Quality Optimisation of Carrot Juice

May 2001

A thesis submitted for the degree of

Doctor of Philosophy

by

Harshul M Vora
(M Sc Food Technology)
(ID No: 3063318-s9512888)

School of Life Sciences and Technology
Victoria University of Technology
Melbourne
Victoria, Australia

Dedicated to

My Parents and Brother

My Special Thanks to

My

Wife Nirzari
Sons Angat and Manan
Sister in law Kanan
Nephew Udit and Niece Anahita
Cousins Pavani, Niraj and Manmath

Friends
Relatives and
Well-wishers

Supervisor

Principal Supervisor
Dr. Darryl M Small
Lecturer in Food Science and Technology
School of Life Sciences and Technology
Victoria University of Technology Werribee Campus (W071)
PO Box 14428, Melbourne City MC, Victoria 8001, Australia
e mail: Darryl.Small@vu.edu.au

Declaration

I hereby declare that all work carried out in this project was performed while I was enrolled as a Ph.D. student in the School of Life Science and Technology, Victoria University of Technology, Werribee Campus. To the best of my knowledge, this work has not been submitted in whole or part for any other degree or diploma in any University and no material contained in this thesis has been previously written or published by another person, except where due reference is made in the text.

Harshul M Vora

May 2001

Acknowledgments

I gratefully acknowledge the continuous and valuable technical guidance and input of my supervisor Dr. Darryl M. Small and also the assistance of Prof Bob Fairclough. Without their help this project would not have been completed. My special thanks go also to Prof W S A Kyle for his valuable technical guidance. Grateful acknowledgment is made to the laboratory and library staff at the Werribee campus of Victoria University of Technology for their continuous support.

Financial support provided by the **A**ustralian **F**ood **I**ndustry **S**cience **C**entre, Werribee (Afisc - currently known as Food Science Australia) and all the efforts of Murray Brown (Scholarship Officer) in organising various valuable industry tours as well as other Afisc scholars for their friendly support is gratefully acknowledged.

Grateful acknowledgment is extended to Henderson Seeds Group Pty Ltd (Bruno Tigani, Grant Munro, Tony Schultz) for the supply of carrot samples; Peter Hagan (Enzymes Australia Pty Ltd) for supplying enzyme samples; Ian Mac (Irymple Citrus Ltd), Kevin Kajawski (Nugan Quality Foods) and Martin O'Donnell (Griffith Producers Co-op Co. Ltd) for their assistance in organising samples of carrot juice concentrates as well as information on present trends of carrot juice in international markets.

The contribution of Professor Bob Hosken (Department of Food Science and Technology, University of Newcastle) and his team of researchers for their assistance in preparation of aseptically packed carrot juice concentrate samples are gratefully

acknowledged. The assistance of Professor Graham Thorpe and his student Mahesh with the mathematical modelling for carrot is gratefully acknowledged.

I wish to express my gratitude to my friends and their families, particularly to Rajiv Dave, Hisham Al-Matubsi, Aslam Khan, Richard Musumeci, Dirk Teale, Easaw Easaw as well as all the members of my sensory panel for their continuous support and generosity.

Last but not the least, I gratefully acknowledge the continuous encouragement and generous support given to me by my family members (wife Nirzari and two sons Angat and Manan, mother Shobhna and my late father Mahendra and late brother Saurabh, sister in law (Kanan) and my nephew (Udit) and niece (Anahita). Without their support, completion of this project would not have been possible.

Harshul Vora

Werribee, Australia

May 2001

Publications and Presentations

Refereed papers

(1) **Harshul M Vora*, William S A Kyle and Darryl M Small 1998** Composition and juicing potential of traditional and newer Australian varieties of carrot. *Food Australia* 50(1): 24-26.

(2) **Harshul M Vora*, William S A Kyle and Darryl M Small 1999** Activity, localisation and thermal inactivation of deteriorative enzymes in Australian carrot (*Daucus carota L*) varieties. *Journal of the Science of the Food and Agriculture* 79:1129-1135.

(3) **Harshul M Vora*, William S A Kyle and Darryl M Small 1999** A comparative study of three blanching methods on whole carrot (*Daucus carota L*) processing. (Manuscript submitted for publication in *Journal of Food Science).*

(4) **Harshul M Vora*, William S A Kyle and Darryl M Small 1998** Effect of enzyme treatment of carrot pulp on juice yield and quality. *Food Australia* 51(4) 146-147.

Abstract

(1) **Harshul M Vora*, William S A Kyle and Darryl M Small 1997** Effect of various blanching treatments on Australian carrots for juice production. (Presented during the 30th AIFST Convention at Perth, Australia).

Conference presentations

(1) **Harshul M Vora*, William S A Kyle and Darryl M Small 1996** A comparative study of the chemical composition and enzyme activity of selected Australian carrot varieties. (Presented during the 29th AIFST Convention at Gold Coast, Australia).

(2) **Harshul M Vora*, William S A Kyle and Darryl M Small 1998** Effect of enzyme treatment of carrot pulp on juice yield and quality. (Presented during the 31st AIFST Convention at Melbourne, Australia).

Abstract

The carrot is one of the most widely consumed root vegetables, both in raw and processed forms and offers various nutritional benefits with the fresh product being low in energy and a good source of fibre, potassium and other minerals. Of all the vegetables, it is one of the richest sources of carotenes especially β-carotene which has a crucial role in vision and is also thought to have benefits due to its anti carcinogenic properties and in protection against heart disease.

In 1990 Australia produced approximately 152,100 tonnes of carrot contributing to 1.12% of global carrot production. Less than half of this production is used domestically as fresh vegetable or in processed forms. Until recently, most of the remaining production has been juiced for the export markets, in particular for Japan and other Asian countries. However, there is potential for increased juice production to service both local and additional international markets. The increasing awareness of and growing demands for carrot juice on world markets, together with increasing consumer concerns regarding the use of preservatives and the trend towards all natural products, provides an opportunity and incentive to further develop juice manufacturing technology to address these consumer demands.

For this strategic investigation on processing suitability, four commercially available Australian carrot varieties (two traditional and two more recently developed) were selected. Nutrient contents have been measured including moisture, protein, crude fibre, pectin, vitamins (B_1, B_2,, C and Carotenes) and minerals (Ca, Fe, K, Mg and Na). Small variations in macronutrients and mineral composition were observed between the varieties. However, marked differences in the levels of α- and β-carotenes were found. Parameters used industrially to indicate juicing potential were also assessed. Considerable variation in yield and colour characteristics of juices prepared from the different varieties was observed.

All four Australian carrot varieties were also investigated for activities of deteriorative enzymes. The activities of three deteriorative enzymes [peroxidase (EC 1.11.1.7), catechol oxidase (EC 1.10.3.1) and pectinesterase (EC 3.1.1.11)] were assayed in juices

prepared from whole carrot as well as from superficial tissues, core, root tip and stem end portions of carrots. The levels and relative distributions vary for the different enzymes and varieties studied. Thermal characteristics of these enzymes in the juices prepared from the four carrot varieties were also assessed. The enzyme catechol oxidase was found to be the least stable and pectinesterase the most stable in each of the varieties. In most cases effective inactivation was achieved within two minutes at 85°C. The enzymes of Top Pak variety showed greater thermal stability. In this variety pectinesterase required treatment at 90°C to ensure rapid inactivation.

Various studies on whole vegetable blanching have been carried out in the past. These have focused on the inactivation of deteriorative enzymes and the time-temperature regime necessary to achieve suitable levels of inactivation. Whole carrot roots of various diameters were subjected to three blanching techniques (boiling water, microwave and steam blanching). The results indicated greater stability of peroxidase enzyme compared to catechol oxidase. Previous studies indicated the greater heat stability of peroxidase when compared to catechol oxidase and have led to the use of peroxidase as indicator enzyme for blanching sufficiency. The current results indicated pectinesterase enzyme to be more heat stable than peroxidase and therefore pectinesterase is recommended as the indicator enzyme for blanching sufficiency.

The effect of the three blanching techniques on nutritive components (vitamin C, carotenes) as well as the physical parameters (weight loss/gain, juice yield, viscosity and colour) of carrot juice were investigated. Amongst the blanching techniques used in this study, microwave blanching resulted in the highest losses of carotenes, as well as reduced juice yields and greater weight losses compared to other methods. Furthermore the microwave blanching of carrots of smaller diameters resulted in burning of carrots with a concomitant effect on juice quality. These observations indicate the unsuitability of microwave blanching in the processing of carrot juice.

Using the carrot varieties, various blanching methods including boiling water, steam and microwave have been evaluated for their efficacy in inactivating the deteriorative enzymes and to establish blanching protocols for commercial processing. Water blanching resulted in a slight weight gain (+1.5%) unlike the weight loss observed in steam blanching (-1.9%). Losses of total carotenes in boiling water blanching were

higher (59.4%) when compared to steam blanching (13%). The juice yield in hot water blanching was lower (27%) compared to steam blanched carrot (34%). Negligible variations in colour were observed between the three blanching techniques used. For each blanching technique carrot diameter was found to be important and mathematical modelling was used to develop a predictive equation relating root diameter to the time required for effective steam blanching. The results of the blanching studies indicate that steam blanching is the preferred method of blanching for processing of carrots for juicing.

Commercially, enzyme preparations are commonly used to facilitate juice extraction, softening, clarification, maceration and liquefaction processes in the food industry. In this study of processing protocols for the production of carrot juice, carrot mash was treated using the commercial enzyme preparations Rohament Max and Rohament PL. The conditions for enzymatic treatments including dosage, dosing time and pH have been optimised. The effect of enzymatic treatment on juice yield, carotene content, relative viscosity and colour were evaluated. The combined application of both the enzyme preparations resulted in the best results. Both the yield and quality of juice were enhanced. The results highlighted the significance of the use of enzymatic treatment for carrot processing and are thus recommended for the commercial preparation of carrot juice.

Various techniques of concentration and evaporation are available for the processing of juices and these techniques include both thermal and non-thermal methods. Amongst the thermal evaporation techniques described, the use of steam has been commonly used. Non-thermal concentration techniques based upon membrane technology include ultrafiltration and reverse osmosis. The juices extracted from carrots with and without enzymatic treatment were subjected to various concentration techniques to prepare carrot juice concentrates. The concentration techniques applied to carrot juice included rising film evaporation (thermal), ultrafiltration and reverse osmosis (non thermal membrane concentration) and combined concentration techniques (reverse osmosis and rising film evaporator). Ultrafiltration techniques were found to be unsuitable for carrot juice concentration. Carrot juice concentrate prepared from enzymatically treated (6.0^{o}Brix) and untreated (4.5^{o}Brix) carrots and concentrated using the reverse osmosis technique achieved almost 3 fold concentration (14.2^{o}Brix) on a single pass before the

of up to 30 days under frozen storage. Changes in carotene contents were also observed during storage. The extent of reduction in both α- and β-carotene was up to 37% observed in juice following the combined concentration technique and after 45 days of storage.

As a result of this strategic study, suitable processing carrot varieties, blanching indicator enzyme, blanching regime, concentration technique and storage conditions have been established. A recommended processing protocol has also been developed for the commercial processing of carrot juice. This approach is suitable for the production of carrot juice concentrate having optimal quality characteristics.

Table of contents

List of tables

List of figures

Abbreviations

Measurements

a*	redness
b*	yellowness
L*	lightness value
µg	microgram
mg	milligram
g	gram
kg	kilogram
ppm	parts per million
°C	degrees Celsius
mL	millilitre
L	litre
mbar	millibar
rpm	revolutions per minute
mm	millimeter
mW	milliWatts
nm	nanometer
v/v	volume to volume
w/v	weight by volume
MWCO	molecular weight cut off
kPa	kiloPascals
TSS	total soluble solids

Chemistry

Ca	calcium
Mg	magnesium
Na	sodium

K	potassium
Fe	iron
Cu	copper
N	nitrogen
mol	moles
M	molar
HCl	hydrochloric acid
NaOH	sodium hydroxide
$CaCl_2$	calcium chloride
H_2O_2	hydrogen peroxide
Na_2HPO_4	disodium hydrogen orthophosphate
NaH_2PO_4	sodium dihydrogen orthophosphate

Instrumental

uv / vis	Ultraviolet and visible
UF	Ultrafiltration
RFE	Rising film evaporator
RO	Reverse osmosis
HPLC	High Performance Liquid Chromatography

General

AOAC	Association of Official Analytical Chemists
Afisc	Australian Food Industries Science Center
FAO	Food and Agriculture Organisation
FAOSTAT	Food and Agriculture Organisation Statistics
ABS	Australian Bureau of Statistics
CFCAB	Californian Fresh Carrot Advisory Board
RDI	Recommended Daily Intake

Carrot varieties

RHO	Red Hot Original
RHC	Red Hot Carotene
RC	Red Count
TP	Top Pak

Mathematical modelling

α	thermal diffusivity of the carrot
T	temperature, [^{0}C]
t	time, [s]
r	radial distance, [m]
z	axial distance, [m]
L	length of the carrot
D	greatest diameter of the carrot

List of appendices

Chapter 1

Introduction

The purpose of this chapter is to provide a brief introduction to carrots, particularly their production and utilization, as background to the literature review and the overall project. With the increasing popularity of fruit juices in global markets, consumption of vegetable juices is attracting considerable interest as healthy food products. Vegetables in the form of juices offer a quick and convenient means of consumption. These vegetable juices and drinks have increasingly been produced and sold through health food and nutrition shops as dietetic food drinks. They have become popular as an ideal vitamin-packed "pick-me-up" and as instant breakfast products.

Vegetables are typically good sources of vitamins, particularly vitamins A and C and of minerals, especially potassium, magnesium, calcium and iron. In addition to their primary value as food crops for people, vegetables also provide feed for cattle, sheep, pigs, and poultry. Further processing of vegetables provides a range of useful materials including sugar and starch, which are added to other food products. Non-nutritive food colourings and additives, alcoholic beverages and fibres are also made from a variety of vegetables. Some of the most popular garden vegetables in the Western world are corn, potatoes (white and sweet), beans, peas, tomatoes, carrots, onions, lettuce, and cabbage. Others include melons, cucumbers, asparagus, turnips, spinach, broccoli, Brussels sprouts, beets, eggplants, squashes, and garlic.

The carrot (*Daucus carota* L) is a root vegetable and belongs to the family *Umbelliferae,* genus *Daucus* and species *carota* and is believed to have originated in Central Asia then spread to Asia, Europe, North Africa and the Mediterranean region (Encyclopaedia Britannica Micropaedia 1995; Mazza 1989; Kalra *et al* 1987; Bajaj *et al* 1978). Although it has been widely cultivated for many centuries, the use of carrot as a food dates only from the early twentieth century. Prior to that time it was used was for medicinal purposes.

The carrot is one of the most widely consumed root vegetables and is used in both raw and processed forms. It offers significant nutritional advantages with the fresh product being low in energy and also a good source of dietary fibre, potassium and other minerals (Cashel *et al* 1989). Of all the vegetables, it is one of the richest sources of carotenes especially β-carotene. These compounds have long been recognised for their role in vision, but more recently carotenes have been claimed to provide protection against a range of serious conditions including cancer and heart disease (Abbey *et al* 1995; Macrae *et al* 1993; Mayne *et al* 1992; Murakoshi *et al* 1992; Moon 1989; Ziegler 1989; Krinsky 1988; Temple and Basu 1988; Olson 1986; Peto *et al* 1981).

In the early part of the twentieth century carrot production was restricted to relatively few countries including USSR, China, Japan, United Kingdom, Poland, France, Italy, Germany and Canada. Today, the carrot is grown in virtually all the countries of the world. In the year 1986, total global carrot production was 12.47 million tonnes, with Europe, Asia, North America, South America, Africa and Oceania producing 4.0, 3.5, 1.4, 0.6, 0.5 and 0.2 million tonnes respectively (Mazza 1989). USSR produced 2.4 million tonnes of carrots in 1986 however the production data of 1996 are unavailable. By the year 1996, this production had increased to 16.48 million tonnes with Europe, Asia, North America, South America, Africa and Oceania producing 6.5, 5.9, 2.4, 2.59, 1.12, 1.98 and 0.8 million metric tonnes respectively (Food and Agriculture Organisation (FAO), 1999). This represents increases in production of 62.5, 68.5, 85, 431, 224 and 400 per cent respectively in ten years. The Oceania region including Australia, New Zealand and the South Pacific islands has shown one of the highest rates of growth for production of carrots during recent years. Australia among the Oceanic region is one of the major producers of carrots.

In 1990 Australia produced approximately 152,105 tonnes of carrots, contributing to 1.12 per cent of the global production of carrot (Table 1.1) and 10.0 per cent and 0.03 per cent of Australian and global production of all vegetables respectively. Carrot production increased by 75 per cent within ten years contributing to 1.40 per cent of the global production of carrot (Table 1.1) and 14.7 per cent and 0.042 per cent of Australian and world vegetables respectively (Australian Bureau of Statistics (ABS) 1996: FAO 1999).

Total land under cultivation for the carrot crop in Australia exceeds 4,500 hectares (ABS 1996).

Table 1.1 Production of carrots and all vegetables in Australia and the world

	Carrots		All vegetables	
Year	**Australia**	**World**	**Australia**	**World**
1990	152,105	13,581,309	1,557,332	461,763,035
1991	158,317	13,905,705	1,513,469	464,511,816
1992	168,917	13,983,982	1,442,585	479,777,864
1993	169,517	15,450,249	1,546,814	508,024,137
1994	194,839	15,681,060	1,586,617	532,663,037
1995	285,539	16,514,535	1,721,947	560,758,211
1996	249,926	17,804,642	1,776,514	591,205,238
1997	257,405	18,660,879	1,781,173	599,038,167
1998	267,000	18,390,051	1,808,500	616,529,400
1999	267,000	18,445,285	1,808,500	628,747,256

Note: 1. All the data are expressed in units of tonnes and were obtained from FAO (1999)

2. Data for 1999 are provisional estimates

Less than half of Australian carrot production is used domestically as either fresh vegetable or in processed forms including frozen and canned carrots as well as prepared salads. Until recently, most of the remaining production has been juiced for export markets, in particular for Japan and other Asian countries. However, there is considerable potential for increased juice production to service both local and additional international markets. The increasing awareness of and growing demands for carrot juice on world markets, together with increasing consumer concerns regarding the use of preservatives and the trend towards all natural products, provides an opportunity and incentive to further develop juice manufacturing technology to address these consumer demands.

In 1994, Australia joined other countries including the United Kingdom, United States of America, Spain, Germany and South American countries as a manufacturer and

exporter of carrot juice. In order to remain competitive in the international market, a continuous supply of a higher quality product is of critical importance. Typically world trade in carrot juice involves juice concentrates. The current manufacturing process for carrot juice concentrate involves thermal processing at various stages and this causes a major deterioration in the important sensory properties of colour and flavour. The processing of juice at higher temperatures may also result in a serious loss of the inherent nutritive value of the carrot. A further consideration is the potential application of newer technologies to replace higher temperature processing and thereby overcome the adverse effects on the quality of the final product. In addition to these issues in the production of carrot juice, factors other than those associated with processing may be important. Those which may influence the quality of the end product include carrot varietal effects as well as post harvest handling.

At the inception of this project in 1995, there were approximately ten processors of carrot juice in Australia. All of these producers exported to Japan and other Asian countries. In due course, other processors including South American, particularly Brazil and Chile entered the market. These suppliers utilised American technologies and gained a competitive edge over Australia for the export of this product to Japan and Asian countries. The inability of Australia to provide product of higher quality and at a cheaper cost in international markets provided opportunities for competitors to become established in the markets of the Asia Pacific region. This has impacted dramatically on the production and marketing of Australian carrot juice in recent years (Personal correspondence with Mr Vimal Maharaj of Cargill Australia Pty Ltd 1998).

Globally, there are many varieties or cultivars of carrot used for processing, and these vary in size, shape, colour and flavour as well as in contents of sugar, β-carotene and total solids. Considerable differences have also been reported in the yield, size, shape, uniformity, colour, flavour, texture, nutritive value, digestibility, protein content, amino acid composition, lead and cadmium contents amongst carrot varieties even when these have been grown under similar conditions (Kalra *et al* 1987; Bajaj *et al* 1978). However, up to this point there has been limited research carried out into the processing of carrot. In

addition, there is very limited information available in the literature regarding enzymes which potentially may have a deteriorative role during carrot juice processing.

Accordingly, the current project was developed as a strategic investigation into the processing of carrot. The primary aim has been to define the processing protocols necessary to produce carrot juice of optimal quality. Within this context, a detailed and systematic study has been undertaken into the parameters influencing the sensory and nutritional qualities of carrot juice as well as carrot juice concentrates.

Four varieties of carrots have been selected from both South Australian and Victorian carrot growing regions for investigation. The varieties selected were Red Hot Original and Red Count (both traditionally used varieties) and Red Hot Carotene 100 and Top Pak (both recently developed juicing varieties) abbreviated as RHO, RC, RHC and TP respectively. These varieties have been investigated and compared for compositional, nutritional and juice quality parameters.

All the four carrot varieties were also investigated for the activities, localisation and thermal inactivation of three deteriorative enzymes (peroxidase, catechol oxidase and pectinesterase) as well as for regeneration of peroxidase. The heat stability of the enzymes from each of the varieties was also investigated as a basis for studies of blanching of carrots. All four carrot varieties were treated under varying conditions using boiling water, microwave energy and steam. The impact of these blanching techniques on deteriorative enzymes, juice quality parameters and nutritive values of carrot juice have been studied.

Blanched carrots after juice extraction were treated using various proportions of commercially produced fungal enzymes containing pectinesterase as well as polygalacturonase activities during the research project. The effect of varying proportions, treatment times and treatment temperatures on juice yield and other juice quality parameters has been reported.

Juices extracted from blanched carrots were concentrated using various approaches including thermal techniques (rising film evaporator) and newer nonthermal

technologies (ultrafiltration and reverse osmosis). The effects of each of these concentration techniques on sensory and nutritional quality parameters of carrot juice have been studied. Unconcentrated and concentrated juice samples produced using the techniques mentioned earlier, have been packed in various packaging materials including both aseptic and nonaseptic methods. The influence of storage at various storage temperatures (-18°C, 2°C and room temperature) for a period of 6 weeks was then compared. The effects of the various treatments were investigated in relation to the sensory and nutritive quality of carrot juice.

Chapter 2

Literature review

2.1 General introduction

2.1.1 Historical origins and development of carrots

Modern varieties of carrots have typically long, smooth, straight, orange coloured roots which are topped by fernlike, bright green foliage. There is some controversy surrounding the origins of the carrot (*Daucus carota* L). The most widely held view is that the species originated in the region now known as Afghanistan and neighbouring lands (Encyclopaedia Britannica Micropaedia 1995). It has also been suggested (Kalra *et al* 1987) that the carrot originated in Central Asia and later spread to Europe particularly around the Mediterranean and also to Northern American countries. However, another theory is that wild carrots (Queen Annes Lace) were natives of Northern American countries and the cultivars developed from these were transported to Europe and Asia (Mazza 1989). The timing and mechanisms of these postulated intercontinental transfers remain unclear.

The carrot roots can be found in a variety of colour including white, yellow, red, purple and orange red colour (Encyclopaedia Britannica Micropaedia 1995). It has been recorded that the purple varieties were one of the first plants cultivated by the Roman surgeon Dioscorides as well as Germanic tribes during the first century AD. At that time carrots were given the name Stafylinos to distinguish it from parsnip which was named Elafoboskon (Mazza 1989). These purple coloured varieties reached China during the 13th century and Japan in the 17th century. In Europe, carrots of red and yellow varieties were cultivated in the 16th century and soon after carrots became popular in India, with their growth expanding to the northern and western parts of the continent. During the early years of the 19th century, carrots were grown domestically in backyards before a French seed breeder Vilmorin (Mazza 1989), initiated commercial cultivation in Europe and Northern American countries. Today carrots are grown extensively throughout the temperate zones of the world.

2.1.2 The utilisation of carrots

Until the early twentieth century, carrots were mainly used as feed for animals. However, since then their consumption by humans has become increasingly popular. Carrot as a vegetable consists of two portions namely the root and the leaves. The leaves are used as cattle feed and horse fodder, while the meal prepared from carrot leaves using spicy flavours is reported to have been used as poultry feed in Java (Bentley and Trimen 1952).

The carrot root is consumed in various forms including raw, semiprocessed and fully processed forms. More than half of the global carrot production is used for making various processed products. The remaining produce is consumed in semiprocessed and raw forms. Globally salads represent the most widely consumed form of semiprocessed carrots (Mazza 1989; Kalra *et al* 1987). Other commercially processed products that are widely consumed include jams, beverage/juice/nectar and dips (Mazza 1989; Kalra *et al* 1987; Saldana *et al* 1980; Saldana *et al* 1976; Stephen *et al* 1976). Processed products of carrots are produced both industrially and domestically. In Africa and the Indian subcontinent, carrot products are processed domestically in a variety of forms, including, stews, curries, preserves, chips and pickles products as the most popularly consumed (Kalra *et al* 1987; Bajaj *et al* 1978). In other parts of the world, the carrot is consumed in various processed forms. In the United States of America, carrot is popularly consumed as processed products particularly carrot juice, slices and soups. Additionally, food products containing carrot such as carrot puree with nutmeg, fruited salads, carrot cakes and pies are popularly consumed (Californian Fresh Carrot Advisory Board (CFCAB) 1996; Saldana *et al* 1980). In Europe, carrot juice, slices and soups are most popular. In Asian countries particularly in Japan, carrot juice in combination with juices from apple, orange and other vegetables are rapidly gaining popularity (Industrial correspondence 1998). Although Australia has become one of the largest processors and exporters of carrot juice in the global market, consumption of this product within Australia was not realised until early 1996. Since then and currently, carrot juice in combination with other vegetables (tomato, celery, beet root) as well as fruits (apple and oranges) has become increasingly popular.

2.1.3 Therapeutic and nutritional value of carrots

Carrot root and its products have been used for medicinal purposes for well over a century (Mazza 1989; Kalra *et al* 1987). For example an infusion of carrot has been used as a folk remedy for the treatment of threadworms. Carrot juice is reported to be a diuretic and to facilitate the elimination of uric acid as well as favourably affecting nitrogen balance. The use of carrot juice to reduce blood sugar has also been reported (Bentley and Trimen 1952).

Carrots offer number of nutritional advantages with the fresh product being low in energy and a good source of fibre, potassium and other minerals. Of all the vegetables, carrots provide one of the richest sources of carotene, especially β-carotene. As well as their role in vision, carotenes have been claimed to provide protection against various forms of cancer and heart disease (Abbey *et al* 1996; Macrae *et al* 1993; Mayne *et al* 1992; Murakoshi *et al* 1992; Moon 1989; Zeigler 1989; Krinsky 1988; Temple and Basu 1988; Olson 1986; Peto *et al* 1981). In addition, it has recently been demonstrated that the juices extracted from raw carrots have antimicrobial properties, particularly against *Listeria* (Beuchart *et al* 1994; Beuchart and Brackett 1990).

2.2 Carrot varieties

Many different varieties or cultivars of carrots are grown around the world and these differ greatly in size, shape, colour, appearance, time of maturity, disease resistance and quality characteristics. Carrot varieties are commonly grouped according to their end use with some varieties being used for the fresh market while others varieties are used for processing. The quality aspects such as appearance, uniformity of size, freshness, maturity and handling compatibility are important for use in the fresh market. These characteristics are more readily achieved in carrot roots that are relatively longer and more slender in shape. Processing varieties are significantly larger in diameter than fresh market varieties and tend to be the varieties that produce higher crop yields (personal correspondence with Henderson Seeds Group 1998; Mazza 1989).

Carrot varieties may also be classified according to their shape and root length. Based on the shape and length of the root, carrot varieties are classed into various groups namely blunt-short, blunt-medium long and blunt-long, pointed-short, pointed-medium long and pointed-long (personal correspondence with Henderson Seeds Group 1997; Mazza 1989). The varieties *Oxheart* and *Chantenay* represent blunt-short and blunt-medium long varieties respectively while *Danver* and *Imperator* represent pointed-medium long and pointed-long varieties respectively (Mazza 1989).

Carrots are also identified according to the colour of the root and this may vary considerably depending on various factors including variety, growing conditions and area of cultivation. The various root colours reported by researchers include white, pale yellow, pinkish yellow, yellow, orange, reddish orange, purple and black (Umele and Gableman 1997; Mazza 1989; Watanabe *et al* 1988; Bajaj *et al* 1978).

2.3 Composition of carrots

Carrots have relatively high moisture content. Other components present in carrot are carbohydrates, proteins, lipids, volatile components, enzymes, vitamins and minerals (Cashel *et al* 1989; Mazza 1989; Kalra *et al* 1987; Munsch *et al* 1986; Bajaj *et al* 1978). All the values reported in the composition section are expressed in units of per cent and on a wet weight basis.

2.3.1 Moisture

In the past there have been various reports on the moisture content in carrot root. The content varies depending on the variety and growing conditions and the values reported range between 86-92 per cent (Jobling *et al* 1997; Khokhar and Pushpanjali 1995; English and Lewis 1992; Cashel *et al* 1989; Mazza 1989; Kalra *et al* 1987; Munsch *et al* 1986; Bentley and Trimen 1952). Ayranci and Tutunculer (1993) investigated the moisture contents of various coloured carrots and reported values ranging between 82.1-87.6 per cent. These workers found that yellow coloured carrots contained higher moisture contents (87.6 per cent) compared to orange (86.6 per cent) and purple coloured carrots were reported to contain the lowest moisture (82.1 per cent). The moisture content of the most commonly available orange colour was reported to be 86.6

per cent, which is within the range of the values reported by other workers in the literature.

In earlier investigations of carrots Kaur *et al* (1976) reported minor variations in moisture contents between carrot varieties. They compared the common varieties *Nantes* (89.9 per cent), *Pusa Kesar* (91.4 per cent) and *Suchet Sharbati* (91.3 per cent). More recently, varieties such as *Nantes, Red Core Danver, Red Core Chantenay* and *Imperator* have been analysed (Mazza 1989; Krarup *et al* 1986) and the moisture contents reported were similar to the values reported previously.

As found for many foods, the moisture in carrot can be classified into three categories. The major part is free moisture which freezes at temperatures of between 0 and -4°C. The other types are weakly bound moisture, which freezes between -4 to -14°C, and strongly bound moisture (Kalra *et al* 1987). Details on locations within carrot tissues and proportion of weakly and strongly bound moisture in carrot tissues remain unclear.

2.3.2 Protein

Proteins are complex organic materials containing sulfur, hydrogen, oxygen, nitrogen and carbon. They are made up of multiple units of amino acids and fulfil many essential roles as enzymes and hormones and facilitate tissue development in the body.

Foods of nonvegetarian origin including meat, beef and pork, milk and eggs are reasonably good sources of proteins and essential amino acids required for normal body functions and growth. Foods of vegetarian origin including grains, legumes, nuts and other seeds are significant sources of protein. Vegetables such as beans, broccoli, cauliflower, mushroom and Brussels sprouts are also good sources of proteins.

In the past limited data have been reported on the analysis of protein content in carrot. They are relatively low in protein with a reported range of 0.77-1.26 per cent (Jobling *et al* 1997; Khokhar and Pushpanjali 1995; English and Lewis 1992; Cashel *et al* 1989; Mazza 1989; Kalra *et al* 1987; Munsch *et al* 1986; Truesdell *et al* 1984; Pruthi *et al* 1980; Bentley and Trimen 1952). Ayranci and Tutunculer (1993) investigated carrots of

different colours for their protein contents. The purple coloured types contained the highest levels of protein (0.86 per cent) compared to orange (0.82 per cent) and yellow (0.80 per cent) carrots. These values are within the range previously reported in the literature. In another investigation carrot varieties *Nantes, Pusa Kesar, Suchet Sharbati, No. 29, Selection 233* and *Me n* were reported to contain protein in the range of 0.76 - 1.05 per cent (Kaur *et al* 1976). Most values reported were within the range of previously reported values however, *Nantes* contained the highest protein (1.1 per cent). Minor variations between *Pusa Kesar, Selection 233* and *Me n* were also reported. Mazza (1989) found 1.0 per cent protein in the varieties *Red Core Danver* and *Imperator*. However, the varieties *Red Core Chantenay* and *Nantes* were reported to contain 0.8 per cent protein. The protein content of the variety *Nantes* was less than the values reported by Kaur *et al* (1976). Although the protein content of carrot varies with varieties as well as growing conditions, carrots are relatively poor sources of protein. For example, it has been stated that one hundred grams of carrot contain less than 2 per cent of the recommended daily intake (RDI) for the human diet (CFCAB 1996). Carrot protein has been reported to particularly contain the amino acids aspartic acid, alanine, serine, glutamic acid, phenylalanine, tyrosine, valine, arginine, leucine, proline, methionine, cystine and glycine (Kalra *et al* 1987).

2.3.3 Dietary fibre

Dietary fibre is generally considered to include all of the nonstarchy polysaccharides, although it is now recognized that some starch may be regarded as fibre and this is referred to as resistant starch. Dietary fibre is typically structural components of the cell walls of plant materials. Cellulose is considered as insoluble dietary fibre. Soluble fibre components include hemicellulose, pectin, gums and lignin. They are found in plant products including cereals, pulses, vegetables, fruits, nuts and seeds. Commonly major sources of dietary fibre for the diet are grains, cereals and their products. Vegetables and fruits are often regarded as poorer sources of fibre content compared to cereals and grains. Amongst vegetables, carrot, celery, lettuce, asparagus and green leafy vegetables contain higher fibre compared to most other vegetables. In fruits, prunes, dates, raisins, plum, citrus, apricot and strawberry are good sources of fibre (Doyle 1995; English and Lewis 1992).

Dietary fibre is increasingly considered to have great significance for humans. Therapeutically, fibre is known to affect faecal bulking thus lowering the risk of diverticular disease of bowel and gastrointestinal cancers (Doyle 1995). In addition, fibre has been claimed to facilitate reduction in ischaemic heart diseases (Khokhar and Pushpanjali 1995).

One of the early reports of the fibre content of carrots gives a value of 1.2 per cent (Anon 1953). More recently, the total dietary fibre content of various vegetables including carrot, cauliflower, gingelly seeds, ladies finger, mustard leaves, tinda and spinach have been reported (Khokhar and Pushpanjali 1995) within the range of 19-24 per cent. These analytical values include hemicellulose, celluloses and pectin components (Khokhar and Pushpanjali 1995). The total dietary fibre of spinach was reported to be higher (24.1 per cent) than that of carrot (21.0 per cent). The dietary fibre contents of carrot vary depending upon varieties and growing conditions with reported values in the range of 0.5-4.4 per cent (Svanberg *et al* 1997; CFCAB 1996; English and Lewis 1992; Pruthi *et al* 1980; Bentley and Trimen 1952). Minor variations in dietary fibre contents in carrots were reported by Krarup *et al* (1986) for the varieties *Imperator, Chantenay* and *Nantes* having values of 0.74, 0.72 and 0.65 per cent respectively. The wide variation in figures for fibre contents in the literature are a direct result of two issues. One is the use of different moisture bases for expression of results. For vegetables which are high in moisture, expression on a dry matter basis will give very much higher apparent fibre values. The second issue is that of the methodology for fibre detemination where there have been rapid changes over recent decades. Newer methods measure more of the components now included in the definitions of dietary fibre and therefore give higher values.

Relatively little data is available on the contents of particular fibre components in foods. In an investigation of pectin in many different fruits and vegetables, Baker (1998), and reported contents the range of 0.09-4.5 per cent. Grapefruit was found to contain the highest pectin content. Values for carrot reported by the same author ranged between 1.17-3.0 per cent which is consistent with the value of 3.0 per cent reported by Khokhar and Pushpanjali (1995).

2.4 Micronutrients

2.4.1 Carotenes

Food carotenes are C_{40} tetraterpenoid compounds and are found universally in the chloroplast of green tissues. Leaves of all the plant species are also found to contain some carotenes. In nature there are at least 563 different carotenoid compounds identified. Amongst these are α-carotene, β-carotene, lutine, zeta carotene, xanthine, lycopene and β-cryptoxanthine. In human diets, some carotenoid compounds are used to produce retinol which is also known as vitamin A. Deficiencies of this vitamin are responsible for skin rashes, night blindness and other eye disorders (Howe 1980). It is also useful in maintaining healthy skin, eyes and provides immunity against infections (Doyle 1995). Carotenes have also been claimed to provide protection against cancer (Macraceae *et al* 1993; Muraksohi *et al* 1992; Krinsky 1988; Moon 1989; Temple and Basu 1988; Oslon 1986). Each carotenoid compound varies in its bioavailability for vitamin A activity in the human body. α-Carotene, β-carotene and β-cryptoxanthine have varying effectiveness as precursors of vitamin A having relative values of 50-54 per cent, 100 per cent and 50-60 per cent for vitamin A activity respectively. However, lutine, zeta carotene, xanthine and lycopene appear to be inactive as precursors of vitamin A (Macrae *et al* 1993; Pesek and Warthesen 1987; Simpson 1983).

Some foods of animal origin including cod liver oil, livers and fatty fishes as well as egg yolk, full fat milk, butter and cheese are sources of carotenes. Amongst the foods of plant origin yellow, red, orange coloured fruits and vegetables including carrots, leafy vegetables, apricot, mango and peaches are rich sources of carotenes. The carrot is considered to be the richest source of carotenes.

Various fruits and vegetables including lettuce, carrots, brussels sprouts, green beans, beet roots, spinach, tomato, red pepper, cabbage, red cabbage, cucumber, onion, potato, cauliflower, squash, sweet potato, nectarine, plum, apricot, asparagus, cantaloupe, oranges, grapefruit, peaches and blueberries have been investigated for carotene content. Varying proportions of carotene isomers including major quantities of α-carotene and β-carotene in the range of 0.27-15.3 mg/100g were reported (CFCAB 1996; Bao and Chang 1994a; Bao and Chang 1994b; Grando *et al* 1992; Cashel *et al* 1989; Dikshit *et*

al 1988; Kim and Gerber 1988; Watanabe *et al* 1988; Chandler and Schwartz, 1987; Pesek and Warthesen 1987; Bureau and Bushway 1986; Krarup *et al* 1986; Hojilla *et al* 1985; Bushway and Wilson 1982; Bajaj *et al* 1978; Baloch *et al* 1977; Umele and Gableman 1997; Sweeney and Marsh 1971; Panalaks and Murray 1970; Bradley and Duck 1968; Laferriere and Gabelmann 1967).

In the past carotenes from carrots have been extensively studied and varying quantities have been reported depending upon the variety, growing conditions and maturity (Umele and Gableman 1997; Bajaj *et al* 1978; Khan *et al* 1975; Bradley and Duck 1968). These isomers identified and reported in carrot include α-carotene, β-carotene, all-trans β-carotene, neo-β- carotene B, neo-β-carotene U, xanthophyll, lutine, zeta carotene, xanthine, lycopene, β-cryptoxanthine, gamma carotene, *cis*-neurosporen, neurosporen and phytofluene. Various carrot varieties have been studied for their carotene contents. Bajaj *et al* (1978) investigated many varieties including *Black carrot, Yellow carrot, Subagh, Nabha, Waryana, No. 29, No. 8-75, S.233-21-75B, Pusa Keasr, No. 10-75B, No. 10-75A, Nantes IARI, S.223, Nantes NSC, Sel. 5A, S.233-75-C, Sel. 5, Sel. 5B, and S.233-22-75A* for carotene contents. β-Carotene contents ranging between 0.40-4.30 mg/100g were reported. Carrots of black and yellow colour were low in β-carotene however red, orange, deep orange and orange red was reported high in β-carotene. In another investigation Krarup *et al* (1986) analysed the β-carotene contents in the varieties *Emperator, Chantenay* and *Nantes*. Values ranging between 4.6-7.2 mg/100g were reported. β-Carotene levels of *Emperator* variety were higher compared to the other varieties. Total carotene contents (7.9-9.3 mg/100g), α-carotene (2.5-3.6 mg/100g) and β-carotene (4.5-6.0 mg/100g) were reported by Bradley and Duck (1968) when varieties such as *Danver 126, Ch. Red Core, Royal Chantenay, Scarlet Nantes, Pioneer, Walthman Hi Color, Bangor F1 BZ, Bergen F1 BZ, Berlicum N, Berlicum R, Casey F1 BZ, Chantenay R, Flakker G, Flakker R, Flaxton F1 BZ, Florence F1 BZ, Fontana F1, Nairobi F1 BZ, Nantes Duck Notabene 370, Nantes Fancy 405, Nantucket F1 BZ, Nantucket F1 BZ, Napoli F1 BZ, Narbpooe, F1 BZ, Nelson F1, BZ, Nelson F1, Rondino F1 BZ* were investigated for carotene content. Total carotenes (1.6-12.6 mg/100g), α-carotene (2.3-4.4 mg/100g) and β-carotene (8.2-11.2 mg/100g) have been reported (Bao and Chang 1994a; Bao and Chang 1994b; English and Lewis 1992;

Grando *et al* 1992; Heinonen 1990; Cashel *et al* 1989; Dikshit *et al* 1988; Kim and Gerber 1988; Kalra *et al* 1987; Truesdell *et al* 1984; Bushway and Wilson 1982; Baloch *et al* 1977).

2.4.2 Other vitamins

Vitamins are organic substances and essentially regulate chemical and biochemical reactions in the body. Vitamins are classified into two groups on the basis of solubility characteristics: water soluble (B complex group and vitamin C) and fat soluble (A, D, E and K).

2.4.2.1 Ascorbic acid

This compound is also known as vitamin C. A deficiency in vitamin C is responsible for the disease known as scurvy which is characterised by multiple hemorrhages. It helps to maintain healthy skin, blood vessels, gums and teeth. In addition to its chemical nature as an antioxidant, it is also helps absorption of iron in the digestive system (Doyle 1995; Howe 1980).

Vegetables and fruits that have a sour taste (citrus, blackcurrant, grapes, mango, tomatoes) are particularly good sources of vitamin C. In addition vitamin C values (mg/100g) of various vegetables have been reported including broccoli (97-110), cabbage (15-100) Brussels sprouts (80-100) parsley (150), cauliflower (38-74), cucumber (13-15), lettuce (2-7), potato (7-19), tomato (9-22) (Hagg *et al* 1994; English and Lewis 1992; Vanderslice *et al* 1990; Cashel *et al* 1989).

Carrots are relatively poor sources of vitamin C. The vitamin C content of carrot varies between varieties with values reported to range between 3.0-7.0 mg/100g (English and Lewis 1992; Vanderslice *et al* 1990; Cashel *et al* 1989; Truesdell *et al* 1977). Krarup *et al* (1986) analysed carrot varieties *Imperator, Chantanay* and *Nantes* for vitamin C contents and reported values of 6.7, 3.6 and 3.5 mg/100g respectively. The RDI values of vitamin C for infants and adults are 25 and 40 mg/100g respectively. Consumption of one hundred grams of carrot is reported to satisfy less than 10 per cent of the RDI (CFCAB 1996).

2.4.2.2 Thiamin

Thiamin is one of the group of B complex vitamins and is also known as vitamin B_1. Deficiency of thiamin causes a disease known as Beri-Beri involving malfunctioning of the nervous system. It is useful in normal functioning of the brain and nervous system as well as appetite and digestion (Doyle 1995; Howe 1980).

Foods of animal origin are not generally high in this vitamin, although lean pork and liver are recognised sources of thiamin. Among the foods of plant origin, whole grains, cereals, pulses and peanuts are rich sources of thiamin. Fruits and vegetables are relatively poor sources of this vitamin. Carrots are reported to contain in the range of 0.6-0.7 mg/100g (English and Lewis 1992; Cashel *et al* 1989; Truesdell *et al* 1977). It has been noted that one hundred grams of carrot satisfies less than 2 per cent of the RDI (CFCAB 1996).

2.4.2.3 Riboflavin

Riboflavin is also a B complex vitamin and is referred to as vitamin B_2. Deficiency of riboflavin is responsible for sores on the skin and corners of the mouth. Riboflavin helps the breakdown of carbohydrates, proteins, fats in the body. It also helps the enzyme regulating activity of the thyroid gland (Doyle 1995; Howe 1980).

Foods of animal origin such as milk, cheese, lean pork kidney and livers are good sources of riboflavin. Among the foods of plant origin, whole grains, cereals and pulses are rich sources of riboflavin. However, fruits and vegetables are poor sources of this vitamin (0-0.4 mg/100g). Carrots are reported to have contents in the range of 0.6-0.7 mg/100g (English and Lewis 1992; Cashel *et al* 1989; Truesdell *et al* 1977). One hundred grams of carrot is reported to satisfy 2 per cent of the RDI (CFCAB 1996).

2.4.3 Minerals

Minerals are inorganic substances and perform a wide range of vital physiological and biochemical functions in the body. Based on quantitative requirements, they are referred as trace elements. Physiological functions contributed by calcium, phosphorus and magnesium are in strengthening of bones and teeth. Salts of potassium and sodium

chlorides are useful in maintaining control of body fluids. Iron and phosphorus are essential components of enzymes and other protein molecules (Doyle 1995; Howe 1980).

Most foods of animal origin and their products including milk, eggs, meat, beef, pork and fish are relatively rich in minerals including sodium, potassium, calcium, magnesium, iron and zinc. Cereals and pulses are good sources of potassium, calcium, magnesium, iron and zinc and poor sources of sodium. Other foods of plant origin and their products including fruits and vegetables are rich sources of sodium, potassium, calcium, magnesium and iron and poor sources of zinc (English and Lewis 1992). The consumption of a wide variety of foods including dairy products, meat, fish, eggs, fruits, vegetables, cereals and cereal products and pulses generally provides sufficient minerals necessary to carrying out body functions.

A variety of minerals have been analysed in carrots. Total minerals contents of up to 1,100 mg/100g have been reported in carrots. Minerals including sodium, potassium, calcium, magnesium, iron, zinc, cobalt, fluoride, copper, manganese, aluminum, barium, strontium, lead and cadmium have been reported in carrot (Cashel *et al* 1989; Kalra *et al* 1987; Bentley and Trimen 1952). Various carrot varieties including *Nantes, Pusa Kesar, Suchet Sharbati, No. 29, Selection 233* and *Me n* were investigated for their mineral contents (Kaur *et al* 1976). The minerals measured include calcium (55.9-72.9 mg/100g), phosphorus (47.3-61.6 mg/100g), iron (2.4-3.1 mg/100g), copper (0.09-0.11 mg/100g) zinc (0.25-0.28 mg/100g), and manganese (0.26-0.35 mg/100g). Among all the varieties *Nantes* was richest in phosphorus, copper and zinc. *Pusa Kesar* was richest in manganese. It has also been reported that carrot is a rich source of potassium (234 mg/100g), sodium and calcium (41 and 30 mg/100g) and a poor source of iron, zinc and magnesium (0.3, 0.2 and 10 mg/100g) (English and Lewis 1992; Cashel *et al* 1989). Each one hundred grams of carrot satisfies up to 2 per cent of RDI for minerals such as calcium and magnesium and less than 2 per cent for iron and zinc (CFCAB 1996).

2.5 Juice quality parameters

In the commercial production and processing of food and food products the issues of primary concern are their sensory qualities particularly taste (contributed by soluble solids including sugars and acids), flavour and colour. The soluble solids are typically measured using hand held instruments known as refractometers and acids are analysed by titration. Colour characteristics are analysed instrumentally using colour analysers and chroma meters.

2.5.1 Soluble solid content

The term total soluble solids is widely used in relation to liquid foods and beverage products and is commonly abbreviated as TSS. This value is generally taken to reflect sugars/carbohydrates of various types (reducing and nonreducing sugars, total sugars) as well as proteins and food acids available in the food system. In commercial situations, TSS is measured by refractometer and results are expressed as °Brix. The results provide an approximate measure of the sugars/ carbohydrate content of a food and primarily include smaller sugar units such as glucose, fructose, galactose, mannose, xylose and maltose. All these sugars collectively impart sweetness to the food products.

In the past very limited research has been carried out for the analysis of sugars in carrots. It is reported that carrot contains both reducing and nonreducing sugars (Kalra *et al* 1987). Kaur *et al* (1976) investigated varieties including *Nantes, Pusa Kesar, Suchet Sharbati, No. 29, Selection 233* and *Me n* for their sugar contents and reported reducing sugar (1.67-3.35 per cent), nonreducing sugar (1.02-1.18 per cent) and total water soluble sugars (2.71-4.53 per cent). English variety *Nantes* was reported to contain the highest content of total soluble sugar (4.53 per cent), free and bound reducing and non reducing sugar (1.0-1.1 per cent). Other varieties *No. 29, Selection 233* and *Me n* were reported to contain similar sugar levels (3.3-3.5 per cent) as well as free reducing (2.1-2.5 per cent) and non reducing (1.0-1.1 per cent) sugars. In another study carrot varieties including *Black carrot, Yellow carrot, Subagh, Nabha, Waryana, No. 29, No. 8-75, S.233-21-75B, Pusa Keasr, No. 10-75B, No. 10-75A, Nantes IARI, S.223, Nantes NSC, Sel. 5A, S.233-75-C, Sel. 5, Sel. 5B, and S.233-22-75A* were investigated for total sugar contents. Values ranging between 3.5-6.8 per cent have been

reported (Bajaj *et al* 1978). These workers also investigated *No. 29, Selection 23 and Pusa Kesar* for total soluble sugar content and reported that *Selection 23* contained the highest total soluble sugars (4.94 per cent) followed *by Pusa Kesar* (4.75 per cent) and *No 29* (4.49 per cent). The values reported were higher in all three varieties compared to the values previously reported by Kaur *et al* (1976). Variety *Yellow carrot* was reported to contain maximum sugars (6.82 per cent) (Bajaj *et al* 1978).

In other investigations, TSS of food products have also been analysed by refractometry and values ranging between 7.0-12.5 °Brix were reported (Industrial correspondence 1998). Mazza (1989) analysed varieties including *Kinko 6, Kinko 8, Nz251, Nantes strong top, Waltham Hi Color, Pioneer F1, Gold Pak, Highpak Elite, Hicolor, Caravella F1, Spartan Bonus F1, Carousal, Danver 126, Scarlet Nantes and Imperator extra long* for TSS contents. A range of soluble solids (6.4-9.3 per cent) was reported. *Spartan Bonus F1* contained highest TSS and other varieties (*Nantes strong top, Waltham Hi Color, Pioneer F1, Gold Pak, Hipak Elite, Spartan Bonus F1, Carousal, Danver 126, Scarlet Nantes* and *Imperator*) contained similar soluble solids *(7.2-7.8* per cent*)*. Mazza (1989) also investigated the varieties *Chantenay, Royal Chantenay* and *Red Core Chantenay* (8.10-10.9 per cent) and *Emperator* and (8.7 per cent) for TSS and reported minor variations between varieties. Carrots of various colours including yellow, orange and purple were also investigated and values reported ranged between 6.0-8.1 per cent. Purple coloured carrots were reported to contain highest TSS levels (Ayranci and Tutunculer 1993).

2.5.2 Acidity

In the past various fruits and vegetables have been analysed for their acid contents. Foods typically contain organic acids including citric acid, oxalic acid, malic acid, tartaric acid, lactic acid, pectic acid, glutamic acid, chlorogenic acid, caffeic acid and many more. These acids collectively form part of soluble solids and are analysed by titration using alkali solutions. Each of these acids is often present naturally as their sodium, potassium or other metal salt forms. Oxalic acid is essentially an acid of vegetables including green leafy vegetables and rhubarb, stems and leaves contain up to 50 per cent of the soluble solids in the form of this compound. Pectic acid and succinic

acid are also widely present in most of the vegetables. Some of the root vegetables also contain chlorogenic acid. Amongst the fruits citric and malic acids are widely present in all the citrus and some berry fruits. Some organic acids such as malic acid and pectic acid are widely present in both fruits and vegetables.

Carrot is low in acid and is reported to contain small quantities of succinic acid, lactic acid, pectic acid, citric acid, malic acid, glycolic acid, ascorbic acid and caffeic acid (CFCAB 1996; English and Lewis 1992; Cashel *et al* 1989; Kalra *et al* 1987; Krarup *et al* 1986; Truesdell *et al* 1977). Quantitatively these acids are expressed in units of per cent in terms of citric acid. Limited investigations have been undertaken in the past to analyse acidity of the juice extracted from carrot. Carrot juice is low in acid and is reported to contain 0.06-0.15 per cent acid (Industrial correspondence 1998; Bawa and Saini 1983; Saldana *et al*. 1976; Stephen *et al* 1976; Stephen *et al* 1974).

2.5.3 Colour

Colour is one of the most important sensory parameters for any food product. It is associated with consumer acceptance or rejection of the product. There are various pigments in food products which are responsible for imparting colour. Some of the most commonly known pigments include carotenes, lycopenes, chlorophyll, and anthocyanin. Carotenoids and lycopenes are responsible for imparting orange and red colours in food products. Chlorophylls and anthocyanins are responsible for imparting green or yellow colours.

The colour of food products can be analysed using various instruments including chroma meters, tintometers and colour analysers. Colour results may be expressed in terms of three individual components in a number of different measurement systems. These include units of *Y x y*, *L a b*, X Y Z and Rd *a b* values. Lightness of the food product has been indicated as L or Rd or *y* or X. Similarly values *a*(Chroma) and *b*(Hue) indicate redness and yellowness of the food product respectively (Howard *et al* 1996; Bao and Chang 1994a; Pesek and Warthesen 1987; Saldana *et al* 1976; Stephen *et al* 1976; Stephen *et al* 1974).

In the past various carrot varieties have been analysed for colour and the visual appearance has been investigated in relation to carotene contents. Various pigments including α-carotene, β-carotene, lycopene, lutine, xanthophyll, β-crypyoxanthine, zetaxanthine, neo β-carotene, phytofluene and gamma carotene and their varying proportions have been reported in relation to particular colour values (Grando *et al* 1992; Kim and Gerber 1988; Watanabe *et al* 1988; Chandler and Schwarts 1987; Pesek and Warthesen 1987; Umele and Gableman 1997; Hojilla *et al* 1985; Munsch and Simrad 1983; Bushway and Wilson 1982; Paulus and Saguy 1980; Bajaj *et al* 1978; Panalaks and Murray 1970). Bajaj *et al* (1978) investigated range of varieties of carrots of various colours and also the pigments associated with these colours. Black and yellow carrots were reported to possess lower quantities of β-carotene whereas red and orange carrots had higher levels of β-carotene.

Carrot varieties *Arizona, Quebec* and *Ontario* were analysed and colour values reported to range between 39.2-30.2 (L), 22.4-8.9 (*a*), 23.2-15.2 (*b*) (Munsch and Simrad 1983). Carrot samples from Arizona were reported to be red-orange and more appealing to the consumer compared to other samples. Similarly in other studies colour values of 43.8 (L), 34.7 (*a*), 26.7 (*b*) (Bao and Chang 1994a); 65.2 (L), 24.3 (*a*), 22.4 (b) (Bao and Chang 1994b); 44.9 (L), 46.3 (Chroma), 34.8 (Hue) (Howard *et al* 1996), 23.7 (Rd), 28.0 (*a*), 34.3 (*b*) (Saldana *et al* 1976), 16.5-20.9 (Rd), 17.8-29.7 (*a*), 21.9-33.5 (*b*) (Stephen *et al* 1974) have been reported.

2.5.4 Juice yield

Juice yield is considered is a factor of paramount economic significance for the juice processing industry. Although the information available is limited, juice yields ranging between 50-70 per cent have been reported, depending upon the method applied for extraction (Bao and Chang 1994a; Sims *et al* 1993; Munsch *et al* 1986; Stephen *et al* 1976).

2.6 Deteriorative enzymes

Vegetables and fruits are living parts of plants and continue to respire after harvest. Deteriorative chemical and biochemical changes can continue to occur during post harvest storage and handling. These changes include deterioration in colour, flavour and texture (Williams *et al* 1986).

Deteriorative changes are often driven by continued enzyme activity after harvest. The rate of these changes often increases if the food surface is bruised or cut. On the other hand, enzymes have also been reported to contribute to certain biochemical changes increasing the solids contents and enhancing colour, flavour, aroma, texture as well as the development of nutritional qualities (Barrett and Theerakulkait 1995; Rahman 1995; Velasco *et al* 1989; Baardseth 1979; Spares and Hicks 1976).

In the past various enzymes present in food have been found to contribute to deteriorative changes in food systems. These enzymes include peroxidase, catalase, catechol oxidase (commonly referred as polyphenoloxidase), pectinesterase (commonly referred as pectinmethylesterase), cellulase, polygalacturonase, thiaminase, lipase, lipoxygenase, ascorbic acid oxidase and protease. Each of these enzymes is normally associated with specific deteriorative changes in food. Lipases, lipoxygenase and protease have been reported to contribute to oxidative rancidity and eventual flavour loss (Barrett and Theerakulkait 1995). Peroxidase, catalase, catechol oxidase, ascorbic acid oxidase and thiaminase are reported to contribute to colour changes (Baardseth and Slinde 1987; Stauffer 1986; Williams *et al* 1986). Ascorbic acid oxidase and thiaminase also appear to cause nutritional changes in food systems. Pectinesterase, polygalacturonase and cellulase are often associated with textural changes in foods (Barrett and Theerakulkait 1995; Williams *et al* 1986; Baardseth 1979; Lee *et al* 1979; Burnette 1977).

A wide range of food products including vegetables and fruits, meat, fish, cereals and pulses have been investigated for the activities and action of deteriorative enzymes. Amongst the vegetables and fruits investigated have been potatoes and radish (Mihalyi and Vamos-Vigyazo 1975), carrot (Bolin and Huxsoll 1991; Plat *et al* 1990; Baardseth

and Slinde 1987; Lee *et al* 1979; Chubey and Nylund 1969), corn cobs, cabbage, Brussels sprouts, peaches, green beans, cucumber (Velasco *et al* 1989), eggplant (Aluko and Ogbadu 1986), green pepper, cauliflower, tomatoes (Castaldo *et al* 1996; Marangoni *et al* 1995; Nath *et al* 1983;), beet root, leek, broccoli, potatoes, onion, carrot, garlic, celery, parsley, squash, green / red pepper (Horvath and Gatai, 1977) spinach, apple, citrus (Hagermann and Austin 1986), red grapefruit (Cameron and Grohmann, 1995), grape, peas, papaya (Cano *et al* 1995), tomatoes (Thakur *et al* 1996) as well as green peas, green beans and carrots (Gunes and Bayindirli 1993).

Carrots have been investigated for the presence and action of some of these deteriorative enzymes. These include peroxidase, catalase, catechol oxidase, pectinesterase cellulase, polygalacturonase, lipoxygenase and ascorbic acid oxidase (Bolin and Huxsoll 1991; Plat *et al* 1990; Baardseth and Slinde 1987; Lee *et al* 1979; Chubey and Nylund 1969). In comparison with other vegetables and fruits, carrots have been reported to contain higher levels of peroxidase and catechol oxidase activities as well as low levels of pectinesterase activities (Bolin and Huxsoll 1991; Plat *et al* 1990; Baardseth and Slinde 1987; Lee *et al* 1979; Dorrell and Chubey 1972; Chubey and Nylund 1969). Lipoxygenase activity has not been detected in carrot (Baardseth and Slinde 1987; Williams *et al* 1986; Baardseth 1979; Lee *et al* 1979).

2.6.1 Peroxidase (EC 1.11.1.7)

Peroxidase is one of the earliest enzymes identified by mankind. It belongs to a large group of enzymes called the oxidoreductases. Peroxidase occurs in nearly all plants and animals as well as microorganisms. It is also considered to be one of the most stable of enzymes in extreme temperatures. It withstands boiling temperatures without permanent inactivation and remains mildly active at freezing temperatures as well as temperatures below -18°C (Ramanuja *et al* 1988; Burnette 1977; Diehl *et al* 1933).

As an oxidoreductase enzyme, the mechanism involves exchanges of hydrogen. Amongst the substrates used in the assay of peroxidase activity, pyrogallol, guaiacol, hydroquinone, catechol, cresol and tyrosine are most widely used. The typical reaction catalysed by peroxidase enzyme is shown (Ashie *et al* 1996; Vamos-Vigyazo 1981).

ROOH	+	AH_2	→	H_2O	+	ROH	+	A
Substrate		Donor Substrate		Water				Free Radical

In the past various food products have been investigated for peroxidase enzyme activity and the role of the enzyme in deteriorative changes in food. Attempts have been made to investigate and understand the mode of action of peroxidase enzyme with limited success. This is due to the presence of multiple isoenzymes referred to as isoperoxidases. As plant foods are complex systems and continue to respire after harvest, the metabolic activities during post harvest storage conditions also continue to occur. Peroxidase enzyme activities during such changes have been reported to increase (Robinson 1991) and cause deteriorative changes. In addition, peroxidase oxidises lipids and chlorophyll and facilitates quicker deteriorative changes when the food system undergoes physical damage such as cutting, brushing, breaking and crushing during processing (Ashie *et al* 1996; Barrett and Theerakulkait 1995; Babic *et al* 1993; Robinson 1991; Burnette 1977; Reed 1975; Lu and Whitaker 1974; Maehly and Chance 1954). Traditionally, peroxidase has been used as the indicator enzyme due to its relatively greater heat stability and ability to regenerate activity once inactivated (Fox 1991; Adams 1981), thereby causing considerable deteriorative changes during extended product storage.

Peroxidase is relatively non specific and a large number of different phenolic and aromatic compounds can act as substrates. Once the food system has undergone physical damage peroxidase enzyme cause oxidiation of phenolic compounds, lipids and chlorophyll resulting in colour changes.

Many vegetables have been assayed for their peroxidase and a wide range of activities have been reported in the literature and this may reflect the fact that the substrates used, the conditions of assay as well as other parameters can influence the results obtained. Ramanuja *et al* (1988) assayed peroxidase activities of vegetables including cluster beans (300-400), horseradish (340-460), lobia (130-170), cabbage (430-490), khol-khol (280-330), cauliflower (130-160), kovia tender (140-220), Japanese radish (160-240), peas (4.5-9), eggplant (32-54), onion (1.5-3.5), ash gourd (3.9) and pumpkin (1.5)

Cluster beans were reported to contain the highest peroxidase activity amongst the vegetables studied. Aluko and Ogbadu (1986) investigated the peroxidase activity of eggplant and reported values of 0.8-1.1 when assayed by the same procedure. This was lower than the value reported by Ramanuja *et al* (1988). Baardseth and Slinde (1987) investigated and reported the peroxidase activities of vegetables including onion (2), leek (12), celery (11), beetroot (39), swede (1281), kale (105), cauliflower (150), cabbage (804), Brussels sprouts (2656), broccoli (406), red pepper (0), green pepper (10), cucumber (12), lettuce (3), parsley (39), snap beans (132), peas (289), tomatoes (24), potatoes (20) and corn (201). Brussels sprouts were reported to have the highest and onion the lowest peroxidase activity. Peroxidase activity was not detected in red pepper. The activities of peroxidase in cabbage and peas reported by Ramanuja *et al* (1988) were found to be much lower than the values reported by Baardseth and Slinde (1987). Vegetables including swede, Brussels sprouts, cabbage, horseradish and cauliflower were reported to contain significantly higher values compared to most other vegetables (Ramanuja *et al* 1988; Baardseth and Slinde 1987).

There have been limited investigations relatively little information is available on peroxidase activity of carrot. It appears that carrots are relatively low in peroxidase activity with values of 8.0 activity units (Baardseth and Slinde 1987).

2.6.2 Catechol oxidase (EC 1.10.3.1)

Catechol oxidase enzyme was first identified well over a century ago. Other names used for this enzyme are tyrosinase, catecholase, polyphenolase, monophenol oxidase, polyphenoloxidase, phenolase, laccase and cresolase. It also belongs to the oxidoreductase group of enzymes. Catechol oxidase is widely distributed in higher plants, fungi, microorganisms, insects as well as animal tissues including humans. In insects this enzyme is involved in sclerotization of exoskeleton thereby offering protection against other organisms. However, in animals and humans it is reported to be involved in skin pigmentation (Mayer 1987).

Amongst the fruits and vegetables, catechol oxidase enzyme is very important in determining the quality of the produce during storage and processing. Catechol oxidase

enzyme has been reported to cause undesirable and deteriorative changes in sensory properties of the food including both browning and development of off flavours (Ashie *et al* 1996; Cano *et al* 1995; Whitaker 1995; Eskin 1990; Velasco *et al* 1989; Lanker *et al* 1988; Vamos-Vigyazo and Markus 1982; Vamos Vigyazo 1981; Mayer and Harel 1979; Reed 1975). In certain plant products including tea, coffee, cocoa, prunes, black raisins and black figs, catechol oxidase enzyme activity plays an important role in producing pigments and providing protection against the sun (Whitaker 1995).

The use of catechol oxidase as an indicator for blanching sufficiency has been suggested by various researchers (Eskin 1990; Reed 1975). However, due to the greater sensitivity of catechol oxidase to heat treatment, peroxidase has generally been considered more suitable as an indicator enzyme (Barrett and Theerakulkait 1995; Eskin 1990; Boyer 1977; Reed 1975).

Almost a century ago catechol oxidase enzyme was first identified and investigated in mushrooms (Soderhall and Soderhall 1989). Over the years, various food products have been investigated for catechol oxidase activity and the role of the enzyme in undesirable and deteriorative changes. Limited success has been achieved in understanding the mode of action of catechol oxidase, partly due to the presence of multiple isoenzymes. In some tissues, as many as thirteen isozymes of catechol oxidase have been described (Robinson 1991).

Catechol oxidase is non-specific in its action and a large number of phenol and aromatic compounds which naturally occur in the plant can act as substrate. Once a food has undergone some form of physical damage such as cutting, brushing, breaking and crushing during processing, catechol oxidase acts on phenolic compounds resulting in colour changes (Mayer 1987).

Catechol oxidase generates oxidised end products including quinones and catechins. The enzyme also oxidises flavanoids to enhance the flavour of the food product. The typical reaction catalysed by catechol oxidase is as follows (Ashie *et al* 1996; Vamos-Vigyazo 1981).

Catechol	+	½ O_2	→	1, 2-Benzoquinone	+	H_2O
Substrate		Oxygen		Colour compound		Water

Many different vegetables have been assayed for their catechol oxidase activity. The results are typically reported in terms of change of absorbance and these have varied widely for different plant tissues. Baardseth and Slinde (1987) reported the catechol oxidase activities of vegetables including onion (14), leek (58), celery (61), beetroot (5), swede (23), kale (147), cauliflower (12), cabbage (12), Brussels sprouts (59), broccoli (40), red pepper (0), green pepper (14), cucumber (2), lettuce (31), parsley (28), snap beans (50), peas (403), tomatoes (0), potatoes (17) and corn (239). Peas were reported to have the highest and cucumber the lowest catechol oxidase activity. However, catechol oxidase activity was not detected in red pepper and tomato. Hsu *et al* (1988) investigated various varieties of potato for catechol oxidase activity and reported the variety Russet Burbank (4-5) to be highest in catechol oxidase activity compared to Atlantis (2-3) and Russet (2.8-3.6). In another investigation Zawistowski *et al* (1988) analysed Jerusalem artichoke for catechol oxidase activity and reported values in the range of 20-130 units.

There have been limited investigations of catechol oxidase of carrot and it appears that they are relatively low in activity. Baardseth and Slinde (1987) reported an activity of 31 units in terms of change of absorbance. Soderhall (1995) and Soderhall and Soderhall (1989) investigated carrot for catechol oxidase activity and reported the values of 960 and 304 units respectively. These activities were much higher than the values reported by Baardseth and Slinde (1987). Again the variation in reported values may partly reflect variations in assay methods.

2.6.3 Pectinesterase (EC 3.1.1.11)

Pectinesterase is now the recommended name for the enzyme commonly referred to as pectin methylesterase, pectin demethoxylase or pectin methoxylase in the literature. This enzyme has been found to occur in all species and tissues of higher plants which have been studied including fruits, vegetables, leaves, stem and roots. Apart from its occurrence in higher plants, a number of fungi and bacteria including plant pathogens are also known to produce pectinesterase (Eskin 1990; Benkova and Markovic 1978).

Pectinesterase was first reported in carrot over a hundred years ago. However, there has been limited research performed regarding the activity of pectinesterase enzyme. Various other fruits and vegetables have been investigated for pectinesterase enzyme activity and its role in deteriorative changes in food.

In vegetables and other plant foods, respiration continues after harvest. Pectinesterase activity during this period is reported to cause ripening changes in fruits. The activity is reported to decline during ripening and is thought to play an important role in senescence of fruit (Eskin 1990; Benkova and Markovic 1976).

Pectinesterase specifically acts on the esterified methoxy groups in pectin polymers producing free methanol and leaving carboxylic groups on the polysaccharide structure. The typical reaction catalysed by pectinesterase enzyme is as follows (Eskin 1990; Benkova and Markovic 1976).

Pectin	+	*n* H_2O	→	*n* methanol	+	pectate
Substrate		Water		alcohol		acid

In the juices of tomatoes, carrot, oranges and other vegetables and fruits, pectinesterase causes cloud loss and separation of solids particles from juice during storage by de-esterification of pectin followed by successive coprecipitation of pectate with insoluble materials. This eventually causes deterioration in the quality of the final product. On the other hand, the desirable use of commercial pectinesterase preparations as processing aids in foods for the purpose of juice extraction, maceration (fruit and vegetables), clarification (apple and grape juices) liquefaction as well as pulp production has been reported (Castaldo *et al* 1996; Marangoni *et al* 1995; Whitaker 1995; Ayranci and Tutunculer 1993; Pilnik and Voragen 1991; Nath *et al* 1983; Horvath and Gatai 1977; Racz and Hajnal 1976).

Many different vegetables have been investigated for pectinesterase activity. Values in the range of 0.25-4.96 activity units have been reported. Racs and Hajnal (1975) investigated various fruits and vegetables and reported the following activities: peaches (1.6), raspberry (1.74), plum (0.82) gooseberry (0.15), apricot (0.17), pear (0.08), cucumber (0.47) and radish (3.21). No activity was detected in strawberry and red

currant. Pectinesterase of cucumber was also analysed and reported to be 0.085 activity units by Hagermann and Austin (1986) which was much lower than the activity reported by Racs and Hajnal (1975). Green apples (0.170), red grapes (0.150), bean sprouts (0.205) and orange (0.145) were reported by Hagermann and Austin (1986). The pectinesterase activities of citrus fruits were reported to be: white grapefruit (2.6-2.9), red grapefruit (3-4), pineapple (3.5-3.8), orange (3.7-4.2) and tangerine (3.1-3.3) (Snir *et al* 1996). Tomatoes have been more extensively investigated for their pectinesterase activity and the result reported by Hagermann and Austin (1986) was 0.305 units. This was much higher compared to the values reported by Marquis and Bucheli (1994) (0.085), and lower than the values reported by Castaldo *et al* (1996) (1-4) and Nath *et al* (1983) (4-5). Oranges, were reported to contain the highest activity of this enzyme however, tomatoes and kiwi fruits contained activities higher than most other fruits and vegetables.

There is very limited information available on the pectinesterase activity of carrots tissues (Heinrichova, 1977). Markovic (1978) investigated and described two extracts of pectinesterase from carrot. The crude extract and purified extracts were reported to contain 1.1 and 5.2 units of pectin methylesterase activity. The results reported from crude extract were similar to the results reported by Racs and Hajnal (1975) (1.56) when carrot was analysed for this enzyme activity.

2.7 Enzyme distribution and localisation

Past research has indicated that plant foods contain many individual enzymes responsible for deteriorative changes. These enzymes include peroxidase, catalase, catechol oxidase, pectinesterase cellulase, polygalacturonase (also referred to as pectinase), thiaminase, lipase, lipoxygenase, ascorbic acid oxidase and proteases (Soderhall 1995; Mayer 1987; Mayer and Harel 1979). Relatively little information is available regarding the locations of these enzymes within particular tissues of the plant.

Research on fruits and vegetables has indicated the presence of peroxidase and catechol oxidase at higher levels in the skin tissues compared to central parts (Mayer 1987). In another investigation, Mihalyi and Vamos-Vigyazo (1975) investigated potatoes, celery

and radish for the distribution of peroxidase and reported the activity in skin at higher levels than central tissues which was in agreement with the results of Mayer (1987). There have been no previous studies of the distribution of catechol oxidase and pectinesterase enzymes reported in plant tissues.

2.8 Approaches used in the processing of vegetables

During the transformation of agricultural produce to food products various food processing operations are utilised. These include washing, peeling, crushing and size reduction, juice separation, blanching, thermal treatments, concentrations (thermal concentration, freeze-drying and membrane concentration), packaging operations (canning and aseptic) and storage. During preparation, foods also undergo a number of value adding procedures which result in to ready to eat and ready to cook food preparations (Heldman and Hartel 1997; Valentas *et al* 1990).

Carrots like most other vegetables and fruits are industrially processed through various processing operations as mentioned earlier to produce juice, concentrates, dried products as well as packaged products. In each of these food processing operations, a variety of different equipment and technologies are available to achieve better quality of final products in a more economical manner.

2.8.1 Blanching

Food materials may undergo chemical and biochemical changes including some which cause deterioration in colour, flavour and textural qualities as a result of enzyme activities during post harvest storage. Control of these changes is critical for the commercial realization of attractive and marketable processed foods. Many plant foods require some form of heat treatment in order to inactivate deteriorative enzymes. This heat treatment is commonly known as blanching and is carried out for most fruits and vegetables prior to the application of further processing steps.

Various sources of heating and heating media have been used in the past for blanching These have included the use of electricity and gas as sources of heating. Cooking through boiling water and steam have been commonly used and described. While steam

and water blanching are most commonly used, the application of microwave technologies has shown modest global growth. The use of microwave technology in combination with steam and hot water for sterilisation of particulate products, aseptic processing and packaging has been described for blanching of vegetables as well as other food processing operations for meat, fish and baked product (Rosenberg and Bogl 1987b; Richardson 1985; Unklesbay *et al* 1983). In previous studies, various blanching methods including boiling water, steam, microwave, pressure cooking and oven cooking on various foods have been investigated and described (Bao and Chang 1994a; Bao and Chang 1994b; Gunes and Bayindirli 1993; Seow *et al* 1992; Munsch *et al* 1986a; Williams *et al* 1986; Muftigul 1985; Warthesen *et al* 1984; Glasscock *et al* 1982; Drake *et al* 1981; Baardseth 1979; Klein *et al* 1979; Mirza and Morton 1977; Collins and McCarty 1969; Kylen *et al* 1960; Oser *et al* 1942; Brinkman *et al* 1941; Olliver 1941).

While the primary aim of blanching is the inactivation of deteriorative enzymes, Poulson 1986 has also discussed other advantages and disadvantages of blanching.

Advantages of blanching

1. Blanching causes an expulsion of entrapped air from vegetable tissues and avoids resultant oxidative changes.
2. As a result of expulsion of entrapped air, volumetric shrinkage of the food material facilitates achievement of correct filling weight in can.
3. Blanching reduces microbial load in food material due to high temperature treatments.
4. Blanching results in softening of food materials due to cooking and facilitates juice extraction.

Disadvantages of blanching

1. Blanching causes nutritional losses in food materials.
2. Blanching causes leaching loss of TSS due to high temperatures.
3. Blanching develops bitterness in some food materials including carrot (Poulson 1986).

2.8.1.1 Water blanching

During water blanching, foods are immersed in boiling water for a suitable period of time to ensure the inactivation of deteriorative enzymes. This is the most commonly used blanching technique due to its thermal efficiency and cost effectiveness. Amongst the sources of heat applied, the steam heated water blanching system is reported to be more cost and energy effective compared to electrically heated systems (Industrial correspondence 1998).

Various approaches have also been described and adopted for commercial water blanching of vegetables. Current water blanching techniques are based principally on immersion of foods in boiling water. The spraying of boiling water onto food is also very commonly used. Various other approaches have been applied for blanching of fruits and vegetables. Seow *et al* (1992) treated carrots using High Temperature Short Time (HTST) and also used Low Temperature Long Time (LTLT) as part of a two stage process known as LTLT/HTST blanching. The latter gave better firmness and dehydration/rehydration characteristics in carrots. In another investigation, a tubular water blancher using steam heated water has been used for vegetable blanching and reported to be more cost effective (Poulson 1986). In other investigations the use of chemical additives including sugar (5 per cent) and sulphite in solutions during carrot blanching have been described (Baloch 1987; Dan and Jain 1971). Sugars enhanced sensory qualities whereas sulphite improved carotenoid stability and hence colour. The use of new blanching techniques was described by Adams (1981) using recycled blanching media as well as by using hot gases. In both these instances the quality of product was reported to be improved although the latter was reported to be relatively expensive.

Boiling water has been investigated for vegetable blanching particularly in terms of the effect on product quality parameters. Green peas and green beans were cut into small pieces and water blanched at temperatures of 70, 80, 90 and 96°C and in a single layer and the effect on various deteriorative enzymes was assessed (Gunes and Bayindirli 1993). In another investigation, carrot, celery and green beans were water blanched in boiling water (Seow *et al* 1992) and the effect of the blanching on physicochemical

properties was reported. Warthesen *et al* (1984) water blanched various vegetables such as cabbage, cauliflower, broccoli, carrots, peas, spinach, zucchini, navy beans, potato and green beans in saucepan using boiling water and reported its influence on sensory and nutritional qualities. The vegetables such as green beans, spinach, peas and broccoli were conventionally cooked in boiling water (Klein *et al* 1979) and the effect on folacin was reported. In another investigation by the same author, spinach was conventionally cooked and the effect on folacin and ascorbic acid were reported. Collins and McCarty (1969) investigated water blanching of potatoes at boiling temperatures for 13 minutes and compared the energy consumption with microwave cooking. Lane *et al* (1985) blanched green beans, squash, purple hull peas and mustard greens using boiling water and compared the retention of nutrients with microwave and steam blanching technique. Vegetables such as carrots, asparagus, cabbage, cauliflower, green beans, peas broccoli and turnips (Brinkman *et al* 1941) were blanched using boiling water and the results were compared with waterless and pressure cooking techniques for their nutrient retention and other quality parameters. In another investigation, vegetables (Olliver 1941) such as cabbage, kale, brussels sprouts, cauliflower heads and peas were blanched using boiling water and the effect on nutritive value of these vegetables was reported.

Various investigations have been carried out and reported on water blanching of carrot. In one study, carrots were cut into small pieces and water blanched (Gunes and Bayindirli 1993) at temperatures of 70, 80, 90 and 96°C and the effect on various deteriorative enzymes was analysed. Bao and Chang (1994a) water blanched carrots in boiling water for 5 minutes and investigated the effect of blanching on colour, water holding and chemical composition. In another investigation, carrots were water blanched in boiling water (Seow *et al* 1992) and effects of the blanching on physicochemical properties were reported. Warthesen *et al* (1984) water blanched carrots in saucepan using boiling water and reported its influence on sensory and nutritional qualities. Carrots were blanched in water at 98°C for 5 minutes (Munsch *et al* 1986a and Munsch *et al* 1986b) and the effect of blanching on degree of grinding, enzymatic maceration, juice yield, minerals and nitrogen content were reported. Similarly Mirza and Morton (1977) investigated the effect of various boiling water blanching techniques on enzyme activity in carrots. Vegetables such as carrot was also

blanched using boiling water (Brinkman *et al* 1941) and the results were compared with waterless and pressure cooking techniques for their nutrient retention and other quality parameters.

2.8.1.2 Steam Blanching

During steam blanching, the foods are exposed to steam for a required period of time to ensure the inactivation of deteriorative enzymes. Following after boiling water blanching this is the most commonly used blanching technique. It is usually performed under pressure and thus is also known as pressure-cooking. Although it is less cost effective, it is more widely used compared to the other method where steaming of food is performed without pressure. The steam blanching technique is reported to have less effluent and pollution problems and is the preferred method as the leaching and nutritional losses are reduced compared to water blanching. However, capital costs are considerably higher in steam compared to water blanching (Poulson 1986).

Various industrial approaches have also been described and adopted for commercial steam blanching of vegetables. Individual Quick Blanching (IQB) of vegetables by spreading them in a single layer on belt and blanching with live steam was developed to improve blanching efficiency. Adding a holding module to suit the blanching of particulate products including peas and carrot cubes (Cumming *et al* 1984; Lazor *et al* 1971) is a further modification. The system was found to be more efficient than IQB and also resulted in enhanced retention of nutritional components.

The effects of steam blanching on various qualitative parameters of vegetables have been investigated. Warthesen *et al* (1984) steam blanched various vegetables including cabbage, cauliflower, broccoli, carrots, peas, spinach, zucchini, navy beans, potato and green beans and reported its influence on sensory and nutritional qualities. In another investigation, Lane *et al* (1985) blanched green beans, squash, purple hull peas and mustard greens using steam and compared the retention of nutrients with microwave and boiling water blanching technique. In 1985 Muftigul also investigated the effect of the steam blanching technique on green beans and compared the effect on colour, chlorophyll and ascorbic acid with other blanching techniques. Vegetables such as carrots, asparagus, cabbage, cauliflower, green beans, peas broccoli and turnips

(Brinkman *et al* 1941) were blanched using steam and the results were compared with waterless and boiling water blanching techniques for their nutrient retention and other quality parameters. In another investigation, vegetables (Olliver 1941) such as cabbage, kale, brussels sprouts, cauliflower heads and peas were blanched using steam and its effect on nutritive values of these vegetables were reported.

A number of studies have been carried out on steam blanching of carrot. Warthesen *et al* (1984) steam blanched carrots in a saucepan and reported the impact on sensory and nutritional qualities. Similarly Mirza and Morton (1977) investigated the effect of various steam blanching techniques on carrot and enzymes inactivation. Brinkman *et al* 1941 also investigated carrot blanching using the steam blanching technique and the results were compared with waterless and boiling water blanching techniques for their nutrient retention and other quality parameters.

The effect of blanching techniques on enzyme inactivation and various quality parameters has been discussed. Higher effectiveness of steam blanching for heat penetration compared to water blanching has been reported. However, few reports are available on the effectiveness of steam blanching on enzyme inactivation. As the leaching losses are reduced in steam blanching, reduced losses of nutrients have been reported (Warthesen *et al* 1984; Glasscock *et al* 1982; Mirza and Morton 1977).

2.8.1.3 Microwave blanching

Microwave blanching is a process in which food is exposed to radiation for a certain period of time before further processing or consumption. More commonly, infrared radiation is used for heating and processing of food. In this technique, food surface is heated using infrared lamps. Ultraviolet light is used more for disinfecting purposes (Stevens *et al* 1990). However, microwave heating is reported to have wider potential significance in food processing (Fellow 1990).

During microwave radiation of food products, various negatively charged oxygen and positively charged hydrogen atoms present in the food change their polarity and dissipate the applied energy as heat (Fellow 1990). In a comparison of the infrared and

microwave energy for food processing it was found that the latter method was more suitable (Fellow 1990).

Microwave energy for food processing was investigated for the first time in 1945 as it was reported to offer time saving during processing (Decareau 1972). Since then the technique has made substantial progress in the field of food processing. Microwave heating of food has been reported to be the most convenient form of food processing compared to other techniques and has been widely used as a research tool for food processing (Richardson 1990; Decareau 1972). Recently the use of microwave energy was reported to be effective in food processing for decontamination of food ingredients, reduction of microbiological load including *Listeria*, extension of shelf life of foods and inhibition of sprouting (Nikdel and MacKellar, 1993; Brody 1992; George and Richardson 1992; Decareau 1990; Richardson 1990; Walker *et al* 1990; Rosenberg and Bogl 1987a; Rosenberg and Bogl 1987b).

The application of microwave blanching to vegetables has been investigated along with the effects on product quality. Higher effectiveness of microwave blanching for heat penetration compared to water and steam blanching has been reported. However, there are relatively few reports on the effectiveness of microwave blanching on enzyme inactivation. As the leaching losses are lower in microwave blanching, reduced losses of nutrients have been reported (Williams *et al* 1986; Lane *et al* 1985; Collins and McCarty 1969; Kylen *et al* 1960). Microwave blanching technology has been used for vegetables including green beans (Muftigul 1985), spinach (Klein *et al* 1981) and potatoes (Collins and McCarty 1969) and the effect on the nutritive values have been discussed. Broccoli, cabbage, cauliflower, green beans, soybeans, spinach, peas, have been blanched using microwave blanching (Kylen *et al* 1960). Microwave blanching techniques have also been applied to green beans, squash, purple hull peas and mustard greens (Lane *et al* 1985), spinach, green peas, green beans, and broccoli (Klein *et al* 1979) and cabbage, cauliflower, broccoli, carrots, peas, spinach, zucchini, navy beans, potato and green beans (Warthesen *et al* 1984).

The use of microwaves for carrot processing has been reported. Warthesen *et al* (1984) blanched carrots in a microwave oven and reported its influence on sensory and nutritional qualities as a comparison with other blanching techniques. Similarly Glasscock *et al* (1982) and Mirza and Morton (1977) investigated the effect of various microwave blanching techniques including enzyme inactivation in carrots.

2.9 The effect of blanching on nutritional and juice quality parameters

Various types of thermal treatment have been used for processing of food commodities and these processes include blanching, pasteurisation, sterilisation and dehydration. Each of these can potentially have deteriorative effects on nutritional and sensory qualities of the food. In the past a number of thermal treatments have been investigated including microwave blanching, infrared blanching, traditional blanching using boiling water and steam, convection cooking, stirfrying and waterless cooking in pressure cooker. These techniques have been applied to many different fruits and vegetables (Rumm-Kreuter and Demmel 1990; Lane *et al* 1985; Martinsen and Ostrander 1984; Warthesen *et al* 1984; Oser *et al* 1942; Brinkman *et al* 1941; Olliver 1941).

2.9.1 Effect of blanching treatments on ascorbic acid

Ascorbic acid is a water soluble vitamin which is relatively stable in the absence of water, air and oxidising agents. In the food processing industry, ascorbic acid plays an important role as an antioxidant which improves keeping quality, colour, stability and palatability of food material (De Ritter 1976).

Extensive investigations have been carried out and data published on the effect of various processing techniques on ascorbic acid content of food. Cooking techniques involving the use of water such as boiling and pressure cooking have been reported to cause increased losses of water soluble vitamins including ascorbic acid due to leaching when compared to steaming (Rumm-Kreuter and Demmel 1990; Lane *et al* 1985; Martinsen and Ostrander 1984; Warthesen *et al* 1984; Oser *et al* 1942; Brinkman *et al* 1941; Olliver 1941). Rumm-Kreuter and Demmel 1990 reported the effect of various cooking techniques including boiling, steaming, oven cooking, and microwave cooking

on vegetables such as spinach, broccoli and green beans, potato, cabbage, celery, pea, cauliflower. The highest retention of ascorbic acid was found using microwave and steaming techniques. Similar reports were made when conventional boiling method of cooking was compared with waterless cooking for cabbage, peas and potatoes (Martinsen and Ostrander 1984). Klein *et al* (1981) studied the retention of ascorbic acid in spinach and the effects of conventional cooking compared with microwave techniques. The results indicated that retention was much higher in microwave cooking when compared to the conventional cooking method. In another investigation it was found that boiling for various cooking times resulted in the maximum loss of ascorbic acid from cabbage, cauliflower, potatoes, Brussels sprouts, broccoli, navy beans, lima beans, zucchini, Swiss chard, peas and spinach. Destruction of between 2 and 38 per cent of ascorbic acid was reported (Olliver 1941). Oser *et al* 1942 compared the use of conventional boiling technique with new improved cooking technique using minimal quantities of water for cooking carrots, peas, potatoes and broccoli. In most cases the loss of ascorbic acid during conventional cooking techniques was much higher compared to the new improved technique. The reported results were similar when Masrizal *et al* (1997) processed beans sprouts, green beans, nappa cabbage, spinach and water spinach using cooking techniques including microwave-steam, stirfry in oil and stirfry in water as well as boiling. The effect of these cooking methods on vitamin C indicated a significant loss of vitamin C in all the cooking methods. Retention of vitamin C was highest in vegetables cooked using microwave-steam cooking method. Similar results were reported when Muftugil (1986) (green beans), Lane *et al* (1985) (green beans, squash, purple hull pea, mustard green), Brinkman *et al* 1941 (beans, pea, spinach, asparagus, broccoli, cauliflower, turnip), Poulson (1986) (asparagus, beans, corn, pea) investigated effect of various cooking methods on retention of ascorbic acid.

Carrot is a poor source of vitamin C. This may be the reason for the limited information that is available for the effect of various cooking methods on vitamin C content of carrots. In early studies Brinkman *et al* (1941) reported the effect of various cooking methods (waterless and saucepan) on ascorbic acid retention in carrots. Increased retention of ascorbic acid during waterless cooking was reported. Oser *et al* 1942

compared the use of conventional boiling technique with new improved cooking technique using minimal quantities of water for cooking carrots. The loss of ascorbic acid during conventional cooking techniques was much higher compared to new improved technique. Similarly, Martinsen and Ostrander (1984) also cooked carrots using waterless cooking and conventional boiling method and reported that carrots cooked with conventional boiling method retained lesser vitamin C compared to waterless technique. Olliver (1941) reported an increase in the loss of vitamin C in carrots with increased boiling times.

2.9.2 Effect of thermal treatment on carotenes

Carotene is a precursor of vitamin A which is a water soluble vitamin. It is very stable in the absence of air, oxidising agent and ultraviolet light. As well as the important role imparted by carotenes in human physiology, it is also important in food industry as additives to develop colour and to increase the nutritive value of the food (Desobry *et al* 1998; Abbey *et al* 1996; Macreace *et al* 1993; Krinsky 1988; Moon 1989; Temple and Basu 1988; Oslon 1986; Peto *et al* 1981; De Ritter 1976). Carotene is generally considered to be relatively stable at higher temperatures compared to many other vitamins and nutrients (Macreace *et al* 1993; De Ritter 1976).

There are extensive investigations carried out and data published on the effect of various processing techniques on carotene content of food. Cooking techniques involving the use of water such as boiling microwaving, pressure cooking, dehydration and stirfrying have been investigated and reported to cause reduced losses of water insoluble vitamins including carotenes and vitamin D and E (Rumm-Kreuter and Demmel 1990; Warthesen *et al* 1984; Oser *et al* 1942; Olliver 1941).

Various reports have previously been described on the effect of various cooking methods on carotene content of foods. Various foods of plant origin such as vegetables (carrots, celery, lettuce, asparagus, cabbage, cauliflower, potatoes, broccoli, navy beans, corn cobs, cabbage, brussels sprouts, peaches, green beans, mung beans, cucumber, green pepper, tomatoes, beet root, leek, spinach, onion, peas) and fruits (apple, banana, citrus, grape, peas, peach, apricot, nectarine, plum, paw-paw) have been investigated for their effect of processing on carotenes (Masrizal *et al* 1997; Granado *et al* 1992; Kim

and Gerber 1988; Chandler and Schwartz, 1987; Hojilla *et al* 1985; Farhangi and Guy Valdon 1981). In most cases, carotene levels remained unchanged however in some instances carotene isomerization and a resultant increase in carotene levels have been reported. These results were further substantiated using chemically pure α- and β-carotenes (Chen *et al* 1995; Marty and Berset 1990). In an investigation carried out by Panalaks and Murray (1970) it was reported the carotene content increased by 72 per cent and 53 per cent in carrots during canning and boiling water cooking respectively. These results were similar to those reported by Bao and Chang (1994b). The carotene content was also investigated in various domestically processed vegetable products including carrot halwa and salad, chavli, spinach and shiplu bhaji, coriander chutney, mayalu pakoda, methi thepla (Dikshit *et al* 1988). The loss of carotene content covering the range of 5-83 per cent was reported (Dikshit *et al* 1988) depending up on the cooking technique applied. Deep frying (pakora) resulted in to the highest loss of carotene however roasting (methi thepla) was reported to result in a minimum loss of carotenes.

2.9.3 Effect of blanching on weight changes and juice yield

Limited information is available on the effect of blanching on weight changes and juice yield, however different blanching methods appear to have different impacts on the weight changes and juice yields on fruits and vegetables. Boiling water blanching have been reported to result in very little change on weight of the food material; however, microwave and steam blanching have reported to reduce losses and increase in weight respectively (Industrial correspondence 1999).

Green beans have been blanched using steam, microwave and conventional boiling water methods and the loss/gain of weights were compared (Muftugil 1986). Higher weight losses occurred for microwave blanching (4.1-4.6 per cent) which is higher than reported by Muftugil (1985) (2.5 per cent). For steam blanching losses of (1.9 per cent) were observed in each variety similar to the reported values of 1.5 per cent. On the other hand, boiling water blanching did result in an increase in weight (1.5-1.6 per cent) compared to reported losses of 1.4 per cent (Muftugil 1985). There are no other reports in the literature on effect of blanching methods on loss/gain of weight in food material.

Apparently contradictory results were found when vegetables (asparagus, green beans, green peas and sweet corn) lost weight when a number of blanching methods were used including boiling water, steam and microwave (Drake *et al* 1981).

Munsch *et al* (1986b) reported average juice yield of 55 per cent from raw carrot and indicated the loss of juice yield during boiling water blanching of carrots. Bao and Chang (1994a) investigated the effect of boiling water blanching on carrots and compared with raw carrots. The results reported the reduced juice yield (to 35 per cent) during blanching which was in agreement with previously reported results by Munsch *et al* (1986b).

2.9.4 Effect of blanching on colour

Very limited information is available in the literature regarding the effect of the various blanching methods on colour of food material although the blanching methods reported to lead to deterioration in colour (Howard *et al* 1996; Muftigul 1985). The effects of different blanching methods (boiling water, steam and microwave) on colour of vegetables (asparagus, green beans, green peas and sweet corn) was reported in an investigation by Drake *et al* (1981). In each of these blanching methods, colour values L^*, a^* and b^* reduced when compared with raw materials (asparagus, green beans, green peas). However in the case of sweet corn, these values were reported to increase. Muftugil (1985) investigated the effect of various blanching methods on green beans and reported increased retention of colour values during microwave compared to conventional boiling water blanching method and indicated that this may be due to a loss of chlorophylls.

Limited reports are available on the effect of various blanching methods on carrots. Mirza and Morton (1977) investigated the effect of water and steam blanching on carrot slices and reported a decrease in colour values. Howard *et al* (1996) investigated the effect of retort temperatures on carrots and reported minimal deterioration in colour with an increase in temperature. Bao and Chang (1994a) blanched carrots using boiling water and compared with boiling acetic acid medium and reported increased redness and

yellowness in both instances, however the latter report found evidence of a brighter colour compared to boiling water.

2.10 Crushing and juicing

During crushing, raw or processed forms are passed through the crushing machine such as juicer and juiced. This is the most common and primary food processing operation performed to any fruit or vegetable. Domestically and industrially, there are various crushing and juicing machines that have been used for juicing. For orange and other citrus fruits, the rotary juice extractor of various types has been reported (Industrial correspondence 1998). Tomatoes are crushed and juice extracted using a series of alternative machines such as hammer mill and rotary turbo finishers. For juicing apples, the apple mill and rotary turbo finishers are commonly used (Industrial correspondence 1998).

Apart from reports on the use of various domestic juicers for research purposes, limited information is available on juicing carrots. However, it is known that various juicers including hammer mill types are used commercially (Industrial correspondence 1998).

2.11 Juice separation

Once the fruit or vegetables are juiced, the separation of juice follows immediately. Various principles including filtration under pressure, gravity separation and pressing have been applied and reported for extraction and separation of juice from the solid materials. In early days decanting separation was used for juice separation. New technological developments include the use of other equipment including belt press, decanter under pressure and centrifuges to achieve similar objectives (Industrial correspondence 1998).

In the past the use of other juice extraction and separation techniques have been reported. The use of belt press (B FRU 1200, B FRU 1750, B FRU 2500) was described by Hartmann *et al* 1996. Colesan and Johrer (1993) reported the use of (Alpha Laval NX309S-31G, Z 23, Z4 D, Z 62) for juice separation. The use of Westfalia centrifugal separator using a pressure technique (Fruit press Bucher HPX 5005 I) was reported by

Beveridge 1994 and performance was compared with the decanter method (Alpha Laval NX309S-31G, Z 23, Z4 D, Z 62,). The latter was reported to have resulted in an improved juice yield and performance.

2.12 Thermal treatment

Thermal treatment is one of the most essential processing operations in the food industry. The prime objective of this treatment is cooking and softening of the food to ease the consumption and destroy all the living organisms which would otherwise cause deterioration in food and risk the health of the consumer. The objective varies depending upon the process. In certain food processing operations, juices crushed from the raw food materials are heated with the objective of inactivating deteriorative enzymes and avoid any sensory changes. However another objective of the thermal treatment was to destroy all the living microorganisms.

The objective of heating food is achieved by applying heat to the food material. Thermal treatment of the food varies depending upon the food and processing protocols. In most cases the liquid and semi-solid foods are heated using various types of heat exchangers including plate heat exchanger, tube heat exchanger, scraped surface heat exchanger (Arthey and Dennis 1990, Fuller 1969). Solid foods are commonly heated in a hot water bath after packing or oven cooking of both hot air or microwave origin.

Two different types of thermal processing have been commonly used in the food industries. The first involves container processing after packing. For this method foods are packed in cans, glass bottles or flexible pouches prior to processing. This is commonly used in the production of thick paste/sauces and particulate products. The second involves processing prior to packaging where foods are processed to a required degree before packing in a container (Industrial correspondence 1998). This is commonly used in aseptically packed products, fresh juices as well as frozen salads and vegetables (Industrial correspondence 1998; Heldman and Hartel 1997; Arthey and Dennis 1990; Valentas *et al* 1990).

In previous times the food material was heated using the batch processes through one of the techniques mentioned earlier. Although the literature available on this development of thermal processing techniques is limited, numerous advancements have taken place on the use of continuous heat exchanger processing over the last two decades. Various manufacturers including TetraLaval and APV have implemented these changes.

Various liquid foods have been thermally processed using different time and temperature regimes depending upon the processing protocols. Most fruit and vegetable juices are treated thermally using the techniques including HTST (High Temperature Short Time), pasteurisation, aseptic packaging and UHT (Ultra High Temperature) (Heldman and Hartel 1997; Arthey and Dennis 1990). Milk products are treated at temperatures of 141°C (UHT) for a short time of up to 30 seconds to achieve the product free of living organisms (Industrial correspondence 1998). Similarly juice products from fruits and vegetable sources are heated at the temperatures of 95°C for up to 60 seconds to achieve similar objectives (Industrial correspondence 1998; Heldman and Hartel 1997; Skudder 1992; Valentas *et al* 1990; Arthey and Dennis 1990; Fuller 1969). In a separate study the use of electric current for the processing of long life products known as Ohmic heating has been reported (Skurray 1986). Liquid foods with particles were reported to have been successfully processed to achieve similar objectives with better textures, colour and flavour of the end product using Ohmic heating (Skurray 1986).

Aseptic processing has been described as the processing and packaging of food material in sterile container and in sterile environment. This is a technique that has been very popularly used for most of the long life juices and dairy products. Various foods have been investigated using aseptic processing techniques in the past and reported to be more suitable compared to other techniques (Industrial correspondence 1998; Heldman and Hartel 1997; Arthey and Dennis 1990; Valentas *et al* 1990).

2.13 Enzymatic treatment

In recent years the use of enzymatic treatments has been widely applied in the food and allied industries. Enzyme technology has been used as a tool by food scientists and

technologists for enhancing economic value in food processing operations. Enzymes are used particularly in processing of juices and beverages, brewing, confectionary, dairying, baking and the meat industry (Tucker 1996; Rombouts and Pilnik 1978).

A number of enzymes including carbohydrases (amylases, cellulases, pectinases), proteases (proteases, rennin, pepsin, papain) and lipases have been extensively used in food processing (Tucker 1996). These enzymes have been used to assist sugar breakdown, clarification, production of fermentable sugars, improving bread quality, bromate replacement in baking, foam stabiliser, pentosan solubilisation, liquification, flavour and colour development, oxygen scavenger, enhance cheese ripening, milk shelf life extension, tenderisation of meat (Tucker 1996; Rombouts and Pilnik 1978).

Globally many companies now produce commercial enzyme preparations containing one or more enzyme activities mentioned earlier. These enzyme preparation have been used during fruit and vegetable processing to facilitate juice extraction, softening, clarification, maceration and liquefaction. Carbohydrase enzymes especially pectinases and cellulases are the most commonly used enzymes in fruit and vegetable processing. Commercially available cellulase preparations contains cellulase and hemicellulase activities however, pectinase preparations typically contain polygalacturonase, cellulase, pectin esterase, pectin lyase and proteases activities (Sreenath *et al* 1986; Foda *et al* 1985; Rombouts and Pilnik 1978). These commercial enzyme preparations include fungal or bacterial enzymes, commonly used for the purpose of maceration and liquefaction (Rombouts and Pilnik 1978). These are generally inactivated under pasteurisation conditions.

2.13.1 Effects of enzymatic treatment

Enzymatic methods have been applied to a number of fruits and vegetables and the effect on various parameters have been investigated. Various commercial preparations as well as enzyme solutions have been used and the effect of these enzymes on increased cloud stability, enhanced juice yield, reduced viscosity and increased extraction of TSS have been reported (Nakamura *et al* 1995; Sreenath *et al* 1986; Foda *et al* 1985; Rombouts and Pilnik 1978).

2.13.1.1 Effect of enzymatic treatment on juice yield

Citrus fruits such as grapefruit, lemon and orange were enzymatically macerated using *Aspergillus aculeatus* DSM 63261 and *Aspergillus aculeatus* LSM 63261 organisms as the source of crude enzyme (Foda *et al* 1985). Increases in the juice yield of 13.7 and 9.4 per cent (orange), 14.9 and 16.0 per cent (grapefruit) and 12.4 and 13.59 per cent (lemon) respectively were reported. Sreenath *et al* (1986) investigated effect of commercial pectinase (Rohament P) as a source of endo-polygalacturonase, cellulase, hemicellulase and protease prepared from *Aspergillus aculeatus* on celery. The effect on softness and tissue disintegration was reported. Vegetables such as potato, garland chrysanthemum, garlic, ginger, spinach and red pepper were enzymatically treated using a crude extract from *Trichospron penicillatum* SNO-3 organism as a source of protopectinase (Nakumara *et al* 1995). Potato was reported to be more effectively macerated compared to other vegetables. Apples have been most widely investigated using the maceration enzymes. The apple varieties such as Golden Delicious, Jonathan, Karmijn were treated for the production of cloud stable juice using pectinase (Rombouts and Pilnik 1978).

2.13.1.2 Effect of enzymatic treatment on TSS contents

Increases in TSS following enzymatic treatment has been previously discussed. The increase in the juice Brix and acidity of 35 and 20 per cent (orange), 30 and 7 per cent (grapefruit) and 32 and 12 per cent (lemon) were also reported (Foda *et al* 1985). In another investigation carrots that were enzymatically treated with Rohament P (RP) (Sreenath *et al* 1986) did result in an increase in the glucose, fructose, arabinose, galactose, xylose and sucrose content in the juice extracted from the mash. Citrus fruits such as grapefruit, lemon and orange were enzymatically macerated using *Aspergillus aculeatus* DSM 63261 as source of crude enzyme (Foda *et al* 1985). An increase in the TSS of 13.8 (orange), 2.3 per cent (grapefruit) and 12.7 per cent (lemon) respectively were reported using this enzyme.

2.13.1.3 Effect of enzymatic treatment on carrot

Limited information is available on the use of such enzymes on carrot processing. Successful attempts were made to prepare carrot juice and puree using commercial enzyme preparation including Rohament PC, Panzym M, Rohament K, Rohament P (RP) (Sreenath *et al* 1986), and Pectinex Ultra SPL (Siliha *et al* 1995) and pure enzyme extracts of polygalacturonase, hemicellulases, cellulase, pectinesterase (Anastasaki *et al* 1987) and protopectinase (Nakamura *et al* 1995).

Sreenath *et al* (1986) investigated the effect of Rohament P (RP) on carrot mash and reported an increase in juice yield of up to 25.5 per cent compared to untreated mash. These results were in agreement with the results reported by Foda *et al* (1985) who treated carrot mash with polygalacturonase enzyme. In a similar investigation Anastasaki *et al* (1987) treated carrot mash with various enzymes and reported an increase in yield of 19 per cent (cellulase), 23 per cent (pectinase), 15 per cent (hemicellulase), 10 per cent (pectin esterase), 50 per cent (Rohament PC), 34 per cent (cellulase and pectinase combination) and 30 per cent (cellulase, pectinase, pectin esterase and hemicellulase combination) respectively.

Foda *et al* (1985) treated carrot mash with the crude preparations of polygalacturonase enzyme and reported the increase in TSS of 14.5 per cent compared to untreated mash. In a similar investigation Anastasaki *et al* (1987) treated carrot mash with various enzymes and reported an increase in TSS of 77 per cent (cellulase), 128 per cent (pectinase), 68 per cent (hemicellulase), 64 per cent (pectin esterase), 143 per cent (Rohament PC), 112 per cent (cellulase and pectinase combination) and 102 per cent (cellualase, pectinase, pectin esterase and hemicellulase combination). Siliha *et al* (1995) investigated the effect of various commercially available pectolytic enzymes such as Panzym M, Rohament K, Pectinex, Celluclast, and Celluclast + Pectinex on various sugars. The levels of sugars such as rhamnose, fucose, arabinose, xylose, mannose, galactose and glucose were reported to have reduced during enzymatic treatment.

2.14 Concentration

Concentration of liquids is one of the most vital food processing operations in the food processing industry. During the concentration process the water from the food material is removed using various evaporation techniques to produce a concentrate. Removal of water from the food product using heat, in most cases steam, is known as thermal concentration. Heat in such cases is applied across stainless steel surfaces to heat the food product under vacuum. It is distinctly different from dehydration based on the differences in final water content and product characteristics. Generally liquid foods are concentrated and remain in a liquid state whereas drying produces solids and semisolids with significantly lower water contents. In thermal concentration, the most commonly known operations are drying and evaporation. In previous studies the concentration of food product was achieved using heat. Earlier studies used a batch process however, continuous processes have been developed recently.

While the thermal concentration process involves the use of heat, other concentration techniques, performed at low, ambient or slightly higher temperatures involve freeze drying/freeze concentration and crossflow membrane concentration are becoming increasingly popular.

2.14.1 Thermal concentration

Thermal concentration by evaporation has application for a variety of liquid food processing operations including fruit and vegetable juices (orange, apple, pineapple and tomato purees and concentrates), dairy products (evaporated milk) and sugar (confectionery). The technique is comparatively less expensive compared to other concentration techniques. Hence it is used more extensively. The concentration process provides an extended shelf life as well as reducing the bulk and hence transportation costs (Heldman and Hartel 1997).

Evaporation technology plays a critical role in the manufacture of variety of products in foods, chemicals as well as in other processing industries. In the past, various evaporators including solar evaporator, batch pan evaporator, jacketed kettle evaporator, natural circulation evaporator, vertical tube evaporator, horizontal tube evaporator,

basket evaporator, forced circulation evaporator, multi stage evaporator, rising film evaporator, falling film evaporator, combination of rising and falling film evaporator, agitated thin film evaporator, plate evaporator, expanding film evaporator, centrifugal thin film evaporator, calandria pan evaporator, vapor compression evaporator have been discussed for their suitability in manufacture of food product concentrates (Mehra 1986). The falling film as the most suitable evaporator and their advantages over other evaporators have been reported (Sandhu and Bhatia 1984; Armerding and Dinnage 1970).

In the past various fruits and vegetable juices for concentration and cubes and slices have been investigated for drying processes. Juices of pineapple, banana, guava, orange and mango were evaporated using the vacuum concentration method in a glass evaporator. Juices from orange, lemon, pineapple, grapefruit, apple, grape, tomato, peach and strawberry have also been concentrated by the vacuum concentration technique (Ramteke *et al* 1993). In other investigations orange juice was concentrated using the vacuum concentration technique (Mohsen *et al* 1986; Moresi and Spinosi 1980; El-Sherbiny and Rizk 1981).

2.14.1.1 Effect of concentration techniques on physico-chemical properties

Sandhu and Bhatia (1984) reported on alterations in physico-chemical properties of concentrates produced from vacuum concentration technique at 50-55°C using a glass evaporator. Juices prepared from banana, mango, orange, guava and pineapple were concentrated and changes in physico-chemical properties reported (Sandhu and Bhatia 1984). Amongst the changes described, an increase in the values of TSS, total solids, pH, acidity, pectin, sugars, ascorbic acid, β-carotene and colour were reported in each of these fruits. A similar pattern for orange juice was reported by El-Sherbiny and Rizk (1981) when various concentration techniques such as freeze concentration and vacuum concentration were applied. In another investigation orange juice was also concentrated by vacuum concentration, centrifugation and freeze concentration techniques (Mohsen *et al* 1986). The effect on TSS, acidity, ascorbic acid, free amino nitrogen, sugars, carotenoids, pulp and pectin methylesterase activity was also studied. Insignificant

changes were found in most parameters in all the three concentration techniques however, reduction in carotenoids, pectinesterase activity as well as pulp contents were reported (Mohsen *et al* 1986).

Previous studies have investigated the effect of concentration techniques on chemical as well as qualitative properties of food material. In one investigation, the extent of sucrose inversion and other changes in chemical properties in banana, mango, orange, guava and pineapple concentrates during vacuum concentration 50-55°C in a glass evaporator have been reported (Sandhu and Bhatia 1984). The effect of volatile contents from orange juice concentrated by various concentration techniques was reported (Mohsen *et al* 1986). Concentration by freezing was reported to result in reduced losses of volatile compounds compared to vacuum concentration.

2.14.2. Low temperature concentration

Many liquid foods contain high amount of water. Water can be removed to prepare concentrate using low temperature concentration techniques. Freeze drying/freeze concentration is one of the most commonly used low temperature concentration techniques. During this process the water component of the food material is frozen to produce ice crystals which are removed using centrifugation (Heldman and Hartel 1997). Although this technique is reported to be uneconomical, this technique causes least thermal damage to the product and results in an improved quality of end product compared to other thermal concentration techniques (Ramteke *et al* 1993; Deshpande *et al* 1982; Casimir and Kefford 1968).

In the past various fruits and vegetable juices have been concentrated using the freeze concentration technique. Orange juice have been concentrated using freeze concentration techniques (Moresi and Spinosi 1980; El-Sherbiny and Rizk 1981; Casimir and Kefford (1968).

2.14.2.1 Membrane concentration

The application of membrane technology for the food and allied industry was introduced in 1960s and increased dramatically in the 1980s. With the membrane technique,

separation of water from the food material takes place under pressures through a membrane. In recent years, the technology has been used in the food, dairy, pharmaceutical and allied industries including water treatment and chemical industries to improve processing economy, quality of product, yields, utilisation of waste, desalination of water, production of medicines as well as development of new products (Nielsen 1992; Kumar *et al* 1992; Cheryan 1991).

Many methods using membranes to concentrate fluids have been described and these vary depending upon the molecular sizes of the food components of interest and and also the required characteristics of the end product. These membrane techniques include microfiltration, nanofiltration, ultrafiltration, reverse osmosis and osmotic distillation. The technologies already commercialised have been used for clarification, fractionation and sterilisation (microfiltration), fractionation, concentration and purification (ultrafiltration), desalting, deacidification (nanofiltration) and demineralisation (reverse osmosis) (Cheryan 1991; Lefebvre 1986; Paulson *et al* 1985).

2.14.2.2 Ultrafiltration

Ultrafiltration is a pressure driven crossflow membrane system that simultaneously concentrates, purifies and fractionates organic components from the food materials. Ultrafiltration is a sieving process which allows constituents from the food material to be separated according to their molecular weight when pressurised influent is passed through the membrane. Membranes separate various constituents according to their molecular weight leaving a residue known as the retentate. The constituents that pass through the membranes are largely low molecular weight compounds.

Various configurations of membranes have been described. They include tubular modules, hollow fibre, spiral wound and plate and frame depending upon their use in the industry. These membranes are made up of different compositions including cellulose acetate, polysulphones, polyamides, polyimides, polycarbonates, cellulose esters and polyester materials (Ramteke *et al* 1993; Cheryan 1991). Ultrafiltration is one of the most widely used techniques for the processing in various food, pharmaceutical and allied industries. Various foods of dairy, fruits and vegetable origin have been

investigated for the suitability and quality of end products using the ultrafiltration technique. In dairy processing, the ultrafiltration technique has been used widely used for the fractionation of milk, production of whey protein concentrate and speciality milk products as well as reducing microbial load at collection sites (Cheryan 1991; Lefebvre 1986).

Amongst the products of plant origin, juices of citrus such as tangerine (Chamchong and Noomhorn, 1991), orange and lemon (Campannelli *et al* 1994; Hernandez *et al* 1992, Koseoglu *et al* 1990) grapefruit (Hernandez *et al* 1992), apple (Constenla and Lozano, 1995; Koseoglu *et al* 1990; Milnes *et al* 1986) and passionfruit (Chao *et al* 1992) have been investigated. Amongst vegetables, production of vegetable juices such as tomato (Koseoglu *et al* 1991; Porretta *et al* 1992) and carrot, celery and cucumber (Koseoglu *et al* 1991) have been reported. The effect of ultrafiltration on the physical and chemical qualities of the juices processed from above mentioned fruits and vegetables have also been reported.

Ultrafiltration has also been widely used in the dealcoholisation of wines, liquors, removal of bitter compounds from citrus and clarification of apple juice (Ramteke *et al* 1993; Hernandez *et al* 1992; Cheryan 1991; Ray 1991; Lefebvre 1986).

2.14.2.2.1. Effect of ultrafiltration on quality of juices

Ultrafiltration of apple juice to produce a clear juice has been widely reported. In a previous study, apple juice was ultrafiltered using a hollow fibre membrane of 30,000-50,000 and 100,000DA. Using this technology, an improvement in objective quality of juice was found when compared to the traditionally used bentonite gelatin filtration technique (Constenla and Lozano 1995). Reduction of haze and turbidity was also reported in juice that was filtered using the ultrafiltration technique. In another investigation ultrafiltration of apple juice did result in a reduction of sugar in permeate (Milnes *et al* 1986). In both investigations, an increase in juice yield, reduction in material and labour costs, ease of cleaning, elimination of the need for pasteurisation compared to results obtained using the traditional thermal concentration technique were reported. Although ultrafiltration does result in an improved quality of the retentate, the

membrane technique does have limitations for achieving higher levels of concentration (Constenla and Lozano, 1995; Milnes *et al* 1986).

The effect of ultrafiltration on orange juice quality parameters has also been reported. When orange juice was ultrafiltered using 50,000 molecular weight cut off (MWCO) membrane it was found that the permeate was free of any suspended solids and pectin and there was an increase in the viscosity of retentate (Hernandez *et al* 1992). Koseoglu *et al* (1990) reported an increase in total solid, ash and total sugars in the retentate of orange and grapefruit juices produced using a hollow fibre ultrafiltration membrane of 50,000 and 100,000 MWCO. Use of the ultrafiltration technique for passionfruit juice was reported in an investigation by Chao *et al* (1992) and an increase in acidity, viscosity and soluble solids was reported. Similar effects were reported when passionfruit juice was subjected to ultrafiltration using a 50,000 MWCO membrane (Chamchong and Noomhorn, 1991). Olle *et al* (1997) reported the loss of flavour components in mango puree following ultrafiltration. The same process has also been used for vegetable juices from tomatoes, carrots, celery and cucumber and the end product was reported to be sterile, fresh tasting, with improved colour, total solids and total sugars in retentate (Porretta *et al* 1992; Koseoglu *et al* 1991).

2.14.2.3 Reverse osmosis

Reverse osmosis is a pressure driven cross-flow membrane system that simultaneously concentrates, purifies and sterilises liquid foods. Reverse osmosis is a sieving process in which water from the food material is separated by passing pressurised influent through the membrane. The constituents that pass through the membranes are permeate which is largely water. In most cases the retentates are concentrates.

Membranes are manufactured in various configuration. They include tubular modules, hollow fibre, spiral wound and flat sheet depending upon their use in the industry. These membranes are made up of cellulose acetate, polysulphones, cellulose esters and polyamide materials (Ramteke *et al* 1993; Cheryan 1991). Reverse osmosis, also known as hyperfiltration, is one of the most widely used techniques for the processing of various foods, for the pharmaceutical and allied industries. A number of foods of dairy

and fruits and vegetable origin have been investigated for the suitability and quality of end products using reverse osmosis technique. In dairy processing, the use of reverse osmosis technique including concentration of milk, production of whey protein concentrate, desalting of whey, and speciality milk products have been reported (Nguyen 1996; Cheryan 1991; Lefebvre 1986; Zadow 1985).

Amongst the products of plant origin, juices of citrus (orange, lemon, grapefruit) (Braddock *et al* 1988), lemon, lime, prune (Matsuura *et al* 1974), pineapple (Bowden and Issac 1989), passionfruit (Chao *et al* 1992), mango (Olle *et al* 1997), red raspberry (Wrolstad *et al* 1993) have been investigated. An increase in juice yield, reduction in material and labour costs, ease of cleaning, elimination of the need for pasteurisation compared to the traditional thermal concentration technique were reported. The use of reverse osmosis for range of other vegetables juices including tomato (Yildiz *et al* 1993; Koseoglu *et al* 1991), carrot (Koseoglu *et al* 1991; Matsuura *et al* 1974), celery cucumber (Koseoglu *et al* 1991) has been reported. The effect of Reverse osmosis on the physical and chemical qualities of the juices processed from above mentioned fruits and vegetables have also been studied.

Reverse osmosis has also been widely used for the dealcoholisation of wines, liquors, preconcentration of fruits and vegetable juices, removal of bitter compounds from citrus and clarification of apple juice (Ramteke *et al* 1993; Hernandez *et al* 1992; Cheryan 1991; Ray 1991; Lefebvre 1986; Robe 1983). As well as improved quality of concentrate, limitations of membrane technique for achieving higher concentrations have also been reported (Campannelli *et al* 1994; Chao *et al* 1992; Kumar *et al* 1992; Chou *et al* 1991; Ben Amar *et al* 1990; Koseoglu *et al* 1990; Walker and Ferguson *et al* 1990; Paulson *et al* 1985; Fukutani and Ogawa 1983).

Limited investigations have been undertaken on the use of reverse osmosis technique for the processing of many vegetables. Reverse osmosis of juices from tomatoes, carrots, celery and cucumber have been investigated and the end products of this process were found to be sterile, fresh tasting and with an improved colour. An increase in juice yield was also reported (Yildiz *et al* 1993; Koseoglu *et al* 1991; Matsuura *et al* 1974).

Reverse osmosis has also been used for the dehydration of vegetables, preconcentration of juices, dealcoholisation of wines and desalination of sea water (Industrial correspondence 1998; Lerici *et al* 1988; Marshall 1985; Robe 1983).

2.14.2.3.1 Effect of reverse osmosis technique on quality of juices

The effect of reverse osmosis on the qualitative aspects of orange, lemon and grapefruit juices was reported by Braddock *et al* (1988). These investigations showed that reverse osmosis resulted in an increase in the brix of orange juice (11.98 to 25.26), lemon (7.70 to 22.52) and grapefruit (8.68 to 25.06). For all three fruit juices the acids, vitamin C pectin and limonene contents were reported to be unchanged and concentrated in retentate however, minor loss of hexose sugars (glucose, fructose and sucrose) were found. Other studies have shown that the flavour of orange juice was improved using reverse osmosis (Walker 1990). Bowden and Isaacs 1989 concentrated pineapple juice using reverse osmosis technique and reported the absence of ascorbic acid in permeate which was in agreement with previously reported results. Various volatile components from mango were investigated in the concentrate produced using reverse osmosis technique (Olle *et al* 1997). The results reported indicated that most volatiles were recovered in the retentate portion of the concentrate.

Yildiz *et al* (1993) investigated the effect of both reverse osmosis and thermal evaporation of tomato juice and reported no changes in acids, pH and calcium contents, however minor variations in iron, copper, and invert sugar were reported. An improvement in the colour values of tomato concentrate prepared from reverse osmosis technique has also been reported in this investigation.

2.14.2.4 Osmotic concentration

Osmotic concentration is more recent membrane processing technology which been has applied in food and allied industries and where maintenance of the product integrity is of considerable importance. Principally, during osmotic distillation, water is separated from the food material as vapour across the hydrophobic membrane using an osmotic pressure-induced vapour pressure gradient as the driving force. In this procedure a brine

solution containing high concentration of salt is used on one side of the membrane to extract water from the food material across the membrane.

As the technology is in the preliminary stages of development, few research reports are available. Juices of a number of fruits including grape, sugarcane, apple and tomatoes have been tested for end products quality using this technique. An enhanced quality of concentrate but with limitations of achieving higher concentration on a single pass have also been reported (Durham and Nguyen 1994; Skurray and Nguyen 1992; Johnson *et al* 1989). The use of osmotic distillation in wine industry has also been reported (Thompson 1991).

2.15 Packaging and storage

A number of packaging technologies have been extensively used over the last two decades for food processing operations. Packaging and storage of foods are of major importance in food industry for the preservation and maintenance of the quality of the final food product. The packaging of foods include flexible pouches, sealed containers, cans and aseptic bags in box/drums. The latest development in aseptic bulk packaging in pallecons has also been investigated.

Although a number of studies have been attempted to develop appropriate packaging for the storage of juice concentrates, most of the research has been of commercial nature and therefore the data in the literature are limited. Industrially, most liquid foods and concentrates are packed in glass bottles, cans and aseptic packaging and stored at room temperatures for long period of times. Products packed and stored using these techniques has been reported to be suitable for consumer consumption. It has also been reported that food concentrates including citrus, apple, carrots, prune and other fruits and vegetables) can be packed in metal/plastic drums with polythene bags and stored at -18°C temperature for both short and long term preservation (Industrial correspondence 1998).

2.16 Aims and objectives

The aims of this current project have been to:

1. Analyse and compare four Australian carrot varieties (two new and two traditionally used) for various compositional parameters (moisture, protein, pectin crude fibre), commercially significant juice parameters (sugar, acid, sugar : acid ratio, juice yield, colour values), micronutrients (vitamin C, carotenes, thiamin and riboflavin) and minerals (calcium, magnesium, sodium, potassium and iron).
2. Characterise each of these carrot varieties in terms of size and shape.
3. Investigate the distribution, thermal inactivation and regeneration of the three deteriorative enzymes, peroxidase (EC 1.11.1.7), catechol oxidase (EC 1.10.3.1) and pectinesterase (EC 3.1.1.11) in each of the four carrot varieties.
4. Determine the effect of blanching of carrot varieties using three different blanching techniques namely steam, microwave and boiling water to establish the most suitable blanching technique for carrot processing.
5. Determine the effect of the blanching techniques on physical parameters (weight, juice yield, colour characteristics), micronutrients (ascorbic acid, total carotenes, α- and β-carotene), inactivation of deteriorative enzymes (peroxidase, catechol oxidase and pectinesterase) and regeneration of peroxidase.
6. Determine the influence of carrot sizes, as measured by carrot diameter, on temperature come-up times.
7. Optimize the treatment of carrot mash using the commercial enzyme preparations Rohament Max (containing pectinase, hemicellulase and cellulase activity) and Rohament PL (containing polygalacturonase activity) and evaluate the effect of enzyme treatment on juice yield, carotene content, relative viscosity and product colour.
8. Investigate the use of both thermal (rising film evaporator) and non-thermal (ultrafiltration and reverse osmosis) concentration techniques and a combination of these treatments for their effectiveness in producing carrot juice concentrates.
9. Establish the suitability of selected concentration techniques for preparing carrot juice concentrate with desirable parameters including taste, flavour and colour.

Chapter 3

Materials and methods

3.1 Chemical reagents

All laboratory chemicals were of analytical reagent grade unless otherwise indicated. The details of the chemicals used, together with the respective suppliers are listed in Table 3.1.

Table 3.1 List of chemicals

Supplier	Chemical
Sigma Chemical Co, USA.	Thiamin hydrochloride (T-4625), Riboflavin (R-4500), L-Ascorbic acid (A-0278), 4-methyl catechol (M-6636), Bromothymol blue (B-8630), β (C-9750) carotene, 2-6, Di-chlorophenol indophenol (D-1878), Tetrahydrofuran (27,038-5, HPLC grade).
BDH Laboratory Supplies, Melbourne.	Standard preparation of minerals solutions (Ca, Mg, Na, K and Fe), Hydrogen peroxide, Amberlite resin IR-120 (Na), Potassium permanganate, Sodium dihydrogen orthophosphate, Disodium hydrogen orthophosphate, Potassium sulphate, Potassium chloride, Calcium chloride anhydrous, Chloroform, Sodium sulphate, Acetonitrile (HPLC grade), Metaphosphoric acid, Sodium acetate.
Ajax Chemicals, Melbourne.	Sodium hydroxide, Pyrogallol, Concentrated Nitric acid, Concentrated Sulphuric acid, Concentrated Hydrochloric acid, Absolute alcohol, Potassium ferricyanide, Glacial acetic acid, Silver nitrate, n-Hexane, Sodium bicarbonate, Magnesium carbonate.
Tecator AB, Sweden.	Kjeltab Cu/3.5
Fluka Chemie GMBH	α (22035) carotene
Rohm Pty. Ltd., Germany.	Industrial grade of enzymes Rohament PL and Rohament Max solutions
Solvay Biosciences Pty. Ltd., Melbourne.	Clarase concentrate

3.2 Equipment

The items of equipment used, together with the details of manufacturers and model numbers are presented in Table 3.2.

Table 3.2 List of equipment

Equipment	Manufacturer	Model No.
pH meter	Hanna Instrument Ltd, Italy	8521
Hand refractometer (0-32°B)	Atago Co Ltd, Japan.	ATC-1
Luminiscence spectrometer	Perkin Elmer	LS 50
Probe thermometer-Digitron	Selby Scientific Supplies Ltd, Melbourne.	3246
Water bath (thermostatically controlled)	Thermoline Scientific Instruments Pty Ltd, Melbourne.	BTC 9090
Cryovac water bath (thermostatically controlled)	W R Grace Australia Ltd, Melbourne.	512
Steriflow programmable retort (modified)	Lanpac International Pty. Ltd, Melbourne.	-
Steam boiler	Simons Co (Aust) Pty Ltd, Melbourne.	VS 580
Rising film evaporator	Armfield Ltd, England.	FT 22
Juice separator	Robot Coupe Pty Ltd, Melbourne.	C 80
Hard Fruit Juicer (Vema)	Robot Coupe Pty Ltd, Melbourne.	2047 XA
Fruit and Vegetable Juicer	Bamix Appliances Ltd, Melbourne.	-
Minolta Chroma Meter	Minolta Camera Co Ltd, Japan.	CR 300
Cary Spectrophotometer (uv/vis),	Varian Australia Pty Ltd, Melbourne.	1E
Atomic Absorption Spectrometer	Varian Australia Pty Ltd, Melbourne.	Spectra AA-400
HPLC	Varian Australia Pty Ltd, Melbourne.	
Tecator Kjeldahl Digester	Tecator AB, Sweden.	1015
Tecator Kjeldahl Distillation unit	Tecator AB, Sweden.	1002

Table 3.2 List of equipment contd.

Equipment	**Manufacturer**	**Model No.**
Vacuum oven	Gallenkamp Ltd, Melbourne.	OVL 570.010J
Vacuum pump	Javac Australia Pty Ltd, Melbourne.	JDS-75
Muffle furnace	Nober instruments, Melbourne.	N3/P
Ultrafiltration unit	Amicon Diaflow, USA.	
Reverse osmosis unit	ACA Australia Pty Ltd, Melbourne.	2521TR
Rotary evaporator vacuum system	HD Scientific Pty Ltd., Melbourne.	Eyela N-N
Oil bath	HD Scientific Pty Ltd., Melbourne.	Eyela 0513650
C_{18} Reverse phase HPLC column,	Alltech Associates (Aust) Pty Ltd, Melbourne.	Vydac 201 TP
HPLC Guard cartridge holder	Varian Australia Pty Ltd, Melbourne.	GCH4
HPLC Guard cartridge C_{18}, 10μ	Varian Australia Pty Ltd, Melbourne.	201GD104T
Reverse osmosis plant for water	Millipore Australia Pty Ltd., Melbourne.	Milli Q Super
Reverse Osmosis membrane cartridge for water	Millipore Australia Pty Ltd., Melbourne.	QPAK kitR5KM61595
Bag filter (45μ)	Pall Filters Aust Pty Ltd.	
Auto Vortex mixer	Selby Biolab, Melbourne.	MT19
Magnetic stirrer	Industrial Equipments & Controls Pty Ltd.	CS76083V
Laminar flow work station	Gelman Sciences Pty Ltd, Melbourne.	HWS180
UHT plant	Microthermics	25DH UHT/HTST

3.3 Carrot samples

3.3.1 Carrot varieties and storage

The carrot varieties used in this study were RHO, RC, TP and RHC. These were selected for investigation as they are currently the leading carrot varieties grown commercially in Australia. Representative whole samples of 22-24 weeks maturity of each of these varieties were obtained from both South Australian and Victorian growing regions and were kindly supplied by Henderson Seeds Pty Ltd, Melbourne. All samples were stored at 2-3°C pending further use.

3.3.2 Sample preparation and handling

3.3.2.1 Carrot washing

Carrots were washed using cold tap water and shaken dry prior to juice extraction.

3.3.2.2 Sectioning of carrots

Fresh carrots were cut longitudinally from stem end to root tip using a clean sharp knife and the core and superficial tissues separated. Similarly, fresh carrots were cut transversely into two equal lengths to provide root tip and stem end portions on samples.

3.3.2.3 Preparation of carrot juice

Juice was extracted from whole raw carrot or selected tissue sections as required, for micronutrient, juice quality parameters and/or enzyme analyses (carotenes, ascorbic acid, soluble solids, acidity, colour, relative viscosity, peroxidase, catechol oxidase, and pectinesterase) using a Bamix laboratory juice extractor. For the larger scale requirement, juice was extracted from both raw and blanched whole carrot (for enzymatic treatments, concentration and sensory evaluation studies) using a Hard fruit extractor (Vema). The extracted juices were collected separately and stored in a refrigerator or cold room at 2-3°C pending analysis. Prior to analysis, carrot varieties were refrigerated overnight to reduce the core temperature. Analysis of juice was undertaken immediately after extraction for components likely to be unstable, particularly ascorbic acid. Other parameters of juice samples were analysed within a period of one hour.

3.3.2.4 Shredded carrots

Shredding of fresh carrots for compositional and micronutrient analyses (moisture, pectin, crude fibre, protein, minerals, thiamin and riboflavin) was carried out manually immediately prior to analyses using a stainless steel domestic grater.

3.4 Analysis of compositional parameters

All the analyses for compositional and micronutrients parameters were performed in triplicate and expressed as g/100g and mg/100g respectively on a wet carrot basis unless otherwise specified.

3.4.1 Analysis of moisture content

The procedure followed was AOAC Method 934.01. Samples of shredded carrot (2 g) were placed in predried and weighed aluminium dishes and dried in a Gallenkamp vacuum oven at 75°C under vacuum (400 mbar) for 6 hours or until constant weight was obtained. The moisture content was calculated from the weight loss on drying to constant weight and expressed as g /100 g carrot on wet weight basis.

3.4.2 Analysis of protein content

The procedure followed was AOAC Method 955.04C. Samples of shredded carrot (2 g) were weighed out on nitrogen free filter paper and placed in glass digestion tubes (250 mL). A digestion catalyst tablet (Kjeltab Cu/3.5 containing 3.5 g potassium sulphate and 0.4 g copper sulphate) and concentrated sulphuric acid (25 mL) were added to the tubes. The digestion tubes were placed in a preheated Tecator digester block and digested at 420°C. Digestion was continued for 30 minutes or until the samples were completely digested and dissolved in acid and the solution in the tubes became clear. The digestion tubes were removed from the digester and cooled. Purified nitrogen free water (Milli Q) (75 mL) was added to each digestion tube. The tube containing the digestion mixture was placed into a Tecator steam distillation unit and NaOH added (40 mL, 40 M) prior to steam distillation for 5 minutes. The distillate was collected in a conical flask containing boric acid indicator solution (25 mL, 4 per cent boric acid in distilled water containing 10 μg bromocresol green and 7 μg methyl red) and the contents of the flask

titrated standardised sulphuric acid (0.2 M) to a neutral grey end point. The samples were analysed in duplicate and nitrogen recovery standards were run using predried (100°C, 2 hours) tryptophan added to the sample. The protein content was calculated and expressed as a percentage on a wet weight basis of carrot using 6.25 as the nitrogen conversion factor.

Calculation of protein content

$$\text{N content (per cent)} = \frac{(\text{mL titrant for sample} - \text{mL titrant for blank}) \times 0.2 \times 1.4007}{\text{weight of sample (g)}}$$

$$\text{Protein content (per cent)} = \text{N content} \times 6.25$$

3.4.3 Analysis of crude fibre content

The procedure followed was that described by Ranganna (1986). Shredded raw carrot samples (10 g) were placed in a round bottom flask and acid washed using sulphuric acid (0.255 M, 200 mL) for 30 minutes under boiling conditions with intermittent stirring, cooled and centrifuged (4000 rpm) for 30 minutes. The acid solution was decanted, the residue washed with water (50 mL) and recovered by centrifugation (4000 rpm). The acid washed residue was transferred to a round bottom flask and alkali washed with sodium hydroxide solution (0.313 M, 200 mL) for 30 minutes before filtering through Whatman No 1 filter paper. The residue on the filter paper was then washed successively with water (50 mL), potassium sulphate (10 per cent w/v, 30 mL), hot water (50 mL) and absolute alcohol (30 mL), dried in a vacuum oven (110°C for 3 hours) and weighed. The crude fibre was transferred to a preweighed and dried Gooch glass filter and heated in a muffle furnace (400°C, 30 minutes), cooled and weighed. The results are expressed as a per cent on a wet weight basis.

3.4.4 Analysis of pectin content

The procedure followed was that described by Ranganna (1986).

3.4.4.1 Acid extraction

Freshly shredded carrot (50 g) was placed in a conical flask (1 L) and acid washed, using hydrochloric acid (0.05 M, 400 mL) for 2 hours at 80-90°C with intermittent stirring. The acid extract was cooled, filtered through Whatman No 4 filter paper and transferred to a volumetric flask (500 mL) and made up to volume using distilled water.

3.4.4.2 Water extraction

Raw carrots were shredded manually using a stainless steel grater. Shredded carrot samples (50 g) were placed in a conical flask (1 L) and washed using distilled water (400 mL) for 2 hours at 80-90°C with intermittent stirring. The extract was cooled, filtered using Whatman No. 4 filter paper and transferred to a volumetric flask (500 mL) and made up to volume using distilled water.

3.4.4.3 Analysis of pectin

Acid and water extracts (50 mL) were transferred into separate conical flasks (500 mL), and in each case distilled water (250 mL) was added prior to neutralisation with NaOH (1 M) using phenolphthalein as indicator. Excess NaOH (10 mL, 1.0 M) was added and the reaction mixture allowed to stand overnight. Acetic acid (50 mL, 1.0 M) and $CaCl_2$ solution (25 mL, 1.0 M) was then added to the reaction mixture. After stirring for 5 minutes, further $CaCl_2$ solution (25 mL, 1.0 M) was added and the mixture stirred for a further 5 minutes. After allowing to stand for 1 hour, the reaction mixture was placed in a boiling water bath for 30 minutes and filtered through a previously prepared filter paper (prepared by wetting the paper in hot water, drying in an oven at 102°C for 2 hours, cooling in a desiccator and weighing in a covered dish). The precipitates on the filter paper were washed with boiling water until free from all chlorides. The filter papers containing the calcium pectate precipitates were transferred to their original weighing dishes, dried overnight in an oven at 100°C, cooled in a desiccator and re-weighed.

Calculation

$$\text{Calcium pectate (per cent)} = \frac{\text{weight of calcium pectate} \times 500 \times 100}{\text{mL of filtrate taken} \times \text{weight of sample (g)}}$$

3.5 Micronutrient analyses

3.5.1 Analysis of total carotenoids

The procedure followed was that described by Wallrauch (1984). Carrot juice (5 mL) prepared from raw or blanched carrots was placed in a clean, dry separating funnel and extraction solvent (petroleum ether:methanol 90:10, 50 mL) added. After vigorous shaking, the solvent layers were allowed to separate for 5 minutes. The juice sample was successively extracted a further three times using fresh solvent (50 mL) to ensure complete extraction of carotenes. All four solvent extracts were combined and further separated by centrifugation (3000 rpm, 15 minutes) prior to evaporation and making up the volume (100 mL) using petroleum ether. The absorbance of the extract was read at 450 nm using a Cary uv/visible spectrophotometer. Considering that a 1% β-carotene solution has an extinction coefficient of 2,500, the results obtained were calculated and expressed as mg L^{-1} of carrot juice.

3.5.2 *Analysis of α- and β-carotenes using HPLC*

The procedure used was that published by Bushway and Wilson (1982) with minor modifications.

3.5.2.1 Preparation of standards

Standard solutions of α- and β-carotenes (10 mg dL^{-1} and 25 mg dL^{-1} respectively) were prepared by dissolving separately the carotenes in a hexane:chloroform (90:10) mixture. Aliquots of these standards (1, 2 and 3 mL) were placed in 100 mL volumetric flasks, the solvents evaporated by flushing nitrogen and the carotenes re-dissolved using 100 mL tetrahydrofuran to provide carotene working standards. 100 mL aliquots of these working standard solutions were used to prepare standard HPLC curves for α- and β-carotenes.

3.5.2.2 Analysis

Carotene extracts from carrot juice samples (10 g) prepared from either fresh or blanched carrots were obtained by extraction with 50 mL tetrahydrofuran, magnesium carbonate (1 g) and sodium sulphate (20 g) in a separating funnel. Each carrot juice sample was successively extracted a further three times using fresh solvent to ensure complete extraction of carotenes. All four extracts were combined, filtered through Whatman No 3 filter paper and evaporated prior to making up to volume (100 mL) using tetrahydrofuran. α- and β-carotene contents were determined by HPLC using a Varian instrument with a Vydac 201 TP reverse phase column operating at 30°C. The mobile phase acetonitrile:tetrahydrofuran:water (85:12.5:2.5 v/v) with a flow rate of 2 mL/min, 10 μL sample injections and the carotenes detected by absorbance at 470 nm were used. Results were expressed as parts per million. The total content of α- and β-carotenes was calculated as the sum of the individual α- and β-carotene contents.

3.5.3 Mineral analyses

Shredded carrot sample (2 g) was placed in a glass digestion tube (250 mL) and water (20 mL) and concentrated nitric acid (5 mL) was added and mixed with sample. The digestion tube was placed into a preheated Tecator digester block and digested at 250°C until the carrot sample was completely digested. The digestion mixture was cooled and transferred to a volumetric flask (100 mL) and the volume adjusted to 10 mL using purified double distilled water. This diluted solution was aspirated for one minute into the oxidised flames of Varian SpectrAA-400 atomic absorption spectrophotometer (Spectra AA-400) and the absorbance read. The appropriate instrument settings for the particular mineral being measured are given in Table 3.3. The mineral concentrations in the sample were determined from the respective calibration curves and the results expressed as mg per 100 g on a wet weight basis.

Table 3.3 Wavelength and slit width adjustments for mineral analysis

	Calcium	Magnesium	Sodium	Iron	Potassium
Wavelength (nm)	422.7	202.6	589.6	248.3	404.4
Slit Width	0.5	1.0	0.5	0.2	0.5

3.5.4 *Analysis of ascorbic acid in carrot juice*

The procedure followed was AOAC Method 985.33. Carrot juice samples (50 mL) prepared from raw or blanched carrot was mixed thoroughly with ascorbic acid extracting solution (50 mL, 3% w/v metaphosphoric acid containing 0.8 mL acetic acid) and filtered through Whatman No. 3 filter paper. The ascorbic acid extract was titrated against standardised 2, 6 di chlorophenol indophenol dye solution (0.25 mg mL^{-1} dye in purified double distilled water containing 0.21 mg of sodium bicarbonate) until a permanent pink colour appeared and remained on mixing. Dye solution standardisation was performed by titration against a standard solution of ascorbic acid (5 mL, 1 mg mL^{-1} in 3% w/v metaphosphoric acid). The results were expressed as mg of ascorbic acid/100 mL carrot juice.

3.5.5 *Analysis of thiamin and riboflavin contents*

The procedures followed were those described by Ranganna (1986).

3.5.5.1 Preparation of resin

Resin (Amberlite IR 120 as sodium salt 100 g) was transferred to a beaker (500 mL) and washed twice, for 15 minutes each, with acetic acid (3% v/v, 200-250 mL). The acid solution was drained off and the resin then washed for 15 minutes with potassium chloride solution (25% w/v, 200-250 mL). The excess potassium chloride solution was decanted and the resin was washed again with acetic acid as before. The acetic acid was drained off and the resin washed with purified double distilled water (200-250 mL) until free of chloride ions. The resin was then dried in an oven at 100°C and stored in a dry container.

3.5.5.2 Preparation of ion exchange column

Dry resin (6 g), prepared using the above method, was mixed with purified double distilled water (50 mL) and the slurry poured into a wide burette fitted with a stopcock and plugged with glass wool. The resin was allowed to settle and the excess water to leave the resin surface covered with water to a depth of 2 mm above the surface of the resins.

3.5.5.3 Extraction of thiamin

Shredded carrot (10 g) was placed in a conical flask (250 mL), mixed with HCl (0.2 M, 75 mL) and heated in a boiling water bath for 30 minutes. After cooling, freshly prepared Clarase enzyme solution (1% w/v, 5 mL) was added to the carrot and acid mixture, mixed and incubated overnight at 37°C. The mixture was transferred to a volumetric flask (100 mL) and made up to volume (100 mL) using distilled water. The mixture was filtered using Whatman No. 1 filter paper and the filtrate (25 mL) passed through the resin column. The column was washed successively with three aliquots of hot water (50 mL) and the water rinses discarded. The column was then washed with two successive aliquots of potassium chloride solution (25% w/v, 10 mL) to elute the thiamin from the resin. The potassium chloride eluates were collected in a volumetric flask (25 mL) and made up to the volume with further potassium chloride solution.

3.5.5.4 Analysis of thiamin

For analysis of thiamin, the eluates were treated as indicated in Table 3.4.

Table 3.4 Analysis of thiamin from eluates

Sequence of component addition	Tube 1 Sample	Tube 2 Standard	Tube 3 Sample blank	Tube 4 Standard blank
Eluate (mL)	5.0	-	5.0	-
Thiamin working std. (mL)	-	5.0	-	5.0
Alk. ferricyanide (mL)	3.0	3.0	-	-
Mix well				
NaOH (15 per cent w/v) (mL)	-	-	3.0	3.0
Mix well				
iso-butanol (mL)	15.0	15.0	15.0	15.0

Following addition of *iso*-butanol, the tubes were mixed vigorously for 90 seconds and the two phases allowed to separate. The bottom phase was removed and dried over anhydrous sodium sulphate (2 g) which was added to each tube and mixed vigorously. Thiamin was analysed fluorimeterically using a Perkin Elmer Luminiscence Spectrometer (LS 50) and excitation emission wavelengths of 360 nm and 435 nm respectively. A working standard of 5 μg thiamin per 100 mL in 25% w/v KCl was used and the results expressed as mg thiamin per 100g carrot.

Calculation

$$\text{Thiamin mg per 100g} = \frac{(A\text{-}B) \times 1\ 25 \times 100 \times 1000}{(C\text{-}D) \times 5 \times V \times W}$$

A = Fluorescence reading of sample solution
B = Fluorescence reading of sample blank
C = Fluorescence reading of thiamin standard
D = Fluorescence reading of standard blank
V = Volume of sample used for adsorption in column
W = Weight of carrot sample (g)

3.5.5.5 Extraction of riboflavin

Shredded carrot (15 g) was transferred to a conical flask (250 mL), mixed with HCl (50 mL, 0.2 N) and placed in a boiling water bath for 60 minutes. The carrot and acid mixture was cooled to room temperature and the pH adjusted to 6.0 using NaOH (1 M). Immediately the pH was adjusted to 4.5 using HCl (1 M), the mixture transferred to a volumetric flask (100 mL) and made up to volume with distilled water.

3.5.5.6 Analysis of riboflavin

For analysis of riboflavin, the extracts were treated as indicated in Table 3.5.

Table 3.5 Analysis of riboflavin from eluates

Sequence of component addition	Tube 1	Tube 2
Filtrate (mL)	10.0	10.0
Water (mL)	1.0	-
Mix		
Riboflavin working std. (mL)	-	1.0
Mix		
Glacial acetic acid (mL)	1.0	1.0
Mix		
Pot. permanganate (3% w/v) (mL)	0.5	0.5
Mix and wait for 2 minutes		
Hyd. peroxide (3% v/v) (mL)	0.5	0.5

Following addition of hydrogen peroxide the tubes were mixed vigorously, sodium dithionite (20 mg) was added to each of the tubes. Riboflavin was then analysed fluorimeterically using a Perkin Elmer luminiscence spectrometer (LS 50) with excitation and emission wavelengths of 470 nm and 525 nm respectively. A working standard of 10 µg riboflavin per 100 mL in distilled water was used and the results expressed as mg riboflavin per 100 g carrot.

Calculation

$$\text{Riboflavin (mg per 100 g)} = \frac{(A-B) \times 0.1 \times 100 \times 1000}{[(C-D) - (A-B)] \times 10 \times W}$$

A = Fluorescence reading of sample solution
B = Fluorescence Reading of blank solution
C = Sample reading + Standard reading
D = Sample reading + Standard blank
W = Weight of sample

3.6 Analysis of juice quality parameters

3.6.1 *Measurement of TSS*

The procedure followed was that described by Ranganna (1986). TSS in juice from raw and blanched carrot was determined by refrectometry using an Atago hand held refractometer calibrated in °Brix within the range 0-32 °Brix. The results are expressed as °Brix.

3.6.2 *Acidity*

The procedure followed was that described by Ranganna (1986). Freshly extracted carrot juice (10 mL) from raw and blanched carrots was titrated with 0.02M NaOH to an end point of pH 7.0 as determined with a precalibrated pH meter (Hanna model 8521). The volume of alkali required was noted and the results were calculated and expressed as per cent w/v of citric acid.

3.6.3 *Colour analyses*

Carrot juice (100 mL) extracted from raw and a blanched carrot was collected in a clean glass beaker (250 mL). The beaker was placed on a white tile. The colour of the juice was measured at a sample depth of 25 mm with a precalibrated Minolta Chroma Meter (CR 300) fitted with a light protection tube (CR-A33e) attachment. The results were expressed in terms of L^*, a^* and b^* values.

3.6.4 *Pulp content*

The pulp content of raw and blanched carrot juice was determined by centrifugation of 25 mL aliquot of juice in 50 mL graduated centrifuge tube at 3000 rpm for 15 minutes. The pulp pellet was measured visually against the tube graduations and the pulp content expressed on a percentage basis (v/v).

3.6.5 *Juice yield*

Juice yield was determined by measuring the volume of juice extracted from 500 g quantities of blanched or raw carrot as described in section 3.3.2.3 and expressed as a percentage (v/w).

3.6.6 *Relative viscosity*

The relative viscosity of carrot juice was determined by time of flow under gravity of a known volume of juice at a prescribed temperature, through an orifice of fixed dimensions. In this procedure the time taken for 40 mL of juice, pre-equilibrated overnight at 2-3°C, to flow freely from a 50 mL burette fitted with a 1 mm orifice stopcock was measured. The runout time for juice extracted from raw carrot was taken as the reference and the viscosities of blanched or other juice samples were measured relative to this runout time from either raw and blanched carrots. Runout time for juice extracted from raw carrot was measured and viscosity expressed as mL sec^{-1}.

3.7 Analysis of deteriorative enzymes

3.7.1 *Preparation of buffers for enzymatic analyses*

Buffers were prepared as described by Dawson *et al* (1986) from the following stock solutions as required and the pH checked prior to the use.
0.1 M sodium dihydrogen orthophosphate (anhydrous): 13.79 g L^{-1}
0.1 M disodium hydrogen orthophosphate (anhydrous): 14.196 g L^{-1}
0.003 M sodium dihydrogen orthophosphate (anhydrous): 0.4137 g L^{-1}
0.003 M disodium hydrogen orthophosphate (anhydrous): 0.4259 g L^{-1}

The buffer solutions (500 mL) were prepared using the formulations shown in the Table 3.6.

Table 3.6 Preparation of buffer solutions for enzyme analyses

Buffer pH at 25°C	Enzyme to be analysed	Concentration of stock buffers used (M)	mL of Na_2HPO_4	mL of NaH_2PO_4
6.8	Catechol oxidase	0.1	245	255
7.0	Peroxidase	0.1	305	195
7.5	Pectinesterase	0.003	420	80

3.7.2 *Spectrophotometric assay of peroxidase enzyme*

The procedure followed was that described by Stauffer (1986). An aliquot (100 µL) of carrot juice extracted from either fresh or blanched carrots was diluted (1:9) with 900 µL of purified double distilled water in an Eppendorf tube (1.5 mL) and stored on ice. For assay, substrate-buffer mixture was prepared by dissolving pyrogallol (0.1250 mg) in

phosphate buffer (50 mL, 0.1 M at pH 7.0). Hydrogen peroxide (0.4 mL, 3 per cent v/v) was then added to a buffer solution and the mixture pre-equilibrated at 30°C prior to assay. Substrate-buffer mixture (2.0 mL) was transferred to a cuvette and placed in a thermostated cuvette holder in a UV/Visible spectrophotometer (Varian Cary 1E). Diluted carrot juice (100 μL) was added to the cuvette with thorough mixing and the change in absorbance at 430 nm recorded over a period of one minute. Enzyme activity was calculated and expressed as change of absorbance min^{-1} mL^{-1} of carrot juice.

3.7.3 *Spectrophotometric assay of catechol oxidase enzyme*

The procedure followed was that described by Stauffer (1986). An aliquot (100 μL) of carrot juice extracted from either fresh or blanched was transferred in an Eppendorf tube (1.5 mL) and stored on ice. Substrate stock solution (5 mL, 4- methyl catechol 0.250 mg/ 25 mL of buffer) was diluted to 25 mL using phosphate buffer. For assay, substrate-buffer (mixture of 1.5 mL buffer and 0.5 mL 4- methyl catechol) was pre-equilibrated at 30°C. For assay, substrate-buffer mixture was prepared by dissolving 4-methyl catechol (0.1 mg) in phosphate buffer (50 mL of 0.1 M at pH 6.8). Substrate-buffer mixture (2.0 mL) was transferred to a cuvette and placed in a thermostated cuvette holder in a UV/Visible spectrophotometer (Varian Cary 1E). Carrot juice (100 μL) was added to the cuvette with thorough mixing and the change in absorbance at 395 nm recorded over a period of five minute. Enzyme activity was calculated and expressed as change of absorbance min^{-1} mL^{-1} of carrot juice.

3.7.4 *Spectrophotometric assay of pectinesterase enzyme*

The procedure followed was that described by Hagerman and Austin (1986). An aliquot (100 μL) of carrot juice extracted from either fresh or blanched was transferred in an Eppendorf tube (1.5 mL) and stored on ice. For assay, substrate-buffer mixture solution (1.0 mL, 0.5 per cent w/v pectin in 0.003 M phosphate buffer, pH 7.5) and purified double distilled water (1.0 mL) were pre-equilibrated at 30°C. Bromothymol blue dye (0.01 per cent w/v in 0.003 M, pH 7.5 buffer) was prepared and stored separately in a brown bottle. Substrate-dye phosphate buffer mixture (2.0 mL) was transferred to a cuvette and placed in a thermostated cuvette holder in a UV/Visible spectrophotometer (Varian Cary 1E). Carrot juice (100 μL) was added to the cuvette with thorough mixing and the change in

absorbance at 620 nm recorded over a period of two minutes. Enzyme activity was calculated and expressed as change of absorbance min^{-1} mL^{-1} of carrot juice.

3.8 Thermal treatment of carrot juice for analysis of residual enzyme activities

Carrot juice (25 mL) extracted from raw carrots was transferred to a conical flask (100 mL) and the flask placed in a shaking water bath preset to 90°C and temperature monitored constantly with a probe thermometer (Digitron 3246) until the juice attained the required temperature. Immediately after the juice attained required temperature (65, 70, 75, 80, 85 or 90°C), the flask was transferred to another water bath preset at the required temperature mentioned above. The juice sample was then held in the bath for a period of ten minutes. The first subsample (1 mL) was withdrawn prior to the transfer of the juice sample into the second bath. Further subsamples were collected at one minute intervals for a period of ten minutes. The subsamples were immediately cooled on ice until analysed for residual enzyme activities as described in sections 3.7.2, 3.7.3 and 3.7.4.

3.9 Regeneration of peroxidase enzyme activity

Following the thermal treatment at all the temperatures, small aliquots of juice were stored over a period of 144 hours at 3°C in Eppendorf tubes. The samples were withdrawn every 24 hours for 144 hours and tested for the regeneration of peroxidase enzyme using the method described in 3.7.2.

3.10 Blanching treatments of carrot

3.10.1 *Water blanching*

3.10.1.1 Determination of optimum hot water blanching conditions

Raw carrots (approx. 500 g) selected to specific diameter ranges (20-21, 25-26, 30-31, 35-36 and 39-40 mm) measured at stem end, were placed in a wire mesh basket and placed in a thermostatically controlled boiling water bath (Thermoline BTC 9090) for a period of 20 minutes. The temperature of the core of the carrot was monitored against time using a data logger (Hobo XT) linked to a thermocouple inserted along the longitudional axis of the carrot to a depth of 25.4 mm from the stem end until the temperature of 90°C was reached. The wire mesh basket was removed from the boiling water bath after the core had reached the required temperature (90°C) to another water

bath preset at the required temperature held for the required time (1, 2 and 3 minutes). The carrots were removed from the bath, cooled, juiced and the juice analysed for residual enzyme activities using the methods indicated in section 3.7.2, 3.7.3 and 3.7.4. The data from data logger were plotted to calculate the time required to attain 90°C at the core and results expressed as time (min) required for the carrot core to reach minimum of 90°C. These results were then used to identify hot water blanching regimes for carrots of different diameter.

3.10.1.2 Treatment of carrots by hot water blanching for further processing studies

Raw carrots (30-31 mm diameter, 500 g) with a temperature probe inserted were placed in chip frying basket and blanched for the above calculated time using method and equipment indicated earlier. Carrots were removed from the basket and cooled for a period of 1 minute on the table. The juice extracted from blanched carrots was analysed for residual enzyme activities using the method indicated earlier. The results were calculated and expressed as the time (min) required for the carrot to reach 90°C.

3.10.2 ***Steam blanching***

3.10.2.1 *Determination of optimum steam blanching conditions*

Samples of raw carrots (500 g) of specific diameters (20-21, 25-26, 30-31, 35-36 and 39-40 mm) measured at stem end were placed into a programmable steam retort. A temperature probe (precaliberated) was inserted into one of the carrots along its longitudional axis to a depth of 25.4 mm from the stem end- core of the carrot to monitor temperature. The retort was closed and the carrots heated using steam at atmospheric pressure. The time required for the carrot core to attain 90°C was measured. Carrots were further continued heating at 90°C for 1 minute, cooled to 60°C and held at this temperature under water for 2 minutes. The steam blanching regime established is described in Table 3.7. The chamber temperature was set to 95°C to effectively achieve 90°C at the centre of the carrot root. The results were expressed as time (min) required for the carrot core to reach minimum of 90°C.

3.10.2.2 Treatment of carrots by steam blanching for further processing studies

Raw carrots (30-31 mm diameter, 500 g) were steam blanched using the steam blanching regime indicated earlier (Table 3.7). The juice was extracted from steam blanched carrots and analysed for residual enzyme activities using the method indicated earlier. The results were expressed as the time required for the carrots to reach 90°C at core under atmospheric steam pressure.

Table 3.7 Time temperature regime for steam blanching

Phase No	Phase Type	Time (minutes)	Total time (minutes)	Chamber temperature (°C)	Pressure (kPa)
1	Initial Heating	7	7	95	101.3
2	Heating	10	17	95	101.3
3	Holding	1	18	95	101.3
4	Cooling	2	20	60	101.3
5	End	0	20	60	101.3

3.10.3 *Microwave blanching*

3.10.3.1 Determination of optimum microwave blanching conditions

Samples of raw carrots (500 g) of specified diameter (20-21, 25-26, 30-31, 35-36, 39-40 and 43-45 mm) measured at the stem end were moistened under a running tap and placed in a single layer on the turntable of a domestic microwave oven (Panasonic Genius series 4) and blanched for varying periods of time (2, 3, 4 and 5 minutes) at 900Watts and an operating frequency of 2450MHz. The temperatures of both core and superficial tissues were measured immediately after heating using a probe thermometer (Digitron 3246). The carrots were then allowed to stand in the oven for a further period of 1 minute holding time prior to juicing for the analysis of residual enzyme activities using the methods indicated in sections 3.7.2, 3.7.3 and 3.7.4. A temperature of 90°C reached after blanching period of 4 minutes was found to be satisfactory for carrots of diameter 30-31 mm or greater and was used in further blanching studies.

Studies on microwave blanching were also carried out at Australian Food Industries Science Centre using a modified microwave oven (Manumaster 3100 I). This microwave oven has been specially constructed with a stainless steel chamber and had no turntable. Experiments were carried out using carrots of selected diameters (30-31 mm, 35-36 mm), variable power settings (800, 900 and 1000 watts) and a fixed heating time (4 min). This microwave oven was found unsuitable for blanching purposes as uneven temperature distributions were observed during heating trials.

3.10.3.2 Treatment of carrots by microwave blanching for further processing studies

Raw carrots (30-31 mm diameter, 500 g) were blanched in a Panasonic microwave oven (Genius series 4) using the method indicated earlier. The juice extracted from blanched carrots was analysed for residual enzyme activities using the method indicated earlier. The results were expressed as time (min) required for the carrot core to reach minimum of 90°C.

3.11 Pilot scale preparation of carrot juice

Fresh carrots (5 kg) were blanched using one of the blanching techniques and regime indicated in sections 3.8, 3.9 and 3.10 above prior to extraction of juice using a hard fruit juicer (Vema 2047 XA). The juice and the pulp were collected and mixed with hot tap water (50-55°C, 50% w/w on initial carrot weight basis) in a stainless steel bucket to prepare carrot slurry. The slurry was then passed through a juice separator (Robot Coupe C80 fitted with a 100 micron screen) to separate juice and pulp. The collected pulp was then recycled through the juice separator for a further two times to recover residual juice. All three juice fractions were combined and passed through 45 μm filter to remove residual suspended particles prior to further processing.

3.12 Enzymatic treatment of carrot pulp

3.12.1 *Preparation of carrot pulp mixture*

Steam blanched carrots (original weight 5 kg) were crushed in a hard fruit juicer (Vema 2047 XA) and the juice and pulp collected in a stainless steel bucket (10 L). Hot water (50°C, 5 L) was added to the juice pulp mixture and the pH adjusted from 5.8-6.2 to 4.8-

5.0 using NaOH (10 M). The mixture was heated with intermittent stirring, to 50-55°C over a period of 30 minutes in a water bath (Cryovac 512) which had been preheated to 60°C.

3.12.2 *Determination of optimum parameters for treatment of carrot*

All the analyses for determination of optimum enzymatic treatment parameters were performed in triplicate and expressed as g/100g and mg/100g respectively on original carrot weight basis unless otherwise specified.

3.12.2.1 Determination of optimum composition of enzyme mixture for treatment of carrot juice

Carrot juice/pulp mixtures prepared as described in section 3.12.1 were treated with a 150 ppm (based on original weight of carrot) enzyme solutions comprising of Rohament Max : Rohament PL enzymes mixed in the following proportions respectively (0 : 100; 75 : 25; 50 : 50; 25 : 75 and 100 : 0). These mixtures were then incubated at 55°C for a further 90 minutes with intermittent stirring prior to further analysis for juice yield and pulp as described in sections 3.6.5, and 3.6.4 respectively.

3.12.2.2 Determination of optimum incubation time for enzymatic treatment of carrot juice

Carrot juice/pulp mixture samples (10 L) prepared as described in section 3.12.1 above were treated with 150 ppm enzyme solution containing Rohament Max and Rohament PL in the proportion of 75:25 respectively. These mixtures were then incubated at 55°C for various time periods (0, 30, 60, 90, 180, 300 minutes) with intermittent stirring prior to further analysis for juice yield and pulp as described in sections 3.6.5, and 3.6.4 respectively.

3.12.2.3 Determination of optimum enzyme dosage rate for treatment of carrot juice

Carrot juice/pulp mixtures prepared as described in section 3.12.1 above were treated with enzyme solution containing 75 parts Rohament Max and 25 parts Rohament PL at additional rates of 0, 75, 150, 300 and 500 ppm (based on original weight of carrots) and incubated at 55°C for 90 minutes. Following incubation, the mixtures were then cooled and analysed for juice yield and pulp as described in sections 3.6.5, and 3.6.4 respectively.

3.12.3 *Routine enzymatic treatment of carrot for subsequent studies*

Steam blanched carrots (original weight 5 kg) were crushed in a hard fruit juicer (Vema 2047 XA) and the juice and pulp collected in a stainless steel bucket (10 L). Hot water (50°C, 5 L) was added to the juice pulp mixture and the pH adjusted to 4.8-5.0. Enzyme solutions (Rohament Max : Rohament PL in the ratio of 75:25 v/v) at 150 ppm level (v/w, expressed as total of both enzymes, based on weight of carrot originally taken for juicing) were added and the mixture incubated at 55°C for 90 minutes with intermittent stirring. The mixture was passed through a juice separator (C80) to separate juice and pulp. The pulp was discarded and the juice retained for concentration studies.

3.13 Juice concentration

3.13.1 *Thermal concentration of carrot juice*

Carrot juice extracted from blanched, enzyme treated/untreated carrots using the methods described in section 3.12.3 was concentrated by thermal evaporation in an Armfield pilot scale single pass rising film evaporator (Model FT 22) using the procedure specified in the manufacturer manual. The conditions employed required a juice flowrate of 6 Lh^{-1}, a boiling temperature of 71°C, an operating vacuum pressure of -85 kPa and a cooling water flowrate of 72 Lh^{-1}. Samples of the concentrate and juice were stored at 4 and –18°C temperatures for shelf life and sensory evaluation studies while samples for carotenes, viscosity and colour determination were stored at 4°C temperature pending analysis. The evaporator was cleaned and sanitised after each concentrate run using the procedure specified by the manufacturer.

3.13.2 *Membrane concentration*

3.13.2.1 Ultrafiltration of carrot juice

Carrot juice extracted from blanched, enzyme treated / untreated carrots using the methods described in section 3.12.3 were collected and concentrated by ultrafiltration at 45°C using an Amicon Diaflow Ultrafiltration unit fitted with hollow fibre membrane cartridge using the procedure specified in the instruction manual. (Amicon Scientific

Australia Ltd). Ultrafiltration trials using two hollow fibre membrane cartridges at different porosities (100,000 MWCO and 30,000 MWCO) were carried out. The conditions employed required a retentate juice flowrate of 60 Lh^{-1}, permeate flowrate of 15 Lh^{-1}, a temperature of <40°C and an operating pressure of 5 kPa. The juice sample passed continuously through the ultrafiltration unit until no further changes in the TSS were observed. Samples of permeate and retentate were stored at 4°C prior to sensory evaluation studies. Cleaning and sanitation of ultrafiltration membrane and equipment was performed after each concentration run using a 1 per cent w/v solution of Ultrasil 56 detergent containing enzymes for a period of 10 minutes using the method specified by the manufacturer (Amicon Scientific Australia Ltd).

3.13.2.2 Reverse osmosis of carrot juice

Carrot juice extracted from blanched, enzyme treated/untreated carrots using the methods described in section 3.12.3 was prefiltered using 5μ pore size cartridge and concentrated at ambient temperature (20-25°C) by reverse osmosis in a Applied Membranes Inc., USA reverse osmosis unit (Series AA, Model No 2521TR) fitted with 100-200 MWCO membrane cartridge according to the procedure specified by the manufacturer. The flow rates of permeate and retentate, operating pressure, permeate and retentate temperature and changes in concentration with time were monitored. Once the juices was completely filtered through the reverse osmosis membrane, the samples of retentate and permeate were collected and analysed for colour, relative viscosity and specific gravity prior to storage or further concentration by thermal evaporator. Routine cleaning and sanitation of the reverse osmosis membrane cartridge and equipment was performed after each concentration run as specified in the instruction manual for the equipment.

3.13.3 *Concentration of carrot juice using combined reverse osmosis and thermal evaporation*

Preparation of carrot juice concentrate by linked reverse osmosis/thermal evaporation process was carried out by first preparing a reverse osmosis concentrate as described in section 3.13.2.2. This preconcentrated juice was then further concentrated by thermal evaporation as described in section 3.13.1.1. The samples of the final concentrate were stored at 4 and –18°C temperatures for shelf life and sensory evaluation studies while

samples for carotenes, viscosity and colour determination were stored at 4°C temperature pending analysis.

3.14 UHT processing of carrot juice concentrate

3.14.1 Commercial carrot juice concentrate, handling and storage

Commercially produced frozen carrot juice concentrate (36°Brix) from RHO variety was procured under frozen condition (-18°C) from Nugan Quality Foods, Griffith, NSW. This concentrate was delivered overnight under frozen conditions to Department of Food Science and Technology, University of Newcastle. Carrot concentrate sample was then UHT treated using a UHT plant (Micronics 25DH UHT/HTST) at various temperatures and aseptically packed in presterilized 125 mL bottles in an ultraviolet irradiated laminar flow chamber.

3.14.2 Establishment of suitable temperature

Carrot juice samples were heat treated at every 5°C intervals between 100- 140°C (100, 105, 110, 115, 120, 125, 130, 135 and 140°C) and packed in to a sterile container under sterile conditions in an ultraviolet irradiated laminar flow chamber. These samples were incubated at room temperature (20 and 37°C) for 2 weeks for the observation of deteriorative changes. For the investigation of the effect of higher temperatures on taste, these samples were tasted by the semitrained panels (15 members) for their comments regarding the taste. The suitable temperature from these trials were selected and used for further commercial aseptic treatment.

3.15 Microbiological analysis of carrot concentrate

3.15.1 *Preparation of peptone water*

Peptone water was prepared by dissolving 1.5 g of peptone powder (Oxoid, West Heidelberg, Australia) in milli Q water (1L), sterilised using autoclave (121°C, 15 minutes) in an autoclave. The peptone water was cooled and placed in a water bath at 37°C prior to use.

3.15.2 *Preparation of microbial media*

Potato dextrose agar powder (Oxoid, West Heidelberg, Australia) was prepared as directed by the manufacturer, sterilised using autoclave (121°C, 15 minutes) in an autoclave. The media was cooled and placed in a water bath at 37°C prior to use.

3.15.3 *Microbial analysis*

Carrot concentrate (1 mL) was diluted with 9 mL of peptone water (0.15 per cent) and mixed uniformly with a Vortex mixer to prepare 10^1 dilution sample of carrot concentrate. Similarly serial dilutions were prepared up to 10^3 for all the carrot concentrate samples (105, 110, 115 and 120°C). Viable numbers of organisms were enumerated using a pour plate technique with 1 mL of the sample and incubation at 37°C for 48 hours.

3.16 Sensory evaluation of carrot juice

Various sensory analyses of carrot juice samples were carried out using a semi-trained sensory panel of 15 members using the form presented in Appendix 3.1.

3.16.1 Training of sensory panelist

All the sensory panel members selected were provided with various concentrations of sugar (5, 6, 7, 8°Brix sugar syrup), acid (0.1, 0.2, 0.3, 0.4 % citric acid) and flavour (0.05, 0.1, 0.15 mL/L) waters. Primary screening of sensory panel members was performed using their capability of distinguishing the sugar and samples provided. The experiments were repeated using another set of sugar (6.5, 7.0, 7.5, 8.0°Brix sugar syrup), acid (0.05, 0.1, 0.15, 0.2 % citric acid) and (0.05, 0.1, 0.15 mL/L) water.

3.16.2 Sensory analysis of carrot juice

Sensory analysis of carrot juice samples were carried out using a hedonic scale by the selectively trained panel members and results were recorded on the form shown in Appendix 3.1. The carrot juice samples were analysed for colour, flavour, taste and mouthfeel.

3.17 Statistical methods

Data were statistically analysed using the one way analysis of variance method (Sigmastat V2 program). Two different approaches were applied. The Tukey test was used for data, which were normally distributed, and Dunns test was used for data not normally distributed (Steel and Torrie 1982).

Chapter 4

Preliminary investigation of the compositional, nutritional and juice quality parameters of carrots

4.1 Abstract

Two traditional and two more recently developed Australian carrot varieties have been selected for this study. Carrot varieties were analysed for a range of compositional parameters and nutrients including moisture, protein, crude fibre, pectin, vitamins (B_1, B_2, C and Carotenes) and minerals (Ca, Fe, K, Mg and Na). The results indicate small varietal variations in macronutrients and mineral composition. However, marked differences in the levels of α and β carotenes were found between varieties. Industrially significant parameters including sugar content, acidity, sugar : acid ratio, juice yield and colour values were analysed and used to indicate juicing potential juicing variety. The results of industrially significant parameters indicate considerable variation in juice yield, flavour and colour characteristics of juices prepared from the different varieties.

4.2 Introduction

Carrot (*Daucus carota* L) is a root vegetable belonging to the family *Umbelliferae*. It is believed to have originated in Central Asia (possibly Afghanistan and neighbouring countries) (Encyclopaedia Britannica 1995) and spread to Asia, North Africa and around the Mediterranean (Kalra *et al* 1987). Although it has been widely cultivated for many centuries, the use of carrots as a food dates only from the early twentieth century. Prior to that time the primary use was for medicinal purposes.

The carrot offers nutritional advantages with the fresh product being low in energy and a good source of fibre, potassium and other minerals. Of all the vegetables, carrot is one of the richest sources of carotenes especially β carotene. As well as their role in vision, carotenes have been claimed to provide protection against cancer and heart disease (Macrae *et al* 1993). In addition, it has recently been demonstrated that raw carrots have antimicrobial properties and particularly against *Listeria* (Beuchart *et al* 1994; Beuchart and Brackett 1990).

In the past there have been various reports on the composition of carrot root. Moisture contents between 86-92 per cent, protein between 0.77-1.26 per cent, dietary fibres between 0.5-4.4 per cent, carotene contents between 0.27-15.3 mg/100g were, thiamin and riboflavin content 0.6-0.7 mg/100g, soluble solids between 1.5 –9.5 per cent, acid between 0.6-0.7 mg/100g have been reported by various previous research reports.

4.3 Aim

This study was undertaken to analyse and compare four Australian carrot varieties (two new and two traditionally used) for various compositional parameters (moisture, protein, pectin crude fibres), commercially significant juice parameters (sugar, acid, sugar : acid ratio, juice yield, colour values), micronutrients (vitamin C, carotenes, thiamin and riboflavin) and minerals (calcium, magnesium, sodium, potassium and iron). This investigation was also aimed at characterising each of these carrot varieties to its own characteristic size and shape.

4.4 Carrot varieties

In the preliminary phase of this study, four Australian carrot varieties were analysed for various compositional, selected nutritional and industrially significant juice quality parameters. The varieties comprised two traditional varieties namely RC and RHO as well as two relatively new varieties RHC 100 and TP. For the investigation representative samples from both South Australian and Victorian growing regions were collected and supplied by Henderson Seeds Pty Ltd, Melbourne. Amongst these varieties RC, RHO and RHC 100 are known to belong to the *Imperator* group and TP to the *Nantes* group in Europe and the US (Personal communication, 1998).

Each of these carrot varieties has a characteristic size and shape (Figure 4.1). Of the newer varieties, RHC was found to be slender and long and similar in size and shape to RC. The traditionally used RHO variety was found to be slender but somewhat shorter as compared to RHC and RC. The newer variety TP was short, stout and differed considerably in size and shape compared to the other varieties used.

Red Hot Original | Red Count | Top Pak | Red Hot Carotene 100

Figure 4.1 **A comparison of root shapes of four Australian carrot varieties**

4.5 Sample preparation and analysis

Prior to analysis, samples of each carrot variety were washed. The analyses carried out were moisture, protein, pectin, crude fibre, minerals, thiamin and riboflavin following the procedures described in Section 3.3.2.4. The analysis of carotene content, ascorbic acid, soluble solids, acidity and colour was carried out on juice which was prepared from the raw fresh carrots as described in Section 3.3.2.3. Each of the analyses was performed in triplicate and the results for the gross compositional parameters moisture, protein, pectin, crude fiber are given in Table 4.1, while the results of micronutrient analyses are presented in Table 4.2.

Table 4.1 Gross composition of Australian carrot varieties (expressed as g/100g carrot)

	RHO	RC	TP	RHC
Moisture	88.3 ± 0.05	88.3 ± 0.02	89.1 ± 0.07	87.7 ± 0.08
Protein	1.10 ± 0.03	1.12 ± 0.02	1.15 ± 0.02	1.19 ± 0.03
Pectin	0.93 ± 0.03	0.96 ± 0.03	0.90 ± 0.01	0.87 ± 0.02
Crude fibre	1.90 ± 0.03	2.23 ± 0.03	2.19 ± 0.04	2.12 ± 0.03

Note: Each value is the mean of 3 determinations and is presented as mean ± SE

4.6.1 *Moisture*

The moisture contents of the four carrot varieties were determined using an AOAC technique described in Section 3.4.1. The moisture contents of the four carrot varieties did not show large variations although all differences were significant ($P<0.05$) except between varieties RHO and RC. The newer varieties ranged between 89.1 ± 0.07 (TP) and 87.7 ± 0.08 (RHC). The overall moisture content in all four carrot varieties ranged between 87.7-89.1 g/100 g. The variety TP contained the highest moisture content (89.10 g/100g) while the moisture content of RHC was found to be the lowest (87.7 per cent).

Various moisture contents for carrots have been reported in the literature. Values in the range of 88.8-92.4 g/100g have been reported (Jobling *et al* 1997; Cashel *et al* 1989; Mazza 1989; Kalra *et al* 1987; Truesdell *et al* 1984; Anon 1953). In another report, Kaur *et al* (1976) analysed various carrot varieties and reported varying contents of moisture content in carrot. *Nantes,* and other commercially nominated varieties *Pusa Kesar, Suchet Sharbati, No 29, Selection 233* and *Me n* were reported to have 89.5, 91.4, 91.9, 92.4, 90.9 and 91.1 g/100 g moisture respectively. The varieties *Pusa Kesar, No 29 and Selection 233* were analysed by Bajaj *et al* (1978) and reported to have moisture contents of 89.5, 89.7, 88.6 g/100g respectively. These results were consistent with the earlier investigation for variety *Pusa Kesar* unlike *No 29* and *Selection 233* which showed moisture values less than the reported value (Kaur *et al* 1976). Bajaj *et al* (1978) also measured moisture contents of various coloured carrot varieties including black carrot, yellow carrot, *Red (Subagh, Nabha, Wryana, No 29, No 8-75, S.233-21-75B*), Deep red *(Pusa Kesar, No 10-75, No 10-75A*), Orange (*Nantes IARI, S 223, Nantes NSC, Sel 5, Sel 233-75-C*), Deep orange (*Sel 5A, Sel 5B, S223-22-75-A*) and reported results in the range of 87.4-91.9 g/100g). The varieties used in the current study showed moisture contents similar to these reported in the literature.

4.6.2 *Protein*

In this study, the protein contents of the four carrot varieties were determined using the Kjeldahl method described in Section 3.4.2. The protein content of each of these carrot varieties was found to be between 1.10-1.19 g/100g. The only difference found to be significant ($P<0.05$) was between RHO and RHC (Table 4.1). However, overall there was little variation observed amongst the varieties.

Various protein contents have been reported in the literature. Protein contents of carrots depend upon variety and size of the root (Kalra *et al* 1987). Most reported values vary between 0.9-1.2g/100g of carrots. Contents in the range of 0.77 - 1.26g/100g have been reported (Jobling *et al* 1997; Cashel *et al* 1989; Mazza 1989; Kalra *et al* 1987; Truesdell *et al* 1984; Anon 1953). In another report, Kaur *et al* (1976) analysed various carrot varieties and reported varying contents of protein in carrot. Variety *Nantes,* contained maximum contents of protein (1.0 g/100g). Other commercially nominated varieties

Pusa Kesar, Suchet Sharbati, No 29, Selection 233 and *Me n* contain lower protein contents of 0.86, 0.79, 0.76, 0.87 and 0.87 g/100g respectively. There is limited information available in the literature regarding the protein content of different carrot varieties. However results reported in the current study were consistent with published values (Cashel *et al* 1989; Mazza 1989; Kalra *et al* 1987; Truesdell *et al* 1984; Anon 1953).

4.6.3 *Pectin*

In this study, the pectin content of the four carrot varieties was determined using the technique described in Section 3.4.4. The results ranged between 0.87-0.96g/100g. The results for variety RC were significantly different from those of RHC (Table 4.1). Again the overall variations in pectin contents are regarded as minor differences.

The pectin contents of carrot varieties have been reported to vary according to variety and size of the root (Baker 1998). There is relatively little literature available on the pectin contents of carrot however, most reported values vary between 0.9 to 1.1 g/100 g of carrots. The results reported during this study were lower than the previously reported value of 1.1 g/100g (Baker 1998).

4.6.4 *Crude fibre*

The crude fibre content of the four carrot varieties was determined using the traditional technique described in Section 3.4.3. In this study, the crude fibre content of the four carrot varieties was found to range between 1.90 and 2.23 g/100 g. Statistically the content for variety RHO (1.9 g/100g) was significantly lower than those of the varieties RHC, RC and TP (Table 4.1).

The crude fibre content of the carrots varieties investigated has been reported to vary depending on the varieties and size of the root. There is relatively little literature available on the fibre contents of carrot however, most reported values vary between 1.0-1.2 g/100 g of carrots (CFCAB 1997; Anon 1953). The results reported during this study were higher than previously reported values (Anon 1953).

4.7 Micronutrients

Carrot varieties were also analysed for selected micronutrients including vitamins (ascorbic acid, riboflavin, thiamin, carotenes) and minerals (Na, K, Mg, Fe, Ca). At least triplicate analyses were carried out and mean values for each of these components are shown in Table 4.2.

Table 4.2 Micronutrient contents of Australian carrot varieties (mg/100g carrot)

	RHO	RC	TP	RHC
Ascorbic acid	1.21±0.04	0.70±0.01	0.60±0.02	0.75±0.02
Thiamin	0.06±0.01	0.08±0.01	0.06±0.01	0.06±0.01
Riboflavin	0.03±0.005	0.03±0.002	0.03±0.002	0.03±0.006
α-carotene	6.85±0.13	5.53±0.09	2.76±0.06	7.42±0.11
β-carotene	7.18±0.13	7.07±0.11	4.79±0.06	7.24±0.10
Total α + β-carotene	14.03±0.13	13.60±0.10	7.55±0.05	14.66±0.105
Sodium	27.0±4.2	42.5±5.2	38.3±2.3	54.0±5.6
Potassium	333±12.2	353±7.1	310±11.3	294±9.2
Magnesium	8.5±0.41	9.6±0.52	3.9±0.29	6.6±0.36
Iron	0.59±0.06	1.03±0.09	0.51±0.03	0.39±0.04
Calcium	41.0±3.0	41.2±3.1	30.4±2.2	37.1±2.4

Note: Each value is the mean of 3 determinations and is presented as mean ± SE

4.7.1 *Ascorbic acid*

The ascorbic acid content of the four carrot varieties was determined using the technique described in Section 3.5.3. The ascorbic acid content of the carrot varieties was found to vary between 0.60-1.21 mg/100 g. Statistical analysis indicated no significant differences in the levels of ascorbic acid between RC and RHC. However the highest level of ascorbic acid (1.21 mg/100 g) was observed in variety RHO and the lowest (0.60 mg/100 g) in variety TP (Table 4.2).

Carrot is considered to be a relatively poor source of ascorbic acid. However, various ascorbic acid contents have been reported in the literature. The RDI for ascorbic acid in Australia is 30 mg/day (women) and 40 mg/day (men) (NHMRC 1991). Ascorbic acid values ranging between 3-8 mg/100g) have been reported in the literature and satisfy 10-30 per cent of RDI for both men and women. CFCAB (1997) reported 5 mg/100g ascorbic acid which is 16 per cent (women) and 12 per cent (men) of the RDI and is higher than the values reported by Cashel *et al* 1989 (4 mg/100g) and lower than 8 mg/100g (Truesdell *et al* 1984) and 6 mg/100g (Mazza 1989). The results obtained in this study were lower than those reported in the literature. Previous studies have reported that ascorbic acid acts as an antioxidant protecting the colour of the carrot during processing (Kalra *et al* 1987; Muftigul 1986; Lane *et al* 1985). In the current study, it was observed that after cutting or juicing, the TP variety darkened more rapidly than other varieties. This observation may be related to the lower levels of ascorbic acid found in this variety.

4.7.2 *Thiamin*

The thiamin contents of the four carrot varieties were determined using the spectrofluorimetric technique described in Section 3.5.5. The RDI for thiamin in Australia are in the range of 0.8-1.1 mg/day (NHMRC 1991). In this study, the values obtained for the thiamin contents of the four varieties ranged between 0.06-0.08 mg/100g. No statistically significant differences in the levels of thiamin were found and the levels satisfy less than 10 per cent of RDI values.

The thiamin contents of carrot varieties in the literature have been reported to vary according to variety. However, carrot is a relatively poor source of thiamin with most reported values varying between 0.06-0.1 mg/100g of carrots. Cashel *et al* 1989 reported 0.07 mg/100g thiamin which is higher than the value reported by Mazza (1989) (0.06 mg/100g). However these values are in agreement with the range of thiamin content reported earlier (0.056-0.11 mg/100g) (Anon 1953). The mean thiamin contents found in the current study is in agreement with the values reported in the literature.

4.7.3 *Riboflavin*

The riboflavin contents of the four carrot varieties were determined using the standard procedure described in Section 3.5.5. The mean riboflavin contents were found to be relatively constant at 0.03 mg/100g regardless of variety and no statistically significant differences were found (Table 4.2). The RDI for riboflavin in Australia are within the range of 1.0-1.7 mg/day (NHMRC 1991) and hence 100g of carrots typically satisfies less than 3 per cent of daily requirements.

Riboflavin contents of carrots have been reported to vary according to variety. Most values vary between 0.05-0.09 mg/100 g of carrots. Cashel *et al* 1989 and Mazza (1989) reported 0.04 mg/100g riboflavin and are in agreement with the range of riboflavin contents described by Anon (1953) (0.05 - 0.9 mg/100g). The mean riboflavin content found in this study is similar for all four varieties (0.03 mg/100g) (Table 4.2) and is less than the values reported in literature.

4.7.4 *Minerals*

The mineral content of the four carrot varieties were determined by atomic absorption spectrophotometry using the procedure described in Section 3.5.4. The total mineral contents of carrots have been reported to vary in the range of 0.5-1.1 g/100 g (Cashel *et al* 1989; Kalra *et al* 1987; Anon 1953) and include calcium, magnesium, sodium, cobalt, iron, zinc, fluoride, aluminium, potassium and barium (Table 4.2).

In the current investigation and in agreement with the literature, the most abundant cation in each of the varieties was found to be potassium (294-353 mg/100 g) followed by sodium (27-54 mg/100 g) and calcium (30-41 mg/100 g) with iron (0.4-1.0 mg/100 g) being the least abundant of the cations analysed. In terms of statistical evaluation, the differences between the mineral contents for the varieties were not significant, with the exception of the calcium level. Here the level in TP was found to be lower than that of other varieties. A previous report (Anon 1953) described the presence of minerals (1.1 mg/100g) including calcium and phosphorus in carrot. Kaur *et al* (1976) investigated a number of varieties of carrots for mineral contents and reported average calcium (55.9-72.9 mg/100g), iron (2.39-3.11 mg/100g), phosphorus (47.3-61.6 mg/100g), copper

(0.09-0.11 mg/100g), zinc (0.25-0.28 mg/100g) and manganese (0.26-0.35 mg/100g) content in carrots. Varieties Nantes, and other commercially named varieties *Pusa Kesar, Suchet-Sharbati, No 29, Selection 233* and *Me n* showed an abundance of calcium and phosphorus compared to other minerals. Similarly Truesdell *et al*, 1984 reported an abundance of potassium (300 mg/100g) compared to all other minerals including calcium (29 mg/100g), iron (0.6 mg/100g), phosphorus (50 mg/100g) and zinc (0.20 mg/100g). Potassium has been reported as the most abundant mineral in carrot followed in decreasing order by sodium, calcium and magnesium (Cashel *et al*, 1989; Kalra *et al* 1987; Anon 1953).

4.7.5 *Carotene*

In this study it was found that for the varieties RHO and RHC, the levels of α- and β-carotenes were very similar (Table 4.2). For the varieties RC and TP β-carotene was significantly higher than α-carotene. The varieties RHC, RHO and RC showed similar total carotene contents (13.6-14.7 mg/100 g). TP on the other hand was found to have a total carotene content of 7.6 mg/100 g, which is significantly lower ($P<0.01$) than that of other varieties. Visual observations showed that the varieties with higher total carotene levels, viz. RHO, RHC and RC showed a stronger, more attractive orange colour than the variety TP. This presumably is related to their higher carotene contents and particularly β-carotene level and is consistent with observations reported in previous studies by Bajaj *et al* (1978).

The carotenes from carrots have been extensively studied. Some studies include include Tee and Lim 1991 (9.9 mg/100g), Cashel *et al* 1989 (13.4 mg/100g), Simon *et al* 1989 (12-14 mg/100g), Dikshit *et al* 1988 (16 mg/100g), Kim and Gerber 1988 (11.14 mg/100g), Swaminathan 1987 (11.3 mg/100g), Hojilla *et al* 1985 (12.4 mg/100g), Borenstein and Bunnell 1966 (16.7 mg/100g), Anon 1953 (12.4 mg/100g). Total carotene contents in the range of 10-20 mg/100 g have been reported. In addition, varying proportions of α- and β-carotenes have been found, with α-carotene generally being found in lower amounts than β-carotene. Investigations carried out during this research also indicated similar results. Variations have been observed amongst all four carrot varieties (Table 4.2). Most importantly the level of β- carotene reported in all the

previous investigations was almost double in most cases and in some cases more than double that of α- carotene. Previously, various carrot varieties have been investigated and the influence of variety on the levels of carotene content has been reported. Mazza (1989) investigated carrot varieties *Imperator*, *Red Core Danvers*, *Red Core Chantenay* and *Nantes* for α- and β- carotene content. Variety *Red Core Danver* reported to contain highest β- (0.730 mg/100g) and α-carotene (0.332 mg/100g). Variety *Imperator* reported to contain higher levels of β- (0.653 mg/100g) and α-carotene (0.271 mg/100g) compared to *Red Core Chantenay* (β- 0.627 mg/100g and α- 0.265 mg/100g) and *Nantes* (β- 0.503 mg/100g and α- 0.180 mg/100g). The varieties *Imperator* and *Red Core Chantenay* were reported to contain similar carotene levels of both α- and β carotenes. In another investigation by Hagg *et al* (1994), carotene contents of domestically grown Finnish varieties *Lapland 1990, Lapland 1991, Lapland 1992* were compared with *Imported 1993* type. The varieties *Lapland 1990* (α- 1.32-3.07 and β- 5.62-9.23 mg/100g), *Lapland 1991* (α- 1.52-3.18 and β- 4.26-7.71 mg/100g), *Lapland 1992* (α- 0.87-2.40 and β- 3.88-11.67 mg/100g). Carotene contents of *Imported 1993* were (α- 1.45-4.37 and β- 5.08-10.91 mg/100g).

In another investigation Bajaj *et al* (1978) reported carotene contents of various colour carrot varieties including Black carrot (Black), Yellow carrot (Yellow), *Red (Subagh, Nabha, Wryana, No 29, No 8-75, S.233-21-75B*), Deep red *(Pusa Kesar, No 10-75, No 10-75A*), Orange (*Nantes IARI, S 223, Nantes NSC, Sel 5, Sel 233-75-C*), Deep orange (*Sel 5A, Sel 5B, S223-22-75-A*) and the carotene content in the range of 0.40 – 4.30ppm. These results were much lower than the value earlier reported by Mazza 1989. The carrot root colour indicated obvious difference in carotene content however the yellow and black varieties contained the lowest carotene contents of 0.4 and 0.5 respectively. Investigations carried out during this current research also indicated varietal differences. The varieties having brighter orange colour (RHO and RHC100) contained higher carotene contents compared to the relatively lighter orange colour (RC and TP) (Table 4.2).

It is noted that carotenes have been analysed using spectrophotometric methods as well as the newer HPLC procedures. Tee and Lim (1991) investigated carotenes from fresh

carrots using both spectrophotometric and HPLC methods. Differences between spectrophotometric results (9.47 mg/100g) compared to those from the HPLC method (10.00 mg/100g) (+5.3 per cent) have been reported. On the other hand, Dikshit *et al* (1988) showed higher carotene values when analysed spectrophotometrically (18.90 mg/100g) compared to HPLC (16.11 mg/100g). The carotenes were also analysed in this investigation using both of the analytical procedures mentioned earlier. The results indicate the juices extracted from all the four carrot varieties contain the total of α- and β-carotene ranging between 12.3-19.0 mg/100 g carrots. The varieties RHO, RHC, RC and TP contain 16.2, 19.0, 14.7 and 12.3 mg/100 g of total carotenes respectively. These values would also include other colour pigments including lutine and chlorophyll compared to the values reported after HPLC analysis. The varieties analysed in this investigation using HPLC method contained RHO (14.07 mg/100g) (-13.14 per cent), RHC (14.66 mg/100g) (-22.8 per cent), RC (13.60 mg/100g) (-8.1 per cent) and TP (7.55 mg/100g) (-47 per cent) respectively. These values were lower than the values analysed using spectrophotometric method consistent with the previous findings reported by Dikshit *et al* (1988).

4.8 Juice Quality

In the commercial production of carrot juice the issues of primary concern to the processor are the ease of processing, juice yield and product stability upon storage. In addition, juice quality factors which influence consumer acceptance include soluble solids content, acidity, colour, flavour and taste. In the current study, the industrially significant juice parameters including TSS, acidity, sugar : acid ratio, juice yield, and colour values (L^*, a^* , b^*) have been measured for each of the four carrot varieties and the results are presented in Table 4.3.

Table 4.3 Juice quality parameters of Australian carrot varieties

	RHO	RC	TP	RHC
TSS (°Brix)	9.8±0.8	7.6±0.69	8.3±0.7	9.8±0.9
Acidity (%)	0.09±0.01	0.08±0.01	0.07±0.01	0.06±0.01
Sugar : acid ratio	109	95	119	163
Juice yield (%)	53.1±01.9	52.1±2.1	52.1±1.8	52.9±1.9
*L**	67.7±0.09	67.7±0.09	62.7±0.10	63.7±0.13
*a**	28.0±0.09	23.1±0.11	20.4±0.12	26.2±0.09
*b**	45.1±0.12	40.2±0.15	33.4±0.13	42.0±0.16

- *L** which indicates black, white or a combination of the two colours (*L** = 0, indicates black and *L** = 100 indicates white).
- *a** which indicates green, red or a combination of the two colours (a* = -80 indicates green and *a** = 100 indicates red)
- *b** which indicates blue, yellow or a combination of the two colours (*b** = -70 indicates blue and *b** = 70 indicates yellow)

Note: Each value is the mean of 3 determinations and is presented as mean ± SE

4.8.1 *TSS contents*

In current commercial practice, TSS content is one of the primary parameters used to assess juice quality. Reference values of 8°Brix are commonly applied for single strength juices. Previous studies have indicated that sugars represent the major component of TSS with both reducing and non-reducing sugars present (Bajaj *et al*, 1987). These sugars include sucrose, maltose and glucose and provide sweetness in the product (Bajaj *et al* 1978). Soluble solids in addition to sugars include low levels of organic acids such as succinic, lactic, ketoglutaric, citric, pectic, glycolic and pyroglutamic acids (Kalra *et al* 1987).

The TSS content of juices from the four carrot varieties were determined using a refractometer as described in Section 3.6.1. In this investigation, the TSS levels in the juices from the four carrot varieties varied between 7.6-9.8°Brix which are similar to the range reported by Mazza (1989). The varieties RHO (9.8°Brix) and RHC (9.8°Brix)

consistently gave juices having much higher (22.5 per cent) TSS values than the commercially accepted reference value (8°Brix) and this is consistent with higher juice yield potential of the varieties (Table 4.3). The TSS values for RC and TP are close to the reference value and indicate a lower juice yield potential. TSS values in the range of 6-9°Brix have been reported in previous studies (Mazza 1989; Bajaj *et al* 1978; Anon 1953). The lower TSS value for RC and TP found in the current study are consistent with current commercial results (Industrial communication 1997) whereas the TSS results of RHO and RHC are quite high compared to commercial practice and this indicates promising juicing potential for these varieties.

In various investigations in the past, varietal effects on TSS contents have been reported. Bajaj *et al* (1978) reported TSS contents of various carrot varieties in the range of 3.52-6.82. Highest TSS contents in variety *Yellow carrot* (6.82) and lowest TSS contents in variety Sel.5A (3.51) have been reported. Other values included: *Black carrot* (5.17), *Subagh* (5.04), *Nabha* (4.82), *Wryana*(4.62), *No 29* (4.49), *No 8-75* (5.95), *S.233-21-75B* (5.83), *Pusa Kesar* (4.75), *No 10-75* (4.48), *No 10-75A* (5.72), *Nantes IARI* (6.28), *S 223* (4.94), *Nantes NSC* (4.79), *Sel 5* (4.77), *Sel 233-75-C* (4.15), *Sel 5B* (5.36), *S223-22-75-A*(5.24) (Bajaj *et al* 1978). In another investigation Mazza (1989) reported TSS contents of varieties which were much higher (6.35-9.30) than those reported by (Bajaj *et al* 1978). Variety *Spartan Bonus* F_1 (9.30) with highest TSS contents and *Scarlet Nantes* (6.35) with lowest were reported. Amongst other varieties *Kinko 6* (6.88), *Kinko 8* (7.13), *Nz 251* (7.09), *Gold Pak* (8.75), *Hipak Elite* F_1 (8.24) have been reported. Similar varieties produced in different areas including *Nantes Strong Top* (7.84), *Nantes Strong Top* (7.95), *Waltham Hicolor* (8.12), *Waltham Hicolor* (8.55), *@Pioneer* F_1 (8.70), *Pioneer* F_1 (8.13), *Hicolor 9* (6.81), *Hicolor 9* (8.72), *Caravella* F_1 (6.83), *@Caravella* F_1 (7.53), *Carousal* F_1 (7.21), *Carousal* F_1 (8.31), *Danvers 126* (7.26), *Danvers 126* (8.22), *Imperator Extra Long* (7.53), *Imperator Extra Long* (8.94) have been reported (Mazza 1989).

4.8.2 *Acidity*

Carrots are typically low in acid. In current commercial practice, acidity level is also one of the parameters used to assess juice quality and reference values of 0.06 per cent

acidity are commonly applied (Industrial communication 1998). The acidities of the four carrot varieties were determined titrimetrically as described in Section 3.6.2. In the current investigation relatively low values were found and there was no statistically significant varietal effects with results falling in the range of 0.06-0.09 per cent (Table 4.3). There are virtually no published values with which these may be compared.

4.8.3 *Sugar : acid ratio*

In current commercial practice, in addition to solids content and acidity, the sugar : acid ratio is also used in the measurement of juice quality. As expected, high levels of sugar and lower levels of acidity increase this ratio and impact positively on the taste of the product. The ratio of sugar to acid, calculated from TSS and acidity measurements, varied widely between the varieties studied (Table 4.3). Here, high sugar levels and low acid levels in juice from RHC showed statistically higher results and ratio than all the other varieties. Therefore it is likely that the taste of juice prepared from this variety would be much more appealing to the consumer.

4.8.4 *Juice yield*

Juice yield is one of the most industrially significant juice parameters for the processor. It indicates the quantity of juice produced per unit quantity of carrot and therefore indicates eventual commercial gains or losses for the processor. In previous studies, juice yield from carrot in the range of 50-55 per cent w/w (Anon 1953) and 52-54 per cent w/w (Munsch *et al* 1986) have been reported. In the current investigation, the juice yield of the four carrot varieties was determined as described in Section 3.6.5. Juice yield from the four carrot varieties varied between 52-54 per cent (w/w). The varieties RHO (53.1 per cent) and RHC (52.9 per cent) consistently gave statistically significantly higher juice yield compared to other varieties. The varieties RC (52.1 per cent) and TP (52.1 per cent) gave similar juice yields which were lower (by 1.7 per cent) than RHO and RHC indicating higher juicing potential of the varieties (Table 4.3). The juice yields, expressed on a direct recovery basis without consideration of TSS for all four varieties and was in the range of 52-55 per cent (w/w) and is in agreement with previously reported values in industrial operations (Industrial communication 1998). Commercially the TSS level is equally as important as yield, as juice is traded in terms

of its strength basis, with single strength juice corresponding to a reference value, for TSS of 8°Brix. Accordingly, measurements of TSS are used as an indicator of potential juice yield from carrots (Industrial communication 1997).

4.8.5 *Colour*

The colour characteristics of the four carrot varieties were determined using the Minolta Chroma Meter as described in Section 3.6.3. The colour parameters of each of these carrot varieties are reported as L^*, a^* and b^* values (Table 4.3). The L^* value represents whiteness or lightness ($L^* = 0$, indicates black and $L^* = 100$ indicates white). The colour value a* indicates green, red or a combination of the two colours ($a^* = -80$ indicates green and $a^* = 100$ indicates red). The other parameter is the value b^* which indicates blue, yellow or a combination of the two colours ($b^* = -70$ indicates blue and $b^* = 70$ indicates yellow) (Ginn *et al* 1998). The colour values of juices obtained from varieties RHC (L* 63.7, a^* 26.2 and b^* 42.0) and RHO (L* 67.7, a^* 28.0 and b^* 45.1) were higher compared to juices from the other varieties RC (L* 67.7, a^* 23.1 and b^* 40.2) and TP (L* 62.7, a^* 20.4 and b^* 33.4) (Table 4.3). The resultant combination of L^*, a^* and b^* colour values in the varieties RHC and RHO compared to other varieties were consistent with visual observation of the juice which had a deep orange colour.

4.9 Conclusion

In the current investigation four Australian carrot varieties (two traditional and two newer varieties) have been analysed for compositional, nutritional and juice quality parameters. In addition these carrot varieties have been assessed for juicing potential.

Carrot contains higher moisture content (88-89 per cent w/w) and reasonably low protein content (1.1-1.2 per cent w/w), pectin (0.87-0.96 per cent w/w) and crude fibre (1.9-2.2 per cent w/w) as described in Table 4.1. Compositional data for all four carrot varieties suggest that RHO, RC and RHC contained lower moisture content compared to TP. However, variety RHC contained higher protein content than other varieties. The pectin and crude fibre contents of RC were higher compared to other varieties. Overall,

the moisture, protein, pectin and crude fibre contents of all the four varieties showed only relatively minor variations.

Carrots are also poor sources of most other nutrients including vitamins (thiamin, riboflavin, ascorbic acid) and typically satisfy a small proportion of the respective RDI values for these nutrients. However, carrots are one of the richest sources of carotenes containing 11-20 mg/100g and satisfying up to 330 per cent of the RDI (CFCAB 1997). The current study also confirms that while carrots are a good source of carotenes, there are compositional differences between the varieties studied with RHO, an older variety and RHC, a newer variety, appearing to offer advantages over RC (older) and TP (newer).

Quality parameters TSS, acidity and colour are important for the juice processor in terms of the quality of final product. Juice yield is also very significant in determining the commercial benefits. For the carrot varieties investigated the results indicate that, of the current widely grown carrot varieties, RHC contains higher TSS (9.8) and sugar : acid ratio (163) as well as higher colour and visual appeal and is therefore preferred over RC for the purpose of juice processing. Of the newer varieties, RHC with its higher TSS and sugar : acid ratio as well as enhanced colour and visual appeal offers greater potential than TP for juicing purposes.

During the study observations were also made regarding the browning action of each of the varieties. Amongst the four varieties, TP variety darkened more rapidly than other varieties. This may be related to the lower levels of ascorbic acid observed with this variety. This further confirms that preference would be given to the varieties RHO, RHC and RC over TP for juicing purposes.

Chapter 5

The location, activity, thermal inactivation and regeneration of deteriorative enzymes from four carrot varieties

5.1 Abstract

Four Australian carrot varieties have been selected for a study of deteriorative enzymes. The varieties included those commonly used for table and also for processing purposes. Carrots were cut longitudinally and the centre (core) was removed from both the pieces to separate core and the superficial tissues (outside). The carrots were also cut transversely into two parts equal lengths. The thicker portion containing leaves is identified as stem end and the tapered thinner portion as root tip. The activities of three deteriorative enzymes (peroxidase, catechol oxidase and pectinesterase) were assayed in juices prepared from whole carrot as well as superficial tissues, core, root tip and stem end. The levels and relative distribution vary for the different enzymes and varieties studied. Thermal inactivation was assessed over a range of temperatures. The enzyme catechol oxidase was found to be the least stable and pectinesterase the most stable in each of the varieties. In most cases effective inactivation was achieved within two minutes at 85°C. The enzymes of TP variety showed greater thermal stability. In this variety pectinesterase required treatment at 90°C to ensure rapid inactivation. It is concluded that pectinesterase should be used as the indicator enzyme in the assessment of blanching sufficiency for processing of carrots.

5.2 Introduction

Fruits and vegetables during post harvest storage undergo various deteriorative changes in quality. These changes include deterioration in colour, flavour, taste and texture and are often catalysed enzymatically. A number of different enzymes have been identified as contributing to deteriorative changes during storage of raw and processed fruits and vegetables. These enzymes include peroxidase, catechol oxidase (commonly referred to as polyphenoloxidase), pectinesterase, lipoxygenase, ascorbic acid oxidase and

thiaminase (Cano *et al* 1995; Marangoni *et al* 1995; Baardseth and Slinde 1987; Stauffer 1986; Williams *et al* 1986; Burnette 1977).

The deteriorative effects of these enzymes have been studied in a wide variety of plant tissues. The enzymes pectinesterase and polygalacturonase have a major role in both development and loss of textural characteristics causing softening of fruits and vegetables. In the juice expressed from fruits and vegetables, pectinesterase enzyme also causes the loss in cloud stability and eventual separation of suspended particles from the liquid juice (Sims *et al* 1993; Hagermann and Austin 1986). Sims *et al* (1993) reported such deteriorative effects of pectinesterase in carrot juice. The enzyme has been found to have an impact in various other fruits and vegetables including tomatoes (Marangoni *et al* 1995; Nath *et al* 1983) and oranges (Hagermann and Austin 1986). Deteriorative effects of peroxidase and catechol oxidase have been reported to be reasonably similar, causing off flavour and colour in raw and processed fruits and vegetables. Catechol oxidase in papaya (Cano *et al* 1995); eggplant (Aluko and Ogbadu 1986); potato (Hsu *et al* 1988); strawberry and orange products (Cano *et al* 1997) have been widely investigated. Surface browning and lignification in raw and partially processed peeled carrots due to peroxidase and catechol oxidase have been reported (Bolin and Huxsoll 1991; Chubey and Nylund 1969). In other fruits and vegetables, peroxidase and associated deteriorative changes have been reported in cabbage (McLellan and Robinson 1987); strawberry and orange products (Cano *et al* 1997); eggplant (Aluko and Ogbadu 1986); and in papaya (Cano *et al* 1995). Lipoxygenase often contributes to rancidity and eventual flavour loss in the varieties of fruits and vegetables (Baardseth 1979). It has also been reported that lipoxygenase activity cannot be detected in carrots (Gunes and Bayindirli 1993; Baardseth 1979; Pinsky and Grossman 1971).

5.3 Aim

This study was undertaken to investigate the distribution, thermal inactivation and regeneration of the three deteriorative enzymes peroxidase (EC 1.11.1.7), catechol oxidase (EC 1.10.3.1) and pectinesterase (EC 3.1.1.11) in each of the four carrot varieties.

5.4 Carrot cutting, identification of tissues, juice extraction and storage, preparation of buffer and substrate, analysis of enzyme activity and location.

Cutting of carrots to separate the core tissue from superficial tissues as well as root tip tissue and stem end tissue was performed as described in Section 3.3.2.2. Carrots were cut longitudinally and the centre (core) was removed from both of the pieces in order to separate core and the superficial (outside) tissues (Figure 5.1). The carrots were also cut transversely into two parts having equal lengths. The thicker portion from which the leaves protrude is identified as the stem end and the tapered, thinner portion is designated as the root tip (Figure 5.1).

The carrots were washed prior to juice extraction and the procedures for preparation of juices are described in Section 3.3.2.1. Clarified juice samples were stored in small plastic tubes (Eppendorf tubes - 1.5 mL) at 2-3°C in the cool room pending the assay of enzyme activities. These assays were commenced within 10 minutes of extraction.

Preparation of various substrate solutions is described in Section 3.7. The juices extracted from whole raw fresh carrots as well as from core and superficial tissues using the method described in Section 3.3.2.1 were assayed spectrophotometrically for peroxidase, catechol oxidase and pectinesterase enzymes activities as described in sections 3.7.2, 3.7.3 and 3.7.4 respectively.

The activities were determined for all four carrot varieties and mean values of triplicate assays are presented in Table 5.1.

5.5 Peroxidase

Various salads and cooking vegetables and fruits have been analysed for peroxidase activity. The deteriorative effects of these enzymes have been studied and reported in a wide variety of plant tissues. These effects of peroxidase and catechol oxidase on quality of fruits and vegetables have been described in various instances. Robinson (1991) described peroxidase and their role in the colour deterioration in vegetables and

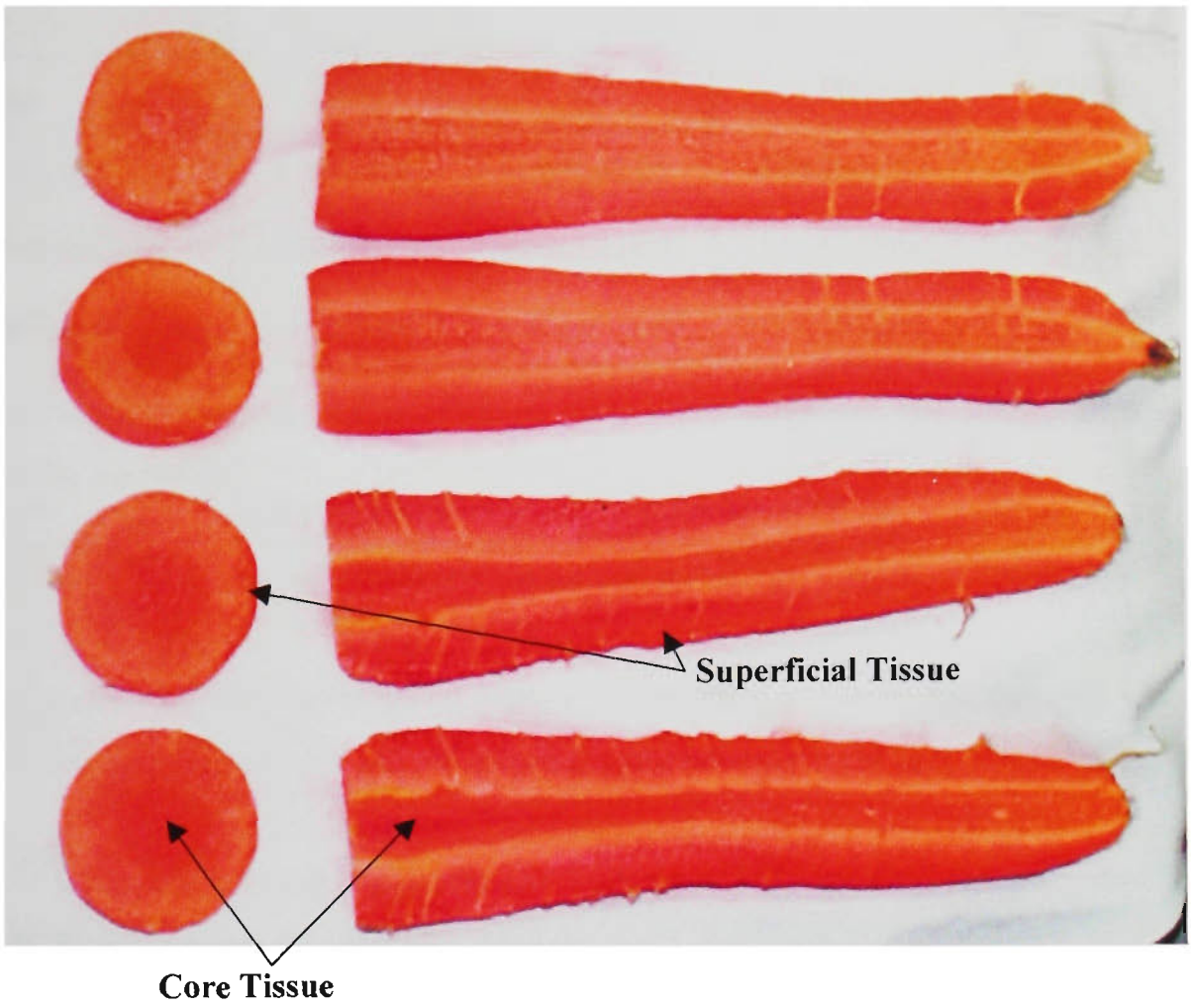

Figure 5.1 Identification of Carrot Tissues used in Enzyme Localisation Studies

Table 5.1 Enzyme activities of carrot varieties

	Carrot varieties			
Enzyme	RHO	RC	TP	RHC
Peroxidase	1.370±0.07	1.000±0.05	0.930±0.05	1.690±0.08
Catechol oxidase	0.006±0.0003	0.006±0.0003	0.008±0.0004	0.006±0.0003
Pectinesterase	0.049±0.0024	0.031±0.0015	0.034±0.0017	0.050±0.0025

Note: All values are the average of triplicate analysis and are expressed as mean ± SE

Activities are expressed as:

Peroxidase	Change of absorbance $sec^{-1}mL^{-1}$ of carrot juice at 430 nm
Catechol oxidase -	Change of absorbance $sec^{-1}mL^{-1}$ of carrot juice at 395 nm
Pectinesterase	µmol of galacturonic acid produced $sec^{-1}mL^{-1}$ of carrot juice at 620nm

fruits. Cano *et al* (1995) investigated papaya for the effect of peroxidase contents on storage and reported increased activity on storage and subsequent changes in flavour.

Various salads and cooking vegetables and fruits have been analysed for peroxidase activity (Baardseth and Slinde 1987; McLellan and Robinson 1987; Williams *et al* 1986; Mihalyi and Vamos-Vigyazo 1975). Various ranges of activities have been reported. Ramanuja *et al* (1988) reported peroxidase values of various fruits and vegetables including cluster beans (40-60 units), horseradish (34-46 units), lobia (25-35 units), cabbage (28-32 units), khol-khol (19-23 units), cauliflower (14.5-16.5 units), kovai (8-16 units), japanese radish (8-12 units), green peas (1.4-2.3 units), french beans (0.5-0.9 units), onion (0.2-0.4 units), pumpkin (0.2 units) and ash gourd (0.15 units). Cluster beans and ash gourd were reported to be highest and lowest in peroxidase activity respectively. Aluko and Ogbadu (1986) reported similar varietal variations in the activity of peroxidase in eggplant. During an investigation five varieties of eggplants were investigated for their peroxidase contents. Purple variety (0.1 units) was reported to contain least peroxidase activity and inferred to be least susceptible to development of off flavour compared to other four varieties which contained higher levels of peroxidase (0.9-1.1 units). In another investigation vegetables including cabbage (8 units), brussels sprouts (2656 units), snap beans (132 units), cucumber, (12 units), green

pepper (10 units), cauliflower (150 units), tomatoes (24 units), beetroot (39 units), leek (12 units), broccoli (406 units), potatoes (20 units), kale (105 units), swede (1281 units), parsley (39 units), onion (2 units), corn (201 units), lettuce (3 units) and peas (289 units) were investigated for their activities of peroxidase enzymes. Similarly, the activity of peroxidase was reported in brussels sprouts and onion to be the lowest in activity (Baardseth and Slinde 1987).

Amongst the vegetables analysed in this study, carrot contained a relatively low peroxidase activity enzyme with the highest reported activity being found with carrot was *Nantes Duke* variety (0.133 ΔA_{420} sec^{-1} in 100 g) (Baardseth and Slinde 1987). The newer variety RHC was found to contain the highest peroxidase activity (1.69 ΔA_{430} sec^{-1} mL^{-1}) compared to the established variety RHO (1.37 ΔA_{430} sec^{-1} mL^{-1})($p<0.05$). Minor variations were observed between the varieties RC (1.0 ΔA_{430} sec^{-1} mL^{-1}) and TP (0.93 69 ΔA_{430} sec^{-1} mL^{-1}) with the least activity found to be 0.93 ΔA_{430} sec^{-1} mL^{-1} (Table 5.1). The peroxidase activities of the varieties analysed during this investigation ranged between 0.93-1.69 ΔA_{430} sec^{-1} mL^{-1} (Table 5.1) which is considerably higher than that reported from varieties (Baardseth and Slinde 1987). The method described by Baardseth and Slinde (1987) used 40g sample and was purified prior to use. The method also used guiacol as a substrate and a wavelength of 420nm. These changes may result in the difference in the activity of peroxidase enzyme as described and compared with the results obtained during this investigation. Similarly the variance in the results of peroxidase activity when analysed using different techniques were reported. Velasco *et al* (1989) investigated corn and reported the peroxidase activity of 3390 units (acetone powder), 3120 units (0-10 per cent ammonium sulphate precipitation technique) 1440 units (10-50 per cent ammonium sulphate precipitation technique), 1600 (dialysis technique) and 1750 units (Ultragel technique) and an absorbance at 420 nm using hydrogen peroxide as substrate. In an investigation the reported values of peroxidase enzyme in the range for green and purple eggplant 2.5-4.8 units which is similar to the range reported by Aluko and Ogbadu (1986).

The peroxidase enzyme along with other enzymes in fruits and vegetables is reported to have been associated with both off flavour development and discolouration. Unprocessed food material when it undergoes physical changes during cutting, peeling

and juice extraction releases phenolic compounds which are then oxidised by peroxidase to cause colour and flavour changes (Ashie *et al* 1996; Barrett and Theerakulkait 1995; Gunes and Bayindirli 1993; Baardseth and Slinde 1987; Williams *et al* 1986, Burnette 1977).

5.6 Catechol oxidase

Various salads and cooking vegetables and fruits have been analysed for catechol oxidase activity. The enzyme catechol oxidase is also commonly known and referred to as polyphenol oxidase in previous reports. The deteriorative effects of this enzyme have been studied and reported in a wide variety of plant tissues. The catechol oxidase enzymes, along with other enzymes, are reported to have been associated with discolouration of the fruits and vegetables. Unprocessed fruits and vegetables when undergoes physical changes during cutting, peeling and juice extraction releases phenolic compounds (catechol, caffeic acid, quinic acid, chlorogenic acid) which are then oxidised by catechol oxidase to cause colour changes (Ashie *et al* 1996; Barrett and Theerakulkait 1995; Baardseth and Slinde 1987; Mayer 1987; Swaminathan 1987; Williams *et al* 1986, Boyer 1977; Burnette 1977).

Varying levels of catechol oxidase activity have been found and reported in different vegetable and fruit tissues (Cano *et al* 1995; Hsu *et al* 1988; Baardseth and Slinde 1987; Reed 1975). Various ranges of activities have been reported. In an investigation vegetables including cabbage (12 units), brussels sprouts (59 units), snap beans (50 units), cucumber, (2 units), green pepper (14 units), cauliflower (12 units), beetroot (5 units), leek (58 units), broccoli (40 units), potatoes (17 units), kale (147 units), swede (23 units), parsley (28 units), onion (14 units), corn (239 units), lettuce (31 units) and peas (403 units) have been investigated and reported for their activities of catechol oxidase enzyme. No activity of catechol oxidase activity was found in tomatoes and red pepper however peas and cucumber showed highest and lowest activity respectively of catechol oxidase enzyme (Baardseth and Slinde 1987). Under the analytical conditions used in this investigation, catechol oxidase activity ranged between 0.006-0.008 (ΔA_{395} sec^{-1} mL^{-1}) in the four carrot varieties (Table 5.1). The newer variety TP was found to contain the highest catechol oxidase activity (0.008 ΔA_{395} sec^{-1} mL^{-1}). The catechol

oxidase activity of the three other varieties RC, RHC and RHO (0.006 ΔA_{395} sec^{-1} mL^{-1}) were identical and statistically lower ($P<0.05$) than that of TP (Table 5.1). The catechol oxidase activities of the varieties analysed during this investigation ranged narrowly between 0.006-0.008 ΔA_{395} sec^{-1} mL^{-1} (Table 5.1) which is considerably lower than that reported in other carrot varieties (Baardseth and Slinde 1987). The method described by Baardseth and Slinde (1987) used 40g sample and was purified prior to use. The method also used guiacol as a substrate and a wavelength of 230nm. These changes may result in the difference in the activity of peroxidase enzyme as described and compared with the results obtained during this investigation. Similarly the variance in the results of catechol oxidase activity when analysed using different techniques were reported. In an investigation the activity of catechol oxidase enzyme in potatoes was analysed using different techniques and the results of 0.92±0.11 units (analysed spectrophotometrically at 420 nm) and 0.96±0.06 units (analysed using column chromatography 725 nm) and a variance of 4 per cent was reported (Hsu *et al* 1988). This activity was lower than the reported value (Baardseth and Slinde 1987) (17 units) which may be due to the techniques used for analysis. In the same investigation Hsu *et al* (1988) also investigated catechol oxidase activity of three varieties of potatoes. The variety Russett Burbank (4.59±0.59 units) was reported to be the highest compared to other Atlantic (2.46±0.46 units) and Russett (3.22±0.47) varieties. Velasco *et al* (1989) investigated corn and reported the catechol oxidase activity of 222 units (acetone powder), 224 units (0-10 per cent ammonium sulphate precipitation technique) 173 units (10-50 per cent ammonium sulphate precipitation technique), 128 (dialysis technique) and 86 units (Ultragel technique) and an absorbance at 420 nm using hydrogen peroxide as substrate.

During the investigation, the rate of colour changes amongst the varieties was measured. The juices extracted from each of the raw carrot varieties were exposed to air for one hour at room temperature to monitor the development of browning. The visual assessment of the juice colour indicated higher levels of browning in the juice extracted from variety TP compared to other varieties. This observation is consistent with the higher catechol oxidase activity in variety TP (Table 5.1).

5.7 Pectinesterase

The relation between the pectinesterase enzyme (commonly known as pectin methylesterase) and the cloud stability of juices from fruits and vegetables has been established (Snir *et al* 1996; Thakur *et al* 1996; Marangoni *et al* 1995; Nath *et al* 1983). Similarly, in another investigation, Marangoni *et al* (1995) demonstrated progressive softening of tomato fruit on storage due to pectinesterase activity.

Amongst the vegetables, tomatoes have been extensively investigated for the activity of pectinesterase enzyme. Marangoni *et al* (1995) investigated pectinesterase from chilled and nonchilled tomatoes and effects of the enzyme on softening of the fruit were established. Pectinesterase activity of 3.3 μmol galacturonic acid min^{-1} mg^{-1} in nonchilled and 1 μmol galacturonic acid min^{-1} mg^{-1} have been reported. These results were slightly lower than the values reported by Nath *et al* (1983) (4.05-4.96 μmol galacturonic acid mL^{-1} using a titration method) and 1.0 –4.0 μmol galacturonic acid sec^{-1} mL^{-1} using extraction and titration method) (Castaldo *et al* 1996) in tomato fruits. Hagermann and Austin (1986) analysed various fruits or the activity of pectinesterase and results reported were green apples (0.83 – 0.89), oranges (3.35 – 4.25), cucumber (1.46 – 1.50, red grapes (0.22 – 0.28) and beans sprouts (1.97 – 2.000 μmol galacturonic acid sec^{-1} mL^{-1}) (Hagermann and Austin 1986) and activities ranging between 0.004-0.105 μmol galacturonic acid sec^{-1} mL^{-1} have been reported. Orange and grape had the highest and lowest activities reported.

During this investigation four carrot varieties were investigated for their pectinesterase activity. Under the analytical conditions used in this investigation, pectinesterase activity ranged 0.031-0.050 in the four carrot varieties (Table 5.1). These values appear to be lower than other reported values (Hagermann and Austin 1986). Minor variations were observed in the pectinesterase activities between newer variety RHC (0.050) and older variety RHO (0.049). Similarly minor variations were observed in the pectinesterase activities between newer variety TP (0.034) and older variety RC (0.031) (Table 5.1). The variety RHC was reported to contain highest and the TP, the least activities of pectinesterase enzyme.

5.8 Localisation of enzyme activity

In the past various investigations have demonstrated a number of locations for deteriorative enzymes (Mayer 1987). It has been hypothesised previously that the catechol oxidase enzyme may be located only in superficial tissues. On the basis of this hypothesis, it has been proposed that a reduction in the interference of catechol oxidase enzyme in the quality of the product, processing of fruits and vegetables can be achieved by peeling operations (Industrial correspondence 1997). No similar suggestion has been reported in the literature regarding peroxidase and pectinesterase. For a food processor, location of these deteriorative enzymes in food material is considered to be important in order to determine the thermal processing parameters.

During this investigation carrots were cut into four separate parts namely core (centre), superficial tissues (outer), root tip and stem end. The activities of the three enzymes in each of these tissues from the four carrot varieties were analysed and the relative distributions of enzyme activity, based up on mean of triplicate assays are summarised in Table 5.2. The results obtained indicate a difference in the distribution of activities between core and superficial tissues for the three enzymes in all four carrot varieties analysed. In all varieties, the activity of peroxidase enzyme in superficial tissues was nearly double than that found in the core tissue. Catechol oxidase activity was only detected in the superficial tissue, in all varieties, while pectinesterase was essentially equally distributed between core and superficial tissues. In comparing the activities between root tip and stem end, each of the enzymes showed uniform longitudinal distribution of activity in all four varieties (Table 5.3).

There is relatively little literature available on localisation of deteriorative enzymes in fruits and vegetables. In a histological study, potatoes, celery and black radish were investigated for the distribution of peroxidase enzymes (Mihalyi and Vamos-Vigyazo 1975). In all three vegetables, a higher level of peroxidase activity was observed in the skin than in the central tissues. This observation is consistent with the results of the current study which indicates the presence of higher levels of peroxidase activity in the superficial tissues of carrot. There appear to have been no previous studies reported on

Table 5.2 The relative distribution of key deteriorative enzymes between core and superficial tissues of carrot varieties

Enzyme	Tissue location	RHO	RC	TP	RHC
Peroxidase	Superficial	69.8	65.8	61.6	72.8
Peroxidase	Core	30.3	34.3	38.4	27.2
Catechol oxidase	Superficial	100	100	100	100
Catechol oxidase	Core	0	0	0	0
Pectinesterase	Superficial	51.2	50.0	52.5	53.4
Pectinesterase	Core	48.8	50.0	47.5	46.6

All values are average of triplicate analyses and expressed in per cent relative terms.

the distribution of catechol oxidase or pectinesterase enzymes in plant tissues. The presence of catechol oxidase in only the superficial tissues is consistent with the hypothesis that this enzyme functions as a protective agent for fruits and vegetables against microbiological spoilage (Mayer 1987).

5.9 Thermal inactivation

Enzymes including peroxidase, catechol oxidase, lipoxygenase and pectinesterase occur widely in plant tissues and are frequently associated with deteriorative changes. Consequently, the deteriorative enzyme, which requires the most rigorous time and temperature conditions for inactivation, is frequently used as an indicator of sufficiency or adequacy of thermal processing operations such as blanching. Based on earlier studies, catalase has frequently been used as an indicator enzyme of blanching efficiency for a range of vegetables and fruits (Williams *et al* 1986; Campbell 1940). Later studies confirmed that catalase and peroxidase in most fruits and vegetables are inactivated at the same temperature (USDA 1975), however, peroxidase required longer treatment times. Other research reported peroxidase to be more stable at higher temperatures and accordingly, is currently preferred as an indicator enzyme in most

plant systems (Gunes and Bayindirli 1993; Ramanuja *et al* 1988; Ramaswamy and Ranganna 1981; Burnette 1977; Collin and McCarty 1969; Zoueli and Esselen 1959). Various studies have been reported on the thermal stability of these enzymes. In a more recent study, lipoxygenase from the vegetables was reported to be more stable at higher temperatures compared to peroxidase and the authors recommended that it should be considered as an indicator enzyme for blanching efficiency (Barrett and Theerakulkait 1995).

Table 5.3 The relative distribution of key deteriorative enzymes between root tips and stem end tissues of carrot varieties

Enzyme	Tissue location	RHO	RC	TP	RHC
Peroxidase	Root tip	50.0	50.0	50.0	50.0
Peroxidase	Stem end	50.0	50.0	50.0	50.0
Catechol oxidase	Root tip	50.0	50.0	50.0	50.0
Catechol oxidase	Stem end	50.0	50.0	50.0	50.0
Pectinesterase	Root tip	50.0	50.0	50.0	50.0
Pectinesterase	Stem end	50.0	50.0	50.0	50.0

All values are the average of triplicate analyses and expressed in per cent relative terms.

Following the extraction of the juice from fresh whole raw carrots, thermal treatment was performed using the procedure indicated in section 3.8. The residual enzyme activities at the end of thermal treatment were measured and conclusions were drawn for the indicator enzyme for carrot processing. Various time and temperature combinations were applied to carrot juice and the thermal inactivation of the enzymes was monitored (Figures 5.2-5.4). In the past similar investigations have been carried out on various other fruits and vegetables to establish suitable indicator enzymes for blanching sufficiency. The results have demonstrated that peroxidase is more stable to higher temperatures compared to catechol oxidase. Regeneration of peroxidase has not been established unlike catechol oxidase. Thus catechol oxidase enzyme has not been investigated. In other investigation, lipoxygenase enzyme is reported to be absent in

carrot (Baardseth and Slinde 1987; Baardseth 1979) and hence not investigated during this research.

5.9.1 Peroxidase

For all varieties the minimum temperature at which complete inactivation of peroxidase enzyme was achieved within the commercially acceptable time frame of 10 minutes was 80°C although varying treatment times were required (RHO-7 minutes, RC-6 minutes, TP-4 minutes and RHC-4 minutes) (See Figure 5.2). All four varieties showed residual activity after 10 minutes treatment at lower temperatures. Varietal variations in the thermal inactivation of peroxidase enzymes were observed. Varieties RHO and RC after treatment at 65-70°C for 1 minute showed reduction of up to 30-60 per cent in activity of peroxidase however, to achieve similar levels of inactivation in the other varieties (RHC and TP) the temperature required was 70-75°C. On further treatment of varieties RHO and RC at temperatures of up to 75°C, residual peroxidase activities of 15 and 40 per cent respectively were observed. Both varieties required 80°C for 1 minute to achieve commercially acceptable residual activity levels of less than 10 per cent. For varieties TP and RHC, it was necessary to further treat the juice at both 80 and 85°C for 1 minute to achieve less than 10 per cent residual enzyme activity.

There have been reports on the thermal inactivation of peroxidase activity in various plant tissues. Ramaswamy and Ranganna (1981), investigated cauliflower slices for the inactivation of peroxidase enzyme and the time of 13.3 minutes at 96.6°C was reported which was lower than the values reported in an investigation by Ramanuja *et al* (1988) (1.0 minute). Ramanuja *et al* (1988) also reported inactivation of peroxidase enzyme activity in various vegetables. Inactivation times of Cluster beans (30.0 minutes), horseradish (23.0), lobia (30.0), cabbage (20.3), khol-khol (15.7), kovai (20.0), Japanese radish (16.9) and French beans (13.6) were reported. McLellan and Robinson (1987) investigated the inactivation of spring cabbage at various temperatures. Inactivation of peroxidase isozyme A3.7 (at 60, 65, 67 and 70°C) and C9.9 (at 53, 56 and 60°C) were investigated. Isozyme C9.9 was reported to be more sensitive to heat compared to A3.7 and inactivation period of 5 minutes (A3.7) at 70°C and 5 minutes (60°C) (C9.9) were

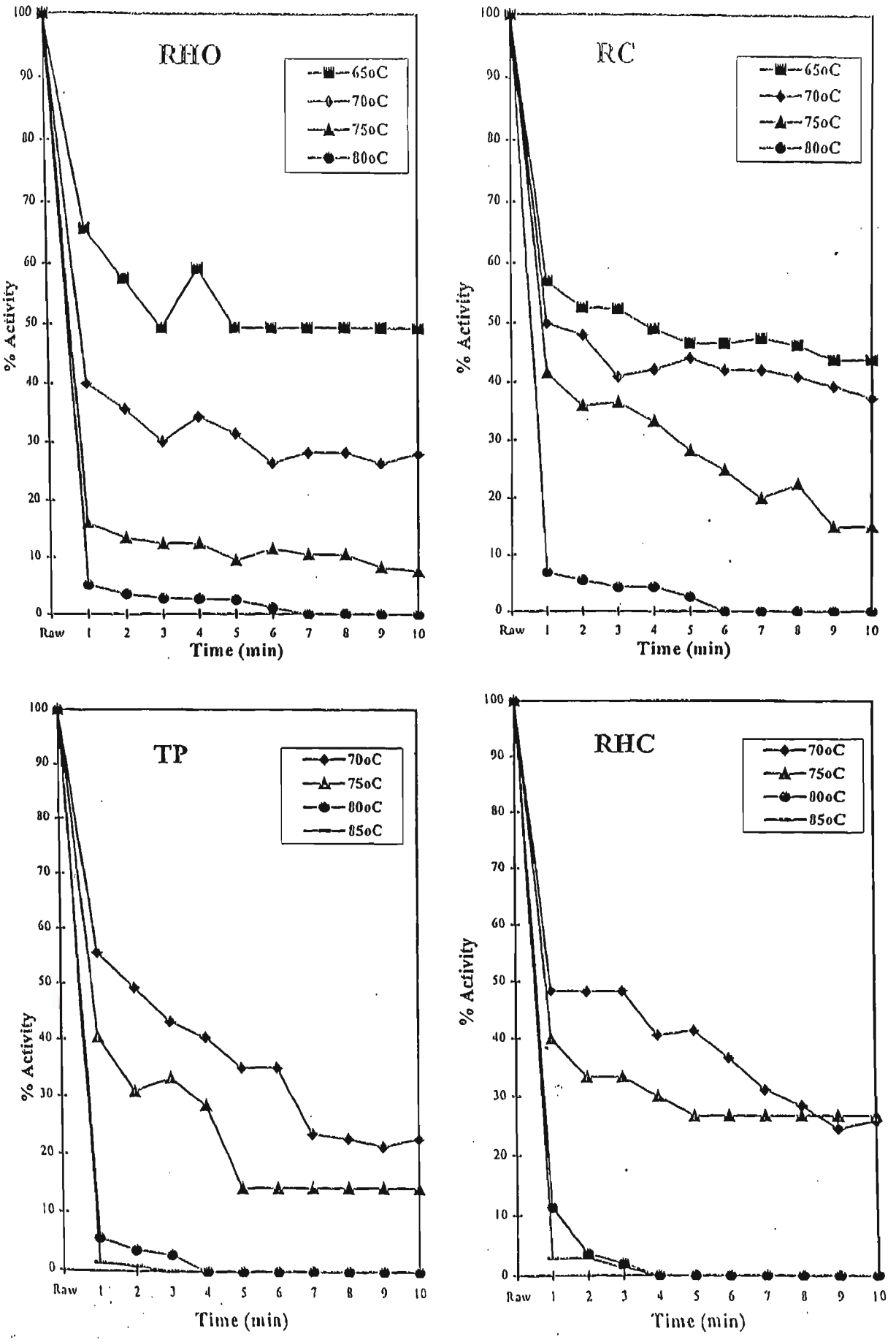

Figure 5.2 Thermal inactivation of peroxidase in juices prepared from carrot varieties

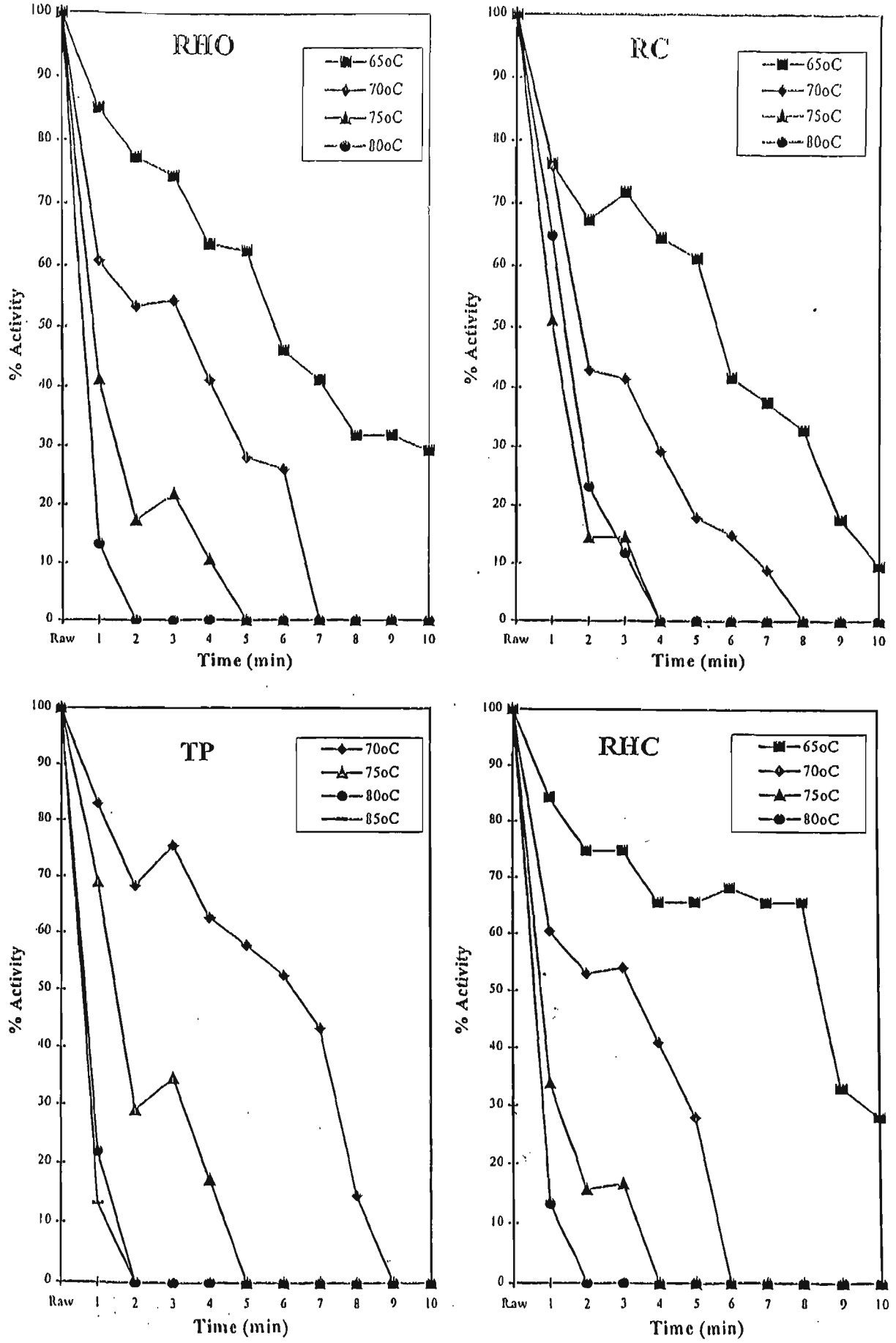

Figure 5.3 Thermal inactivation of catechol oxidase in carrot juices

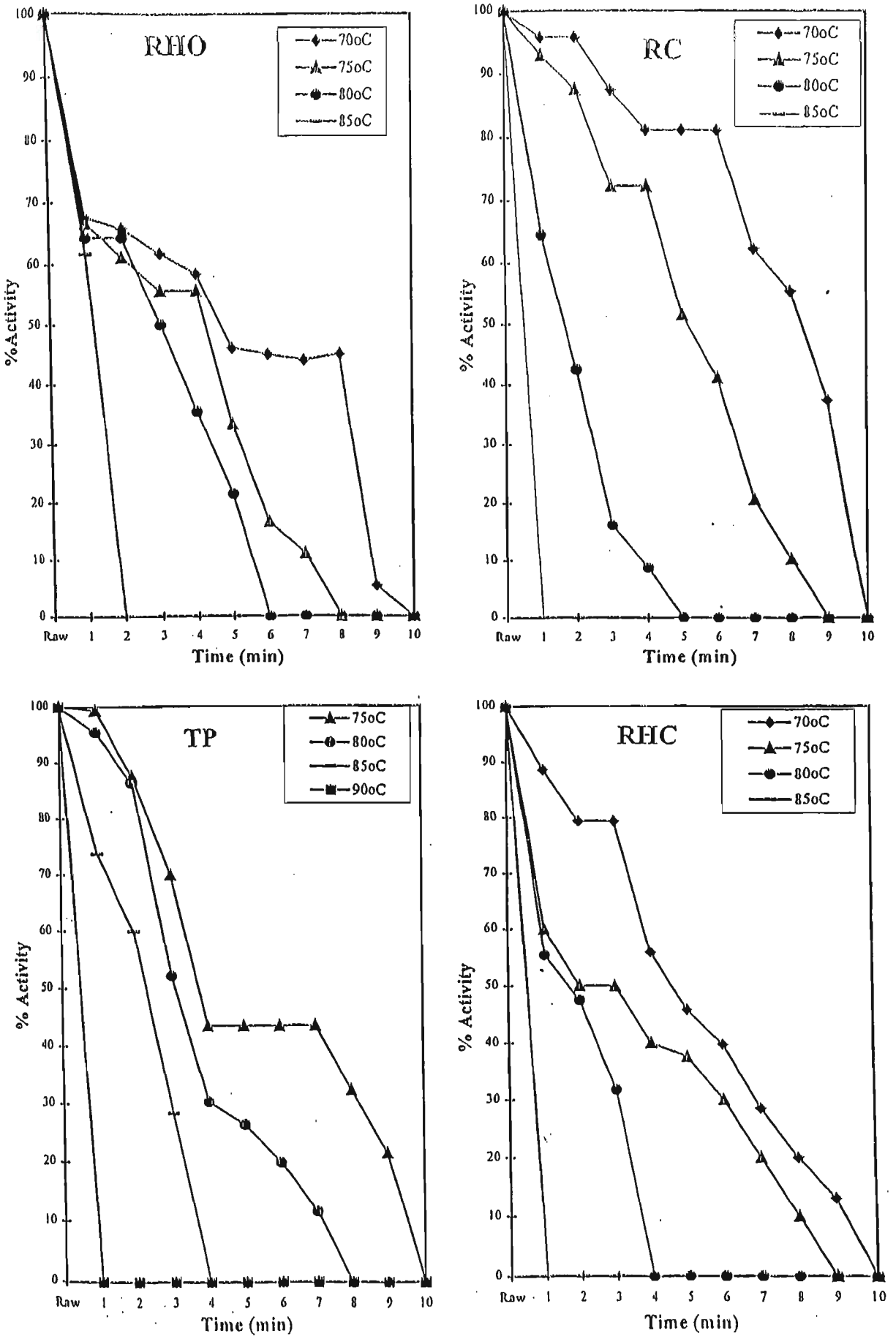

Figure 5.4 Thermal inactivation of pectinesterase in carrot juices

reported. Inactivation times of Cluster beans (30.0 minutes), horseradish (23.0), lobia (30.0), cabbage (20.3), khol-khol (15.7), kovai (20.0), Japanese radish (16.9) and French beans (13.6) were reported. McLellan and Robinson (1987) investigated the inactivation of spring cabbage at various temperatures. Inactivation of peroxidase isozyme A3.7 (at 60°C, 65°C, 67°C and 70°C) and C9.9 (at 53°C, 56°C and 60°C) were investigated. Isozyme C9.9 was reported to be more sensitive to heat compared to A3.7 and inactivation period of 5 minutes (A3.7) at 70°C and 5 minutes (60°C) (C9.9) were reported.

There is relatively little literature available on the inactivation of peroxidase in carrot. The only previous study of peroxidase inactivation in carrot involved treatment of cubes (2-3 mm size). A commercially acceptable level of inactivation was achieved with treatment at 85°C for 8 minutes or 90°C for 6 minutes (Gunes and Bayindirli 1993). The inactivation curves for the carrot varieties obtained in the current study (Figure 5.2) are similar to previous reports for peroxidase from other plants. The time required for the inactivation of peroxidase enzyme in carrot during this investigation was within the range reported in other vegetables by previous researches (Ramanuja *et al* 1988). There was an increase in the residual activity of peroxidase enzyme observed in variety RHO (65 and 70°C at 4 minutes), RC (70 and 75°C at 5 and 8 minutes), TP (70 and 75°C at 6 and 3 minutes) and RHC (70 and 75°C at 5 and 3 minutes) during the inactivation. Such increases in the residual enzyme activity in carrot were similar to those reported in celery, black radish and potatoes (Mihalyi and Vamos-Vigyazo 1975).

5.9.2 *Catechol oxidase*

For all varieties the minimum temperature at which complete inactivation of catechol oxidase enzyme was achieved was 70°C in an acceptable time frame of 10 minutes although varying treatment times were required (RHO-7 minutes, RC-8 minutes, TP-9 minutes and RHC-6 minutes) (See Figure 5.3). Varieties RHO, RC and RHC showed residual activity after 10 minutes treatment at 65°C temperatures. Varietal variations in the thermal inactivation of catechol oxidase enzymes were observed. Varieties RHO and RC after treatment at 65-70°C for 1 minute showed reduction of up to 10-40 per cent in activity of catechol oxidase however, to achieve similar levels of inactivation in other

varieties RHC and TP the temperature required was 70-75°C. On further treatment of varieties RHO and RC at temperatures of up to 75°C, residual catechol oxidase was of 40 per cent activity observed within 1 minute. Both varieties required 80°C for 3 minute to achieve commercially acceptable residual activity of less than 10 per cent. Varieties TP and RHC were further treated at 80°C and 85°C for 2 minutes to achieve less than 10 per cent residual enzyme activity. The thermal inactivation of catechol oxidase enzyme has not previously been extensively investigated in fruits and vegetables due to its relatively low thermal stability when compared with peroxidase (Ramanuja *et al* 1988; Williams *et al* 1986; Mihalyi and Vamos-Vigyazo 1975).

5.9.3 *Pectinesterase*

For all varieties the minimum temperature at which complete inactivation of pectinesterase enzyme was achieved within 10 minutes was 85°C although varying treatment times were required (RHO-2 minutes, RC-1 minutes, TP-4 minutes and RHC-4 minutes) (See Figure 5.4). Varietal variations in the thermal inactivation of pectinesterase enzymes were observed. Varieties RHO and RC after treatment at 70-75°C for 1 minute showed reduction of up to 20-25 per cent in activity of pectinesterase however, to achieve similar levels of inactivation in other varieties RHC and TP the temperature required was 70-75°C for 2-3 minutes. On further treatment of varieties RHO and RC at temperatures of up to 80°C, complete inactivation was achieved at 6 and 5 minutes respectively. The variety TP needed the highest temperature treatment of 90°C for 1 minute to achieve complete inactivation. The variety RHC needed 85°C for 1 minute was completely inactivated.

There is limited information available on thermal inactivation of pectinesterase enzymes. In an investigation Nath *et al* (1983) thermally treated tomatoes at various temperatures in the range of 62-90°C and established inactivation time of 6.6 minutes at 80°C. There is insufficient information available in literature on thermal inactivation of carrot. However time and temperature of regime of current investigation is similar to the one reported in inactivation studies of tomato pectinesterase. Similarly pectinesterase enzyme isolated from red grapefruit pulp (Cameron and Grohmann 1995) was thermally inactivated at 95°C within 30 seconds. Time and temperature regime required for

inactivation of pectinesterase enzyme from red grapefruit pulp was more rigorous than the current research of carrot. This may due to reduced interference of other components during thermal inactivation studies.

5.10 Enzyme regeneration

For all the four carrot varieties, the time temperature regime was carefully chosen from thermal inactivation data (Refer Figures 5.2-5.4), for regeneration studies. Juice subsamples after treatments as indicated earlier were stored at 2-3°C and the regeneration of peroxidase, catechol oxidase and pectinesterase enzyme activity were measured at 48, 96 and 144 hours of storage as indicated in Section 3.9.

In plant tissues, partial reversibility of peroxidase inactivation following heat treatment has been clearly established (Williams *et al* 1986; Diehl *et al* 1933). Various studies have been undertaken on peroxidase regeneration. Adam (1978) reported substantial regeneration of peroxidase enzyme from horseradish after heat treatment at 70°C.

There have been no reports indicating reversibility of the inactivation of catechol oxidase and pectinesterase. During storage at room temperature or in a deep freezer (-18°C) regeneration of peroxidase occurred within 1-2 days or several months respectively (Pinsent 1962). In order to achieve irreversible inactivation, vigorous and prolonged heat treatments have been recommended (Esselen and Anderson 1956). During the current study, thermal treatments applied to carrot juice of different varieties were sufficiently vigorous to prevent any regeneration within 6 days (144 hours) when stored at 2-3°C. Samples of juice were analysed for regeneration of peroxidase activity following treatment at 80 (RHO and RC) and 85°C (TP and RHC). No regeneration was found to occur in any of the varieties when the juice was stored for up to 144 hours after heat treatment (Table 5.4).

Table 5.4 Regeneration of deteriorative enzymes

Enzyme	24 Hours	48 Hours	96 Hours	144 Hours
Peroxidase	–ve	–ve	–ve	–ve
Catechol oxidase	–ve	–ve	–ve	–ve
Pectinesterase	–ve	–ve	–ve	–ve

–ve indicates absence (no regeneration) of enzyme activity

5.11 Conclusion

It has been well established that active enzyme systems cause deteriorative changes in foods even at subzero temperatures and low moisture levels. Foods are commonly heat treated to inactivate such enzymes. Peroxidase has traditionally been used as an indicator enzyme for thermal processing of fruits and vegetables as it is a relatively more heat stable enzyme when compared to catechol oxidase (Williams *et al* 1986; Adams 1978). On the other hand it has been suggested that palmitoyl CoA hydrolase may be an index enzyme for foods susceptible to deterioration by lipid degradation (Barrett and Theerakulkait 1995; Baardseth and Slinde 1987). The investigations found higher thermal stability of this enzyme than peroxidase. This enzyme has not been investigated in this current research work as there are reports indicating the absence of this enzyme in carrots.

In the current study, deteriorative enzymes from all the four carrot varieties were investigated for their thermal stability. Deteriorative enzymes of TP have been found to be more heat stable than those of other varieties requiring higher temperatures for inactivation. The study also indicated that amongst the enzymes investigated from carrots, pectinesterase was more stable than either peroxidase or catechol oxidase in each carrot variety at higher temperatures. These results have been in agreement with the results of studies on fruits such as citrus as well as vegetables such as tomatoes and potatoes (Snir *et al* 1996; Marangoni *et al* 1995; Nath *et al* 1983).

Current commercial practice for processed and minimally processed fruits and vegetable products indicate the use of peroxidase enzyme as the indicator for blanching efficiency (Industrial correspondence 1998). The current studies have indicated that pectinesterase is more heat stable than peroxidase and it may be a more appropriate enzyme to use as an indicator of blanching efficiency. As all the four carrot varieties have shown that pectinesterase is more heat stable than peroxidase, for further investigations this will be used as the indicator enzyme for blanching efficiency instead of peroxidase. It is likely that these results have more widespread application and similar practices might also be followed for processing of other fruits and vegetables including citrus, potato and tomato.

Chapter 6

A comparative study of blanching methods for carrots

6.1 Abstract

Carrot is a nutritious vegetable offering considerable potential for juice processing. Four popular table and processing varieties of Australian carrots have been studied for their processing potential. Various blanching methods including boiling water, steam and microwave have been evaluated for their efficacy in inactivating the deteriorative enzymes and to establish blanching protocols for commercial processing. Different diameters of carrots have been evaluated to establish temperature come up times. Regeneration of peroxidase and the effect of blanching method on weight loss/gain, yield, ascorbic acid, α and β carotenes and total carotene content have been analysed following blanching treatments. Steam blanching appears to be more suitable for carrot processing.

6.2 Introduction

Vegetables and fruits are living plant materials (Williams *et al* 1986) and as such continue to respire during post-harvest storage conditions. During storage deteriorative reactions occur which are often due to enzymic catalysis. The results of the chemical and biochemical changes include deterioration in colour, flavour and textural qualities. The control of these changes is critical to the final quality of food products for consumer acceptance. Accordingly, many plant foods are heat treated before processing to inactivate enzymes using a process commonly known as blanching.

It has been reported that enzymes including peroxidase, catalase, lipoxygenase, pectinesterase and catechol oxidase are responsible for undesirable quality changes during processing and storage of vegetables and fruits. Peroxidase and catechol oxidases are responsible for colour and flavour changes. Various investigations have been carried out to establish a suitable indicator enzyme for assaying sufficiency of the blanching process (Gunes and Bayindirli 1993; Williams 1986; Adams 1981). Traditionally, peroxidase has been used as indicator enzyme for testing the tissues of a range of different plants due to its heat stability and ability to regenerate activity once inactivated

(Pilnik and Voragen 1991; Adams 1981), thereby causing considerable deteriorative changes during extended product storage. However, the results reported in Chapter 5 on the thermal inactivation of the deteriorative enzymes peroxidase, catechol oxidase and pectinesterase from carrot varieties have indicated pectinesterase to be a more heat stable enzyme than peroxidase.

In a number of previous studies, various mechanisms of controlling enzymatic reaction in foods have been reported. Thermal treatments (blanching), dehydration (reduction of water activity) and chemical preservation (eg sulphites) have commonly been utilised (Ashie *et al* 1996). Amongst heat treatments, techniques including the use of boiling water, steam, microwave energy, pressure cooking and oven cooking have been investigated. Enzyme inactivation and the effect of blanching methods on changes on various physical and nutritional characteristics have been investigated for various fruits and vegetables. Bao and Chang (1994a, 1994b) (carrot), Howard *et al* (1996) (Carrot), Seow *et al* 1992 (Carrot, celery, green beans), Munsch *et al* (1986) (Carrot), Baardseth (1979) (Carrots), reported the effect of various blanching techniques on carotene, ascorbic acid and colour of carrots. Glasscock *et al* 1982 (Carrot, broccoli, cauliflower, green beans, zucchini), Oser *et al* 1942 (potato, parsnip), Warthesen *et al* (1984) (Broccoli, potato, peas, cauliflower, turnips, cabbage, carrots celery, green beans, zucchini), Muftigul (1985) (green beans), Klein *et al* (1979) (Spinach), Mirza and Morton (1977) (Carrot), Gunes and Bayindirli 1993 (Carrot), Drake *et al* (1981) (Asparagus, green beans, green peas, sweet corn), Farhangi and Guy Valdon 1981 (Mung beans), Kylen *et al* (1960) (Broccoli, peas, cauliflower, cabbage),Collins and McCarty (1969) (Potatoes) and Brinkman *et al* (1941) (Asparagus, broccoli, peas, cauliflower, turnips, cabbage, carrots) reported the effect of varying cooking techniques on colour, flavour, texture, chlorophyll, vitamins (ascorbic acid, carotenes), minerals and physical parameters have been reported.

While the primary aim of blanching is the inactivation of deteriorative enzymes, other advantages and disadvantages have been described by Williams *et al* (1986). These are:

Advantages

1. Blanching causes an expulsion of entrapped air from plant tissues and avoids resultant oxidative changes.
2. As a result of expulsion of entrapped air, volumetric shrinkage of the food material facilitates achievement of correct filling weight in can.
3. Blanching reduces microbial in food material load due to high temperature treatments.
4. Blanching results in softening of food materials due to cooking and facilitates juice extraction.
5. Blanching also assists in the creation of an effective vacuum during exhausting in the canning process.

Disadvantages

1. Blanching causes nutritional losses in food materials.
2. Blanching causes leaching loss of total solids due to high temperatures.
3. Blanching develops bitterness in some food materials including carrot (Poulson 1986).

6.3 Aim

In this study the changes in physical parameters (weight, juice yield, colour (L^*, a^* and $^*b$ values)), micronutrients (ascorbic acid, total carotenes, α- and β-carotene), inactivation of deteriorative enzymes (peroxidase, catechol oxidase and pectinesterase) and regeneration of peroxidase were investigated using four Australian carrot varieties and three blanching techniques. In addition, the influence of carrot sizes, as measured by carrot diameter on temperature comeup times, has been compared for each of the blanching procedures.

6.4 Blanching

The carrot varieties were blanched using hot water blanching, steam blanching and microwave blanching techniques as described in Sections 3.10.1, 3.10.2 and 3.10.3 respectively. The studies reported here include the temperature comeup times for various sizes of carrots and the effect of blanching methods on weight changes, juice yield, colour characteristics and nutrient composition.

6.4.1 Selection of carrot size

Various sizes of carrots (measured at the stem end portion of the carrot) were treated using the three blanching techniques in order to determine the impact of carrot size. A microwave blanching technique was used for the selection of suitable carrot sizes for further investigation. This size of the carrot was then used for further investigation. Carrot samples within the size range 20-21, 24-25, 30-31, 35-36, 39-40 and 43-45 mm were heated in a microwave oven as described in Section 3.10.3 for various time periods (1-5 minutes) and the temperatures measured in both superficial and core tissues. The smaller sizes of carrots (20-21, 24-25 mm) were found to be burnt within 2 minutes leaving other sizes insufficiently heat treated at the core. Similar observations were carried out when carrots were blanched using other blanching techniques. These results raised the necessity of selecting a particular size of the carrot for further investigations. These phenomena were not observed in other blanching techniques employed for heat treatment of carrots. Carrots within the diameter size range of 30-31 mm were found to be the most suitable and were selected for further experimentation.

6.4.2 Temperature comeup time

Carrots of various diameters measured at the stem end portion were selected for the investigation of temperature comeup time for each of the blanching technique. The temperature comeup times were measured on a continuous basis in boiling water bath using a Hobo data logger, in the steam retort using a thermometer probe and in the microwave oven using probe thermometer as described in Sections 3.10.1.1, 3.10.2.1 and 3.10.3.1 respectively. For all carrots of different diameters, the time taken to achieve 90°C temperature at the core was measured for all three blanching methods

(Figures 6.1-6.8). Temperatures were also measured in the superficial tissues of carrot during microwave blanching to investigate the heating pattern. Microwave blanching (Figures 6.1-6.6) of thinner carrots (20-21 and 25-26 mm) resulted in a core temperature of 90°C within 1 minute of heating at which time the superficial tissues had only reached 75°C. Heating for 3 minutes resulted in 90°C at the surface with the core reaching 98°C. Heating for 4 minutes resulted in carrots developing a burnt appearance, especially for those of smaller diameters of 20-21 and 25-26 mm. This observation indicates that this blanching technique has limited potential for this vegetable. Carrots of diameter 30-31 and 35-26 mm attained temperatures of 90°C in 1 minute of heating at which time the temperature in superficial tissues had reached 75°C. Heating for 4 minutes resulted in core temperatures of 97-98°C and superficial tissues had temperatures of 95°C. For thicker carrots (39-40 mm), a core temperature of more than 90°C was achieved within 1 minute with surface temperatures only reaching up to 69°C. Carrots of 43-45 mm diameter required heating of up to 2 minutes to achieve 90°C temperature at the core tissues. For all carrots of diameters greater than 25-26 mm, heating for 4 minutes was required to achieve a temperature of 90°C in superficial tissues.

Similar results were observed during steam blanching of carrots. For smaller diameter carrots (20-21mm), a temperature of 90°C was achieved within 11 minutes of heating. For carrots of larger diameters viz 25-26, 30-31, 35-36 and 39-40 mm longer heating times of 10, 13, 15, 19 and 21 minutes respectively were required to achieve core temperatures of 90°C (Figure 6.8).

During hot water blanching, thus carrots of smaller diameter (20-21 mm) achieved core temperatures of 90°C within 8 minutes whereas larger diameter carrots of 25-26, 30-31, 35-36 and 39-40 mm attained a similar temperature after 10, 12, 17 and 22 minutes respectively (Figure 6.7). As expected, an increase in time of heating was observed with an increase in diameter of carrots.

In each of the blanching techniques studied, the time taken to attain the required temperature was lower for the smaller diameter carrots and greater for large carrot sizes. Microwave blanching is known for its rapid heating of food products. In these trials

microwave heating, in contrast to the traditionally used blanching methods (steam and boiling water), resulted in the core of the carrots being heated first with the heat subsequently being conducted to the superficial tissues (Figures 6.1-6.6).

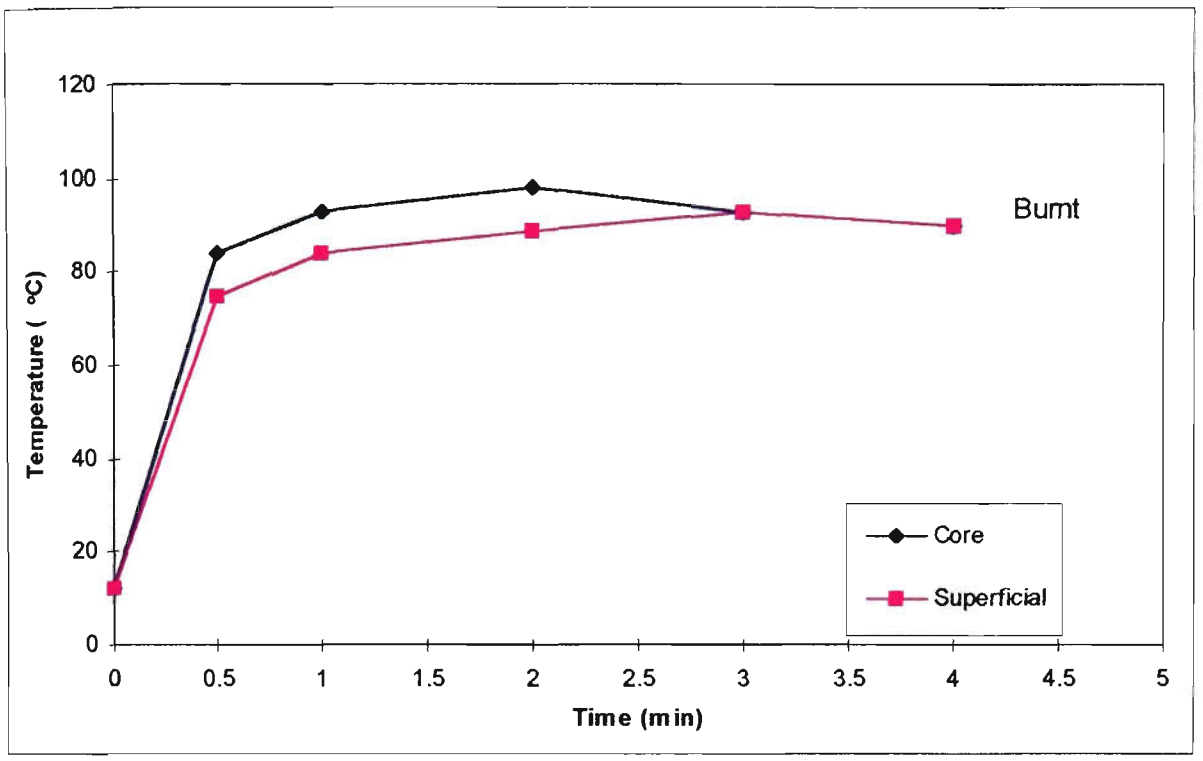

Figure 6.1 Temperature comeup time of carrot (20-21 mm ●) tissues during microwave blanching

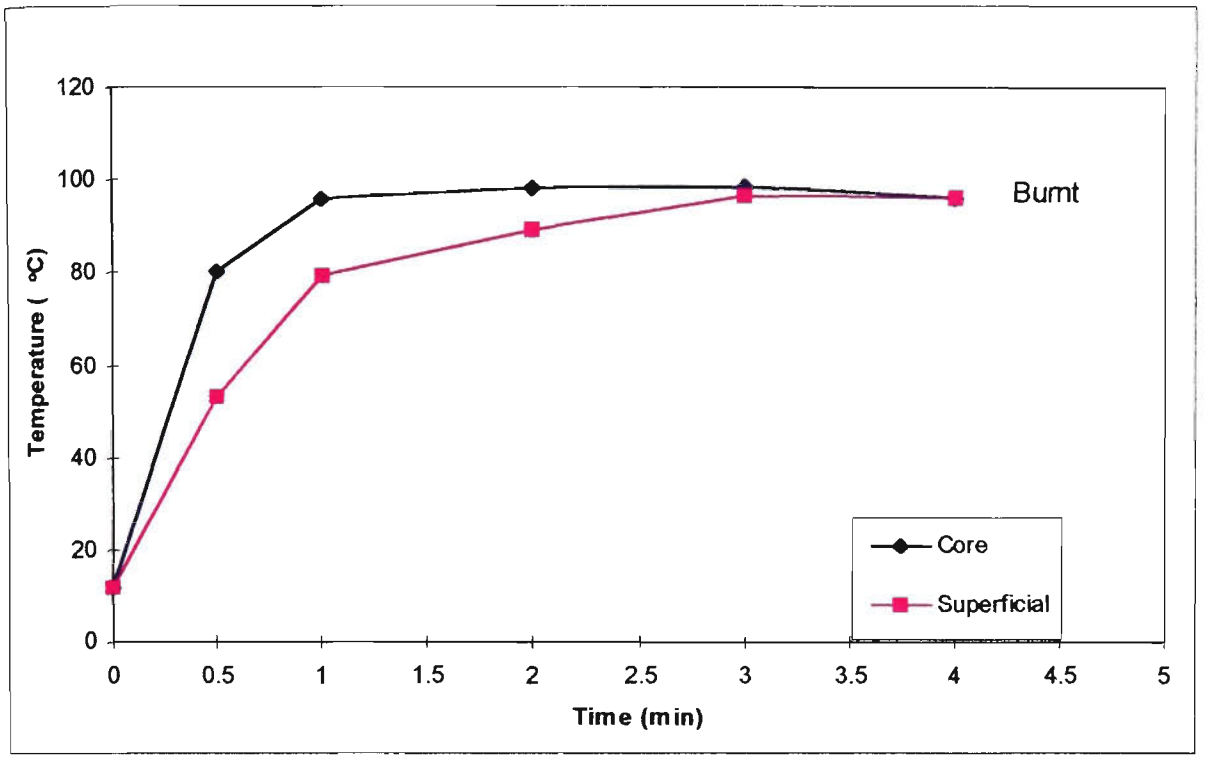

Figure 6.2 Temperature comeup time of carrot (24-25 mm ●) tissues during microwave blanching

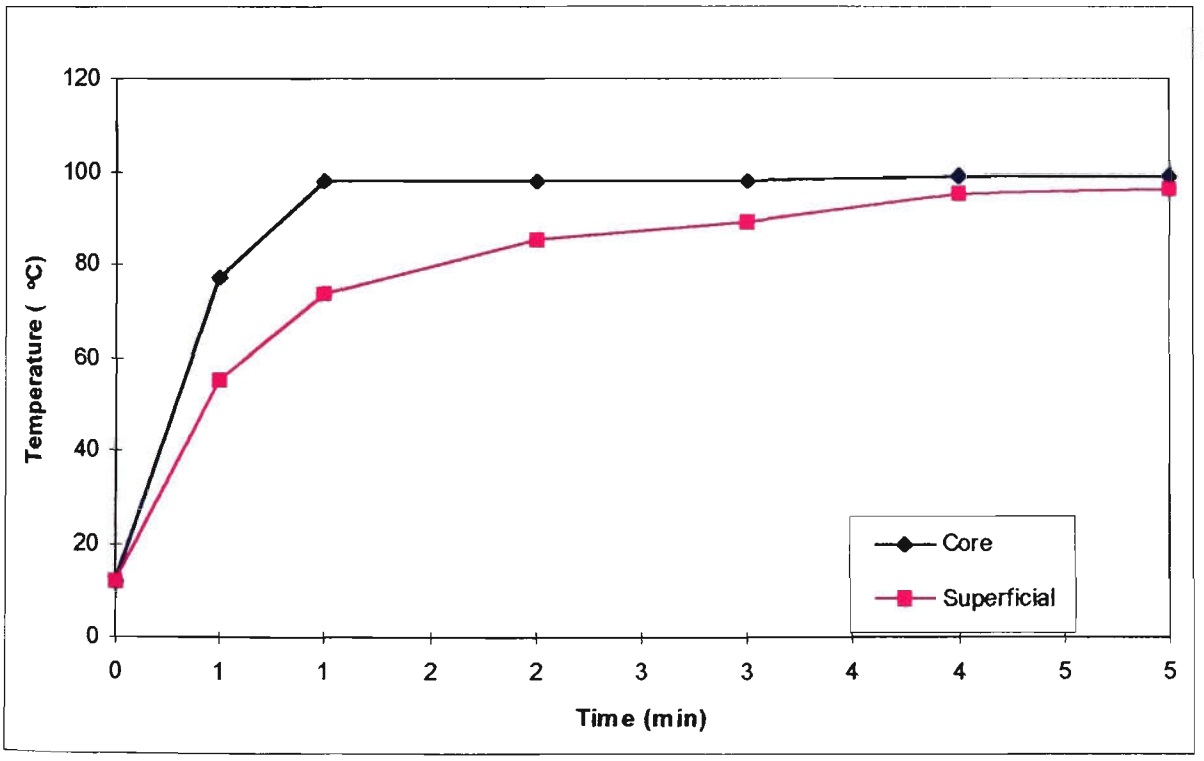

Figure 6.3 Temperature comeup time of carrot (30-31 mm ●)tissues during microwave blanching

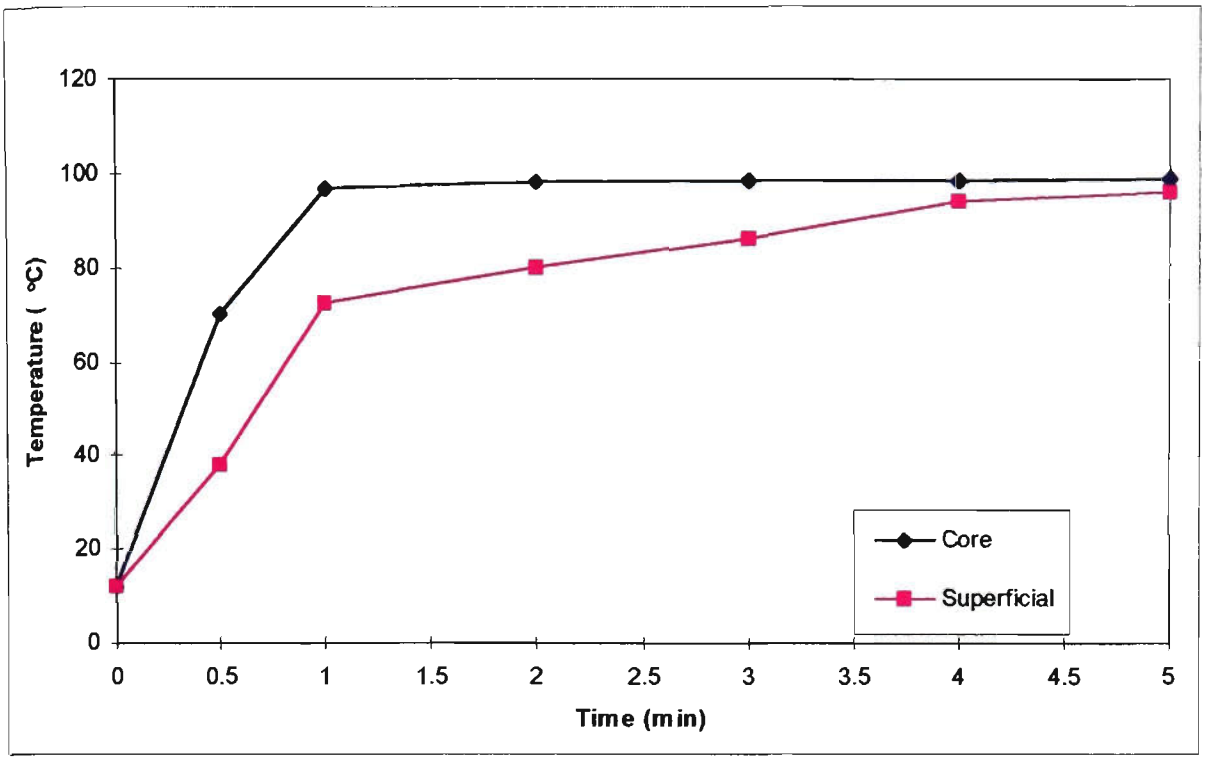

Figure 6.4 Temperature comeup time of carrot (35-36 mm ⬣) tissues during microwave blanching

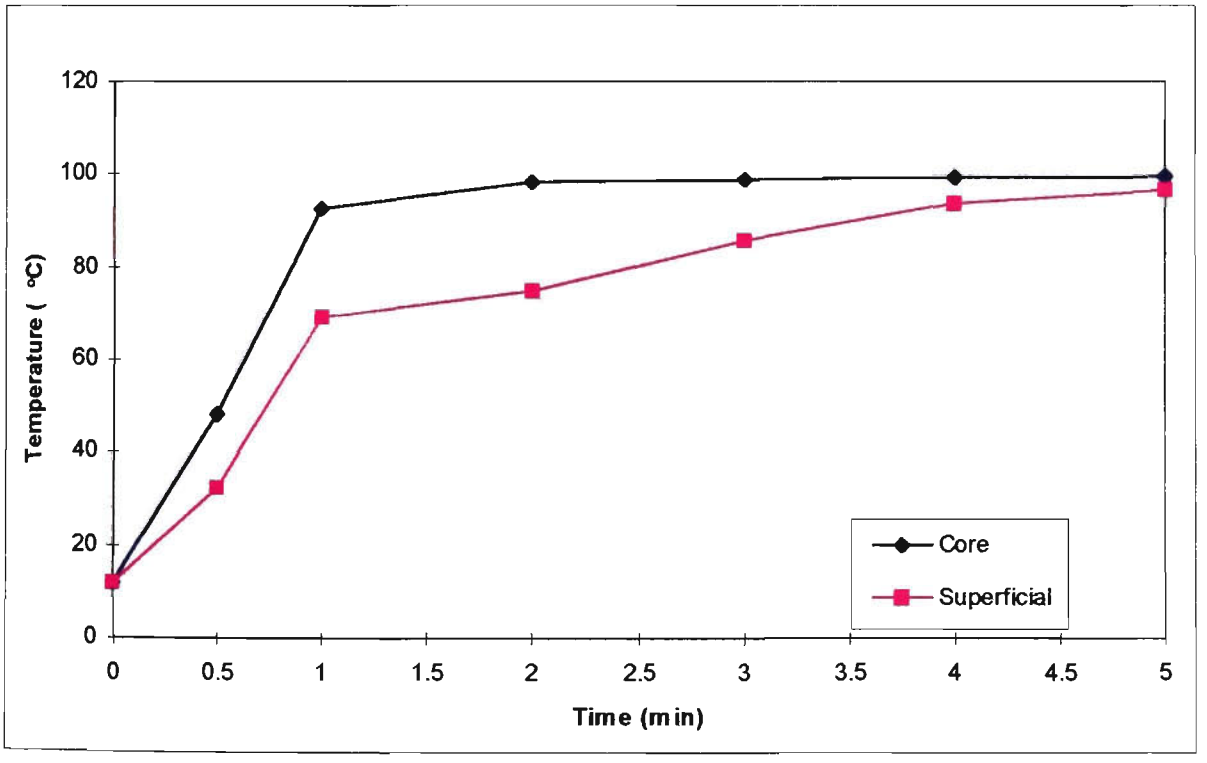

Figure 6.5 Temperature comeup time of carrot (39-40 mm ⬣) tissues during microwave blanching

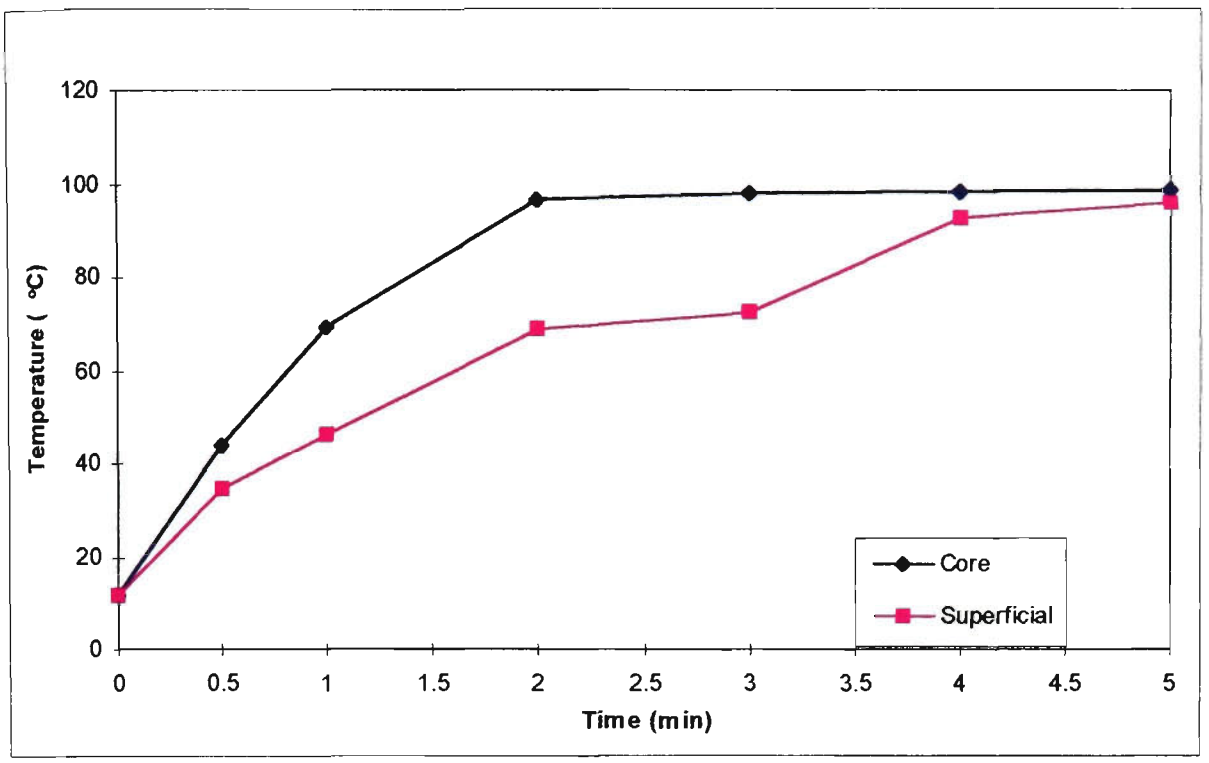

Figure 6.6 Temperature comeup time of carrot (43-44 mm ●) tissues during microwave blanching

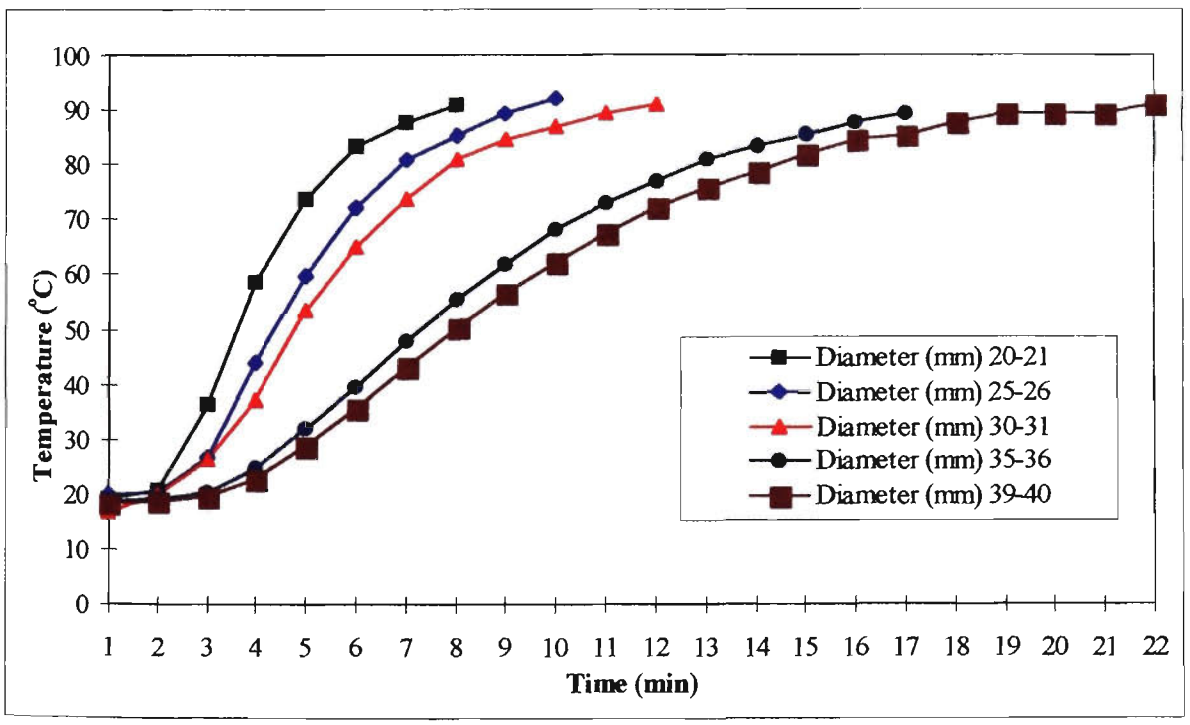

Figure 6.7 Temperature comeup times for carrot tissue during water blanching

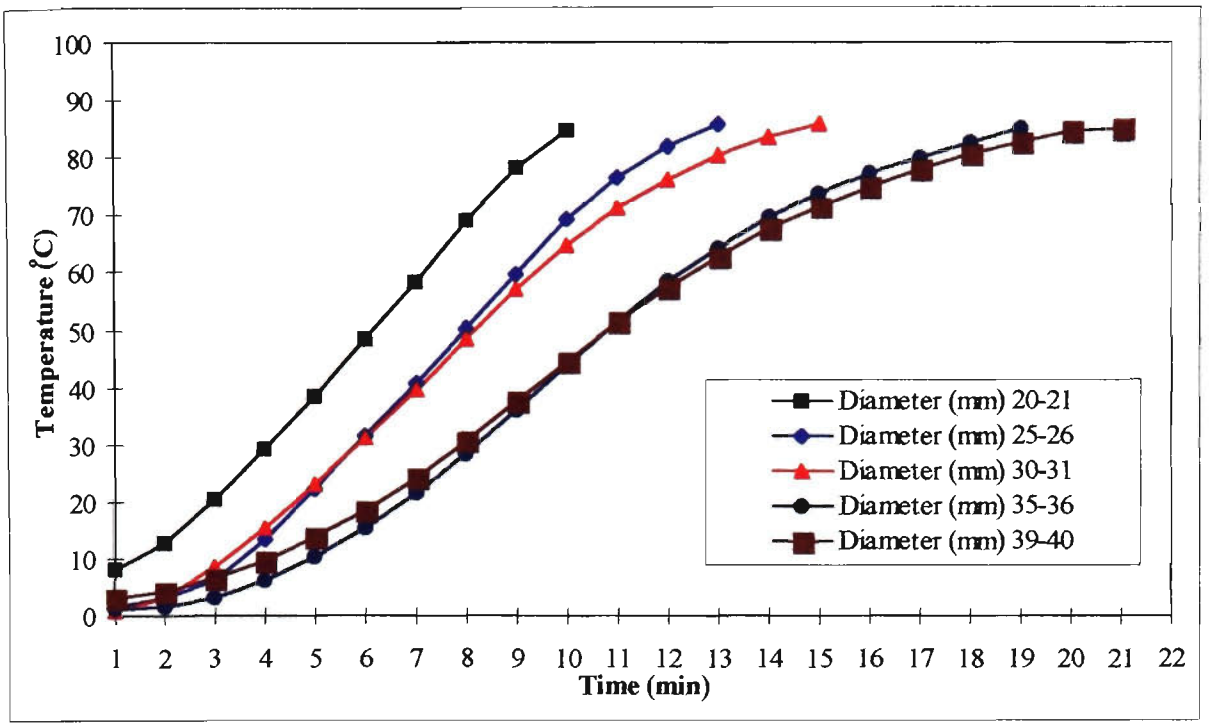

Figure 6.8 Temperature comeup times for carrot tissue during steam blanching

In general, the results reported here confirm those obtained in previous studies (Ryynanen and Ohlsson 1996). Steam and hot water blanching methods were also investigated for blanching carrots. The temperature comeup times varied with size of the carrot used for investigation. Amongst the sizes of carrots used for the investigation, smaller diameters have been suitable for blanching studies in hot water and steam blanching techniques and unsuitable for microwave blanching. Carrots of diameters 30-31 mm and larger have been investigated in all the three blanching techniques employed. Additionally the sizes of most carrots available and used for investigation were in the range of 30-31 mm. Due to these the diameters of 30-31 mm were used for further studies. The temperature come up time comparison in the carrot of 30-31mm diameter has been compared from the figures above. For carrot blanching using microwave technique, the temperature of 90°C at superficial was considered. Carrot blanched using microwave attained temperatures of 90°C within 3.5 minute at the superficial tissues. The temperature come up time for boiling water (12 minutes) and steam treatments (15 minutes) indicating the highest heat penetration for the microwave technique compared to other techniques.

6.5 Mathematical modelling

The data obtained experimentally during blanching treatment of carrots using water, steam and microwave blanching techniques showed that diameter size influenced the time required to achieve a suitable temperature for enzyme inactivation. In order to assess the possibility of being able predict comeup relationships for carrots of varying diameters, a simulation study was undertaken. The data presented in Figure 6.7 was used for this purpose.

In development of the model a number of issues were taken into account. Firstly, it was considered that the steam blanching technique involves a change of phase from water vapour to water and the results might be influenced by the quality of steam used for the experiments. Microwave blanching on the other hand involves heat transfer by radiation as well as conduction. Thus for an initial investigation, conditions prescribed for water blanching were used for the simulations. A comparison was then made between the simulated results as predicted mathematically and the experimental results obtained for water blanching.

6.5.1 Numerical procedure

6.5.1.1 Governing equation for heat transfer in carrot

As a first approximation, all the heat transferred to the carrot was assumed to be through conduction. The carrot is represented by an axi-symmetric geometry with a decreasing radius from the stem end. The governing equation for heat transfer is represented as:

$$\frac{\partial T}{\partial t} = \alpha\left(\frac{\partial^2 T}{\partial r^2} + \frac{1}{r}\frac{\partial T}{\partial r} + \frac{\partial^2 T}{\partial z^2}\right) \qquad \text{(equation 1)}$$

where

α is the thermal diffusivity of the carrot [m^2s^{-1}]

T is the temperature [°C]

t is time in seconds [s]

r is the radial distance in meters [m] and

z is the axial distance in meters [m].

6.5.1.2 Boundary and initial conditions

For water blanching the physical boundary of the carrot has a temperature of 100°C. In addition, the carrot was assumed to have an initial temperature of 20°C. These conditions are represented graphically in Figure 6.9.

6.5.1.3 Method of solution

The governing Equation (1) was solved explicitly after discretisation using finite differences. Due to the assumption of axi-symmetry only one half of the carrot was solved. Since r is equal to zero at the symmetry axis the term, $\frac{1}{r}\frac{\partial T}{\partial r}$, would become infinite at the symmetry axis. In order to overcome this problem the *L'Hospitals* rule was employed to represent this term at the symmetry axis. Thus:

$$\lim_{r \to 0} \frac{1}{r}\frac{\partial T}{\partial r} = \frac{\partial^2 T}{\partial r^2} \qquad \text{(equation 2)}$$

The FORTRAN program used to solve the problem is presented in Appendix 6.1.

Initially the thermal diffusivity of the carrot, α, was fixed at 1.0×10^{-7} m^2s^{-1}. It is also noted that experimentally, the temperature probe was inserted along the longitudinal axis of the carrot to a depth of 25.4 mm from the stem end. The length of the carrots used varied considerably. Therefore for this study, an aspect ratio of L/D=5 was used for all the simulations where L is the length of the carrot and D is the greatest diameter. Furthermore, for the simulations, the temperature along the symmetry axis and at a distance of 25 mm from the stem end was monitored and plotted against time (Figure 6.9) in order to allow a comparison with the experimental results. For the unsteady calculations a time step of 0.1 s was chosen after carrying out a time step refinement study. On reducing the time step to 0.01 s no significant change was observed in the results. Initially the carrot was divided into 1 mm (axial) by 0.5 mm (radial) cells. The results for this case are plotted as black lines in Figure 6.9. Upon reducing the cell size to 0.5 mm (axial) by 0.25 mm (radial), almost grid-independent solutions were obtained

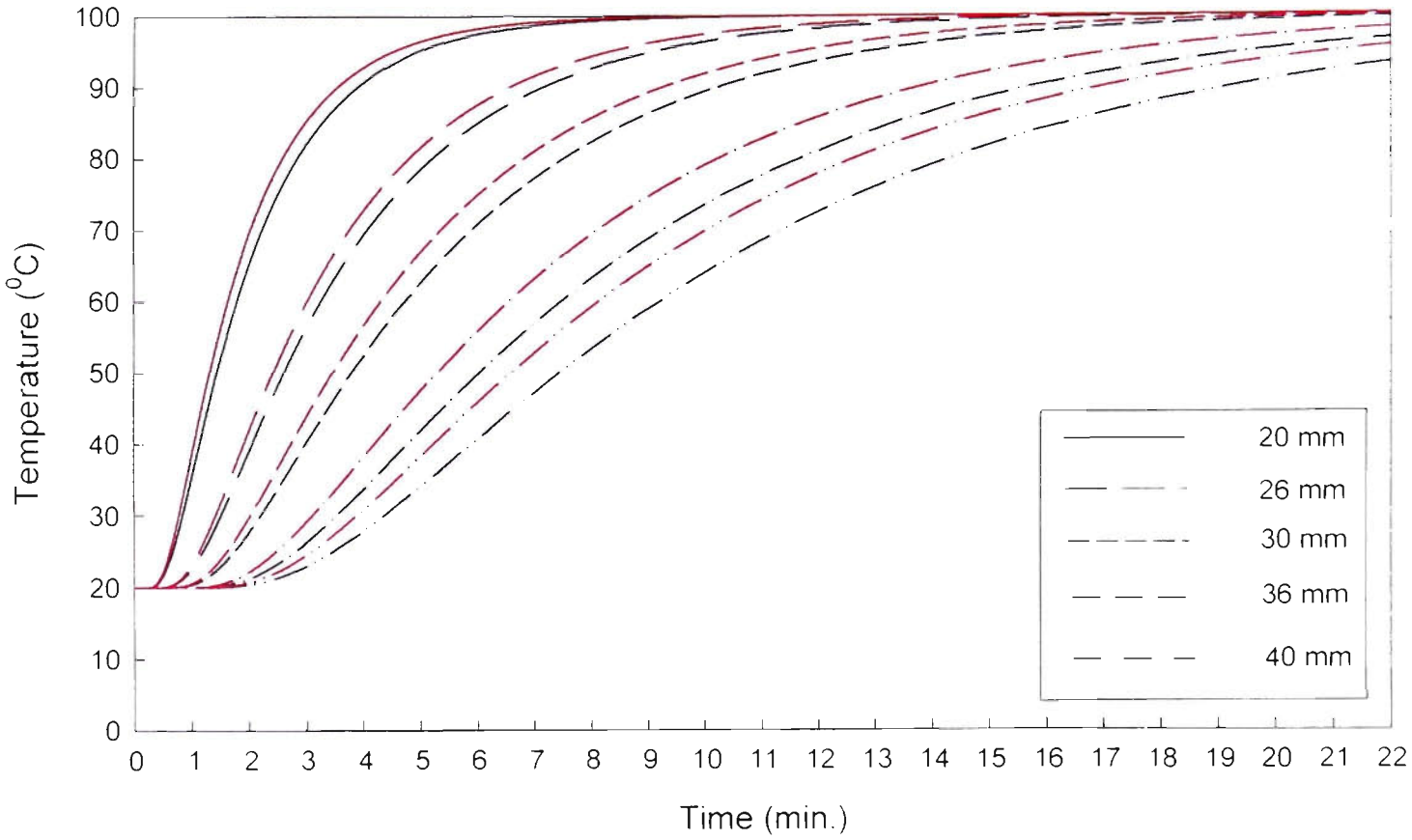

Figure 6.9 The predicted results obtained using the mathematical model for temperature of carrot s during blanching. The curves show the effect of varying the radius of the carrot as measured at the widest part of the root. Note that the red curves are based upon the parameters of dt=0.1 s, dr=0.25 mm, and dz=0.5mm and for the black curves dt=0.1 s, dr=0.5 mm, and dz=1.0mm, where t is time, r is the radius and z is the length of the carrot root

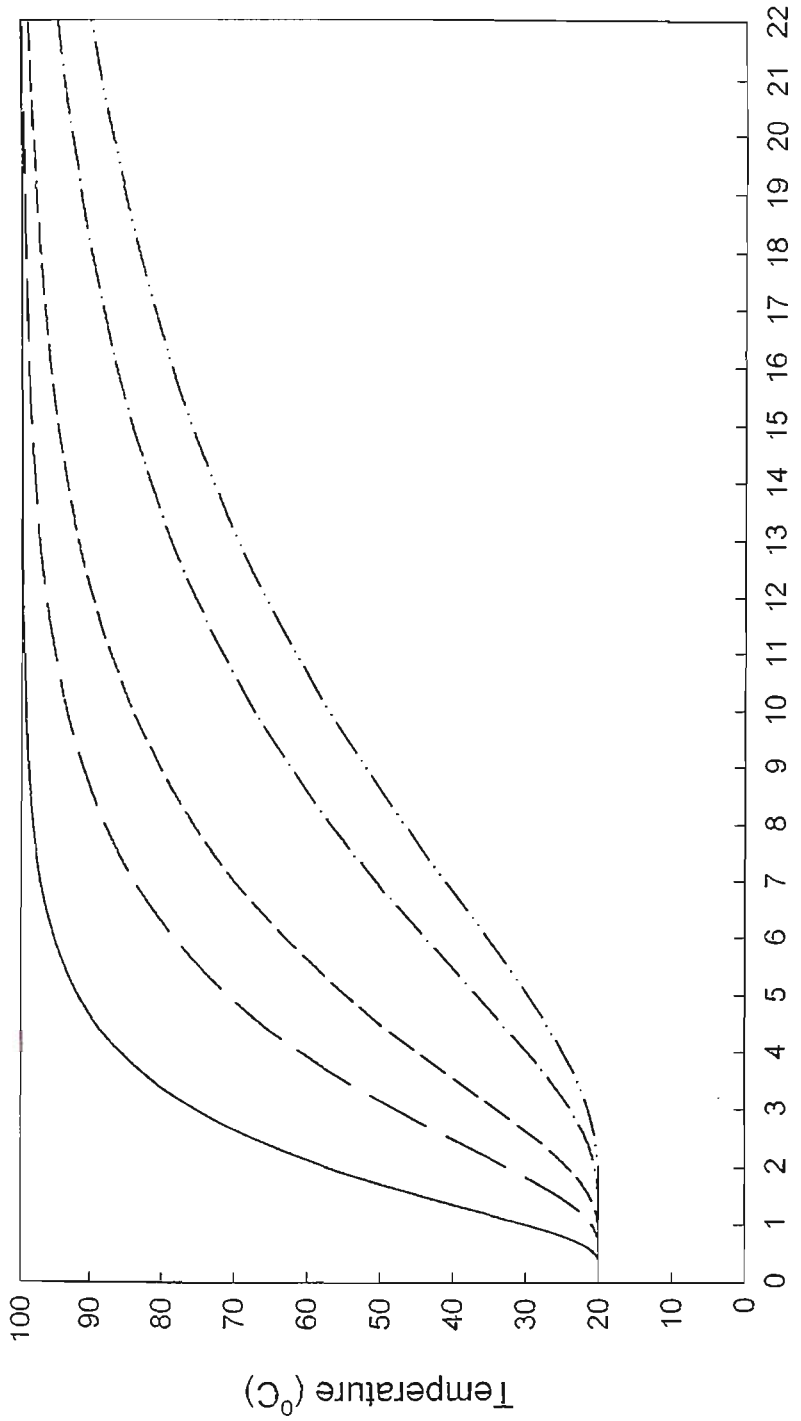

Figure 6.10 **The predicted results obtained using the mathematical model for temperature of carrot s during blanching. The curves show the effect of varying the radius of the carrot as measured at the widest part of the root. Note that the legend for this figure is the same as that for Figure 6.9.**

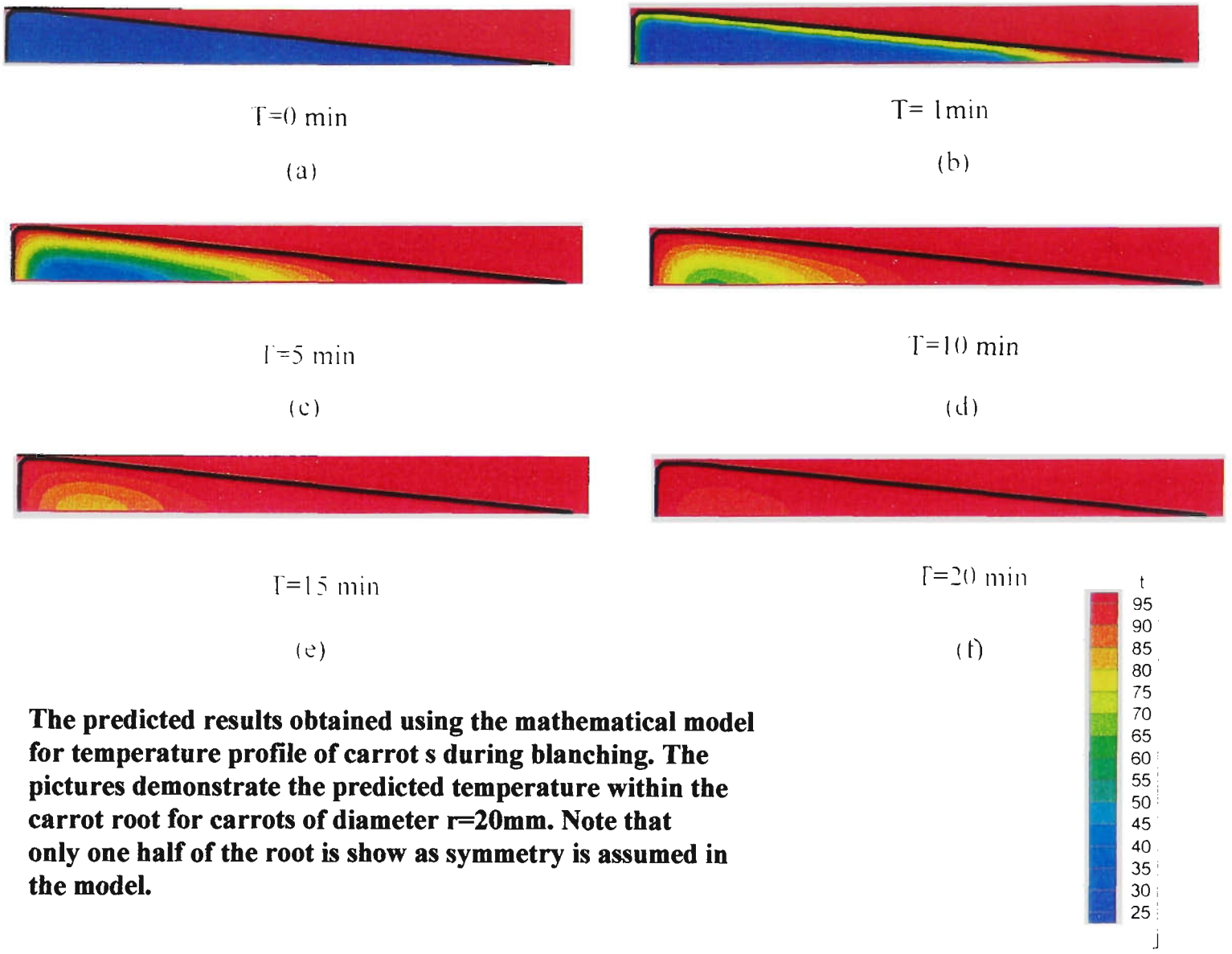

Figure 6.11 **The predicted results obtained using the mathematical model for temperature profile of carrot s during blanching. The pictures demonstrate the predicted temperature within the carrot root for carrots of diameter r=20mm. Note that only one half of the root is show as symmetry is assumed in the model.**

with the time step fixed at 0.1 s. This was checked by using a smaller cell size for one of the simulations and no difference in results was obtained.

Reasonable agreement with the experimental results (compare Figure 6.10 with Figure 6.9) has been obtained. With an increase in the diameter of the carrot, the time required for the core of the carrot to reach a steady state increases. In the experiments the time required for the carrot core to reach 90°C was monitored for each case. A comparison between the time required from the experimental and simulated results for each diameter is presented in Table 6.1. The simulations consistently under predict this time although the predictions are better for the larger diameters. The thermal diffusivity value selected for the simulations as well as the assumption of a constant aspect ratio can have a significant effect on these predictions. In order to investigate the effect of variation in the thermal diffusivity, its value was changed to a lower value of 0.8×10^{-7} m^2s^1. With this value, the results compare more favourably with the experimentally obtained results as can be seen from Figure 6.11 and Table 6.1. Figure 6.11 shows a typical variation in temperature with time in the form of temperature contours for the largest carrot diameter simulated.

Table 6.1 Time required for the core to reach 90°C: Comparison between experimental and simulated results.

Diameter of carrot (approx.) mm	Time required (Experimental) min	Time required (Numerical) min $\alpha=1.0\times10^{-7}$ m^2s^{-1}	Time required (Numerical) min $\alpha=0.8\times10^{-7}$ m^2s^{-1}
20mm	8	3.5	4.8
26mm	10	7	8.5
30mm	12	10	12
36mm	17	14	18
40mm	22	17.5	22

The equation developed appears to be useful as it successfully predicts the experimental data. There is relatively little published scientific data with which this model can be compared. The only values for thermal diffusivity of carrot tissues available are those reported by Kubota *et al* (1981) who experimentally determined values of 0.103

cm^2/min for carrot. They found similar results for other root vegetables in the range of 0.097 to 0.101 cm^2/min.

6.6 Enzyme inactivation and regeneration

The initial selection of appropriate processing times and temperatures for the different carrot varieties were based on the results of the earlier trials carried out on juice extracted from the four carrot varieties. Details of these experiments have been discussed in Chapter 5 and the blanching techniques applied to the carrots are described in Sections 3.10.1, 3.10.2 and 3.10.3. The carrot varieties RHO, RHC, RC were blanched for 1, 2 and 3 minutes at 90°C at core tissues in a boiling water bath while the variety TP was blanched for 1, 2 and 3 minutes at 95°C at core in a boiling water bath. This heating regime was based on the results obtained during the enzyme inactivation trials on various carrot varieties as indicated in Chapter 5. The residual activities of the deteriorative enzyme pectinesterase, peroxidase and catechol oxidase were measured using the techniques described in Sections 3.7.3, 3.7.4 and 3.7.5 respectively. Treatment at 90°C for 2 minutes for the carrot varieties RHO, RHC, RC was found to be sufficient to inactivate all three enzymes while treatments of 95°C for 1 minute for variety TP was sufficient to inactivate the three enzymes in this variety.

As indicated in Chapter 5, the enzyme pectinesterase was found to be the most heat stable of the deteriorative enzymes during processing of carrot juice. Treatment at 85°C for 2 minutes was sufficient to inactivate all deteriorative enzymes including pectinesterase in juice extracted from three of the four varieties tested RHO, RHC and RC. The enzymes of TP variety showed greater thermal stability requiring treatment at 95°C to ensure inactivation. In this study, blanching of whole carrots (30-31 mm dia) required 90°C for 2 minutes in boiling water to inactivate pectinesterase in most cases (RHO, RHC and RC). Pectinesterase from TP was inactivated when the carrots were treated at 95°C for 1 minute. Thus, as might be expected, the temperature required during hot water blanching of whole carrots was higher than that required for juice. Gunes and Bayindirli (1993) blanched carrot slices (20-30 mm diameter) using a hot water at 70, 80, 90 and 96°C and reduction in activity of more than 90 per cent was

reported within 9 minutes at 90 and 96°C and 7 minutes in boiling water respectively. There was no regeneration of peroxidase activity reported in this experiment

Microwave blanching experiments were carried out using a domestic microwave oven. Comparisons were made for the efficiencies of microwave blanching using two different microwave ovens. A domestic microwave oven was compared with a modified microwave oven suitable for experimental studies and available at the Australian Food Industries Science Centre. In preliminary studies, the modified microwave oven was used at 900 and 1000Watts. The modified microwave had stainless steel linings in the chamber and was without a turntable assembly. The use of this unit was found to result in cold patches in the carrot. Hence it was considered unsuitable for the blanching operation and it was not used for further studies. It is recognised that microwave ovens have a tendency to heat food unevenly leaving cold and overheated patches. This causes qualitative changes in food during storage (Ryynanen and Ohlsson 1996; Rosenberg and Bogl 1987a). In preliminary phases of the studies reported here, dipping of carrots in water prior to microwave blanching was found to facilitate speedy and uniform heating. The dipping of carrots did not improve the heating of carrots in modified microwave. Similarly during microwave blanching of the three carrot varieties (RHO, RC and RHC), core tissues attained 90°C within 2 minutes thus completely inactivating all the three enzymes. Variety TP required additional heating for 1 minute to attain core temperatures of up to 95°C for inactivation of all the three deteriorative enzymes. Additional treatment of 3 minutes at 900Watts for larger carrots (31-31, 35-36, 39-40 and 43-45mm) was necessary to achieve the required temperature.

Carrots blanched using each of the three techniques were crushed to extract juice. The juice samples from each of the varieties were analysed at various time intervals for the regeneration of peroxidase enzyme activity using the technique described in Section 3.7.3. The results indicated no regeneration of peroxidase activity during storage periods of up to 144 hours (6 days) after blanching treatment in any of the carrot varieties.

The blanching of carrots in various forms (cubes, frozen, slices, whole) has been studied using various blanching methods. Carrots have also been blanched using various techniques including microwave (Warthesen *et al* 1984; Glasscock *et al* 1982; Mirza

and Morton 1977); pressure cooking and boiling water (Warthesen *et al* 1984; Mirza and Morton 1977). Critical observations were carried out and the effect of various blanching techniques was compared. There were no differences observed in flavour and colour of carrots between microwave and boiling water blanching (Warthesen *et al* 1984; Mirza and Morton 1977). Amongst the other blanching techniques, waterless cooking of carrots compared to open pan cooking resulted in a more satisfactory product (Warthesen *et al* 1984). The retention of ascorbic acid during pressure cooking, microwave and steam blanching for carrot and other vegetables was similar whereas low retention occurred with boiling water blanching (Warthesen *et al* 1984).

Various industrial approaches have been adopted for the commercial blanching of vegetables. Individual Quick Blanching (IQB) of vegetables by spreading them in a single layer on the belt and blanching with live steam was developed to improve blanching efficiency (Lazor *et al* 1971). This has been further modified by adding a holding module to suit the blanching of particulate products including peas and carrot cubes (Cumming *et al* 1984; Lazor *et al* 1971). The system was found to be more efficient than IQB and also resulted in enhanced retention of nutritional components. Seow *et al* (1992) treated carrots using HTST and a two stage LTLT/HTST blanching methods. The latter gave better firmness and dehydration / rehydration characteristics in carrots.

Various other industrial approaches have been adapted for blanching carrots and improving the final quality of blanched product. The use of chemicals including sugar (5 per cent) (Baloch 1987) and sulphite (Dan and Jain 1971) in blanching solutions during carrot blanching have also been described. Sugar enhanced sensory qualities whereas sulphite improved carotenoid stability and hence resultant colour. The use of a new blanching technique was described by Adams (1981) using recycled blanching media as well as by using hot gases. In both these instances the quality of product was reported to be improved however the use of hot gas was reported to be relatively expensive.

While steam and water blanching are most commonly used, the application of microwave technologies has shown modest global growth. The use of microwave technology in combination with steam and hot water for sterilisation of particulate

products (Rosenberg and Bogl 1987b), aseptic and packaging processing (Richardson 1985; Unklesbay *et al* 1983) has been described for blanching of vegetables as well as other food processing operations for meat, fish and baked products.

6.7 Effect of blanching

Blanched carrots were crushed to extract juice using a Bamix laboratory juice extractor, the juice was weighed and the yield calculated on a weight basis against the original weight of carrots as described in Section 3.6.5 respectively. The juice was then analysed for total vitamin C, total carotenes, α- carotene and β- carotenes, relative viscosity, juice yield, colour and weight changes using the techniques as described in Section 3.5.1, 3.5.2, 3.5.3, 3.6.3 and 3.6.6. The effect of the various blanching techniques on the above mentioned juice quality parameters were analysed and summarised in Table 6.2.

6.7.1 Effect of blanching on weight changes and juice yield

All four carrot varieties were investigated for juice yield and weight changes following blanching by each of the three methods using techniques indicated in Section 3.3.2.3 and 3.6.5. As indicated in Chapter 4, juice yield from raw carrot averaged 52-54 per cent in all four varieties which is similar to literature reported values of 55 per cent (Munsch *et al* 1986). Blanching of carrots resulted in reduced juice yields (Table 6.3) in all carrot varieties although steam blanching gave higher juice yield compared to the other blanching methods. Blanching of raw carrot using steam resulted in reduced yield losses (35 per cent) when compared hot water blanching (46 per cent) and microwave blanching (52 per cent). The results of juice yield obtained during this research are in agreement with previous investigations in which carrots were blanched using boiling water (Bao and Chang 1994a) and resulted in juice yield which was less than that of unblanched carrot. In another investigation the effect of various processing techniques including boiling water, microwave-steam, stir fry with oil as well as stir fry with water on changes in juice yield of beans sprouts, green beans, nappa beans, spinach and water spinach has been reported. The reduction in the yield in the range of 10-18 per cent was reported. The combined technique of microwave and steam processing was reported to yield highest juice content compared to the other technique (Masrizal *et al* 1997).

Weight changes during blanching have been described in Table 6.2. Higher weight losses occurred for microwave blanching (4.1-4.6 per cent) which is higher than reported by Muftugil (1985) (2.48 per cent). For steam blanching losses (1.9 per cent) were observed in each variety similar to the reported values of 1.48 per cent. On the other hand, boiling water blanching resulted in increased weight (1.5-1.6 per cent) compared to reported losses of 1.41 per cent (Muftugil 1985).

All the four carrot varieties have been investigated for the effect of various blanching treatments on of weight changes juice yield and relative viscosity and compared with raw carrots. The comparative narration of weight changes, juice yield and relative viscosity (3.6.6) of four carrot varieties investigated have been described in Table 6.2. Amongst the individual varieties, the juice yield from TP was found to be higher for each of the blanching methods used. Juice yield of TP variety during steam blanching (35.1 per cent) was highest compared microwave blanching (26 per cent) and hot water blanching (34.1 per cent). Minor variations in the juice yield between the varieties have been observed during microwave blanching and steam blanching (Table 6.3). Structurally, TP variety is short and stout and different than other varieties, which are long and slender. This may contribute to the higher juice yield. In each of the blanching methods, carrot residues appeared to have increased juice holding capacity when compared to unblanched carrots, possibly causing a reduction in juice yield.

Table 6.2 Effect of various blanching methods on quality parameters of carrot juice

	Blanching method			
Parameters	**Raw**	**Hot water**	**Microwave**	**Steam**
Weight change	-	1.5	-4.4	-1.9
Juice yield*	52-53	28±0.5 (46%)	25±0.5 (52%)	34±0.5 (35%)
Relative viscosity	1.0	1.2	1.8	1.2
Total carotene	-	59.4 (- 40.6)	69.7 (- 30.3%)	87 (-13%)
α-carotene	-	64.2 (- 35.3%)	61.8 (- 38.2%)	81.9 (- 18.1%)
β-carotene	-	65.3 (- 34.7%)	61 (- 39%)	72.1 (- 27.9%)
Ascorbic acid	1.303	10.16 (7.8 fold)	6.32 (4.9 fold)	7.38 (5.7 fold)
Colour *L**	70.0	65.4	66.7	65.6
*a**	20.2	11.5	12.3	15.3
*b**	36.8	22.9	29.8	29.8

All values of weight changes, juice yield, are expressed as per cent changes.

All values of total carotene, α- and β-carotenes are expressed as per cent residual activities and in parentheses indicate per cent reduction.

All the values of ascorbic acid are contents. Values in parentheses indicate fold increase.

Values in parentheses indicate per cent reduction in juice yield compared to the original yield values.

Values are expressed as the mean of 3 determinations ± SE

Table 6.3 Effect of various blanching methods on quality parameters of carrot juice extracted from four carrot varieties

Variety	Blanching	Juice yield (%)	Wt changes (%)	Rel. viscosity
RHO	Raw	52.0	-	1.0
	WB	26.4	+ 1.3	1.2
	MWB	25	- 4.12	1.8
	SB	33.6	- 1.7	1.2
RHC	Raw	53.0	-	1.0
	WB	29.3	+ 1.3	1.2
	MWB	26.0	- 4.35	1.8
	SB	33.2	- 1.9	1.2
RC	Raw	53.0	-	1.0
	WB	23.6	+ 1.3	1.2
	MWB	25.0	- 4.12	1.8
	SB	34.2	- 1.8	1.2
TP	Raw	52.0	-	1.0
	WB	34.1	+ 1.5	1.2
	MWB	26.0	- 4.4	1.8
	SB	35.1	- 1.9	1.2

All values of weight changes and juice yield are expressed as % changes. Positive (+) and negative (-) values indicate gain and loss respectively.

WB- Hot water blanching, MWB- Microwave blanching, SB- Steam blanching

6.7.2 Effect of blanching on juice viscosity

The effect of blanching on the viscosity of juice extracted from carrot blanched using various blanching techniques was investigated using the technique described in Section 3.6.3. There was no difference between the relative viscosities of juices extracted from both steam and hot water blanched carrot (1.2) although both juices had a higher relative viscosity compared to juice extracted from fresh (raw) carrots with a relative viscosity of (1.0). The microwave blanched carrot juice resulted in a much higher relative viscosity compared to other blanching techniques. The loss of water, due to evaporation, in the microwave blanching technique was higher than the other techniques and this may have contributed to such an increase in relative viscosity in juice extracted from microwave blanched carrots.

6.7.3 Effect of blanching on juice colour

The effect of blanching technique on colour of the carrot juice was investigated using the procedure described in Section (3.6.3). The L^* value reduction in hot water blanching (6.5 per cent) was maximum. The steam and microwave blanching showed 6.3 and 62.8 per cent reduction in L^* value respectively. The reduction in a^* and b^* values was greater for water blanching compared to other methods. In the past a range of approaches to colour measurement have been used to study the effect of blanching on the colour of vegetables (Howard *et al* 1996; Muftigul 1985). Mirza and Morton (1977) investigated the effect of blanching on carrot slices and reported decreases in colour parameters in agreement with these experiment results. In an investigation described by Chen *et al* (1995), carrot juice was heated under four different processing conditions. For all treatments the carrot juice was acidified and heated to 105°C, and in three other processes unacidified juice was heated to 120°C, 110°C and 121°C in the can. The retort processing in the can at 121°C resulted in the highest reduction of colour values $^*L$ (29 per cent), $^*a$ (51 per cent) and $^*b$ (22 per cent) (Luh *et al* 1969). The other processing techniques resulted in an average reduction in colour value of L^* (9 per cent), a^* (27 per cent) and b^* (7.5 per cent). The reduction in colour parameter obtained in this investigation were much lower than the values reported earlier.

6.7.4 *Effect of blanching on nutrients*

6.7.4.1 Ascorbic acid

The ascorbic acid content of carrots treated using the three blanching techniques was analysed using the procedure indicated in Section 3.5.3. The ascorbic acid content appears to have increased during blanching. The increase in Vitamin C between the blanching techniques varied significantly where microwave blanching showed the lowest increase in ascorbic acid (4.9 fold). Steam blanching showed increase of 5.7 fold and water blanching showed the highest increase (7.8 fold). These results are in partial agreement with the research carried out by Warthesen *et al* (1984) in which the effect of various cooking techniques on retention of Vitamin C was investigated. The results described by Warthesen *et al* (1984) showed significant increase in Vitamin C contents during all the processing techniques (boiling water, steam, microwave and pressure cooking) in most vegetables analysed (Broccoli, cabbage, carrot, cauliflower, green beans, peas, spinach and zucchini). Amongst the processing techniques described, microwave blanching technique showed highest increase in the Vitamin C content (Warthesen *et al* 1984). In contrast however, vegetables processed using microwave-steam, stir frying with oil, stir frying with water and boiling water resulted in various levels of destruction of vitamin C. The vitamin C retention of the vegetables investigated varied between the cooking techniques applied (Masrizal *et al* 1997). The retention values were highest for vegetables processed by microwave-steaming or stir frying with oil (1.31-1.83 folds) compared to vegetables processed in boiling water (Masrizal *et al* 1997). In another investigation (Klein *et al* 1981), spinach was processed using microwave and conventional techniques (Pressure cooking of vegetable using minimal water) and was reported to have lost 54 per cent of vitamin C content using the latter when compared to the microwave technique (52 per cent). Ascorbic acid is normally used as the yardstick for the processing of fresh vegetables and fruits. This is due to the antioxidative nature of ascorbic acid. There have been various reports of the effect of blanching on ascorbic acid content. Reduction in ascorbic acid content during water, steam and microwave blanching of spinach (Rumm-Kreuter and Demmel 1990), green beans (Muftigul 1985), yellow squash, mustard green, purple beans, green beans, (Lane *et al* 1985), cabbage, cauliflower, potato, parsnip (Olliver 1941), asparagus, green beans, green peas, sweet corn (Drake *et al* 1981) have been reported. Retention of

ascorbic acid during microwave blanching has been reported to be the maximum when compared to other blanching methods.

The current studies showed increases in the apparent ascorbic acid content of the juice produced from blanched carrot. An inverse relationship between the oxidation-reduction potential and vitamin C content has been observed in fresh and frozen milk. A lower oxidation-reduction potential inhibits the oxidation of ascorbic acid. Raw carrot was found to have a redox potential of 192.8 mV while blanched carrot exhibited a redox potential of 29.7 mV. Disruption of cell walls during blanching appears to release ascorbic acid. In our study, extraction of juice from blanched carrot may have resulted in released ascorbic acid reducing the oxidation-reduction potential of the blanched carrot, thus effectively protecting ascorbic acid in carrot.

6.7.4.2 Carotene

The effect of blanching technique on the total and α- and β-carotene content of carrot juice has been investigated using the procedure described in Section 3.5.1 and 3.5.2 and results summarised in Table 6.2. Amongst the blanching techniques used, steam blanching resulted in lowest destruction unlike other methods showing negligible difference. The loss of total carotenes during steam blanching (13 per cent) was lower than for hot water blanching (40.6 per cent) and microwave blanching (30.3 per cent) (Table 6.2). There have been various reports of the effect of blanching on carotene contents (Bao and Chang 1994b; Kim and Gerber 1988; Hojilla *et al* 1985; Farhangi and Guy Valdon 1981). These results were higher then the reported value by Bao and Chang (1994b) and lower than the value reported by Oser *et* al (1942) when carrots were cooked using minimal quantity of water. Increased losses were reported during hot water blanching of carrots using water blanching (Bao and Chang 1994b).

The effect of these blanching methods on α-and β-carotenes was evaluated. During steam blanching minimum loss of total of α-and β-carotenes (overall 13 per cent) (18.1 and 27.9 per cent respectively) which was lowest when compared to hot water (40.6, 35.8 and 34.7 per cent respectively) and microwave blanching methods (30.3, 38.2 and 39.0 per cent respectively) (Table 6.2). There was a little difference between water and

microwave blanching in overall retention of carotenes. However, retention of specific α-carotene was higher than β-carotene during steam blanching.

Carotene has been widely investigated in various plant foods for the effect of various processing techniques used. Extensive data have been published on the effects of various processing techniques, including boiling water, microwaving, pressure cooking, dehydration, boiling water, a combination of microwave-steam, stir frying in oil as well as stir frying with water, on carotene content of foods. Most procedures have been reported to cause only relatively small losses of water insoluble vitamins including carotenes and vitamins D and E (Desobry *et al* 1998; Masrizal *et al* 1997; Rumm-Kreuter and Demmel 1990; Lane *et al* 1985; Warthesen *et al* 1984; Oser *et al* 1942; Brinkman *et al* 1941; Olliver 1941).

The results of previous studies reported have indicated that in most cases levels of carotenes after processing have remained unchanged however, the carotene isomerization and resultant increases in carotene contents have been reported (Granado *et al* 1992; Kim and Gerber 1988; Chandler and Schwartz, 1987; Hojilla *et al* 1985; Oser 1942). These results were further proved using chemically pure α-and β-carotenes (Chen *et al* 1996; Marty and Berset, 1990). An investigation reported the increase of 72 per cent and 53 per cent in carotene content of carrots during canning and water cooking respectively. These results were similar to those reported by Bao and Chang (1994b). In another investigation, carotenes were investigated in various domestically processed vegetable products including carrot halwa and salad, chavli, spinach and shiplu bhaji, coriander chutney, mayalu pakoda, methi thepla. The loss of carotene contents in the range of 5-83 per cent was reported (Dikshit *et al* 1988).

6.8 Conclusion

Carrots of different diameters (measured at the stem end portion) were investigated for the effect of carrot sizes on temperature comeup times. The results obtained indicated that the temperature comeup times for each of the sizes investigated were dependent on the diameter of carrots. The larger diameter carrots required prolonged heat treatments to attain the required temperature for all blanching techniques investigated. The

variations in the carrot diameters also showed an impact on the thermal degradation of deteriorative enzymes.

A comparison of stimulated Mathematical results to the experimentally obtained data for water blanching show reasonable agreement with the numerical simulations. The stimulated and experimental results agree quite well for the carrot core to reach a temperature of 90°C by using a thermal diffusivity of 0.8×10^{-7} m^2s^{-1} for the carrot.

Various studies have been carried out in the past on the inactivation of deteriorative enzymes and the time temperature regime necessary to achieve levels of inactivation. These studies have indicated the higher heat resistance of peroxidase when compared to catechol oxidase and the use of peroxidase as indicator enzyme for the blanching sufficiency. Results obtained during this study indicated higher heat resistance of peroxidase enzyme compared to catechol oxidase which is similar to the earlier reported results. During this study, identification of suitable indicator enzyme for the blanching sufficiency was indicated. Our results indicated the necessity of more aggressive heat treatments to inactivate pectinesterase in whole carrots when compared to peroxidase and catechol oxidase. In the past there are no reports of similar studies in carrots however, the results obtained during this investigation were similar to those reported in tomatoes (Nath *et al*, 1983) which indicated higher heat resistance of pectinesterase enzyme.

The effect of various blanching techniques on nutritive components (vitamin C, carotenes) as well as the physical parameters (weight loss/gain, juice yield, viscosity and colour) of carrot and carrot juice were also investigated. Microwave blanching and it's convenience, economic importance and shortcomings compared to other blanching methods have been well established in the past. Amongst the blanching techniques used in this study, microwave blanching resulted in an increased loss of carotenes, reduced juice yield and greater weight loss (Table 6.2) compared to other methods. Furthermore the microwave blanching of carrots of smaller diameters resulted in burning of carrots (Figures 6.1 and 6.2) with a concomitant effect on juice quality. These observations suggest that the unsuitability of microwave blanching in the processing of carrot juice. Water blanching resulted in a slight weight gain (1.5 per cent) unlike the weight loss observed in steam blanching (1.9 per cent). Losses of total carotenes in boiling water

blanching were higher (40.6 per cent) when compared to steam blanching (13 per cent). The juice yield in hot water blanching was lower (27 per cent) compared to steam blanched carrot (30.4 per cent). Negligible variations in colour were observed between all the three blanching techniques used. These results suggest that steam blanching is the more preferred method of blanching for processing of carrots for juicing purpose.

Chapter 7

Effect of enzyme treatment of carrot pulp on juice yield and quality

7.1 Abstract

In the production of juices from fruits and vegetables, commercial enzyme preparations may facilitate juice extraction, softening, clarification, maceration and liquefaction. As part of a study of processing protocols for the production of carrot juice, carrot mash has been treated using the commercial enzyme preparations Rohament Max (RM) and Rohament PL (RPL). The conditions for enzymatic treatments including dosage, dosing time and pH have been optimised. The effect of enzymatic treatment on juice yield, carotene content, relative viscosity and colour were evaluated. The combined application of the two preparations gave the best results. Both the yield and quality of juice were enhanced indicating that the inclusion of an enzymatic treatment step offers significant potential benefits.

7.2 Introduction

Enzymes are biological catalysts and play an instrumental role in catalysis of biochemical reactions in all living organisms. They are relatively specific and effective in small quantities under mild reaction conditions. Enzymes have been used empirically over a long period of time by housewives and chefs in some forms of processing. For example, the addition of mucous membranes of calves as a source of enzyme for improvement of milk quality as well as the addition of saliva as a source of salivary amylase for the degradation of complex sugars to glucose and the use of enzymes for the production of fermented liquors have been established in the literature (Jennylynd and Simpson 1996). Commercially some of the early uses of enzyme preparations in food manufacture have included α-amylase for preparation of wine, malted beverages and bread baking as well as proteases for meat tenderisation (Jennylynd and Simpson 1996).

Currently, commercial enzyme preparations are routinely used in a wide range of food, feed and other industrial processing applications (Tucker 1996). The major applications of these enzymes within the food industry are for juices and beverages, brewing, dairying, baking and meat processing operations. The nature of the enzyme preparations used during food processing is based upon the type of food being processed with the principal enzymes used belonging to various groups of enzymes including the carbohydrases, proteases and lipases. The enzymes belonging to the group of proteases include the activities of proteolytic enzymes such as papain. The use of these enzymes in food processing operations include the chill proofing of beer and ale, clarification of juices, preparation of chocolate, cheese and whey processing and tenderisation of meat. Enzymes belonging to the lipase group are used for processing operations such as modification of di- and tri- glycerides in chocolate manufacture and preparations of novel ingredients for use in the dairy industry. Some other commonly used commercial enzyme preparations used in food processing operations include arabinase, limonase and naringinase (Jennylynd and Simpson 1996).

Enzymes belonging to the group of carbohydrate hydrolases which are commonly used industrially, include amylase (production of fermentable sugars), pectinase (fruit juice clarification), invertase (sucrose hydrolysis in confectionery production, flavour development in fruit juices), lactase (reduction of lactic acid content of milk), glucose oxidase (prevention of Maillard reactions, oxygen scavenger in fruit juices), polygalacturonase, pectinesterase and pectin lyase (cloud stability in juices) and cellulase (increase in juice yield) (Tucker 1996). The use of these enzymes in fruit and vegetable processing includes the treatment of mash to facilitate total or partial liquefaction of fruit/vegetable flesh for preparation of juices and nectars as well as to increase juice yield and release flavours and colours (Baumann 1981). They are also used in the preparation of juices to reduce viscosity and facilitate concentration as well as aiding clarification and filtration (Rombouts and Pilnik, 1978) and product stabilization (Jennylynd and Simpson 1996).

In most cases, commercial enzyme preparations containing carbohydrase, protease and lipase activities are prepared from fungal or bacterial sources and these have been

widely used in food processing operations. Lipases from plant and animal origin have to date had limited use in food processing however, the use of carbohydrases and proteases from these sources has been well documented (Jennylynd and Simpson 1996).

In previous studies, the effects of enzymes including commercial pectinase on carrot and celery juices (Sims *et al* 1993; Anastasakis *et al* 1987; Sreenath *et al* 1986) have been reported. Polygalacturonase has been used for orange, apple, lemon and carrot (Foda *et al* 1985) and hemicellulase and cellulase for carrots (Industrial correspondence 1998). Other commercial enzyme preparations have been applied for various fruits and vegetables including apple, citrus, melons, carrots, potatoes, red pepper and spinach (Sreenath *et al* 1986; Baumann 1981; Horvath and Gatai 1977). The use of commercial enzyme preparations including Rohament PC as a source of cellulase and hemicellulase enzyme has been compared to other preparations including pectinase, cellulase, hemicellulase, and pectinesterase for the processing of carrots (Anastasakis *et al* 1987). In another investigation the use of Rohament PC (cellulase and hemicellulase), Rohament K (pectinase, cellulase, hemicellulase) and RPL (polygalacturonase) has been described for the processing of carrot (Handschuh 1996; Nakamura *et al* 1995; Sims *et al* 1993) and tomatoes (Sreenath *et al* 1986). The use of polygalacturonases and their effects on cloud stability, juice yield, viscosity and degree of maceration for various vegetables including potatoes, carrot, onion, garlic, squash, parsley, celery, cauliflower and cucumber has also been described by Foda *et al* (1985) and Horvath and Gatai (1977).

In the current study, carrot mash has been treated using the commercial enzyme preparations RM (containing pectinase, hemicellulase and cellulase activity) and RPL (containing polygalacturonase activity). The treatment conditions including enzyme application levels, incubation time and pH have been studied and the effects of enzymatic treatment have been evaluated in relation to juice yield, carotene content, relative viscosity and product colour.

7.3 Aim

This study was aimed at optimising of the treatment of carrot mash using the commercial enzyme preparations RM (containing pectinase, hemicellulase and cellulase activity) and RPL (containing polygalacturonase activity) and evaluating the effect of enzyme treatment on juice yield, carotene content, relative viscosity and product colour.

7.4 Sample preparation, blanching, juice extraction and analysis of juice quality parameters

Samples of the enzyme preparations RM (containing pectinase, hemicellulase and cellulase activities) and RPL (containing polygalacturonase activity) manufactured by Rohm International, Germany, were supplied by Enzymes Australia Pty. Ltd, Ringwood, Victoria.

For this study, steam blanching of carrots (approx. 500g) was performed using the technique described in Section 3.3.2.3. Blanched carrots were crushed to extract juice using a Bamix laboratory juice extractor as described in Section 3.10.2. Preparation of carrot-pulp mixture after the blanching and juice extraction of carrot was carried out using the method described in Section 3.12.1.

All enzyme treatments were performed at 55°C and a pH of 4.8-5.0 for 90 minutes using the method described in Section 3.12.3 unless otherwise indicated. The pulp content, juice yield, relative viscosity, colour and α-and β-carotene levels of juice obtained from enzyme treated carrot mash samples were analysed using techniques described in Sections 3.6.4, 3.6.5, 3.6.6, 3.6.3 and 3.5.2 respectively.

7.5 Effect of enzyme preparation composition, enzyme concentration and treatment time on juice yield

During this investigation, carrot mash prepared from steam blanched carrots was treated with various proportions of both commercial enzyme preparations (RM and RPL) at

various application levels using varying incubation times to determine the optimum treatment conditions.

7.5.1 Effect of varying proportions of the two enzyme preparations

In order to investigate the effect of both preparation compositions (ie proportions of RM and RPL), the dosage levels and treatment times recommended by the manufacturer were used as the starting point. These levels or concentrations of enzyme for preliminary trials on fruit and vegetables for RPL were 200-1000 ppm, while for RM the level was 100-200 ppm. These treatment levels are expressed on a v/w basis relative to the original weight of carrots used for juicing. The recommended treatment times ranged between 30-300 minutes.

During the preliminary investigations, various proportions of the two commercial enzyme preparations (ranging from 0 per cent - 100 per cent of each enzyme) were to evaluate the effect on the release of cell contents and evaluated as juice yield and the results are shown in Table 7.1. A treatment time of 180 minutes was applied for this initial investigation. In each case the total level of addition of enzyme, ie of RM and RPL together, was held constant at 150 ppm. It was observed that each enzyme treatment resulted in a marked increase in juice yield when compared with untreated mash. For example, the juice yield from carrots treated with only RPL was 58.6 per cent compared to the untreated yield of 36 per cent. This represents a yield increase of 62 per cent. Previous investigations on the effect of enzyme treatment on juice yield have reported increases in the juice yield of up to 19.6 per cent when carrot samples were treated with polygalacturonase enzyme at 50°C temperatures and 0.5 per cent v/v levels for 60 minutes (Foda *et al* 1985; Horvath and Gatai, 1977). In another investigation, carrot was treated using various enzyme preparations of cellulase, pectinase, hemicellulase pectinesterase and their combinations and compared with the commercial preparation RPC (polygalacturonase and cellulase) (Anastasakis *et al* 1987). Increases in the juice yield of up to 54.3 per cent was reported when treated at 50°C, for 30 minutes at 2 per cent enzyme levels. In the current investigation carrots were treated using much lower enzyme levels than those previously reported where up to 250 ppm was utilised. However the results obtained here (58.3 per cent) were similar to those

reported by (Foda *et al* 1985 and Horvath and Gatai, (1977). A marked increase in the juice yield was observed with increasing proportions of RM. The juice yield from untreated carrot (36 per cent) was increased up to 81 per cent in treated carrot samples. Maximum juice yield of 81 per cent was achieved with 100 per cent RM addition. The commercial enzyme RPL contains high activity of the enzyme polygalacturonase.

Table 7.1 Effect of varying proportions of enzyme preparations on juice yield and pulp content

Enzyme proportion (%)		Juice yield (%)	Pulp content (%)
RPL	RM		
Untreated	Untreated	36.0	< 0.2
100	0	58.6	< 0.2
75	25	54.7	< 0.2
50	50	72.1	< 0.2
25	75	72.4	< 0.2
0	100	81.0	> 1.0

RPL: Rohament PL, RM: Rohament Max

The results indicated in this table are means of 3 replicate trials.

For most treatments the juice contained relatively small amounts of sediment (pulp) (<0.2 per cent v/v). However, treatment with 100 per cent RM resulted in a pulp content in excess of one per cent. This level is considered unacceptable under current commercial juice manufacturing practices and therefore treatment with 100 per cent RM is not recommended. Treatments with enzymes in proportions 25:75 and 50:50 of RPL:RM resulted in only minor variations in juice yield and pulp levels. Enzymatic treatment using only RPL resulted in a lower juice yield of 58.6 per cent. The results obtained during this study indicated that the commercial enzyme preparation RM was more effective in liquefying carrot mash when compared to RPL. For further enzymatic treatment investigations, an enzyme mixture of RPL : RM (25 : 75) was used.

7.5.2 Effect of enzyme application levels on juice yield and pulp content

Four different levels of enzyme mixture were applied to the carrot mash in order to investigate the effect of enzyme concentration on juice yield and pulp content and the results are shown in Table 7.2. Each of the treatments resulted in a marked increase in juice yield when compared to the untreated mash.

Table 7.2 Effect of enzyme application level on juice yield and pulp content in carrot juice

Enzyme application level (ppm)	Juice yield (%)	Pulp content (%)
Untreated	36.0	< 0.2
75	61.4	< 0.2
150	74.3	< 0.2
300	72.3	0.5
500	82.2	> 2.0

Enzyme mixture added comprised of 25 : 75 of RPL : RM.

The results indicated in this table are mean of 3 replicate trials.

Several workers have investigated the effect level of enzyme additions on juice yield (Foda *et al* 1985; Horvath and Gatai, 1977). Increases in the juice yield ranging from 2.4 per cent (at 18.3 ppm enzyme addition level) up to 19.6 per cent (at 183 ppm enzyme addition level) were reported when carrot samples were treated with polygalacturonase enzyme at 50°C for 60 minutes (Foda *et al* 1985). Similar results were also reported by Horvath and Gatai, (1977). Anastasakis *et al* 1987 investigated juice extraction from coarsely shredded carrot using various preparations of cellulase, pectinase, hemicellulase, pectinesterase and their combinations and compared these with the commercial preparation RPC (polygalacturonase and cellulase for varying period of times. An increase in juice yield of up to 54.3 per cent (at 2000 ppm enzyme addition levels) was reported when carrot was treated at 50°C, for 30 minutes. In the current investigation carrots were treated at much lower levels than those reported in the literature. However the results obtained indicated juice yields considerably higher than those reported by Foda *et al* (1985) and Anastasakis *et al* (1987). A marked

enhancement in the juice yield was observed with the increase in level of enzyme used. The juice yield increased up to 82 per cent (2.28 fold) in carrot samples treated with enzyme at 500 ppm level, compared to the untreated carrot for which the juice yield was 36 per cent. A maximum juice yield of 82 per cent was achieved when pulp was treated with enzyme at the 500 ppm level. The pulp content of juice remained low (<0.2 per cent) at enzyme addition of up to 150 ppm but an increase in pulp level was obtained when 300 and 500 ppm of enzyme were used. These levels of pulp contents are considered unacceptable under current commercial practice of juice manufacturing and therefore treatment at 500 ppm level is not recommended. During this investigation only minor variations between the juice yield of the samples treated with 150 and 300 ppm were observed and thus it is concluded that an enzyme treatment of 150 ppm is suitable for carrot juice processing.

7.5.3 Effect of enzymatic treatment times on juice yield

This experiment was carried out using the enzymatic treatment parameters described from earlier studies (Section 7.2.1 and 7.2.2). Thus the effect of enzymatic treatment time on juice yield was investigated using a 150 ppm enzyme application level and enzymes in the ratio of 25:75 (RPL:RM) and the results are presented in Table 7.3.

It was observed that the juice yield from blanched carrots increased with increasing treatment times. The carrot mash was treated for various times including 30, 60, 90, 180 and 300 minutes and compared with an untreated control sample. The results indicate an apparent increase in the juice yield of 42 per cent over the untreated sample. The subsequent increase in juice yield with increasing treatment times was also observed. The other treatment times 60, 90, 180 and 300 minutes showed an increase in juice yields of 101 per cent, 216 per cent, 220 per cent and 237 per cent respectively. The increases at treatment times between 180 and 300 minutes were marginal in comparison with that achieved with the first 90 minutes of treatment. The pulp content in the juice

Table 7.3 Effect of enzyme treatment time on carrot juice yield

Treatment time (min)	Juice yield (%)
0 (Untreated)	36.0
30	51.2
60	71.9
90	77.6
180	79.1
300	85.4

Enzyme mixture added comprised of 25 : 75 of RPL : RM.

The results indicated in this table are mean of 3 replicate trials.

was also measured in each of the samples. No difference was observed in pulp levels for the different treatment times.

The effect of varying treatment times on the juice yield during the enzymatic treatment of carrots has been reported (Foda *et al* 1985). In their investigation, Foda *et al* (1985), reported an increase in juice yield ranging from 2.5 per cent following 10 minutes treatment to 7.6 per cent increase after 90 minutes treatment time using polygalacturonase at 50°C temperatures. In an another investigation, carrot was treated using various solutions of cellulase, pectinase, hemicellulase, pectinesterase and their combinations, and compared with the commercial preparation Rohament PC (polygalacturonase and cellulase) for periods of 10, 15 and 300 minutes (Anastasakis *et al* 1987). An increase in the juice yield of up to 48.7 per cent (at 2000 ppm enzyme addition) was reported (Anastasakis *et al* 1987) when carrot was treated at 50°C, for 10 minutes using Rohament PC. Further increases in treatment times up to 300 minutes tended to reduce the juice yield by 4.3 per cent.

In the current investigation the juice yields obtained from carrots were higher than those reported by Foda *et al* (1985) and Anastasakis *et al* (1987). A marked increase in juice

yield was observed with increase in treatment time particularly up to 90 minutes digestion time. Thereafter only minor increases in juice yield were observed for samples treated at 180 and 300 minutes. Whether or not an addition of 90 minutes treatment period to achieve only minor increases in juice yield can be justified in relation to current industrial practices is debatable. Thus a treatment or digestion time of 90 minutes is considered optimal and would be recommended for commercial carrot juice processing. This is consistent with the general recommendations of the enzyme manufacturer of up to 90 minutes for vegetable and fruit processing applications.

7.6 Effect of enzymatic treatment on juice quality parameters

The effect of enzymatic treatment on the juice quality parameters including viscosity, colour and carotene content are shown in Table 7.4. The juices extracted from enzymatically treated and untreated carrot samples were analysed for their viscosity using the technique described in Section 3.6.6. Enzymatically treated juice was found to have a relative viscosity of 0.96 which was marginally lower than that of the untreated juice sample (1.0). These results are consistent with earlier studies which have reported reductions in the relative viscosity due to the removal of pectin and cellulose in the juice (Sreenath *et al* 1986; Baumann 1981).

Earlier investigations have also reported that hydrolysis of pectin and subsequent reduction in viscosity also influences the concentration processing of enzymatically treated juice samples compared to an untreated sample. This reduction in the viscosity due to degradation of long chain carbohydrates is accompanied by enhanced processing behaviour during concentration by evaporation. Sreenath *et al* (1986) treated tomato fruit and celery leaves in 0.05M citrate buffer at 4.5 pH using RP enzyme as the source of endo-polygalacturonase activity at 0.01 per cent (w/v) incubated at 40°C. A reduction in viscosity and enhanced concentration characteristics of tomato juice were reported as a result of the enzymatic treatment. Similarly Anastasakis *et al* (1987) reported the treatment of carrot in 1.0 M ammonium sulphate buffer at 5.0 pH using RPC, cellulase and pectinase enzymes at 1.0 per cent (w/v) levels incubated at 50°C. Cleavage of cellulose fibres and cell walls and resultant increase in juice yield was reported.

In the current study, the colour characteristics of the enzymatically treated and untreated samples were analysed using the Minolta Chroma Meter as described in Section 3.6.3. The value of L^* is a measure of the whiteness or lightness of the samples while a^* value indicates greenness/redness and b^* value indicates blueness/yellowness in the sample (Ginn *et al* 1998). A reduction in the L^* colour values was observed for the treated samples (52.0) compared to the untreated sample (54.0). The values of a^* and b^* were also found to be reduced in the treated sample when compared to the untreated sample. The reduction in values of L^*, a^* and b^* indicate a move towards a darker and more orange coloured juice. These results are in agreement with visual observations of the samples. Sims *et al* (1993) investigated the effect of commercial enzyme Rohament K (pectinase and hemicellulase activities at 0.45mL/kg, 50 °C) for varying times on colour of carrots at various pH levels, time and temperatures. The results indicated the increase in colour values of a^* (30, 60 and 120 minutes treatment time and 9, 11 and 18.5 per cent respectively) and b^* by (30, 60 and 120 minutes treatment time and (10, 10 and 11.5 per cent respectively).

Table 7.4 The effect of enzymatic treatment on carrot juice quality parameters

Juice quality parameter		Untreated	Enzyme treated
Relative viscosity		1.00	0.96
Colour	L^*	54.2	52.0
	a^*	21.8	17.2
	b^*	33.5	28.4
Carotene (ppm)	α	2,955	10,773
	β	3,159	14,036

The effect of enzyme treatment on carotene content of the resultant carrot juice was also investigated and the results are presented in Table 7.4. A marked increase in the extraction of α-carotene (3.6 fold) from enzyme treated carrot was observed when compared to untreated carrot. A similar increase in β-carotene (4.4 times) was also observed. This probably reflects enhanced extraction as the enzyme hydrolyses the cell

wall structures, thereby releasing the cellular contents into the resultant juice. The higher levels of these vitamin A precursor compounds are clearly advantageous from a nutritional perspective. The observations of enhanced yields are in agreement with others reported in the literature. Sims *et al* (1993) treated carrot pulp using Rohament K enzyme containing predominantly pectinase and hemicellulase activities at a level of 0.45 ppm. The reaction time of 2 hours at pH 5.0 and 50°C temperature resulted in an increase in the extraction of carotene of up to 20 per cent. Similar results have also been reported in carrot (Handschuh 1995) as well as tomatoes (Horvath and Gatai 1977) when these were treated with commercial enzyme preparations. During the current investigation a much higher increase in extraction of β-carotene was observed compared to α-carotene. These results are in agreement with those reported earlier when carrot as well as other vegetables were treated using commercial enzymes (Rombouts and Pilnik 1978).

In previous studies the application of various enzyme preparations to the processing of fruits and vegetables have been reported. These include studies of polygalacturonase preparations from *Aspergillus aculeatus* (Foda *et al* 1985) and crude preparations of cellulase, hemicellulase, pectinase as well as pectin esterase and these have been used to treat citrus fruits, carrots and celery (Anastasakis *et al* 1987). Other commercial enzyme preparations containing cellulase/pectinase (Anastasakis *et al* 1987; Sreenath *et al* 1986) have been used either singly or in combination for maceration of celery and carrots. Significant increases in the juice yields have been reported. In the current investigation, when only one enzyme preparation was used, RM gave much higher yields than RPL (81 per cent compared to 58.6 per cent). However, the high yield with RM resulted in unacceptably high levels of pulp content. When varying combinations of the two enzyme preparations were used, lower yields were obtained and levels of pulp were acceptable. The best yields were achieved when RPL and RM were used in proportion of 25:75 and therefore this is the recommended treatment for carrot juice production.

7.7 Conclusion

It is well known that the commercial preparations used for maceration containing the activities of pectinesterase, cellulase, hemicellulase and polygalacturonase have the

potential to significantly increase juice yields and pulp contents in the fruits and vegetable juicing systems. These enzymes also facilitate disintegration of fruits and vegetables to release nutrients and other valuable compounds from the solid matrices. In the present study promising results were obtained for the juice yield and pulp contents during the application on carrots. The yield of the juice increased from 36 per cent in untreated carrots to 82 per cent when treated at 150 ppm and 25 : 75 proportions of enzymes RPL and RM (Tables 7.1 and 7.2). The yields at 150 ppm level of treatment were much higher than reported previously when using levels of 250 ppm polygalacturonase (Foda *et al* 1985) and RPC, cellulase, pectinesterase (Anastasakis *et al* 1987).

This investigation also found high levels of carotene extractions in enzyme treated samples when compared to untreated samples. The α-carotene level increased from 2,955 ppm to 10,773 ppm (3.64 times) however β-carotene increased from 3,159 ppm to 14,036 ppm (4.4 times) when treated with enzyme at 150 ppm in proportions of 25 : 75 (RPL : RM) for 90 minutes at 50-55°C. The yields of carotene levels obtained during this investigation were much higher than reported earlier using polygalacturonase (Foda *et al* 1985) and RPC, cellulase and pectinesterase (Anastasakis *et al* 1987). There were minor changes observed in colour and viscosity of juice following enzyme treatment.

The enzyme treatment regime used for this investigation has resulted in much higher yields of juice, higher carotene levels and minor changes in colour in comparison with control treatments. Thus the application of this enzyme treatment regime is highly recommended for industrial processing of carrot juice.

Chapter 8

Concentration, packaging and sensory evaluation of carrot juice

8.1 Abstract

Both thermal and non-thermal concentration techniques were applied to the carrot juice extracted from enzymatically treated and untreated carrots after steam blanching. The carrot juice expressed from blanched carrots was concentrated using a Rising Film Evaporator (RFE), Ultrafiltration (UF) and Reverse Osmosis (RO) procedures as well as a combination of both membrane and thermal concentration techniques. The carrot concentrates were packed in plastic containers and stored at –18°C. The concentrate was also processed using Ultra High Temperature processing and aseptically packed in presterilized containers. Carrot juice concentrate prepared from enzymatically treated and untreated carrots and concentrated using RO technique attained almost 3 fold concentration on single pass prior to blocking of the membrane. The enzymatic treatment showed no impact on level of concentration indicating the limitations of RO in achieving higher concentration levels. Carrots juice concentrate prepared by combined concentration technique (RO and RFE) resulted in the highest concentration levels compared to using thermal concentration (RFE). The effect of the various concentration techniques on colour values of carrot juice did not indicate any noticeable differences. Carotene levels of the carrot juice during concentration using RO technique were negligibly reduced to 99.1 per cent (-0.9 per cent). This trend was not observed in the carrot juice concentrate prepared using thermal and combined concentration technique. Minor apparent increases in carotene contents were observed during the concentration using RFE and combined concentration techniques. The α-carotene was found to have been reduced (0.3-0.7 per cent) for each of these techniques compared to β-carotene, the levels of which were slightly higher for RFE and combined concentration techniques.

The effect of storage period on the sensory characteristics as well as colour values and carotenes indicated mixed responses. The commercial sample was found to be unacceptable for taste, flavour and mouthfeel in contrast to the high acceptability of

samples prepared using the combined concentration techniques. There were minor changes observed in panel preference for each of the sensory parameters throughout the storage period of 45 days.

There were minor variations in colour values observed in most concentration techniques until 30 days of storage period. The extended storage period of 45 days resulted in increased L^*, a^* and b^* values indicating deterioration (darkness) in colour. This suggests the limited storage stability of carrot juice of up to 30 days under frozen conditions. Subsequent changes in carotene contents were also observed during storage. The reduction of up to 37 per cent in both α- and β-carotene levels was observed following the application of the combined concentration technique, within 45 days of storage.

8.2 Introduction

The technique of thermal concentration is also commonly known as evaporation. It is achieved principally by exploiting the relative volatility of water in comparison with that of other solutes (Fellows 1990b). The term evaporation is commonly used when the resultant product remains in liquid or semisolid state (Armerding 1966). The water component of the food product is removed partially thereby increasing the content of TSS including sugars, acids and vitamins in the product. Similarly concentration can also be achieved using non-thermal techniques such as membrane techniques in which water is removed leaving the soluble solids in the liquid mass.

In the past various techniques of concentration / evaporation have been investigated by researchers. These techniques include both thermal and non-thermal concentration techniques which have been applied to the juices of various fruits and vegetables. Amongst the thermal evaporation techniques described, the use of steam has been most widely applied (Fellows 1990b). Of the non-thermal concentration techniques, membrane technology (separation by diffusion across a membrane based on the molecular weights) including UF and RO as well as freeze concentration (separation achieved using differences in freezing points) (Armerding 1966; Ramteke *et al*, 1993; Cheryan 1991) have been most widely described.

8.2.1 Advantages and disadvantages of concentration

Various advantages and disadvantages of evaporation techniques have been described in the literature (Ramteke *et al*, 1993; Fellows 1990b; Armerding 1966). These include:

Advantages

1. Provides microbiological and enzymatic stability to the resultant product by reducing the water activity.
2. Operational economy in packaging, transportation and distribution to the product.
3. Provide enhanced colour and flavour stability to the product.

In addition to these advantages, evaporation also has application and is widely used to pre-concentrate food products prior to further processing operations such as drying, freezing and sterilization (Fellows 1990b).

Disadvantages

1. Loss of colour and flavour from certain food products in case of thermal evaporation techniques.
2. Loss of heat sensitive nutrients from the food products in case of thermal evaporation techniques.

The development of concentration techniques has advanced rapidly with continued emphasis on development of equipment which maintain the final quality of food products without adverse effects. The focus also has been given to efficiency, sanitation, ease of cleaning, operational simplicity, high capacity and robust construction as well as economics in designing the equipment. The literature available has been limited as most of the research performed by the equipment manufacturers (Mehra 1986) has remained unpublished as it has been used for commercial purposes in improving the quality of final products.

In this investigation both thermal and non-thermal concentration techniques have been applied to the carrot juice extracted from blanched carrots. Steam blanching treatment to the carrots was employed using the technique described in Section 3.10.21.1. The carrot was crushed and the juice was separated using the pilot scale equipment as described in Section 3.11. Carrots were enzymatically treated using a technique described in Section 3.12.3. The carrot juice expressed from blanched carrots was concentrated using a RFE (Ch 3, section 3.13.1), UF (Ch 3, section 3.13.2.1), RO techniques (Ch 3, section 3.13.2.2) and a combination of membrane and thermal concentration techniques (Ch 3, section 3.13.3). UHT processing and aseptic packaging of carrot juice was performed using the facilities of the Department of Food Technology, University of Newcastle following the methods described in Section 3.14.1. Microbiological analysis of carrot juice was performed using procedures outlined in Section 3.15. Sensory evaluation of carrot juice was performed using a technique described in Section 3.16. Other parameters including viscosity, TSS, colour and carotenes were analysed using the techniques described in Sections 3.6.6, 3.6.1, 3.6.3 and 3.5.2 respectively.

8.3 Aim

This phase of the study was designed to

1. Investigate the use of both thermal (RFE) and non-thermal (UF and RO) concentration techniques and their combination to produce carrot juice concentrate.
2. Establish the suitability of concentration technique and compare with industrially prepared carrot juice concentrate for sensory parameters such as taste, flavour and colour and mouthfeel.

8.4 Thermal concentration

Principally in thermal concentration technique the latent heat produced by the heat source (steam in most cases) is transferred to the food material under vacuum to raise the temperature to boiling point and vaporise water. The vapours are then removed from the surface of food material leaving the residual solutes in concentrated state (Fellows 1990b).

Most of the previous research carried out has not been published as this information is used for improvement in the process and quality of the food products. Thermal concentration techniques are the most commonly used industrial technique for the concentration of liquid foods including juices of fruits and vegetable as well as milk. The commonly used equipment are rising film and falling film evaporators (Industrial correspondence 1999) concentrating the liquid food to higher levels of TSS under vacuum using steam.

Various types of concentration equipment have been investigated and described in the past on various liquid foods. Previous studies utilized solar energy to vaporise moisture from seawater for the production of salt have been described (Armerding 1966). The technique was then developed further using a heat of any kind and source. The most commonly used source of heat until today remains steam. Various instruments and equipment were also developed. Amongst the equipment development, Open kettle, Jacketed kettle, Vacuum cooking, Calandria pan, Tubular evaporator, Forced circulation evaporator, Falling Film evaporator, Heat Pump evaporator, Indirect Heat Pump evaporator, Centrifugal Thin Film evaporator, Plate Type evaporator, Expanding Flow evaporator, have been described (Armerding 1966). In another report evaporation equipment such as Short Tube vertical evaporator, Horizontal Tube evaporator, Basket Type evaporator, Long Tube vertical evaporator, RFE, Falling Film evaporator, Rising/Falling Film evaporator, Agitated Thin Film evaporator, Plate Type evaporator, Vapour compression evaporator and Horizontal Spray Film evaporator and their advantages as well as disadvantages have been described (Mehra 1986).

In previous investigations, the use of vacuum concentration equipment for juice expressed from orange has been described (El-Sherbiny and Rizk 1981, Mohsen *et al* 1986)

8.5 Non-thermal concentration

There are various types of low temperature concentration techniques described in literature including freeze concentration and membrane concentration (Ramteke *et al*, 1993; Cheryan 1991; Fellows 1990b). Industrially, most commonly used low temperature concentration techniques are freeze concentration and membrane

concentration techniques. The developments of low temperature concentration techniques have been described (Ramteke *et al*, 1993; Cheryan 1991; Fellows 1990b).

8.5.1 *Freeze concentration*

The process of freeze concentration technique involves crystallization of water component from liquid foods by passing through a low temperature column followed by removal of ice crystals using centrifugal forces (Deshpande *et al* 1982). This is one of the commonly used low temperature concentration techniques for temperature sensitive food products. This is often considered to be more expensive compared to other thermal techniques (Deshpande *et al* 1982; Karel 1975).

8.5.2 *Membrane concentration*

The membrane concentration technique involves a crossflow filtration technique employed to separate components of the liquid food product on the basis of molecular weight. The food product is passed under pressure through the membrane of a particular MWCO separating solutes from water (Cheryan 1991) (Figure 8.1). This is one of the most rapidly developing and widely used low temperature concentration techniques used for the temperature sensitive food products. This is often considered to be relatively expensive in comparison with thermal techniques because high establishment costs are incurred (Cheryan 1991).

There are various membrane concentration techniques developed depending on the targeted final products and the molecular weights of compounds needing separation. The membrane concentration techniques have been widely used in various processing operations including food, feed and pharmaceuticals. Microfiltration techniques are used in filtration operations, using membranes in the range of 100,000 – 500,000 MWCO retaining suspended particles from the liquid foods in the retentate. UF technique is used in separation and purification operations, using the membrane in the range of 500 – 100,000 MWCO retaining macromolecules including proteins, pectin, enzymes and fats from the liquid foods in the retentate. Nanofiltration and RO techniques are used in desalination and deacidification operations, using the membrane in the range of 100 – 500 MWCO retaining macromolecules from the liquid foods in the retentate (Figure 8.1) (Cheryan 1996, Paulson *et al* 1985).

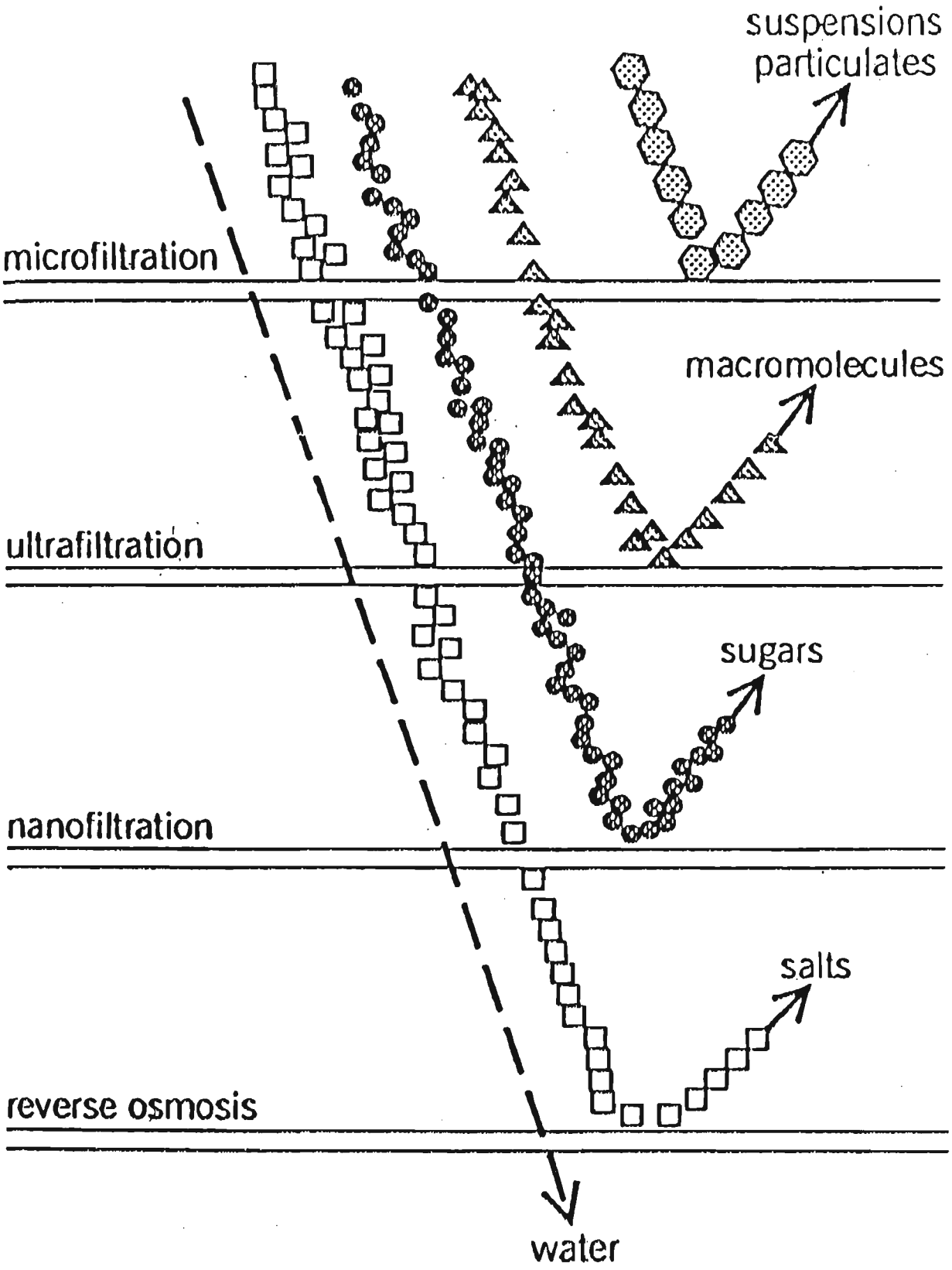

Figure 8.1 **A comparison of selectivities of membranes for various separation techniques (Cheryan 1991)**

8.6 Concentration of carrot juice

In the studies reported here for carrot juice concentration, a range of pilot scale equipment was used and these are depicted in Figures 8.2 to 8.7. The equipment included that used for blanching (Figures 8.2 and 8.3), crushing and juice separation (Figures 8.5 and 8.6), concentration by RFE (Figure 8.7) as well as UF and RO (Figure 8.4).

8.6.1 *Thermal concentration of carrot juice*

The carrot juice extracted from blanched carrots was concentrated using RFE equipment (Figure 8.7). The carrot juice was introduced at 4°C temperature and placed under vacuum pressure (-0.8 bars) to form a thin film and at 71°C evaporation occurs. Carrot juice in a single pass was evaporated from 4.5°Brix to 21-22°Brix resulting in a 4-fold increase in concentration.

Various attempts were made by varying temperature and TSS of the juice in anticipation of achieving higher TSS levels in the concentrated final product. The initial level for juice was varied between 5.0, 5.5 and 7.0 and the resultant concentrate TSS levels of 21.6 (4 fold), 22.0 (4 fold) and 28.0 (4 fold) were achieved respectively. Similarly the initial temperatures of juice was increased to 20°C to observe the effect on fold concentration. There was no change observed. These results indicate the limitation of the RFE method to concentrate carrot juice to more than 4 fold (Table 8.1).

A similar experiment was performed using carrot juice extracted from enzymatic treatment. Carrot juice extracted from enzymatically treated and blanched carrots were concentrated using RFE equipment under similar processing conditions. During concentration, carrot juice of 6.0 TSS level was evaporated up to 26-27°Brix in a single pass resulting in a 6-fold increase in concentration (Table 8.1). In the past, various research and industrial trials have been carried out for the effect of enzyme treatment on levels of TSS during evaporation process. The theory has been established that the juices treated with pectolytic enzymes have no residual pectin and thus attain higher

Figure 8.2 Hot water bath used for water blanching of carrots

Figure 8.3 Retort system used for steam blanching of carrots

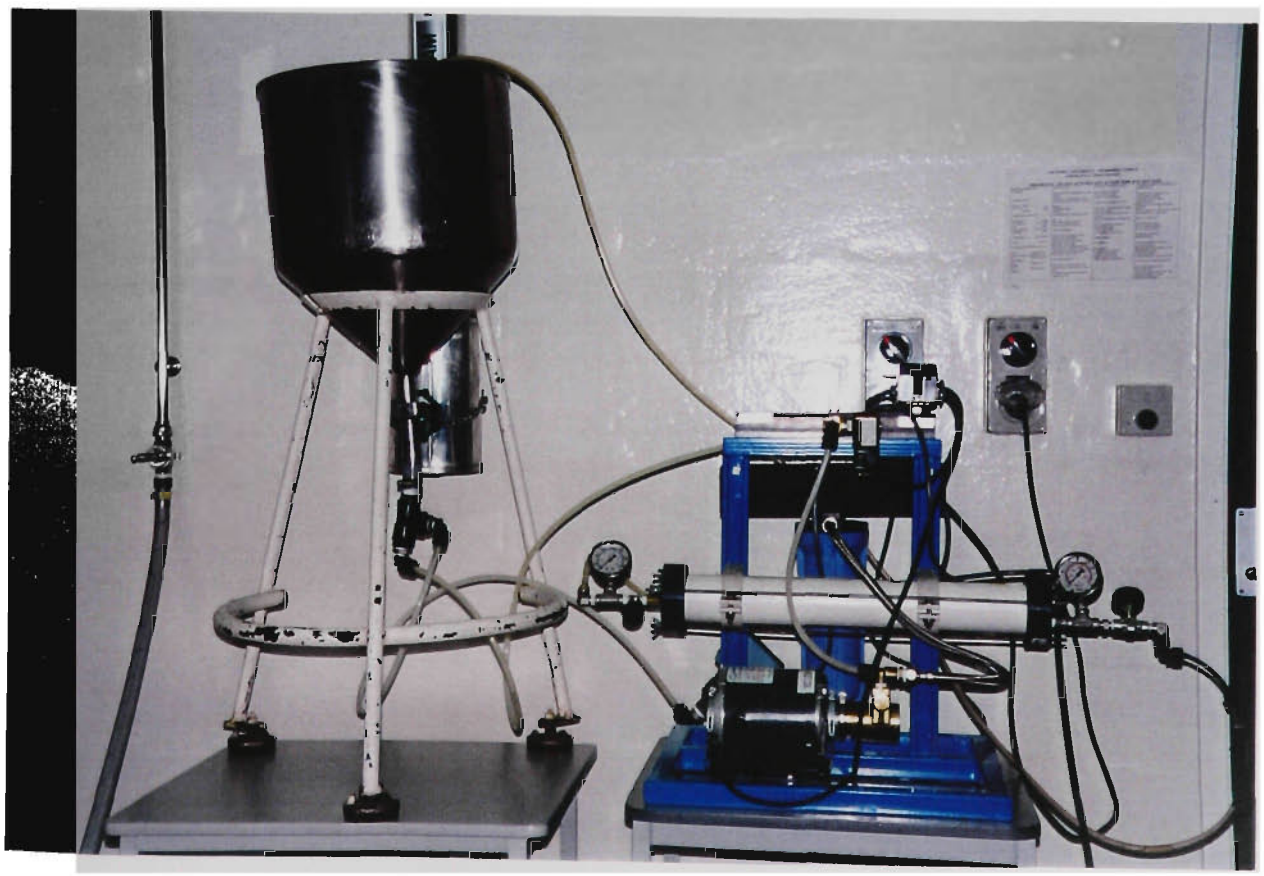

Figure 8.4 Membrane apparatus used for concentration of carrot juice

Figure 8.5 Apparatus used for preparation of carrot juice

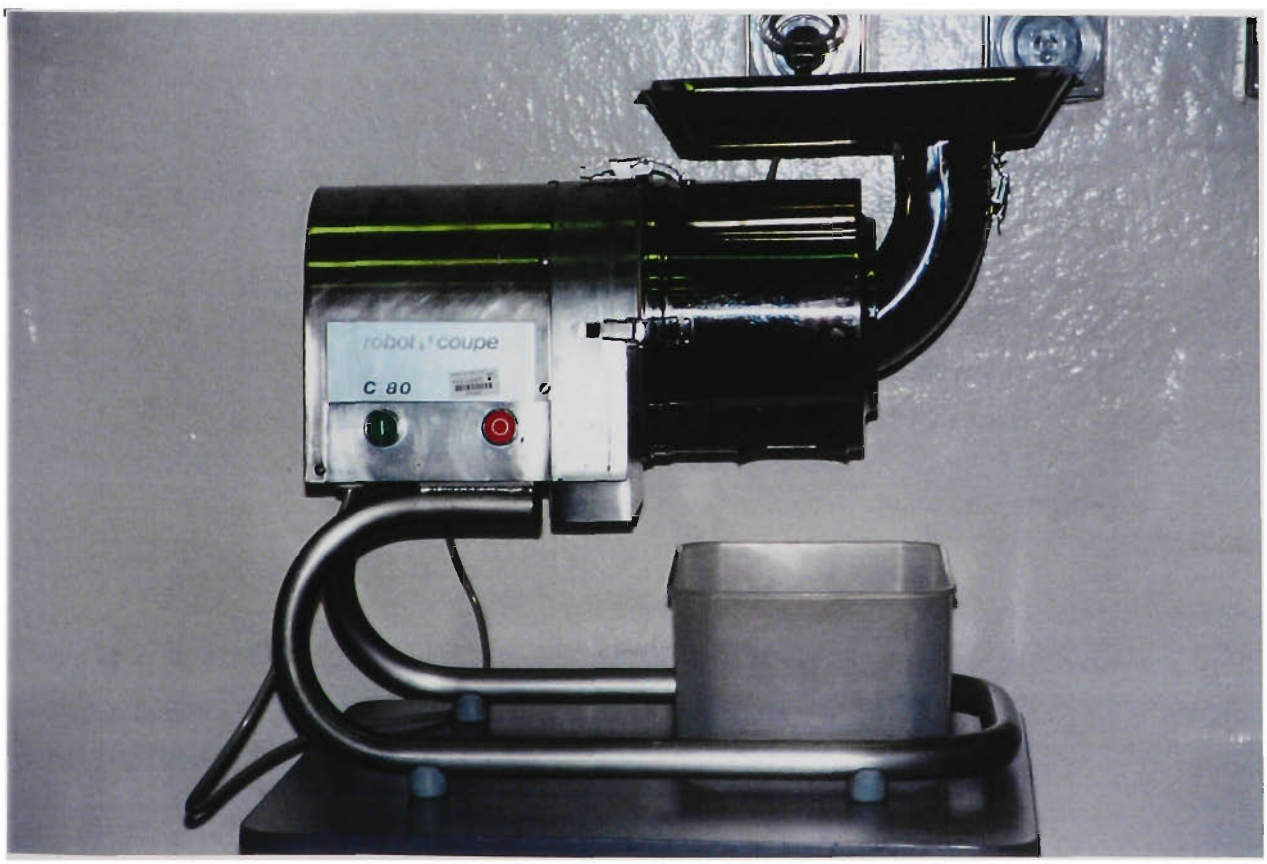

Figure 8.6 Apparatus used for separation of carrot juice and pulp

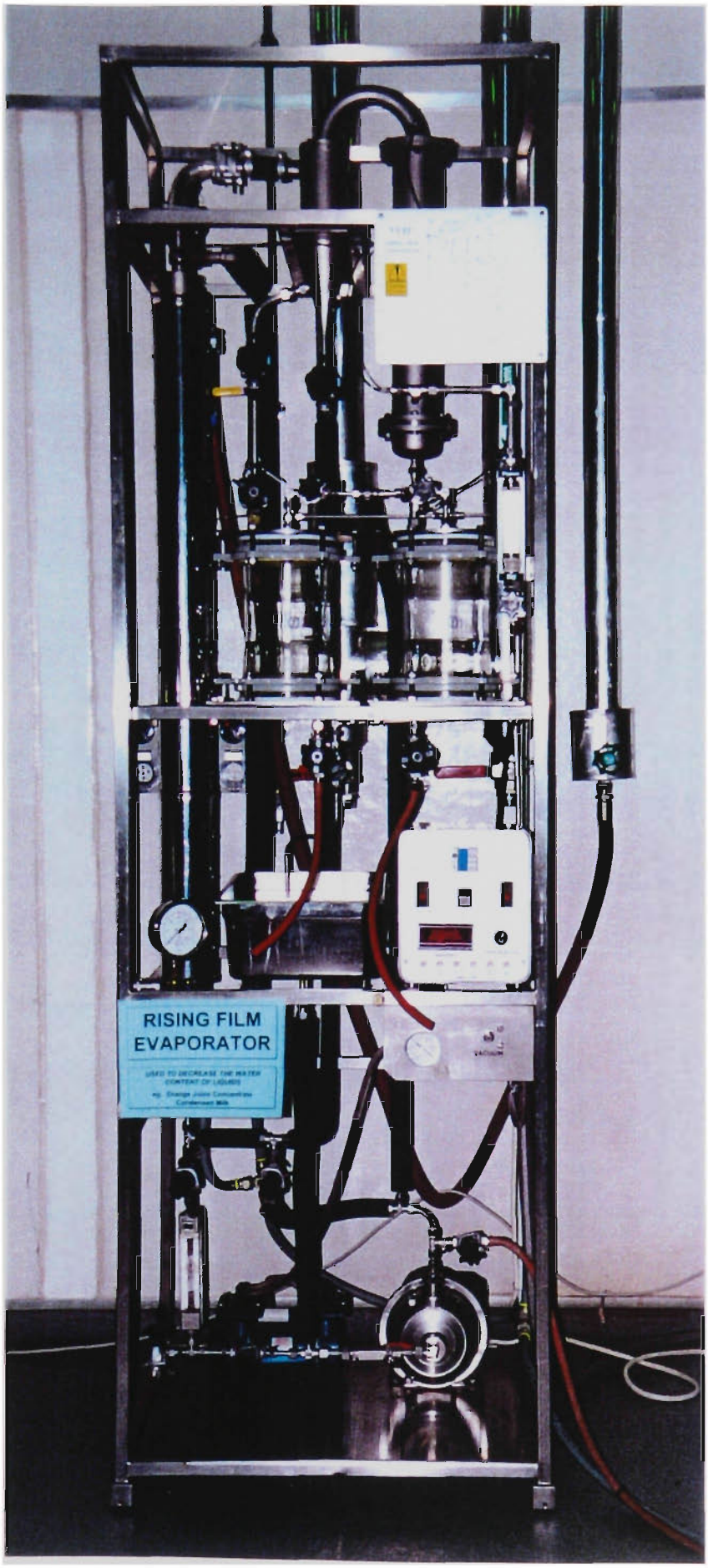

Figure 8.7 RFE used for concentration of carrot juice

Table 8.1 Effect of various concentration methods on quality characteristics of carrot juice[1]

Concentration Technique[2]	Relative Viscosity[3]	TSS (°Brix)	Colour Characteristics[4]	Relative carotene contents (%)[5]		
				α	β	Total
Juice	1.0 (**1.0**)	4.5 (**6.0**)	*L** 32.78 (**32.46**) *a** 15.87 (**15.07**) *b** 26.61 (**26.85**)	100 (**100**)	100 (**100**)	100 (**100**)
RO	2.95 (**1.75**)	14.2 (**14.2**)	*L** 32.61 (**31.97**) *a** 16.51 (**14.23**) *b** 26.86 (**25.66**)	98.7 (**99.3**)	99.4 (**99.6**)	99.1 (**99.5**)
RFE	26.3 (**10.4**)	21-22 (**26-27**)	*L** 32.49 (**31.52**) *a** 16.76 (**13.38**) *b** 26.87 (**24.92**)	98.5 (**99.6**)	102.8 (**101.3**)	100.7 (**100.5**)
RO and RFE	30.8 (**10.4**)	26.4 (**35.2**)	*L** 32.71 (**31.63**) *a** 16.59 (**13.31**) *b** 26.69 (**24.83**)	99.1 (**99.7**)	101.8 (**101.4**)	100.5 (**100.6**)

1. Values in the bracket show the results for enzymatically treated carrots. The values are the averages obtained from three separate trials.
2. Abbreviations referring to concentration methods and combinations are: RO - reverse osmosis and RFE - rising film evaporation.
3. Viscosity is expressed in relative terms with that for untreated juice corresponding to 1.0.
4. Colour characteristics are the mean of triplicate determinations obtained with the Minolta Chroma Meter.
5. Carotene contents are expressed in relative terms with reading after 15 days storage corresponding to 100%.

levels of TSS during evaporation (Industrial correspondence 1998; Handschuh 1996). Carrot juice was enzymatically treated and concentrated using thermal treatments and increased TSS was reported in the final product (Handschuh 1996). Various fruit products including prune, carrot and apple juices (Industrial correspondence 1998) have been used for evaporation using enzymatic treatments. Similar results of increased levels of TSS have been attained (Industrial correspondence 1998).

8.6.2 Non-thermal concentration of carrot juice

8.6.2.1 UF of carrot juice

The carrot juice extracted from blanched carrots was concentrated using UF equipment fitted with 10,000 and 30,000 MWCO membranes. The juice of 4.5°Brix while passing through UF membranes attained a maximum TSS of 10.5° of retentate and 5.5°Brix in permeate under continuous recirculation for up to 45 minutes. The loss of TSS in permeate indicate that varying types and molecular weights of soluble solids are present in carrot juice.

In the past various fruit juices have been used for concentration using the UF technique. In a previous investigation, passion fruit juice (Chao *et al* 1992) was concentrated using the UF technique. The partial recovery of total solids of up to 97 per cent of sugar and 91.8 per cent of organic acid was reported indicating the loss of 3 per cent sugar and 8.2 per cent of acid. Similar results were reported when the apple juice was concentrated using metallic UF membrane (Thomas *et al* 1986). Results obtained in this investigation were similar to those previously reported in the literature.

Under commercial manufacturing practices, the juice products are concentrated to higher levels of TSS to achieve the increased shelf life and logistical convenience. UF of carrot juice is usually performed at lower temperatures and lower pressure compared to RO and offers greater retention of temperature sensitive nutrients. However, the technique dose not offer achievement of sufficiently high levels of TSS in the final product for commercial use in the concentration of carrot juice.

The use of UF in other food processing operations such as debittering of citrus fruits juices (Hernandez *et al* 1992a, Koseoglu *et al* 1990) and removal of cloud from apple and pear juices (Constenla and Lozano 1995) have also been reported.

8.6.2.2 RO of carrot juice

The carrot juice extracted from blanched carrots was concentrated using RO equipment fitted with 200 MWCO membranes under ambient temperature conditions. The juice of 4.5°Brix was concentrated using RO membrane under pressure, measuring continuously the changes in pressure, temperature, permeate flowrate and TSS as indicated in Tables 8.1 and 8.2. During the process, an increase in temperature and levels of TSS as well as pressure were observed. Extended RO of carrot juice resulted in the reduction of the flowrate. This may be due to the concentration polarisation on the membrane caused by the solutes present in the carrot juice. The juice of 4.5°Brix concentrated to 10.2°Brix within 30 minutes of operation increasing the pressure across the membrane to 300 psi and a temperature up to 24.3 °C of permeate. Further processing for 60 minutes resulted in the permeate having a TSS of 14.2°Brix and temperature of 39.8°C without a noticeable increase in the pressure (Table 8.2). There was no loss of TSS observed in the permeate indicating the molecular weights of soluble solids of carrot juice to be higher than 200.

Similar RO concentration techniques were also employed to the carrot juice extracted from enzymatically treated carrots. The juice of 6.0°Brix was concentrated using RO membrane under pressure, measuring continuously the changes in pressure, temperature, permeate flowrate. During the experiment similar pattern of changes in temperature, pressure and TSS was observed. Flowrate of retentate and TSS values were higher however temperature values were lower compared to enzymatically untreated carrot juices. This may be due to the reduced resistance offered by the membrane as pectic substances during enzymatic treatment were disintegrated caused by the pectic and cellulolytic enzymes. The time required to concentrate the carrot juice to 14.2°Brix in the case of enzymatically treated carrot was less (15 minutes) compared to untreated carrot juice (Table 8.2).

Table 8.2 Changes in TSS, temperature and pressure during RO of carrot juice

Time	Permeate flowrate (ml/min)	Pressure (psi)	TSS (°Brix)	Temperature (°C)
11.00	0.0	0.0	4.5/**6.0** [1]	4.8/**7.9**
11.15	150/**160**	260/**260**	8.2/**7.2**	15.5/**13.7**
11.30	130/**176**	300/**290**	10.2/**8.2**	24.3/**20.1**
11.45	110/**144**	300/**310**	11.0/**10.4**	30.1/**27.1**
12.00	104/**130**	300/**310**	12.6/**13.0**	34.0/**32.5**
12.15	80/**80**	300/**310**	13.0/**14.2**	36.0/**36.4**
12.30	40/-	300/-	14.2/-	39.8/-

1. Initial juice – 4.5°Brix and **6.0°Brix** in case of enzymatically treated carrot juice.
   Processing conditions: (a) Initial juice temperature – 4.1°C
   (b) Retentate Flowrate – 4200 mL/min
2. Values in **bold** express the results for enzyme treated carrot juice.

RO of carrot juice is usually performed at lower temperatures and higher pressure than UF and offers greater retention of temperature sensitive nutrients compared to thermal concentration technique. RO technique also results in a higher level of TSS of the final product compared to UF technique and thus is more suitable for the concentration of carrot juice.

In the past the use of RO in various food processing operation including filtration, debittering and concentration have been reported. Yildiz *et al* (1993) reported concentration of tomato juice (5.22 per cent TSS) using RO technique up to 20.3 per cent TSS levels. Hernandez (1992a) and Walker (1989) also reported the loss of alcohol, aldehyde and ester based aroma compounds during UF retaining hydrocarbon based aroma compounds, pectins and other sugars. Similar results have been reported when pineapple juice (Bowden and Isaacs 1989), mango puree (Olle *et al* 1997), citrus

juices (Braddock *et al* 1988) and Raspberry juice (Wrolstad *et al* 1993) were concentrated using RO techniques.

8.6.3 Concentration of carrot juice using combined techniques (RO and RFE)

Preparation of carrot juice concentrate by linking combined concentration techniques in order of RO and thermal evaporation process was also carried out. The use of RO concentration technique was used as a process of preconcentration prior to further concentration using RFE technique. Carrot juice was preconcentrated using RO process from 4.5°Brix to 14.2°Brix. This juice concentrate prepared from RO process was then further concentrated by thermal evaporation to 26.4°Brix in a single pass (Table 8.1).

The carrot juice prepared using enzymatically treated carrot was also concentrated using combined concentration technique using processing parameters as indicated earlier. Carrot juice prepared from enzymatically treated carrots was preconcentrated using RO process from 6.0°Brix to 14.2°Brix prior to concentration using RFE. This juice concentrate prepared from RO process was then further concentrated by thermal evaporation to 35.2°Brix in a single pass.

The combined concentration techniques performed on carrot juice prepared from untreated carrots for attainment of higher TSS content in carrot juice does not offer any additional advantage over the traditionally used thermal concentration techniques. The carrot juice extracted using enzymatic treatment was found to achieve higher levels of TSS (35.2) compared to untreated carrot juice (26.4). This may be due to the degradation of pectic substances caused by the pectic and cellulolytic enzymes.

8.7 Effect of various concentrate techniques on quality of carrot juice

Concentration techniques have been under constant review and improvements by commercial industries. In this investigation the juices from both enzymatically treated and untreated carrots were concentrated using various concentration techniques including thermal and non-thermal. The effect of each of these concentration techniques on various juice quality parameters is reported in Table 8.1.

8.7.1 Effect of various concentration techniques on relative viscosity of carrot

Carrot juice and concentrates of both enzymatically treated and untreated carrots, prepared from various concentration techniques were analysed for relative viscosity (Table 8.1) using the technique described in Section 3.6.6. The relative viscosity of juices extracted from both enzymes treated and untreated carrot juices (1.0) were used as a reference for comparison with the concentrates and reconstituted juices. The carrot juice concentrates prepared from enzymatically treated and untreated carrots using RO had relative viscosity of 1.75 and 2.95 respectively. The TSS content of the juice contributes to the viscosity. In this instance the TSS of carrot juice concentrate prepared by RO were 14.2 for both enzymatically treated and untreated carrots (Table 8.1). Although, enzymatically treated carrot juice concentrate had 14.2°Brix, the pectin hydrolysis caused by the enzymes used (pectolytic and cellulolytic) could have resulted in the reduced relative viscosity compared to enzymatically untreated carrot juice.

Similarly, the carrot juice concentrates prepared from enzymatically treated and untreated carrots using thermal evaporation had relative viscosity of 10.4 and 26.3 respectively. The TSS present in the juice contributes to the viscosity. In this instance the TSS content of carrot juice concentrate prepared by thermal evaporation for enzymatically treated and untreated carrots were 35.2 and 26.4 respectively. The higher TSS of carrot concentrates in this instance increased the relative viscosity compared to the juices as well as the concentrates prepared by RO procedure. Although, concentrates prepared form enzymatically treated and thermal evaporation procedures possess higher TSS levels, the viscosity (10.4) was lower than enzymatically untreated concentrate (26.3). This was more obvious in the combined concentration technique. Increased TSS levels in enzymatically untreated (26.4) carrot juice showed the increase in viscosity (30.8). However the increase in TSS levels in enzymatically treated carrot juice (35.2) carrot juice had much less effect on changes in viscosity compared to other treated sample (10.4). This may be due to the pectin hydrolysis caused by the enzymes used (pectolytic and cellulolytic) and could have resulted in the reduced relative viscosity compared to enzymatically untreated carrot juice. The results achieved by the combined concentration procedures were similar to that for thermal evaporation procedures (Table 8.1).

8.7.2 Effect of various concentration techniques on TSS of carrot juice

Carrot juice and concentrates of both enzymatically treated and untreated carrots, prepared using various concentration techniques were analysed for relative viscosity (Table 8.1) using the technique described in 3.6.1. The carrot juice prepared from enzymatically treated and untreated carrots had 4.5 and 6.0°Brix respectively. The carrot treatment using cellulolytic enzymes cleaved the cell wall and assisted the increased extraction of soluble materials causing increase in TSS of the juice. These juice samples from both enzymatically treated and untreated carrots were concentrated using RO technique and had maximum TSS of 14.2°Brix. The enzymatic treatment of carrot did not have any effect on the level of concentration achieved. This may be due to a physical separation of water from solutes using membrane. When these carrot juices were concentrated using thermal techniques, carrot juices of enzymatically treated and untreated samples reached up to 35.2 and 26.4°Brix respectively. Similar results were achieved during the concentration of carrot juice using combined concentration techniques (Table 8.1). The higher levels of TSS that were achieved in enzymatically treated samples may be due to a reduced interference of pectins.

8.7.3 Effect of various concentration techniques on colour of carrot juice

The colour values of juices extracted from both enzymatically treated and untreated were analysed using a technique described in Section 3.6.3. The colour values of carrot juice concentrates prepared using various concentration techniques were also analysed. The colour parameters of each of these samples were analysed and reported as L^*, a^* and b^* values (Table 8.1). L^* value represents whiteness or lightness ($L^* = 0$, indicates black and $L^* = 100$ indicates white). The colour value a* indicate green, red or combination of the two colours ($a^* = -80$ indicates green and $a^* = 100$ indicates red) however the value b^* indicate blue, yellow or combination of the two colours ($b^* = -70$ indicates blue and $b^* = 70$ indicates yellow) (Ginn *et al* 1998). (The higher a^* and b^* values in combination with good brightness (L^*) characteristics).

The carrot juice prepared from enzymatically untreated carrots showed L^* values of 32.78, a^* value of 15.87 and b^* value of 26.62. These values were slightly higher compared to juices from enzymatically treated carrots (L^* 32.46, a* 15.07) however the

colour value b^* (26.85) was slightly higher in case of enzymatically treated samples (26.62). The values obtained in this investigation suggest that there was negligible colour difference between enzymatically treated and untreated carrot juices. The untreated juice was slightly darker and more orange in colour compared to treated samples. On the other hand the untreated sample was slightly higher in yellowness (brighter) compared to untreated samples.

Similarly the carrot juice concentrated using RO technique showed L^* values of 32.61, a^* value of 16.51 and b^* value of 26.86 (Table 8.1). These values were slightly higher compared to juices from enzymatically treated carrots (L^* 31.97, a^* 14.23 and b^* 25.66). The values obtained in this investigation suggest that there was negligible colour difference between enzymatically treated and untreated carrot juices concentrated using RO technique. The untreated juice was slightly darker and more orange in colour compared to the treated samples. The colour values of carrot juice concentrates produced from thermal technique were also analysed. The result obtained was similar to the one indicated earlier. The carrot juice concentrated using thermal concentration technique showed L^* values of 32.49, a^* value of 16.76 and b^* value of 26.87 (Table 8.1). These values were slightly higher compared to juices from enzymatically treated carrots (L^* 31.52, a^* 13.38 and b^* 24.92). Similarly the colour values for the carrot juice concentrate prepared from combined concentration technique was also measured. Results indicate the L^* value (32.71), a^* and b^* values (16.59 and 26.69) have been observed which were slightly lower that enzymatically treated carrots (L^* 31.63, a^* 13.31 and b^* 24.83) (Table 8.1). The values obtained in this investigation suggest that there was negligible colour difference between enzymatically treated and untreated carrot juices concentrated using RO technique.

8.7.4 Effect of various concentration techniques on carotene of carrot juice

The effect of concentration technique on the total and α-, β- and total carotene of carrot juice concentrates have been investigated using the procedure described in Section 3.5.1 and 3.5.2. None of the concentration techniques used resulted in considerable destruction of carotene from carrot juices. The carotene levels for each of these techniques have been expressed in relative terms (as a percentage) because the initial carotene contents varied between batches. The changes in α- carotene and β-carotene

during RO process were not significant corresponding to an insignificant loss of total carotenes (-0.95 per cent). The results obtained in enzymatically treated carrot juice indicated that the loss of α- carotene (-0.7 per cent) and β-carotene (-0.4 per cent) were also not statistically significant. There was negligible difference in carotene losses observed between the enzymatically treated and untreated carrot juice concentrates during RO process (Table 8.1). Such negligible loss can be attributed to relatively low levels of oxidation occurring during the RO process.

The effect of thermal evaporation techniques on the carrot juice concentrate was also investigated. The loss of total α- carotene during thermal evaporation process was -1.5%. On the other hand, there appeared to be a slight increase in the levels of β-carotene (+0.6 per cent) observed in the juices concentrated using thermal evaporation process (Table 8.1). The average of total carotene content was slightly higher (+0.65 per cent). The results obtained in enzymatically treated carrot juice indicated a minor loss of α- carotene and an increase for β-carotene so that overall changes in total carotene were not statistically significant (Table 8.1).

Similarly, the effect of combined concentration techniques (RO and thermal evaporation) carrot juice concentrate was investigated. Again the results showed effectively no differences (Table 8.1).

Between the concentration processes applied, there was little apparent difference in the carotene loss / gain observed. In most cases the retention of α-carotene levels was slightly lower compared to β-carotene possibly indicating higher sensitivity of α-carotene to the higher levels of temperatures and oxidation conditions. The retention of α- carotene was higher during the thermal evaporation and combined evaporation processes compared to RO indicating to the increased sensitivity to oxidation. β-carotene in all the cases have been increased only slightly (up to 1.8 per cent). The retention of β-carotene appeared to be higher during the thermal evaporation and combined evaporation processes compared to RO probably due to increased sensitivity to oxidation.

Carotene has been widely investigated in various plant foods for the effect of various processing techniques used. Though the data published on the effect of concentration techniques on carotene levels have been very limited, the extensive literature is

published on effect of various blanching techniques including boiling water, microwaving, pressure cooking, dehydration, combination of microwave-steam, stir frying in oil, stir frying with water and boiling water on carotene (Masrizal *et al* 1997; Rumm-Kreuter and Demmel 1990; Lane *et al* 1985; Warthesen *et al* 1984; Oser *et al* 1942; Brinkman *et al* 1941; Olliver 1941). The results of previous studies reported have indicated that in most cases levels of carotenes after processing have remained unchanged however, the carotene isomerisation and resultant increases in carotene contents have been reported (Granado *et al* 1992; Chandler 1989; Kim and Gerber 1988; Chandler and Schwartz, 1987; Hojilla *et al* 1985; Oser 1943). Results achieved during this investigation suggest that the likely isomerisation of carotenes during the thermal and combined concentration processes could have resulted in to the increase in carotene levels unlike RO where no isomerisation occurred due to lower temperatures used during the process.

8.8 Packaging and storage of carrot juice concentrates

8.8.1 Aseptic packaging

Commercially produced frozen carrot juice concentrate (36°Brix) from RC variety was procured under frozen condition (-18°C) from Nugan Quality Foods, Griffith, New South Wales. This concentrate was delivered overnight under frozen conditions to Department of Food Science and Technology, University of Newcastle. Carrot concentrate sample was then UHT treated using a UHT plant (Micronics 25DH UHT/HTST) at various temperatures (90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140°C) and aseptically packed in presterilized 125 mL bottles in an ultraviolet irradiated laminar flow chamber. These samples were then stored at ambient temperature.

8.8.2 Other techniques

Carrot juices and concentrates produced using enzymatically treated and untreated carrots were packed in 1L HDPE containers. The headspace of these containers was charged with nitrogen gas to avoid surface oxidation. These containers were then stored at -18°C until further use.

8.9 Microbiological studies of carrot juice concentrates

Microbiological analysis of carrot juice concentrates on both commercial samples (90, 95, 100, 105, 110, 115, 120, 125, 130, 135 and 140°C) packed aseptically in 125 mL sterile container and frozen samples (prepared in food processing room using RO, rising film and combined RO and RFE techniques) was performed using a technique described in Section 3.15. Carrot juice concentrate samples were diluted using sterile water to single strength levels (8°Brix) prior to plating for assessment of total plate count as well as for yeast and moulds.

The results indicated no microbiological growth from any carrot juice sample.

8.10 Sensory evaluation

Sensory analysis of carrot juice samples were carried out on both aseptically packed commercial samples and frozen samples using a semi trained panel consisting of twenty members. The results were recorded on the form presented in Appendix 3.1.

8.10.1 Training of sensory panel

All the 20 sensory panel members selected were provided with various concentrations of sugar (5, 6, 7, 8°Brix sugar syrup), acid (0.1, 0.2, 0.3, 0.4 per cent citric acid) and flavoured (0.05, 0.1, 0.15 mL/L) waters. Primary screening of sensory panel members was performed using their capability of distinguishing the sugar and samples provided and using the chart shown in Appendix 3.1. The experiments were repeated using another set of sugar (6.5, 7.0, 7.5, 8.0°Brix sugar syrup), acid (0.05, 0.1, 0.15, 0.2 % citric acid) and (0.05, 0.1, 0.15 mL/L) water. The selected panel members were then provided with carrot juice samples.

8.10.2 Sensory analysis of carrot juice

Carrot juice samples prepared form various carrot juices concentrates were subjectively analysed using a semi trained sensory panel of 20 members. The sensory responses were noted on response chart using hedonic scale of 1-5 as described in Appendix 3.1. The sensory parameters analysed were colour, flavour, taste and mouthfeel. Carrot juice prepared in the food laboratory (using RO, RFE and combined concentration

techniques) and commercially prepared concentrates were compared for the above mentioned sensory parameters.

Aseptically packed carrot juice sample was subjectively analysed for taste and flavour and compared with the sample prepared in food laboratory using RFE. The sensory response indicates that aseptically packed commercial sample was less preferred with the taste (1.1) and flavour (1.8) when compared to the sample prepared in the food laboratory for taste (4.1) and flavour (3.6) respectively. Thus the aseptically prepared carrot juice sample is less preferred and is not considered further for storage and sensory analysis.

8.11 Effect of concentration technique on quality of carrot juice

Effect of concentration technique on sensory parameters including taste, flavour, colour and mouthfeel as well as carotene contents were analysed.

8.11.1 Effect of concentration techniques and storage on sensory quality of carrot juice

The carrot juice concentrates prepared from various concentrations techniques were diluted using water to 8°Brix prior to analysis. The 20-member semi trained sensory panel performed the analysis. These samples were also analysed for colour values and carotene retentions and results are reported in Table 8.3. The samples after preparation were analysed immediately and are reported as 0 period results. Carrot juice concentrates were also stored and analysed for up to 45 days of storage with regular intervals of every 15 days.

Sensory responses obtained for the samples at the start of the storage studies indicate that the taste, flavour and mouthfeel of commercial carrot juice sample were least preferred (1.1, 1.85, 2.40) compared to RO (3.20, 3.55, 3.00), RFE (4.10, 3.55, 3.80) and RO and RFE (4.35, 3.45, 4.0). The acceptability of these sensory parameters for varying storage periods was also investigated. In most cases minor variations were

Table 8.3 Effect of storage period under freezing conditions on characteristics of carrot juices processed using different concentration techniques

Juice sample[1]	Storage period (days)	Sensory scores[2]				Colour characteristics[3]			Relative carotene contents (%)[4]	
		Taste	Flavour	Colour	Mouthfeel	*L**	*a**	*b**	α	β
Commercial	0	1.10	1.85	3.70	2.40	31.21	16.25	26.5	100	100
	15	1.20	1.90	3.90	2.60	31.19	16.10	26.41	100	100
	30	1.55	2.00	4.00	2.40	31.27	16.25	26.46	88.9	84.1
	45	1.65	2.40	4.10	2.60	51.81	23.85	37.98	63.9	79.5
RO	0	3.20	3.55	3.45	3.00	32.61	16.51	28.86	100	100
	15	3.25	3.50	3.35	3.10	33.06	16.07	28.98	100	100
	30	3.90	3.60	3.20	3.60	33.55	16.57	28.51	94.0	87.7
	45	3.20	3.60	3.45	3.30	53.47	24.87	39.95	86.2	75.7
RFE	0	4.10	3.55	2.95	3.80	32.49	16.76	26.87	100	100
	15	4.20	3.70	3.00	3.80	33.10	16.39	28.65	100	100
	30	4.30	3.90	3.00	4.00	33.06	16.46	29.01	72.2	67.6
	45	4.10	3.50	3.00	3.80	54.26	24.87	42.20	89.0	84.1
RO and RFE	0	4.35	3.45	3.15	4.00	32.43	16.11	28.82	100	100
	15	4.40	3.50	3.10	4.10	32.41	16.09	28.65	100	100
	30	4.10	3.60	3.40	3.80	32.81	16.01	27.49	66.9	85.2
	45	4.20	3.50	3.00	3.90	53.70	23.93	40.38	63.0	63.1

For footnotes to this table see next page

Table 8.3 continued

Footnotes:

1. Abbreviations referring to concentration methods and combinations are: RO - reverse osmosis and RFE - rising film evaporation.
2. The average score obtained during sensory evaluation. Participants were asked to score each sample on a scale from 0 to 5, with zero corresponding to the least and 5 to the most desirable score in each case.
3. Colour characteristics are the mean of triplicate determinations obtained with the Minolta Chroma Meter.
4. Carotene contents are expressed in relative terms with reading after 15 days storage corresponding to 100%.

observed until 30 days of storage period for taste, flavour and mouthfeel characteristics. In the case of taste, flavour and mouthfeel, a moderate increase in the acceptability was observed in samples prepared from RO and RFE technique as well as commercially prepared samples (Table 8.3). Similar trend was observed for flavour characteristics for the sample prepared from combined concentration technique. On the other hand, the panel responses to taste and mouthfeel reduced within 30 days of storage period. Thus in most cases the moderately increased acceptability of taste, flavour and mouthfeel were observed in all the carrot juice samples up to 45 days of storage period. These results obtained were also analysed statistically. Statistical analysis showed a varying degree of inconsistency in sensory results for taste, flavour, colour and mouthfeel. There is no literature available to further compare these results.

The colour of carrot juice samples was also analysed both using sensory panel as well as the Minolta chroma meter. The results indicate that the commercial carrot juice was found to be more readily preferred (3.70) compared to other concentration techniques (Table 8.3). The panel response to colour increased with the storage period up to 45 days in most cases except combined concentration technique of RO and RFE (Table 8.3). A sudden decrease in response to colour characteristics (L^*, a^* and b^* values) was observed between 30 and 45 days of storage period. This decrease was not consistent with the results obtained by the sensory panel. Increase in L^*, a^* and b^* values during the storage period between 30 and 45 days indicate the onset of browning of juice colour.

In an earlier investigation, tomato juice was concentrated using RO and compared with evaporative concentration techniques (Yildiz *et al* 1993) The colour of tomato juice processed using thermal concentration was more readily accepted compared to RO technique.

Colour values responded during sensory analysis in this investigation did not match the results reported in the literature. However, in this investigation minor variations in the colour characteristics were reported. Colour values of carrot juice commercially processed using thermal concentration technique was most preferred compared to any other technique.

8.11.2 Effect of concentration techniques and storage on carotene content of carrot juice.

Carrot juice concentrates prepared by various concentration techniques were also analysed for the effect of storage period on carotene contents. The results were expressed in terms of percentage retention as described in Table 8.3.

For all concentration techniques, carotene content remained stable and unchanged up to the storage period of 15 days. The deterioration of carotene contents in all cases was observed during the storage period when extended from 15 days. The changes in carotene retention varied depending up on the concentration technique used.

The juices prepared by the commercial concentration technique indicated the loss of 11.1 per cent (α) and 15.9 (β) carotenes between 15 and 30 days storage period. Extended storage period of up to 45 days resulted in to further loss of carotene by 36.1 per cent (α) and 20.5 per cent (β). Similarly RO technique resulted in carotene losses of 6.0 per cent (α) and 12.3 (β) carotenes between 15 and 30 and the loss of 13.8 (α) and 24.3 per cent (β) during extended storage period. Combined concentration techniques using RO and RFE indicated a similar trend however the loss of carotene was higher than most other techniques. Carrot juice during combined technique lost 33.1 per cent (α) and 14.8 per cent (β) within 30 days and 37.0 per cent (α) and 36.9 per cent (β) during extended storage period of 45 days. Juice prepared by RFE technique showed varying results. In this technique loss of carotene was higher [27.8 per cent (α) and 32.4 per cent (β)] during the storage period between 15 and 30 days which was then reduced to 11.0 (α) and 15.9 per cent (β) respectively.

Thus in most cases the loss of carotene was observed between storage period of 15 to 45 days unlike RFE. The phenomenon cannot be readily explained however this may be due to the isomerisation of carotenes during thermal processing. The results of previous studies indicated that in most cases levels of carotenes after processing have remained unchanged however, the carotene isomerisation and resultant increases in carotene contents have been reported (Granado *et al* 1992; Chandler 1989; Kim and Gerber 1988; Chandler and Schwartz, 1987; Hojilla *et al* 1985; Oser 1943).

Carotene has been widely investigated in various plant foods for the effect of various processing techniques used. Though the data published on the effect of concentration techniques on carotene levels have been very limited, the there is extensive literature on effect of various blanching techniques including boiling water, microwaving, pressure cooking, dehydration, combination of microwave-steam, stir frying in oil, stir frying with water and boiling water on carotene (Masrizal *et al* 1997; Rumm-Kreuter and Demmel 1990; Lane *et al* 1985; Warthesen *et al* 1984; Oser *et al* 1942; Brinkman *et al* 1941; Olliver 1941).

8.12 Conclusion

In this study carrot juice concentrate prepared from enzymatically untreated carrots and concentrated using RO technique attained almost 3 fold concentration on single pass prior to blocking of membrane. The juice of 4.5°Brix was concentrated up to 14.2°Brix. This concentration technique was also applied to the carrot juice prepared from enzymatically treated carrot. The juice of 6.0°Brix was concentrated up to 14.2°Brix reducing the fold of concentration to almost 2.5. The increase in the initial juice TSS from 4.5 to 6.0 was due to the enzymatic treatment applied to carrots. The enzymatic treatment showed no impact on level of concentration indicating the limitations of RO technique in achieving higher concentration levels.

Carrot juice concentrate was also prepared using thermal concentration (RFE) and combined concentration technique (RO and RFE). The carrot juice prepared using combined concentration technique resulted in the highest levels of TSS (26.4°Brix) compared to RFE (21-22°Brix) achieving 6 and 5 fold concentration respectively. These fold concentration did not change when carrot juice was concentrated using enzymatically treated carrots where the highest level of TSS achieved for the combined concentration technique was 35.2 and for RFE 26-27°Brix.

The effect of the concentration techniques on colour values of carrot juice did not indicate any noticeable difference. Carotene levels of the carrot juice during concentration using RO technique were not significantly reduced to remain at around

99.1 per cent (-0.9 per cent). This trend was not observed in the carrot juice concentrate prepared using thermal and combined concentration techniques. Minor increases in carotene contents were observed during the concentration using RFE and combined concentration techniques. The α-carotene was found to have been reduced (0.3-0.7 per cent) in each of these techniques compared to β-carotene which were slightly higher in RFE and combined concentration techniques (Table 8.1).

The effect of storage period on the sensory characteristics as well as colour values and carotenes were also investigated. Sensory results indicated mixed responses to the quality parameters. The result clearly indicates that the commercial sample was unacceptable for the taste, flavour and mouthfeel. The sample prepared using the combined concentration technique showed highest preference for all of the sensory parameters except colour, which was most preferred in the commercial sample. There were minor changes observed in panel preference for each of the sensory parameters throughout the storage period of 45 days.

There were minor variations in colour values observed in most concentration techniques until 30 days of storage period. The extended storage period of 45 days resulted in increased L^*, a^* and b^* values indicating deterioration (darkness) in colour. This suggests the limited storage stability of carrot juice of up to 30 days under frozen conditions. There were subsequent changes in carotene contents were also observed during the storage. The reduction in both α- and β-carotene up to 37 per cent was observed using the combined concentration technique within 45 days of storage.

Chapter 9

General discussion and conclusion

The carrot is one of the most widely consumed root vegetables, both in raw and processed forms and offers several nutritional advantages with the fresh product being low in energy. Carrot is high in fibre and also contains high levels of minerals such as potassium and other minerals and one of the richest sources of carotenes especially β-carotene.

9.1 Carrot production in Australia

Australia annually produces approximately 150,000 tonnes of carrot, contributing to 1.1 per cent of the global production of carrot. With recent increases of carrot production, Australia now contributes to 1.40 per cent of the global carrot production. Most of the crop produced in Australia is consumed as processed product.

9.2 Carrot processing industry

Carrot in Australia like most other countries are consumed in all forms including raw, semi processed and processed products. In 1995, there were about 10 processors of carrot juice in Australia. All of the product was exported to Japan and other Asian countries. Over time, South American countries, particularly Brazil and Chile, increased production of carrot juice through the adoption of American processing technology. As a result, they gained a competitive edge in the Asian market. Additionally, Australia's inability to produce and deliver this product at a cheaper cost in the international market assisted other competitors to establish a presence in the Asia Pacific region. This resulted in a dramatic reduction in carrot juice production in Australia. However the consumption of carrots in raw and semi processed forms continued to be increasingly popular.

This project was aimed towards the strategic investigation of processing carrot to define the processing protocols necessary to produce juice and optimise quality. In this investigation four commercially available Australian carrot varieties (two traditional

and two newer varieties) were investigated for compositional, nutritional and juice quality parameters. The suitability of these carrot varieties for juicing was investigated. During the project the enzymatic analysis, thermal processing, concentration of carrot juice and detailed and systematic study was undertaken for the parameters affecting the sensory and nutritional quality of carrot juice and carrot juice concentrates.

9.2.1 Composition

Relatively little compositional variation was observed between older and newer varieties. However, moisture content of these carrot varieties ranged between 87.7 and 89.1 per cent with only minor differences between the varieties. Protein contents all the carrot varieties ranged between 1.10 per cent - 1.19 per cent with minor difference between the varieties. Pectin content of all the carrot varieties ranged between 0.87 per cent - 0.96 per cent and crude fibre content between 1.90 per cent - 2.23 per cent with minor variations between the varieties.

9.2.2 Micronutrients

Nutritionally, carrot is a poor source of most other nutrients including vitamins (thiamin, riboflavin and vitamin C) and satisfies less than 2 per cent of the RDI values for these nutrients. However, it is the richest source of carotenes and contains 11-20 mg/100 g and satisfy up to 330 per cent of the RDI (CFCAB 1997). Varieties RHO, RHC and TP (0.06 g/100g) contained lower quantities of thiamin compared to RC (0.08 g/100g) however riboflavin content (0.03 g/100g) in all the varieties was similar. Carrots are high in potassium content (between 294 – 333 ppm), calcium (30 – 41 ppm), sodium (27 –54 ppm) and low in magnesium (3.9 – 9.6 ppm) and iron (0.39 – 1.03 ppm).

9.2.3 Juice quality parameters

The industrially significant juice quality parameters such as TSS, acidity and colour are important for the juice processor in terms of the quality of final product and juice yield which improve the commercial benefits. Commercially significant variations in juice quality parameters were observed for different varieties of carrot investigated. Variety RHO contains higher TSS (9.8) and sugar : acid (163) ratio as well as higher colour and

visual appeal and the new variety RHC showed potential for processing and was preferred over RC for the purpose of juice processing. Of the newer varieties, RHC (163) with its higher TSS and sugar : acid ratio as well as enhanced colour and visual appeal is preferred over TP (119) for juicing purposes.

During the study observations were also carried out regarding the browning rates of each of the varieties. Amongst all the four varieties, TP variety darkened more rapidly than other varieties. This may be related to the lower levels of vitamin C observed with this variety.

Considering compositional, nutritional and juice quality parameters and rate of browning, varieties RHO, RHC and RC indicates higher preference for juicing purpose compared to TP. This indicates the unsuitability of TP for juice processing and consumption compared to other three varieties. The current study also confirms that while carrots are a good source of carotenes, there are compositional differences between the varieties studied with RHO, an older variety and RHC, a newer variety, appearing to offer advantages over RC (older) and TP (newer).

9.3 Deteriorative enzymes

It has been well established that active enzyme systems cause deteriorative changes in foods even at subzero temperatures and low moisture levels. Most commonly indicated deteriorative enzymes are peroxidase, catechol oxidase and pectinesterase. These enzymes cause deteriorative changes including colour, and flavour and textural respectively. Foods are commonly heat treated to inactivate such deteriorative enzymes. Amongst these enzymes, peroxidase is a relatively more heat stable enzyme when compared to catechol oxidase. Thus peroxidase has traditionally been used as an indicator enzyme for sufficiency of thermal processing of fruits and vegetables.

9.3.1 Enzyme activities and localisation

During this study, all the three deteriorative enzymes including peroxidase, catechol oxidase and pectinesterase were quantitatively analysed from each of the four carrot varieties. The variety RHC contained highest contents of peroxidase followed by variety

RHO. However, there were minor variations observed between RC and TP varieties with the earlier containing higher peroxidase contents.

The distribution of these enzymes between carrot tissues core and superficial tissues were also analysed. Distribution between core and superficial tissues showed different pattern for the three enzymes. Peroxidase enzyme was found to be higher (2-3 times) in superficial compared to core tissues in all the varieties. Catechol oxidase enzyme activities were relatively lower in all the carrot varieties with highest activities in variety TP compared to other three varieties. However the activity of catechol oxidase in all three other varieties (RHO, RHC, RC) were similar. The enzyme catechol oxidase was detected only in superficial tissues. Activities of pectinesterase were found to be very low in all the four carrot varieties. The varieties RHO and RHC indicated minor variations in pectinesterase activity however the activities in RC and TP were similar. The distribution of pectinesterase enzyme in variety RC was uniform in both superficial and cores tissues however minor variations were observed in other varieties. In comparing the activities between root tip and stem end, each of the enzymes showed a uniform distribution in all four varieties.

9.3.2 Thermal inactivation of deteriorative enzymes

In this study, deteriorative enzymes from all the four carrot varieties were investigated for their thermal resistance. Amongst the varieties, deteriorative enzymes of TP were found to be more heat resistant than those of other varieties requiring higher temperatures for inactivation. Thermal inactivation study also indicated pectinesterase to be more heat stable than both peroxidase and catechol oxidase in each carrot variety requiring increased time of processing at higher temperatures.

Current commercial practice for processed and minimally processed fruits and vegetables products indicated the use of peroxidase enzyme as the indicator for blanching efficiency. This study carried out on carrot varieties has indicated that pectinesterase to more heat stable than peroxidase and may be a more appropriate enzyme to be used as indicator for blanching efficiency. Thus pectinesterase was used as indicator for all the further studies. The available literature also indicate the wide spread presence of pectinesterase enzyme in most fruits and vegetables. Thus during the

processing of fruit and vegetables pectinesterase may be used as indicator of blanching sufficiency instead of traditionally used peroxidase.

9.4 Carrot blanching and processing

The processing of all the vegetables and fruits comprises time and temperature regimes in relation to the size to achieve required quality of end product. In this study, carrots of different diameters (measured at the stem end portion) were investigated for the effect of carrot sizes on temperature come-up times. The results obtained indicated that the temperature come-up times for each of the sizes investigated were dependent on the diameter of carrots. The larger diameter carrots required prolonged heat treatments to attain the required temperature for all blanching techniques investigated. The variations in the carrot diameters also showed an impact on the thermal inactivation of deteriorative enzymes.

The effect of various blanching techniques on nutritive components (vitamin C, carotenes) as well as the physical parameters (weight loss/gain, juice yield, viscosity and colour) of carrot and carrot juice were also investigated. Microwave blanching and convenience, economic importance and shortcomings compared to other blanching methods have been well established in the past. Amongst the blanching techniques used in this study, microwave blanching resulted in an increased loss of carotenes, reduced juice yield and greater weight loss compared to other blanching methods. Furthermore the microwave blanching of carrots of smaller diameters resulted in burning of carrots with a concomitant effect on juice quality. These observations suggest unsuitability of microwave blanching in the blanching of carrot. Other quality parameters investigated including carotenes, vitamin C, colour characteristics between the blanching techniques suggest steam blanching to be more preferred method of processing of carrots for juicing purpose.

Enzyme distribution studies were undertaken to establish presence or absence of these deteriorative enzymes from various portions of carrot. These could also assist to establish blanching protocols. The results obtained in this study indicate enzyme pectinesterase to be the most heat stable enzyme and present in all tissues of carrot. As a

result of this finding it is recommended that it is necessary to heat carrot up to 95°C for one minute (pectinesterase inactivation temperature) for core tissues.

9.5 Enzymatic treatment

Commercial enzyme preparations containing activities of pectinesterase, cellulase, hemicellulase and polygalacturonase were used to treat blanched carrots. The effect of these enzymes for maceration of fruits and vegetable tissues and their significant contribution in increasing juice yield and pulp contents in the fruits and vegetable systems has been well recognised. These enzymes also facilitate disintegration of fruits and vegetables to release nutrients and other valuable compounds from the food systems. In the present study promising results were obtained for the juice yield and pulp contents during the application of these enzymes on carrots. As a result of the enzymatic treatment using 150 ppm and 25:75 proportions RPL and RM, significant increases in the juice yield and carotene content were achieved. There were minor changes observed in colour and viscosity of juice before and after enzyme treatment.

For the industrial processor, use of such enzymes is of great commercial significance. The increase in juice yield and increased extraction of nutrients are of great commercial benefits. Thus the application of this enzyme treatment regime is highly recommended for industrial processing of carrot juice.

During enzymatic treatment, pH of the carrot mass was reduced to 4.8 –5.0 using citric acid. This reduction in the pH commonly increases the sourness of carrot juice reducing the taste preference against the untreated carrot juice. The enzymatic treatment of carrot may still be continued to achieve the aforesaid benefits with the different end use of the product. The pH adjusted juice can either be neutralised to natural carrot juice pH of 5.8-6.2 or be used in preparation of juice blends with other fruits and vegetable juices. The juice mixtures of carrot with fruits and vegetables such as orange, apple, lemon, celery, spinach, tomato and ginger are gaining increasing popularity in Australia and in Asian countries including Japan.

9.6 Concentration and storage of carrot juice

In this study, carrot juice concentrate was prepared from the variety RHO using various concentration techniques including thermal and non-thermal (membrane). The samples investigated were carrot juice concentrates prepared from RO, RFE, combined concentration (RO and RFE) as well as commercial concentrate. The commercial concentrate was prepared from thermal concentration technique (RFE).

While concentrating carrot juice using the UF technique (10,000 and 30,000 MWCO membrane), loss of sugars and acids in permeate were observed. Because of this limitation, further investigations on carrot juice concentration using UF were not pursued.

Carrot juice concentrate prepared by RO attained almost 3 fold (4.5 to 14.2°Brix) concentration in single pass. Similar experiments using enzymatically treated carrots attained a 2.5 fold (6.0 to 14.2°Brix) concentration in a single pass. The enzymatic treatment showed no impact on level of concentration using RO. This indicates the limitations of this technique in achieving higher concentration levels in single pass. The use of multiple RO systems using various MWCO sizes membranes may assist in achieving higher concentrations. The carrot juice prepared from enzymatically treated carrot juice and combined concentration technique resulted in the highest levels of TSS (26.4°Brix) compared to the RFE technique (21-22°Brix) achieving 6 and 5 fold concentration respectively. The extent of concentration did not change when juice was prepared using enzymatically treated carrots. However, highest level of TSS achieved for the combined concentration technique was 35.2 and for RFE was 26-27°Brix.

For the preparation of concentrate carrot juice the level of fold concentration achieved during thermal and combined concentration techniques were significantly higher compared to membrane techniques. Under commercial operations, achievement of higher concentrations is of a great logistical convenience resulting into the reduction of the freight costs. This also assists in increasing the shelf life of the product during storage. Additionally, thermal and combined concentration techniques using RFE did not indicate any noticeable impact on colour, carotene levels and sensory characteristics such as taste, flavour and mouthfeel. The sensory characteristics such as taste, flavour

and mouthfeel for commercial sample clearly indicate poorer acceptability compared to other concentration technique. Thus the use of thermal or combined concentration techniques are highly recommended for the processing of carrot juice.

The effect of storage period on the sensory characteristics as well as colour values and carotene indicated mixed responses to the quality parameters. The sample prepared using combined concentration technique showed highest preference for all the sensory parameters except colour, which was most preferred in commercial sample. There were minor changes observed in panel preference for all the sensory parameters throughout the storage period of 45 days. The extended storage period of 45 days resulted in increased *L*, a* and b** values resulting in deterioration (darkness) in colour. This suggests the limited storage stability of carrot juice of up to 30 days under frozen conditions.

9.7 Processing protocols

As a result of this strategic study on carrot juice processing, certain processing protocols have been established (Figure 9.1). A typical flow diagram of carrot juice processing based upon existing commercial practice is shown. In addition process flow charts for both enzymatically untreated and treated carrots have also been proposed.

9.8 Further studies

This study carried out contains various processing parameters applied during processing of carrot juice. Processing protocols also have been established. However further studies are still required to continually achieve improved quality as well as economics. Future investigations recommended for further work are as described below.

1. Investigate new and improved carrot varieties for suitability of juice processing. There are opportunities for genetically modified varieties which containing none of the deteriorative enzymes indicated in this study.

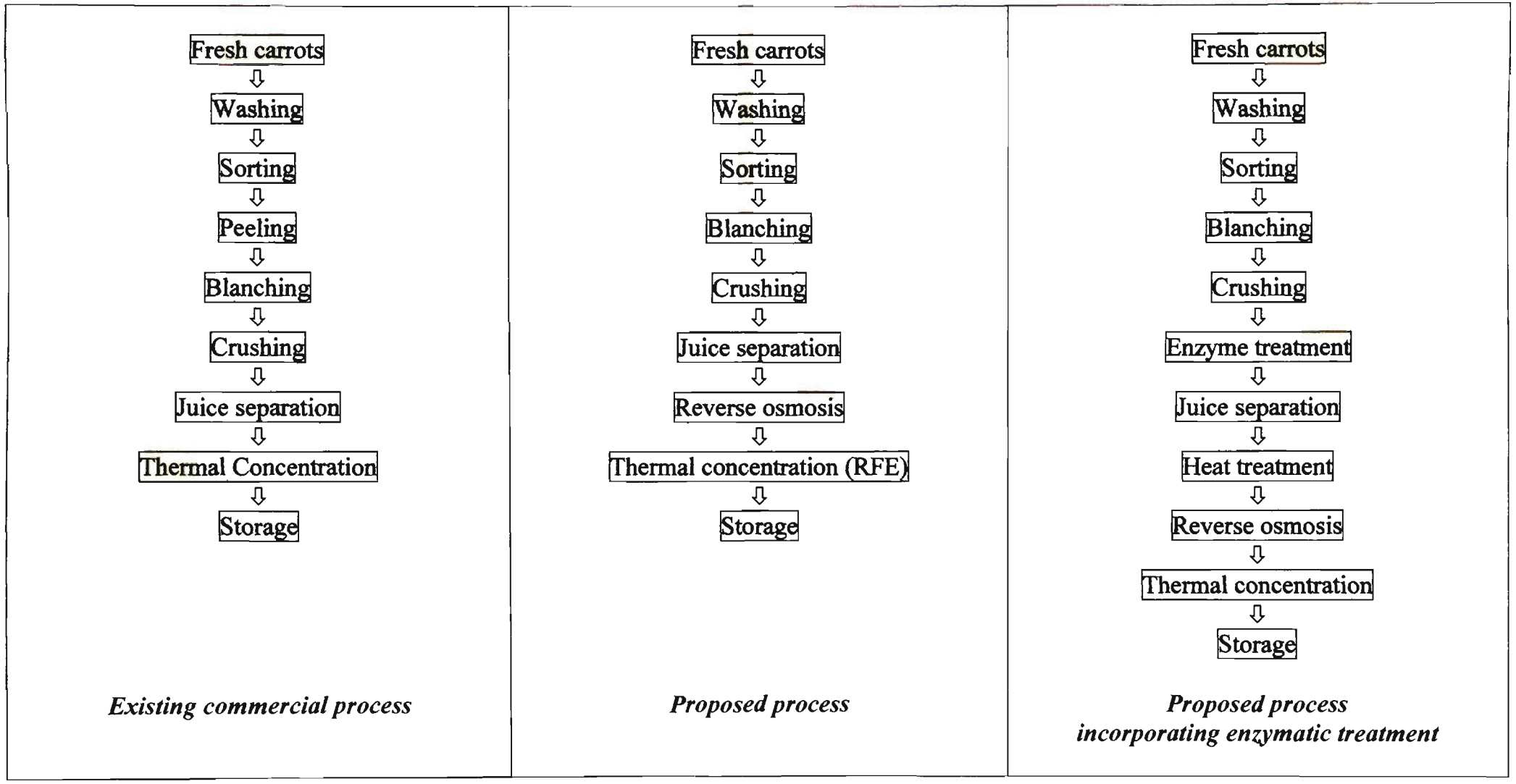

Figure 9.1 A comparison of the existing and proposed processes for production of juice concentrate from fresh carrots

2. There are increasing opportunities for the use of improved and continuous microwave blanch technology which may assist in improving efficiency and economics of carrot juice processing.
3. The use of a series of RO membranes to achieve higher levels of concentration. This will certainly assist logistics of transportation.

9.8.1 Use of by-products

Carrot juice processing, as shown in this study, results in very high quantities of waste material which are rich in fibre and nutrients including carotenes. This waste material after drying can be used in manufacture of breakfast cereals, muesli bars as well as cattle feed.

References

AOAC 1990 *Official Methods of Analysis.* 15th edition. Association of Official Analytical Chemists, Arlington, Virginia.

Abbey M, Noakes M, Nestle P J 1995 Dietary supplementation with orange and carrot juice in cigarette smokers lowers oxidation products in copper-oxidized low-density lipoproteins. *Journal of the American Dietetic Association* **95** 671-675.

Adams J B 1978 The inactivation and regeneration of peroxidase in relation to the high temperature-short time processing of vegetables. *Journal of Food Technology* **13** 281-297.

Adams J B 1981 Blanching of vegetables. *Nutrition and Food Science* **6** 11-13.

Aluko R, Ogbadu G H 1986 Analysis of eggplant varieties for enzymes related to their organoleptic properties. *Tropical Science* **26** 163-171.

Anastasakis M, Lindamood J B, Chism G W, Hansen P M T 1987 Enzymatic hydrolysis of carrot for extraction of a cloud stable juice. *Food Hydrocolloids* **1** 247-261.

Anon 1953 Wealth of India, a dictionary of Indian raw materials and industrial products. Council of Scientific and Industrial Research, III 19-23.

Armerding G D 1966 Evaporation methods as applied to the food industry. In: *Advances in Food Research Vol 15* ed C O Chichester, Mark E M, Stewart G F. Academic Press, New York, pp 303-358.

Arthey D, Dennis C 1990 *Vegetable processing.* Glasgow, Blackie; New York. VCH Publisher. pp 42-67.

Ashie I N A, Simpson B K, Smith J P 1996 Mechanism for controlling enzymatic reaction in foods. *Critical Reviews in Food Science and Nutrition* **36** 1-30.

Australian Bureau of Statistics 1996 *Year Book Australia* (ABS 1301.0), Australian Bureau of Statistics, Canberra.

Ayranci G, Tutunculer A 1993 Preparation and storage stability of juices from different carrot species. *Die Nahrung* **37** 433-439.

Baardseth P 1979 Enzymatically induced quality changes in fresh and frozen carrot. *Acta Horticulturae* **93** 67-74.

Baardseth P, Slinde E 1987 Enzymes and off flavours: palmitoyl Co-A hydrolase, lipoxygenase, α-oxidation, peroxidase, catalase activities and ascorbic acid content in different vegetables. *Norwegian Journal of Agricultural Science* **1** 111-117.

Babic I, Amiot M J, Nguyen-The C, Aubert S 1993 Changes in phenolic content in fresh ready to use shredded carrots during storage. *Journal of Food Science* **58** 351-356.

Bajaj K L, Kaur G, Brar J S, Sukhija B S 1978 Chemical composition and keeping quality of carrot (*Daucus carota* L) varieties. *Plant Foods for Man* **2** 159-165.

Baker R A 1998 Reassessment of some fruit and vegetable pectin levels. *Journal of Food Science* **62** 225-229.

Baloch A K 1987 Effect of sulphur dioxide and blanching on stability of carotenoids of dehydrated carrots. *Journal of the Science of Food and Agriculture* **40** 179-187.

Baloch A, Buckel K, Edward R A 1977 Effect of processing variables on the quality of dehydrated carrot. I- Leaching losses of carotenoid content. *Journal of Food Technology* **59** 238-243.

Bao B, Chang K C 1994a Carrot juice color, carotenoids, and nonstarchy polysaccharides as affected by processing conditions. *Journal of Food Science* **59** 1155-1158.

Bao B, Chang K C 1994b Carrot pulp chemical composition, color, and water holding capacity as affected by blanching. *Journal of Food Science* **59** 1159-1161.

Barrett D M, Theerakulkait C 1995 Quality indicator in blanched, frozen, stored vegetables. *Food Technology* **45** 62-65.

Baumann J W 1981 Application of enzymes in fruit juice technology. In: *Enzymes and Food Processing*, ed Birch G G, Blackborough N, Parker K J. Applied Science Publishers, London, pp 129-147.

Bawa A S, Saini S P S 1987 Effect of method of preservation on storage quality of carrot juice. *Indian Food Packer* **1** 42-46.

Ben Amar R, Gupta B B, Jaffrin M Y 1990 Apple juice clarification using mineral membrane: fouling control by backwashing and pulsating flow. *Journal of Food Science* **55** 1620-1625.

Benkova R, Markovic L 1976 *Advances in Carbohydrate Chemistry and Biochemistry.* Academic Press, New York, pp 323-385.

Beuchart L R, Brackett R E, Doyle M P 1994 Lethality of carrot juice to *Listeria monocytogenes* as affected by pH, sodium chloride and temperature. *Journal of Food Protection* **57** 470-474.

Beuchart L R, Brackett R E 1990 Inhibitory effects of raw carrots on *Listeria monocytogenes*. *Applied and Environmental Microbiology* **1** 1734-1742.

Beveridge T 1994 Decanting centrifuge application in fruit juice processing. *Fruit Processing* **12** 390-394.

Bolin H R, Huxsoll C C 1991 Control of minimally processed carrot (*Daucus carota*) surface discoloration caused by abrasion peeling. *Journal of Food Science* **56** 416-418.

Borenstein B, Bunnell R H 1966 Carotenoids: properties, occurrence and utilisation in foods. *Advances in Food Research* **15** 195-275.

Bowden R P, Isaacs A R 1989 Concentration of pineapple juice by reverse osmosis. *Food Australia* **41** 850-851.

Boyer R F 1977 A spectrophotometric assay of polyphenoloxidase activity. *Journal of Chemical Education* **54** 585-586.

Braddock R J, Nikdel S, Nagy S 1988 Composition of some organic and inorganic compounds in reverse osmosis-concentrated citrus juice. *Journal of Food Science* **53** 508-512.

Bradley G A, Dyck R L 1968 Carrot color and carotenoids as affected by variety and growing conditions. *American Society for Horticulture Science* **93** 402-407.

Brinkman E V S, Halliday E G, Hinman W F, Hamner R J 1941 Effect of various cooking methods upon subjective qualities and nutritive values of vegetables. *Food Research* **7** 300-305.

Brody A L 1992 Microwave food pasteurization, sterilisation and packaging. *Food Technology International Europe* 67-71.

Bureau J L, Bushway R J 1986 HPLC determination of carotenoids in fruits and vegetables in the United States. *Journal of Food Science* **51** 128-130.

Burnette F S 1977 Peroxidase and its relationship to food flavor and quality: A review. *Journal of Food Science* **42** 1-6.

Bushway R J, Wilson A M 1982 Determination of α- and β-carotene in fruit and vegetables by high performance liquid chromatography. *Canadian Institute of Food Science and Technology Journal* **15** 165-169.

Cameron R G, Grohmann K 1995 Partial purification and thermal characterization of pectinmethylesterase from red grapefruit finisher pulp. *Journal of Food Science* **60** 821-825.

Cano M P, Hernandeze A, DeAncos B 1997 High pressure and temperature effects on enzyme inactivation in strawberry and orange products. *Journal of Food Science* **62** 85-88.

Cano P M, Begona De Ancos, Lobo G 1995 Peroxidase and polyphenoloxidase activities in papaya during postharvest ripening and after freezing/thawing. *Journal of Food Science* **60** 815-817.

Campannelli G, Bottino A, Munari S, Lister D G, Maschio G, Becchi I 1994 Use of membrane process in the clarification of orange and lemon juices *Food Engineering* 473-483.

Cashel K, English R, Lewis J 1989 *Composition of Foods, Australia.* Australian Government Publishing Service, Canberra, 13A1-146C.

Casimir D J, Kefford J F 1968 Low temperature evaporation. *CSIRO Food Preservation* **28** 20-26.

Castaldo D, Servillo L, Loiudice R, Balestrieri C, Laratta B, Quagliuolo L, Giovane A 1996 The detection of residual pectin methylesterase activity in pasteurised tomato juice. *International Journal of Food Science and Technology* **31** 313-318.

CFCAB (Californian Fresh Carrot Advisory Board) 1996 An information bulletin.

Chamchong M, Noomhorn A 1991 Effect of pH and enzymatic treatment on microfiltration and ultrafiltration of Tangerine juice. *Journal of Food Process Engineering* **14** 35-39.

Chandler B V 1989 Fruit juice review. *Food Australia* **41** 926-928.

Chandler L A, Schwartz S J 1987 HPLC separation of cis-trans carotene isomers in fresh and processed fruits and vegetables. Journal of Food Science **52** 669-672.

Chao H, Wen S, Fang T 1992 Effect of ultrafiltration and reverse osmosis on the quality retention of passion fruit juice concentrate. *Journal of the Chinese Agricultural Chemical Society* **30** 413-431.

Chen B H, Peng H Y, Chen H E 1995 Changes of carotenoids, colour and vitamin A contents during processing of carrot juice. *Journal of Agricultural and Food Chemistry* **43** 1912-1918.

Chen B H, Peng H Y, Chen H E 1996 Stability of carotenoids and vitamin A during storage of carrot juice. *Food Chemistry* **57** 497-503.

Cheryan M 1996 *Ultrafiltration Handbook.* Technomic Publishing, Lancaster PA, ISBN 87762-457-459.

Cheryan M 1991 Reverse osmosis in food processing. *Indian Food Industry* **6** 30-37.

Chou F, Wiley R C, Schlimme D V 1991 Reverse osmosis and flavour retention in apple juice concentrate. *Journal of Food Science* **56** 484-487.

Chubey B B, Nylund R E 1969 Surface browning of carrots. *Canadian Journal of Plant Science* **49** 421-426.

Colesan F, Johrer P 1993 Belt presses, decanters and disk stack centrifuges for the processing of fruits and vegetables. *Fruit Processing* **8** 292-302.

Collins J L, McCarty I E 1969 Comparison of microwave energy with boiling water for blanching whole potatoes. *Food Technology* **23** 63-66.

Constenla D T, Lozano J E 1995 Effect of ultrafiltration on concentration of apple juice colour and turbidity. *International Journal of Food Science and Technology* **30** 23-30.

Cumming D B, Stark R, Timbers G E, Cowmeadow R 1984 A new blanching system for the food industry. II Commercial design and testing. *Journal of Food Processing and Preservation* **8** 137-150.

Dan A, Jain N L 1971 New blanching medium for dehydration of carrots (*Daucus carota* L). *Indian Food Packer* **4** 10-14.

Dawson R M C, Elliot D C, Elliot W H, Jones K M 1986 *Data for Biochemical Research*, Third Edition. Oxford University Press, Oxford, pp 417-448.

Decareau R V 1972 The progress of microwave blanching. *Food Production Management* 12-13.

Decareau R V 1990 Microwave use in food processing. *Food Technology International Europe* 69-72.

De Ritter E 1976 Stability characteristics of vitamins in processed foods. *Food Technology* **1** 48-54.

Deshpande S S, Bolin H R, Salunkhe D K 1982 Freeze concentration of fruit juices. *Food Technology* **36** 68-82.

Desobry S A, Netto F M, Labuza T P 1998 Preservation of β-carotene from carrots. *Critical Reviews in Food Science and Nutrition* **38** 381-396.

Diehl H C, Dingle J H, Berry J A 1933 Enzymes can cause off flavours even when foods are frozen. *Food Industry* 300-301.

Dikshit S N, Udipi S A, Rao A, Manohar V 1988 Separation of carotenoids and estimation of beta-carotene content of selected Indian food and food preparations by HPLC. *Journal of Food Science and Technology* **25** 39-41.

Dorrell D G, Chubey B B 1972 Acceleration of enzymatic browning in carrot and parsnip roots by induced suberization. *Journal of the American Society of Horticultural Science* **97** 110-111.

Durham R J, Nguyen M H 1994 Hydrophobic membrane evaluation and cleaning for osmotic distillation of tomato puree. *Journal of Membrane Science* **87** 181–189.

Drake S R, Spayd S E, Thompson J B 1981 The influence of blanch and freezing methods on the quality of selected vegetables. *Journal of Food Quality* **4** 271-278.

El-Sherbiny G. A, Rizk, S S 1981 Orange concentrates prepared by different techniques I-Quality attributes, *Agricultural Research Review*, **59** 1-14.

Encyclopaedia Britannica Micropaedia 1995. 15th edition, no. 2, p 903.

English R, Lewis J 1991 *Nutritional Values of Australian Foods*, Australian Government Publishing Service, Canberra.

Eskin M N A 1990 Biochemical changes in raw foods: fruits and vegetables. *Biochemistry of foods*; 2nd edition, Academic Press. Ch. 2, pp 69-165. ISBN 0-12-242351-8.

Esselen W B, Anderson E E 1956 Thermal destruction of peroxidase in vegetables at high temperatures. *Food Research* **21** 322-326.

FAO 1999 *FAOSTAT Agriculture Database; domain Agriculture production; data collection Crops Primary*; last update 3 November 1999, [Online]. http//www.apps.fao.org/cgi-bin/nph-db.pl?subset=agriculture Accessed 30 November 1999.

Farhangi M, Guy Valdon L R 1981 Effect of acidified processing and storage on carotenoids (provitamin A) and vitamin C in mung beans sprouts. *Journal of Food Science* **46** 1464-1466.

Fellows P J 1990a *Food Processing Technology:* Microwave and Infrared Radiation. Chapter 17, VCH Publishers, ISBN No. 3-527-26626-7, pp343-355.

Fellows P J 1990b *Food Processing Technology:* Evaporation. Chapter 12, VCH Publishers, ISBN No. 3-527-26626-7, pp250-266.

Foda M S, Hussien M F, Rizk I R S, Gabriel, A Y, Bash S I 1985 Studies on production of fruits and vegetable juices using polygalacturonase from *Aspergillus aculeatus*. *Egyptian Journal of Food Science* **13** 143-150.

Fukutani K, Ogawa H 1983 A comparison of membrane's suitability and effect of operating pressure for juice concentration by reverse osmosis. *Nippon Shokubin Kogyo Gakkaishi* **30** 626-641.

Fuller R 1969 Heat exchange in the food industry. *Food Technology in Australia* **9** 448-453.

George R M, Richardson P S 1992 Reheating instruction for microwave ovens. *Food Technology International Europe* 63-66.

Ginn P W, Hosken R W, Cole S J, Ashton J F 1998 Physicochemical and sensory evaluation of selected Australian UHT processed soy beverages. *Food Australia* **50** 347-351.

Glasscock S J, Axelson J M, Palmer J K, Phillips J A, Taper L J 1982 Microwave blanching of vegetables for frozen storage. *Home Economics Research Journal* **11** 149-158.

Granado F, Olmedilla B, Blanco I, Hidalgo E R 1992 Carotenoid composition in raw and cooked Spanish vegetables. *Journal of Agricultural and Food Chemistry* **40** 2135-2140.

Gunes B, Bayindirli A 1993 Peroxidase and lipoxygenase inactivation during blanching of green beans, green peas and carrots. *Lebensmittel Wissenschaft und Technologie* **26** 406-410.

Hagermann A E, Austin P J 1986 Continuous spectrophotometric assay for plant pectin methyl esterase. *Journal of Agricultural and Food Chemistry* **34** 440-444.

Hagg M, Ylikoski S, Kumpulainen J 1994 Vitamin C and α- and β-carotene contents in vegetables consumed in Finland during 1988-1989 and 1992-1993. *Journal of Food Composition and Analysis* **7** 252-259.

Handschuh B 1995 Making of carrots into juice and puree. *Fruit Processing* **9** 278-280.

Hartmann E, Lenggenhager T, Keller M 1996 New findings when extracting with Becher HPX 5005 I fruit presses. *Fruit Processing* **12** 482-486.

Heinonen M I 1990 Carotenoids and provitamin A activity of carrot cultivars. *Journal of Agricultural and Food Chemistry* **38** 609-612.

Heinrichova K 1977 Isolation, characterisation and mode of action of exo-D-galacturonase from carrot. *Collection of Czechoslavakian Chemical Communications* **42** 3214-3221.

Heldman D R, Hartel R W 1997 *Principles of Food Processing*. Chapman and Hall, New York, ISBN 0-412-99451-8.

Hernandez E, Couture R, Rouseff R, Chen C S, Barros S 1992a Evaluation of ultrafiltration and adsorption to debitter grapefruit juice and grapefruit pulp wash. *Journal of Food Science* **57** 664-667.

Hernandez E, Chin S C, Shaw P E, Carter R D, Barros S 1992b Ultrafiltration of orange juice: Effect on soluble solids, suspended solids and aroma. *Journal of Agricultural and Food Chemistry* **40** 986-988.

Hojilla M P, Garcia, V V, Raymundo L C 1985 Thermal degradation of β-carotene in carrot juice. *ASEAN Food Journal* **1** 157-161.

Horvath K Z, Gatai K 1977 Application of endo-polygalacturonase to vegetables and fruits. *Acta Alimentaria* **6** 355-376.

Howard L R, Braswell D D, Aselage J 1996 Chemical composition and colour of strained carrots as affected by processing. *Journal of Food Science* **61** 327-330.

Howe P S 1980 Vitamins Ch. 10, In: *Basic Nutrition in Health and Disease*, W B Saunders Company, Philadelphia, ISBN 0-7216-4796-0.

Hsu A F, Thomas C E, Brauer D 1988 Evaluation of several methods for estimation of total activity of potato polyphenol oxidase. *Journal of Food Science* **53** 1743-1745.

Industrial correspondence 1997, 1998, 1999 (Henderson Seeds Pty Ltd. and Australian Food Processing Industries).

Jennylynd J, Simpson B K 1996 Application of enzymes in food processing. *Critical Reviews in Food Science and Nutrition* **36** 437-463.

Jobling J J, Patterson B D, Moradi S, Joyce D 1997 A non-destructive method for measuring the water potential of fruits and vegetables. *Postharvest Biology and Technology* **10** 1-8.

Johnson R A, Valks R H, Lefebvre, M S 1989 Osmotic distillation, A low temperature concentration technique. *Australian Journal of Biotechnology* **3** 206-207.

Kalra C L, Kulkarni S G, Berry S K 1987 The carrot (*Daucus carota* L)- A most popular root vegetables. *Indian Food Packer* **19** 46-73.

Karel M 1975 Concentration of foods. In: *Principles of Food Science, Part II: Physical Principles of Food Preservation,* ed Karel M, Fennema O R, Lund D B. Marcel Dekker Inc, New York.

Kaur G, Jaiswal S P, Brar J S, Nandpuri K S, Kumar J C 1976 Physico-chemical characteristics of some important varieties of carrot (*Daucus carota* L*). Indian Food Packer* **30** 5-8.

Khan N A, Khalil J K, Khan I 1975 Changes in the carotene contents of carrots during maturity, processing and storage under different temperature conditions. *Pakistan Journal of Scientific Research* **27** 112-114.

Khokhar and Puhspanjali 1995 Dietary fibre composition of Indian foods and intake of Indian vegetarian population. *Journal of Human Nutrition and Dietetics* **8** 447-467

Kim H, Gerber L E 1988 Influence of processing on quality of carrot juice. *Korean Journal of Food Science and Technology* **20** 683-690.

Klein B P, Kuo C H Y, Boyd G 1981 Folacin and ascorbic acid retention in fresh raw, microwave and conventionally cooked spinach. *Journal of Food Science* **46** 640-641.

Klein B P, Lee H C, Reynolds P A, Wangles N C 1979 Folacin contents of microwave and conventionally cooked frozen vegetables. *Journal of Food Science* **44** 286-288.

Koseoglu S S, Lawhon J T, Lucas E W 1990 Use of membrane in citrus juice processing. *Food Technology* **44** 90-97.

Koseoglu S S, Lawhon J T, Lucas E W 1991 Vegetable juices production with membrane technology. *Food Technology* **45** 124-130.

Krarup H A, Ruiz V C, Manquian T N 1986 Quality and chemical composition of 3 varieties of carrot cultivars in Valdivia. *Agro-Sur* **14** 99-107.

Krinsky N I 1988 Carotenoids and cancer in animal models. *American Institute of Nutrition* 123-126.

Kubota K, Fujimoto M, Suzuki K, Takasaki K, Hosaka H 1981 A study on the thermal diffusivity of spherical root vegetables. *Journal of Japanese Society of Food Science and Technology [Nippon Shokuhin Kogyo Gakkaishi;* 28 68-73.

Kumar G C, Kanawjia S K, Ladkani B G, Singh 1992 Recent advances in the processing of fruit juices. *Indian Dairyman* **44** 167-179.

Kylen A M, Charles V R, McGrath B H, Schleter J M, West L C, Van Duyne F O 1960 Microwave cooking of vegetables. *Journal of the American Dietetic Association* **39** 321-326.

Laferriere L, Gabelmann W H 1967 Inheritance of colour, total carotenoids, alpha carotenes and beta carotenes in carrots, *Daucus carota* L. *Proceedings of American Society of Horticultural Science* **93** 408-419.

Lane R H, Boschung M D, Abdel-Ghany M 1985 Ascorbic acid retention of selected vegetables blanched by microwave and conventional methods. *Journal of Food Quality* **8** 139-144.

Lanker T, Flurkey W H, Hughes J 1988 Cross reactivity of polyclonal and monoclonal antibodies to polyphenoloxidase in higher plants. *Phytochemistry* **27** 3731-3734.

Lazor M E, Lund D B, Dietrich W C 1971 IQB -A new concept in blanching. *Food Trade Review* **25** 684-686.

Lee C Y, Bourne M C, Van Buren J P 1979 Effect of blanching treatments on the firmness of carrots. *Journal of Food Science* **44** 615-616.

Lefebvre M S 1986 Membrane technology and the food industry. *ASEAN Food Journal* **2** 51-57.

Lerici C R, Mastrocola D, Nicoli M C 1988 Use of direct osmosis as fruit and vegetables dehydration. *Acta Alimentaria Polonica* **14** 35-40.

Lu A T, Whitaker J R 1974 Some factors affecting rates of heat inactivation and reactivation of horseradish peroxidase. *Journal of Food Science* **39** 1173-1178.

Luh B S, Antonakos J, Daoud H N 1969 Chemical and quality changes in strained carrots canned by the aseptic and retort process. *Food Technology* **23** 103-107.

Macrae, R, Robinson R K, Sadler M J 1993 Carotenoids- Properties, Determination and Physiology. In: *Encyclopaedia of Food Science, Food Technology and Nutrition* Vol. 1, Academic Press, London, pp 707-718.

Maehly A C, Chance B 1954 The assay of catalase and peroxidase. *Methods of Biochemical Analysis* **1** 357-424.

Marangoni A G, Jackman R L, Stanley D W 1995 Chilling associated softening of tomato fruit is related to increased pectinmethylesterase activity. *Journal of Food Science* **60** 1277-1281.

Markovic O 1978 Pectinesterase from carrot (*Daucus carota* L). *Specialia* **15** 561-562.

Marquis H, Bucheli P 1994 Inhibition of tomato pectin methylesterase by partially purified kiwi pectin methylesterase inhibitor protein. *International Journal of Food Science and Technology* **29** 121-128.

Marshall S C 1985 The reverse osmosis process. *The Australian Journal of Dairy Technology* **40** 86-87.

Martinsen C S, Ostrander J G 1984 Waterless cooking- Influence of energy consumption and nutrient retention. *Journal of Consumer Studies and Home Economics* **8** 305-312.

Marty C, Berset C 1990 Factors affecting the thermal degradation of *all trans* β-carotene. *Journal of Agricultural and Food Chemistry* **38** 1063-1067.

Masrizal M A, Giraud D W, Driskell J A 1997 Retention of vitamin C, iron and β-carotene in vegetable prepared using different cooking methods. *Journal of Food Quality* **20** 403-418.

Matsuura T, Baxter A G, Sourirajan S 1974 Studies on reverse osmosis for concentration of fruit juices. *Journal of Food Science* **39** 704-711.

Mayer A M 1987 Polyphenol oxidases in plants-Recent progress. *Phytochemistry* **26** 11-20.

Mayer A M, Harel E 1979 Polyphenol oxidase in plants. *Phytochemistry* **18** 191-215.

Mayne S T, Janerich D T, Greenwald P, Chorst S, Tucci C, Zaman M B, Melamed M R, Kiely M, McKneally M F 1992 Dietary β carotene and lung cancer risk in US nonsmokers. *Journal of National Cancer Institute* **86** 33-38.

Mazza G 1989 Carrots. In: *Quality and Preservation of Vegetables,* ed Eskin N A M. CRC Press, Florida, pp 75-120.

McLellan K M, Robinson D S 1987 The heat stability of purified spring cabbage peroxidase isozymes. *Food Chemistry* **26** 97-107.

Mehra D K 1986 Selecting evaporators. *Chemical Engineering* **3** 56-72.

Mihalyi K, Vamos-Vigyazo L 1975 Determination, localization and heat inactivation of peroxidase in some vegetables. *Acta Alimentaria* **4** 291-308.

Milnes B A, Breslau B R, Reardon P C 1986 The application of ultrafiltration to apple juice processing. *Process Biochemistry and Technology* **8** 8-11.

Mirza S, Morton I D 1977 Effect of different types of blanching on the colour of sliced carrots. *Journal of the Science of Food and Agriculture* **28** 1035-1039.

Mohsen S M, El-Hashiny F S A, Gawad A A 1986. Effect of concentration methods on orange juice properties and it's volatile components. *Egyptian Journal of Food Science* **14** 191-204.

Moon R C 1989 Comparative aspects of carotenoid and retenoids as chemopreventive agents for cancer. *Journal of Agricultural and Food Chemistry* **119** 127-134.

Moresi M, Spinosi M 1980 Engineering factors in the production of concentrated fruit juices. *Journal of Food Technology* **15** 265-276.

Muftigul N 1985 Effect of different types of blanching on the colour and the ascorbic acid and chlorophyll contents of green beans. *Journal of Food Processing and Preservation* **10** 69-76.

Munsch M H, Simard R E, Girard J M 1986a Effect of blanching, degree of grinding and enzymatic maceration on the yield and quality of carrot juice. *Lebensmittel Wissenschaft und Technologie* **19** 229-239.

Munsch M H, Simard R E, Girard J M 1986b Effect of blanching, degree of grinding and enzymatic maceration on the mineral and nitrogen content of carrot juice. *Lebensmittel Wissenschaft und Technologie* **19** 240-248.

Munsch M H, Simard R E 1983 Relationships in colour and carotene content of carrot juice. *Canadian Institute of Food Science and Technology Journal* **3** 173-178.

Murakoshi M, Nishino H, Satoni Y, Takayasu J, Hasegawa T, Tokuda H, Iwashima A, Okuzumi J, Okabe H, Kitano H, Iwasaki R 1992 Potent preventive action of α-carotene against carcinogenesis: spontaneous liver carcinogenesis and promoting stage of lung and skin carcinogenesis in mice are suppressed more effectively by α-carotene than β-carotene. *Cancer Research* **52** 6583-6587.

NHMRC 1991 *Recommended Dietary Intakes for use in Australia.* Australian Government Publishing Service, Canberra, ISBN 0-644-1380-2.

Nakamura T, Hours R A, Sakai T 1995 Enzymatic maceration of vegetables with protopectinases. *Journal of Food Science* **60** 468-472.

Nath N, Rao A V, Gupta R G 1983 Thermal resistance of pectinmethyl esterase in juice of Pusa-Ruby tomatoes. *Indian Food Packer* **5** 30-38.

Nguyen M H 1996 Australian developments in membrane processing of liquid foods. *Food Australia* **48** 232-234.

Nielsen W K 1992 Membrane filtration technology. *Food Technology International Europe* 99-102.

Nikdel S, Mackellar D G 1993 Continuous pasteurization of citrus juice with microwave heating. *Fruit Processing* **12** 433-435.

Olle D, Baron A, Lozano Y F, Sznaper C, Baumes R, Bayonove C, Brillouet J M 1997 Microfiltration and reverse osmosis affect recovery of mango puree flavour compounds. *Journal of Food Science* **62** 1116-1119.

Olliver M 1941 Effect of cooking on the nutritive value of vegetables. *Chemistry and Industry* **60** 586-596.

Olson J A 1986 Carotenoids, vitamin A and cancer. *Journal of Nutrition* **116** 1127-1130.

Oser B L, Melnick D, Oser M 1943 Influence of cooking procedure upon retention of vitamins and minerals in vegetables. *Journal of Consumer Studies and Home Economics* **8** 115-122.

Panalaks T, Murray T K 1970 The effect of processing on the content of carotene isomers in vegetable and peaches. *Canadian Institute of Food Science and Technology Journal* **3** 145-151.

Paulson D J, Wilson R L, Spatz D D 1985 Reverse osmosis and ultrafiltration applied to the process of fruit juice. In: *Reverse osmosis and Ultrafiltration*, pp 325-344.

Paulus K, Saguy I 1980 Effect of heat treatment on the quality of cooked carrots. *Journal of Food Science* **45** 239-245.

Pesek C A, Warthesen J J 1987 Photodegradation of carotenoids in a vegetable juice system. *Journal of Food Science* **52** 744-746.

Peto R, Doll R, Buckley J D, Sporn M B 1981 Can dietary beta carotenes materially reduce human cancer rate? *Nature* **290** 201-208.

Pilnik W, Voragen A G J 1991 The significance of endogenous and exogenous pectic enzymes in fruits and vegetables. In: *Food Enzymology,* ed Fox F P. Elsevier Applied Science, Vol 1, pp 303-335.

Pinsent B R W 1962 Peroxidase regeneration and its effect on quality in frozen peas and thawed peas. *Journal of Food Science* **27** 120 124.

Pinsky A, Grossman S M 1971 Lipoxygenase content and antioxidant activity of some fruits and vegetables. *Journal of Food Science* **36** 571-572.

Plat D, Ben-Shalom N, Levi A 1990 Changes in Pectic Substances in Carrot during dehydration with and without blanching. *Food Chemistry. Elsevier Science Publishers Ltd England.* ISBN No. 0308-814\90.

Porretta S, Capri G, Dall'aglio G, Ghizzoni C 1992 Use of UF for preparing improved tomato pulp. *International Journal of Food Science and Technology* **27** 427-433.

Poulson K P 1986 Optimization of vegetable blanching. *Food Technology* **6** 122-129.

Pruthi J S, Saxena A K, Manan J K 1980 Studies on the determination of optimum conditions of preservation of fresh vegetables in acidified sulphited brine for subsequent use in Indian style curries etc. *Indian Food Packer* **36** 9-16.

Racz M, Hajnal K 1976 Determination of pectinmethylesterase, polygalacturonase and pectic substances in some fruits and vegetables Part II – Study into the pectolytic tissue enzymes of Jonathan apple and some other fruits and vegetables. *Acta Alimentaria* **5** 189-204.

Rahman S 1995 *Food Properties Handbook*, CRC Press, Boca Raton, Florida.

Ramanuja M N, Vibhakara H S, Jayaraman K S 1988 Studies on the occurrence, partial purification and effect of heating on peroxidase in some vegetables. *Journal of Food Science and Technology* **25** 201-204.

Ramaswamy H S, Ranganna S 1981 Thermal inactivation of peroxidase in relation to quality of frozen cauliflower (var Indian Snowball). *Canadian Institute of Food Science and Technology Journal* **14** 139-143.

Ramteke R S, Singh N I, Rekha M N, Eipeson W E 1993 Methods for concentration of fruit juices, A critical evaluation. *Journal of Food Science and Technology* **30** 391-402.

Ranganna S 1986 *Handbook of Analysis and Quality Control of Fruits and Vegetable Products*, 2nd edition. Tata McGraw Hill Publishing, India, pp 31-65.

Ray M A 1991 The use of grape derived concentrate and aroma in winemaking. *Australian and New Zealand Wine Industry Journal* **6** 272-274.

Reed G 1975 *Enzymes in Food Processing.* Academic Press, New York, pp 237-254.

Richardson P 1985 Microwave food processing, *Technical Bulletin* (58) Campden Food Preservation Research Association, UK.

Richardson P 1990 Microwave heating: the food technologist's toolkit. Food *Technology International Europe* 65-68.

Robe K 1983 Hyperfiltration methods for preconcentrating juice save evaporation energy. *Food Processing* 100-101.

Robinson D S 1991 Peroxidases and their significance in fruits and vegetables. In: *Food Enzymology,* ed Fox P F. Elsevier Applied Science, London, Vol 1, pp 399-426.

Rombouts F M, Pilnik W 1978 Enzymes in fruit and vegetable juice technology. *Process Biochemistry* **8** 9-13.

Rosenberg U, Bogl W 1987a Microwave thawing, drying and baking in the food industry. *Food Technology* **6** 85-91.

Rosenberg U, Bogl W 1987b Microwave pasteurization, sterilization, blanching and pest control in the food industry. *Food Technology* **6** 92-99.

Rumm-Kreuter D, Demmel I 1990 Comparison of vitamin losses in vegetables due to various cooking methods. *Journal of Nutritional Science and Vitaminology* **36** S7-S15.

Ryynanen S, Ohlsson T 1996 Microwave heating uniformity of ready meals as affected by placement, composition and geometry. *Journal of Food Science* **61** 620-624.

Saldana G, Stephens T S, Lime B J 1976 Carrot beverages A research note. *Journal of Food Science* **41** 1243-1245.

Saldana G, Meyer R, Lime B J 1980 A potential processed carrot product A research note. *Journal of Food Science* **45** 1243-1245.

Sandhu K S, Bhatia B S 1984 Physico-chemical changes during preparation of fruit juice concentration. *Journal of Food Science and Technology* **22** 202-206.

Seow C C, Ng K B, Bourne M C 1992 Effect of blanching treatments on physicochemical properties of dehydrated carrots, celery and green beans. *ASEAN Food Journal* 7 184-188.

Siliha H, El-Zoghbui M, Labib A, Askar A 1995. Effect of enzymatic treatment of carrot puree. *Fruit Processing*. **10** 318-322.

Simon W P, Wolff X Y, Peterson C E, Kammerlohr D S, Rubatzky V E, Strandberg J O, Bassett M J, White J M 1989 High carotene mass carrot population. *Hortscience* **24** 174-175.

Simpson K 1983 Relative value of carotenoids as precursors os vitamin A. *Proceedings of the Nutrition Society* **42** 7-17.

Sims C A, Balaban M O, Matthews R F 1993 Optimization of carrot juice color and cloud stability. *Journal of Food Science* **58** 1129-1131.

Skudder P 1992 Long life products by Ohmic heating. *International Food Ingredients* **4** 36-41.

Skurray G R, Nguyen M H 1992 An evaluation of osmotically distilled grape juice. *The Australian Grapegrowers and winemakers: An annual technical issue*. 27-28.

Snir R, Koehler P E, Sims K A, Wicker L 1996 Total and thermostable pectinesterase in citrus juices. *Journal of Food Science* **61** 379-383.

Soderhall I 1995 Properties of carrot polyphenoloxidase. *Phytochemistry* **39** 33-38.

Soderhall I, Soderhall K 1989 Purification of prophenol oxidase from *Daucus carota* cell culture. *Phytochemistry* **28** 1805-1808.

Sreenath H K, Kogel F, Radola B 1986 Macerating properties of a commercial pectinase on carrot and celery. *Journal of Fermentation Technology* **64** 37-44.

Stauffer C E 1986 *Enzyme Assays for Food Scientists*. AVI Publishing, New York, pp 202-219.

Steel R G D, Torrie J H 1982 *Principles and Procedures of Statistics*. McGraw-Hill Kogakusha, New York.

Stephen T S, Saldana G, Brown H E 1974 Process for preparing carrot juice. US Patent 3787589.

Stephen, T. S, Saldhana, G, Lime, B J 1976. Neutralised carrot juice of acid treated carrots- A Research note. *Journal of Food Science* **41** 1245- 1246.

Stevens C, Khan V A, Tang A Y, Lu J Y 1990 The effect of ultraviolet radiation on mold rots and nutrients of stored sweet potatoes. *Journal of Food Protection* **53** 223-226.

Svanberg M S J, Nyman E M G L, Andersson R, Nilsson T 1997 Effect of boiling and storage on dietary fibre and digestible carbohydrates in various cultivars of carrots. *Journal of the Science of Food and Agriculture* **73** 245-254.

Swaminathan M 1987 Browning reactions in foods. *Food Science Chemistry and Experimental Foods.* The Bangalore Printing and Publishing Company, India, pp95-101.

Sweeney J P, Marsh A C 1971 Effect of processing provitamin A in vegetables. Journal of American dietetic association. *Food processing and vitamin A 238-242.*

Tee E S, Lim C L 1991 Carotenoid composition and content of Malaysian vegetables and fruits by the AOAC and HPLC methods. *Food Chemistry* **41** 309-339.

Temple N J, Basu T K 1988 Does β-carotene prevent cancer? A critical appraisal. *Nutrition Research* **8** 685-701.

Thakur B R, Singh R K, Tieman D M, Handa A K 1996 Tomato product quality from transgenic fruit with reduced pectin methylesterase. *Journal of Food Science* **61** 85-87.

Thomas R L, Westfall P H, Louvieri Z A, Ellis N D 1986 Production of apple juice by single pass metallic membrane ultrafiltration. *Journal of Food Science* **51** 559-563.

Thompson D 1991 The application of osmotic distillation for wine industry. *The Australian Grapegrower and Winemaker* **4** 11-14.

Truesdell D D, Whitney E N, Acosta P B 1984 Nutrients in vegetarian foods. *Journal of the American Dietetic Association* **1** 28-35.

Tucker G A 1996 Enzymes- Developing enzyme based processes. *International Food Ingredients* 23-26.

Umele N, Gabelman W H 1997 Inheritance of root colour and carotenoid synthesis in carrots. *Journal of the American Society of Horticulture Science* 7: 453-460.

Unklesbay N, Davis M E, Krause G 1983 Nutrient retention of portioned menu items after infrared and convective heat processing. *Journal of Food Science* **48** 869-873.

USDA 1975 *Enzyme inactivation test (frozen vegetables).* Technical inspection procedures for the use of USDA inspectors. U.S Department of Agricultural Marketing Services Washington, DC.

Valentas K J, Levine L, Clark P J 1990 Overview of food industry. In: *Food Processing Operations and Scale-Up.* Ch 2, Marcel Dekker, New York.

Vamos-Vigyazo L 1981 Polyphenol oxidase and peroxidase in fruits and vegetables. *Critical Reviews in Food Science and Nutrition* **15** 49-127.

Vamos-Vigyazo L, Nadudvari-Markus V 1982 Enzymatic browning, polyphenol content, polyphenol oxidase and peroxidase activities in pear cultivars. *Acta Alimentaria* **11** 157-168.

Vanderslice J T, Higgs D J, Hayes J M, Block G 1990 Ascorbic acid and dehydroascorbic acid content of food as eaten. *Journal of Food Composition and Analysis* **3** 105-118.

Velasco P J, Lim M H, Pangborn R M, Whitaker J R 1989 Enzymes responsible for off flavor and off aroma in blanched and frozen stored vegetables. *Biotechnology and Applied Biochemistry* **11** 118-127.

Walker, J B 1990 Reverse osmosis concentration of juice products with improved flavour. US Patent 4959237.

Walker J B, Ferguson R R 1990 Process to make juice products with improved flavour. US Patent 4933197.

Walker S J, Bows J, Richardson P, Banks J G 1990 Effect of recommended microwave cooking on the survival of *Listeria monocytogenes* in chilled retail products. *Tech. Memorandum No. **548**.* Campden Food and Drink Research Association, UK.

Wallrauch S 1984 Carotenoids. *Flussiges Obst* **51** 64-66.

Watanabe K, Saito T, Hirota S, Takahashi B 1988 Carotenoid pigments in Red, Orange and Yellow Root of carrot (*Daucus carota* L.) cultivars. *Nippon Shokuhin Kogyo Gakkaishi* **35** 315-320.

Warthesen J J, Vickers Z M, Whitney-West S, Wolf I D 1984 Cookery methods for vegetables: Influence on sensory quality, nutrient retention and energy consumption. *Home Economics Research Journal* **13** 61-79.

Whitaker J S 1995 Polyphenol oxidase. In: *Food Enzymes Structure and Mechanism*, Ch. 9, ed Wong D W S. Chapman and Hall, New York, pp 271-307.

Williams D C, Lim M H, Chen A O, Pangborn R M, Whitaker J R 1986 Blanching of vegetables for freezing- which indicator enzyme to choose. *Food Technology* **3** 130-140.

Wrolstad R E, McDaniel M R, Durst R W, Micheals N, Lampi K A, Beaudry E G 1993 Composition and sensory characterisation of red raspberry juice concentrated by direct osmosis and evaporation. *Journal of Food Science* **58** 633-637.

Yildiz F, Bozkurt M, Wiley R C 1993 Comparison of reverse osmosis and evaporation processing on the quality characteristics of tomato concentrates. *Food Control* **4** 155-158.

Zadow J G 1985 Reverse osmosis investigation-Project objectives. *The Australian Journal of Dairy Technology* **9** 84-85.

Zawistowski J, Biliaderis C G , Murray E D 1988 Isolation and some properties of an acidic fraction of polyphenol oxidase from Jerusalem artichoke (*Helianthus tuberosus* L.). *Journal of Food Biochemistry* **12** 23-35.

Ziegler R 1989 A review of the epidemiological evidences that carotenoids reduce risk of cancer. *Journal of Nutrition* **119** 116-122.

Zoueli M E, Esselen W B 1959 Thermal destruction rates and regeneration of peroxidase in green beans and turnips. *Food Research* **24** 119-133.

Appendix 3.1 Score sheet for sensory evaluation of carrot juice samples

Taste

Score	Taste property	A	B	C	D	E
1	Bitter					
2	Slightly bitter					
3	Moderate					
4	Slightly sweet					
5	sweet					

Panellist's preference of the taste

0% 25% 50% 75% 100%

Flavour

Score	Flavour property	A	B	C	D	E
1	No flavour					
2	Very mild					
3	Moderate					
4	Strong					
5	Very strong					

Panellist's preference of the flavour

0% 25% 50% 75% 100%

Colour

Score	Colour property	A	B	C	D	E
1	Very pale orange					
2	Pale orange					
3	Orange					
4	Bright orange					
5	Orange red					

Panellist's preference of the colour

0% 25% 50% 75% 100%

Mouthfeel

Score	Mouth feel property	A	B	C	D	E
1	Very watery					
2	Watery					
3	Moderate					
4	Slightly thick					
5	Very thick					

Panellist's preference of the mouth feel

0% 25% 50% 75% 100%

Appendix 6.1 – Fortran program for mathematical modelling

This program has been written for assessing the temperature profile in a carrot.
Program written by Prof.Graham Thorpe and Mahesh Prakash 10/08/1999

```
      include 'comcar.h'
      real length
      open(6,file='carin.dat',status='old')
      open(8,file='cartec.dat',status='old')
      OPEN(7,FILE='carmon.dat',STATUS='old')
      read(6,'(/)')
      read(6,*)length,radius
      write(*,*)"length radius"
      write(*,*)length,radius
      read(6,*)
      read(6,*)dz,dr
      write(*,*)"dz dr"
      write(*,*)dz,dr
      nz=length/dz+1
      nr=radius/dr+1
      nzm1=nz-1
      read(6,*)
      do 30 i=2,nzm1
   30 read(6,*)rz(i)
      write(*,*)"rz(i)"
      do 40 i=2,nzm1
   40 write(*,*)rz(i)
*
*     Determining the radial and axial distances with respect to postion
*     rr(j) represents the value of radius at a particular radial location.
*
      do 10 j=1,nr
   10 rr(j)=dr*(j-1)
      do 20 i=1,nz
   20 zz(i)=dz*(i-1)
      write(*,*)"rr(j)"
      do 50 j=1,nr
   50 write(*,*)rr(j)
*
*       Initialise temperatures
*
      do 1 j=1,nr
      t(1,j)=100.0
    1 t(nz,j)=100.0
c
        do 2 j=1,nr
        do 2 i=2,nzm1
    2   t(i,j)=20.0
```

```
*
*    Determine the radii along the length of the carrot
*    rz(i) represents the variation in radius in the axial direction
*
     do 3 i=2,nzm1
   3  nrz(i)=rz(i)/dr
*
*    Setting the value of temperature along the periphery of carrot
*         do 4 i=2,nzm1
         nrmax=nrz(i)+1
         do 4 j=nrmax,nr
   4      t(i,j)=100.0
*
*    Thermal diffusivity, alpha
*
     alpha=1.0e-7
     dt=0.1
*
*    Begin numerical calculations
*
     time=0
     do 12 k=1,12000
     time=time+dt
     do 5 i=2,nzm1
     nrmax=nrz(i)
      do 5 j=1,nrmax
      if(j.eq.nrmax) t(i,j)=100.0
      if(j.gt.1) then
      dt2dr2=(t(i,j+1)-2*t(i,j)+t(i,j-1))/dr**2
      dt2dz2=(t(i+1,j)-2*t(i,j)+t(i-1,j))/dz**2
      dtdr=(t(i,j+1)-t(i,j-1))/(2*dr)
      tnew(i,j)=t(i,j)+alpha*dt*(dt2dr2+1/rr(j)*dtdr+dt2dz2)
     else if (j.eq.1) then
      dt2dr2=(2*t(i,j+1)-2*t(i,j))/dr**2
      dt2dz2=(t(i+1,j)-2*t(i,j)+t(i-1,j))/dz**2
      dtdrbyr=dt2dr2
      tnew(i,j)=t(i,j)+alpha*dt*(dt2dr2+dtdrbyr+dt2dz2)
     end if
     t(i,j)=tnew(i,j)
   5  continue
     write(7,*)time,t(25,1)
  12    continue
*
*    outputs for plotting using tecplot
*
     write(8,*)'title="carrot plots"'
     write(8,*)'VARIABLES="rr" "zz" "t"'
     write(8,*)'ZONE F=POINT, I=', nz, ', J=', nr
     write(*,*)nr,nz
     do 7 j=1,nr
```

```
      do 7 i=1,nz
    7 write(8,11)zz(i),rr(j),t(i,j)
      stop
*
*     format statements
*
   11 format(1pe18.11,2x,1pe18.11,2X,1pe18.11)
      end
common
      1 rr(1000),rz(1000),zz(1000),nrz(1000),
      1 t(1000,1000),tnew(1000,1000)
c     Total length and greatest radius in metres
      length radius
      0.2    0.02
      dz     dr
      0.001  0.0005
      rz(i)
```

0.02	0.017	0.014	0.011	0.008	0.005	0.002
0.02	0.017	0.014	0.011	0.008	0.005	0.002
0.02	0.017	0.014	0.011	0.008	0.005	0.002
0.02	0.017	0.014	0.011	0.008	0.005	0.002
0.02	0.017	0.014	0.011	0.008	0.005	0.002
0.02	0.017	0.014	0.011	0.008	0.005	0.002
0.02	0.017	0.014	0.011	0.008	0.005	0.002
0.02	0.017	0.014	0.011	0.008	0.005	0.002
0.02	0.017	0.014	0.011	0.008	0.005	0.002
0.02	0.017	0.014	0.011	0.008	0.005	0.002
0.019	0.016	0.013	0.01	0.007	0.004	0.001
0.019	0.016	0.013	0.01	0.007	0.004	0.001
0.019	0.016	0.013	0.01	0.007	0.004	0.001
0.019	0.016	0.013	0.01	0.007	0.004	0.001
0.019	0.016	0.013	0.01	0.007	0.004	0.001
0.019	0.016	0.013	0.01	0.007	0.004	0.001
0.019	0.016	0.013	0.01	0.007	0.004	0.001
0.019	0.016	0.013	0.01	0.007	0.004	0.001
0.019	0.016	0.013	0.01	0.007	0.004	0.001
0.019	0.016	0.013	0.01	0.007	0.004	0.001
0.018	0.015	0.012	0.009	0.006	0.003	
0.018	0.015	0.012	0.009	0.006	0.003	
0.018	0.015	0.012	0.009	0.006	0.003	
0.018	0.015	0.012	0.009	0.006	0.003	
0.018	0.015	0.012	0.009	0.006	0.003	
0.018	0.015	0.012	0.009	0.006	0.003	
0.018	0.015	0.012	0.009	0.006	0.003	
0.018	0.015	0.012	0.009	0.006	0.003	
0.018	0.015	0.012	0.009	0.006	0.003	
0.018	0.015	0.012	0.009	0.006	0.003	