and were examined using light microscopy for their microstructure in frozen state before freeze drying, during the sublimation drying stage, in the desorption drying stage and in the final dried form. It was reported that in the raw potatoes, the polyhedral cells were intact and orderly, and, a thickening of the cell wall was observed after Ca^{2+} soaking.

In the samples pre-treated with Ca^{2+}, the increased cell rigidity reduced the tissue damage during FD, and the samples could preserve the porous structure. The volume of the Ca^{2+}pre-treated samples was well preserved due to the improved strength in cell structure while the raw FD slices showed warping.

5.6 Quality of freeze dried material

A number of quality parameters frequently used by researchers for the evaluation of the quality of freeze dried fruit and vegetable materials are listed in Table 6. Some of these parameters and their interactions are further discussed.

5.6.1 Bulk density:

Bulk density of the FD material decreased with deceasing moisture content while in conventional drying e.g. fluid bed and tray driers, infrared assisted air drying and osmotic dehydration combined with conventional air drying) bulk density increased (Koç, Eren *et al.,* 2008). This indicates that freeze dried materials require less storage space compared to dried material obtained by other dehydration processes.

5.6.2 Rehydration properties

According to Ratti (2001) rehydration is the most studied quality parameter in the literature. Most of the literature analysed the effect of pre-treatments on rehydration. Lately, more attention has been drawn towards the effect of drying methods on rehydration properties. A number of quality parameters related to rehydration was found in literature.

Rehydration Ratio (RR) of the dried material is calculated by the ratio between the equilibrium moisture content of rehydrated material and the moisture content of dehydrated material ((Krokida and Marinos-Kouris 2003). RR of freeze-dried foods is in general 4–6 times higher than air-dried foods. The RR of air dried fruit and vegetable is around 1-4 (Krokida and Marinos-Kouris 2003). Due to the high RR, FD products are excellent for ready-to-eat instant meals or soups (Ratti 2001).

The dry matter holding capacity (DHC) index measures the dry matter loss during rehydration, expressing the ability of the material to retain soluble solutes and thus showing the extent of tissue damage and its permeability to soluble material. These indices range from 0 to 1.

Water absorption capacity (WAC) index provides information on the water uptake capacity soluble solids inside the matrix, thus expressing the total damage caused by drying and rehydration processes in the material structure (Marques *et al.,* 2009).

To quantify the amount of water absorbed and the losses of soluble compounds and to fully characterize the rehydration of the freeze-dried fruits, WAC, DHC and rehydration ability (RA) were proposed by Lewick (1998).

FD quinces showed the highest porosity when compared to the products dried using other conventional processes such as conventional drying in fluid bed and tray driers, infrared assisted air drying and osmotic dehydration combined with conventional air drying (Koc *et al*., 2008). Increased porosity of FD material allowed rapid rehydration.

5.6.3 Shrinkage:

Freeze dried material showed limited shrinkage compared to dried material resulting from conventional drying in fluid bed and tray driers, infrared assisted air drying and osmotic dehydration combined with conventional air drying (Koc *et al.,* 2008).

5.6.4 Glass transition temperature:

The freeze-drying process causes changes in some products and produce amorphous and hygroscopic structures. These structures are sensitive to changes in their physical, chemical and microbiological characteristics (de Oliveira Alves *et al.,* 2010). The physical state of amorphous materials may change from a solid glassy state to a liquid-like rubbery one when the glass transition temperature (Tg) is reached. Tg is defined as the temperature at which an amorphous system changes from the glassy to the rubbery state (Karmas 1992); (Roos 1991). Tg of food products has been reported as an important parameter to observe for the control of deterioration mechanisms during processing, and as an indicator of food stability (Ratti 2001). Processing temperatures that are higher than Tg, leads to considerable level of change in quality of foodstuffs (Peleg 1996).

Food powders containing amorphous sugars experience stickiness and caking when the powder is exposed to temperatures above the powder's glass transition temperature (Tg), which is a function of the moisture content or the water activity of the powder. (Foster *et al.,*

2006) have confirmed that the glass transition-related flow changes (sticking, crystallization and collapse) and the rate of change of the cohesiveness are directly dependent on the extent to which the glass transition temperature (Tg) is exceeded (T − Tg). Please refer to (de Oliveira Alves *et al,.* 2010)) for further details on structure, collapse and shrinkage of FD material.

The effect of water activity and glass transmission on the textural and optical properties of freeze-dried banana and apple slices has been studied by (Moraga, Talens *et al.,* 2011). This study identified the critical water activity that causes the glass transition as 0.04–0.08 and 0.23–0.26 for apple and banana, respectively.

Freeze-dried Peki (*Caryocar brasiliense* Camb; a Brazilian fruit) was pre treated by adding alcohols and sugars to the fruit pulps and freeze-dried by de Oliveira Alves *et al*.(2010). The pre-treatment with sucrose and ethanol improved the freeze-dried product and yielded a lower number of collapsed structures. The pulp pre-treated with sucrose was amorphous and stable, while the drying process was accelerated by ethanol. The application of ethanol resulted in protecting the structure of the final product from collapsing.

Table 8– Test parameters used for the evaluation of quality in FD products

Test parameter	Indicator of	Commodity	Reference
Water activity	Browning	Acerola Muscadine pomace	(Marques, Prado *et al.*, 2009) (Vashisth, Singh *et al.*, 2011)
Glass transition temperature	Shrinkage quality	Acerola Muscadine pomace	(Marques, Prado *et al.*, 2009) (Vashisth, Singh *et al.*, 2011)
Residual moisture content	Stability during storage	Potato apple, Courgette Muscadine pomace	(Donsì, Ferrari *et al.*, 1998) (Vashisth, Singh *et al.*, 2011)
Bulk density	rehydration of the samples	Carrot	(Lin, Durance *et al.*, 1998)
Rehydration potential	Porosity	Carrot Acerola	(Lin, Durance *et al.*, 1998) (Marques, Prado *et al.*, 2009)
Rehydration rate	Porosity	Potato apple, Courgette	(Donsì, Ferrari *et al.*, 1998)
Texture	Indicates case hardening	Carrot	(Lin, Durance *et al.* 1998)
Colour	Detecting browning, changes to pigments	Carrot	(Lin, Durance *et al.*, 1998)
Bioactives	Stability and quality of the product	Carrot – vit C, CaroteneAcerola – vitC Muscadine pomace- total phenolics, antioxidant capacity	Lin *et al*., 1998 (Marques, Ferreira *et al.*, 2007) (Vashisth, Singh *et al.*, 2011)
Sensory evaluation	Preliminary information on consumer preference		(Marques, Ferreira *et al.*, 2007)

5.6.5 Optical properties

The optical properties of FD material depend on the product type, the type of pigments present in products and the pre-treatments applied to the products.

A study demonstrated that air drying at 30°C resulted less colour change compared to 70°C air drying and freeze drying of green bell peppers and pumpkin. Freeze drying lead to more intense colour changes in green pepper. In addition, the chroma of dried pumpkin decreased significantly with the freeze drying, while the hue angle was maintained constant as compared with the fresh vegetables. With air drying the colour saturation of pumpkins increased and the hue angle decreased with the increase of temperature (Guiné and Barroca 2011).

The changes in colour parameters; lightness, hue and chroma were closely associated with the changes in pigment concentration of freeze dried Roselle (*Hibiscus sabdariffa* L.) extracts, which is a natural colorant. The colour of roselle extract was stable when moltodextrin (3g/100g) was used as a stabilizer (Duangmal *et al.,* 2008).

Increase in water activity changed in the optical properties (L, a and b values) of freeze dried apple and banana. An increase in the translucency of samples and the development of browning were detected at a water activity of over 0.430. It is reported that the water activity needs to be controlled for the control of browning reactions (Morga *et al*., 2011).

5.6.6 Texture:

The texture related mechanical response (the maximum force attained or the area under the curve in the puncture test) of freeze dried apple and banana slices was dependent on the water content. Moraga *et al.,* (2011) concluded that monitoring of both glass transition and water activity could be of use for preventing the changes in texture.

5.6.7 Bio-actives

Generally, bioactive content is affected by the conventional drying processes compared to the FD. A commercial sample of dry quandong had been evaluated by Konczak and Roulle (2011). The total oxygen radical scavenging capacity of the dry quandong reached 2027.97±275 μM TEq/g DW, and this was 11.1% lower than that of a fresh quandong (2281.41±221 μM TEq/g DW). The ORAC-L values of the fresh and dry quandong were 132.02±1.3 and 39.98±1.0 μMTEq/g DW, respectively. These results indicate significant losses of lipophilic antioxidants in quandong during the commercial drying process (Konczak and Roulle 2011). However, the drying conditions were not available in this publication.

Three drying technologies, vacuum belt drying (VBD), hot air drying (HAD), and FD were evaluated for the processing of muscadine pomace by Vashisth *et al.,* 2011. The total phenolic content and antioxidant activity of the VBD samples were not significantly ($P > 0.05$) different compared to those of freeze dried samples. VBD products possessed high total phenolic content and were dried four times faster than the FD process.

Peki fruit (*Caryocar brasiliense* Camb); a Brazilian fruit) products were pre treated by adding alcohols and sugars to the fruit pulps and freeze-dried by de Oliveira Alves *et al.* (2010). The highest total carotenoid contents were found in samples pre-treated with alcohol. In these treatments, the alcohol concentration influenced carotenoid preservation. Increasing the sugar concentrations in the freeze-dried peki pulps reduced the total carotenoid contents.

Mahinda *et al.* (2010) evaluated far-infrared radiation (FIR) drying and FD for the dehydration of citrus press-cakes which is a waste product from citrus processing. FIR-dried samples showed slightly lower extraction yield and total phenolic content compared to freeze dried samples. Almost equal level of total flavonoid and antioxidant activities were observed in samples dried with both technologies. However, significantly lower drying times were observed with FIR-drying compared to freeze drying.

Heat treatment (100°C for 2hrs) increased the antioxidant activity as compared with freeze dried kumquat extracts. However, heat treatment (100°C for 2hrs) and oven drying (55°C) of kumquat flesh decreased the antioxidant activities compared to freeze drying (Chih-Cheng *et al.,* 2008). As demonstrated in above research studies the effect of freeze drying on the retention of bioactive compounds vary with the products.

6.0 Conclusions

6.1 Freeze drying of finger lime

A literature search (patents and scientific publications) was conducted using ISI web of Knowledge and Food Science and Technology abstracts. Patents or research articles on freeze drying of finger limes were not retrieved.

The bioactives of finger limes were widely studied and reported in literature by Dr. Izabela Konczak and her colleagues of CSIRO Food and Nutritional Sciences at North Ryde. According to their studies finger limes contains both hydrophilic and lipophilic bio actives. Total phenolics of the hydrophilic fraction were lower in finger limes compared to blue berries (cv. Biloxi). Gallic acid, chlorogenic acid and cyaniding 3-glucosed were among bioactives of the hydrophilic fraction while vitamin E and Lutein were the predominant bioactives in the lipophilic fraction. Finger limes were rich in ascorbic and citric acids. The antioxidant activity of the hydrophilic fraction (ORAC_H) was lower than that of the blue berries but the antioxidant activity of the lipophilic fraction (ORAC_L) was comparable to blue berries and avocado. The ORAC_H contribution of towards the total antioxidant activity of finger lime was higher (> 3 folds) than the ORAC_L contribution.

Finger limes could be considered as a high value product due to its seasonal production, perishable nature and the current market demand. Freeze drying could have a high potential to extend the shelf life of this exotic citrus fruit. However, it may be necessary to conduct a cost benefit analysis to evaluate the cost effectiveness.

Research articles reviewed on freeze drying of fruit and vegetable products provided constructive information to plan future freeze drying experimental trials. During the experimental stage quality of the final products will be evaluated using selected quality parameters discussed in this review.

6.2 Impact of patents

The patents on freeze drying of citrus create may have an impact on the use of blanching treatments (JP60070030-A; JP91020220-B / 1985). If financial or legal decisions are to be made in this area, it is recommended that the services of a patent attorney be sought to confirm the freedom to operate.

6.3 Proposed pre-treatments and formulations for freeze drying of finger lime products

Proposed project plans were provided to Sunfresh Marketing Co-op Limited in September 2011. The detailed experimental plans for the Activity 1 include the following:

1. **Establishing the product characteristics (quality evaluation parameters)**

   Untreated frozen finger lime, whole fruit, slices, caviar and peel will be freeze dried and will be used to establish the quality evaluation parameters.

   It is advisable to keep the product formulation simple as possible close to natural condition if possible to avoid labelling issues. The product label needs to have all additives listed. Additives will be added at stage 3 if the untreated products have defects such as warping.

2. **Blanching and /or ultrasound treatment to reduce the oil levels in peel, whole fruit and slices.**

A. Whole finger limes and peel product will be dipped in hot water at 80°, 90°and 100°C for 30 sec, 1, 3 and 5 min time periods to remove peel oil

B. Whole finger limes and peel product will be ultrasonically treated at a two selected treatment condition in water at room temperature for 30 sec, 1, 3 and 5 min time periods to remove peel oil.

C. Whole finger limes and peel product will be ultrasonically treated at a two selected treatment condition in water at at a selected temperature for 30 sec, 1, 3 and 5 min time periods to remove peel oil.

D. Control sample with no pre-treatment.

   Resulting finger lime products (2A- 2C) will be freeze dried and the amount of oil remaining in the product will be assessed. Samples will also be provided to Sunfresh to evaluate the taste and flavour.

3. **The application of calcium ions may improve product quality if the freeze dried samples showing the signs of warping*. If warping is observed in stage 1 following experiments will be conducted.***

A. Finger lime slices will be dipped in 0.5% calcium chloride solution for 10 min and excess liquid will be removed. Resulting product will be frozen and freeze dried.
B. Untreated control samples will be freeze dried.

Calcium treated samples and untreated samples will be compared for the signs of Warping. Samples (3A &3B) will be provided to Sunfresh for assessment of appearance and tasting. If the taste of the calcium treated samples are not appealing the level of calcium salt application will be re evaluated at 0.1 and 0.3% level.

4. **Inclusion of sugars or dextrin could be considered *if finger lime slices show tissue damage after rehydration at stage 1.***
   A. Finger lime slices will be dipped in sucrose and trehalose solutions at concentrations of 1%, 5% and 10% for 10 min prior to freezing and then freeze dried.

   B. Untreated control samples will be freeze dried

   Treated and untreated samples (4A & 4B) will be compared for the signs of tissue damage after rehydration. Samples will be provided to Sunfresh for assessment of appearance and tasting.

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