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DEVELOPMENT OF A FUNCTIONAL BEVERAGE FROM THE KEI APPLE FRUIT *DOVYALIS CAFFRA .*

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DIE ONTWIKKELING VAN 'N FUNKSIONELE DRANKIE VAN DIE KEIAPPEL VRUG (*DOVYALIS CAFFRA*)

Agtergrond en Motivering

Oor die afgelope twee dekades het die belangstelling in die gesondheidsvoordele van polifenole, spesifiek met betrekking tot chroniese siekte, gegroei. Die Keiappel (*Dovyalis caffra*) is 'n inheemse plant aan Suid-Afrika en is bes moontlik 'n ryk bron van polifenole. Hierdie studie het dit ten doel gehad om 'n funksionele drankie te ontwikkel van die Keiappel met 'n aantoonbare voordeel in terme van voedingswaarde, wat ter selfde tyd ook deur verbruikers op grond van sintuiglike kwaliteite aanvaar sou word. Die langertermyn doel van die projek is ook om die inkomste van kleinboere in landelike gebiede, sowel as stedelinge te verbeter deur die verbouing van Keiappels aan te moedig om uiteindelik ekonomiese voordeel daaruit te haal. 'n Deeglike literatuurstudie is onderneem en sluit onderwerpe soos funksionele voedsel, neigings in die ontwikkeling van drankies, wetgewing, polifenole as funksionele bestanddele met 'n fokus op vrugte en die lot van polifenole in die liggaam in. 'n Oorsig oor vrugteprosesserings- en preserveringstegnieke, asook wetgewing word gevolg deur 'n oorsig oor die ontwikkeling van nuwe produkte en die soort verbruikers wat in funksionele voedsels belang sou stel. Daar is ook gekyk na die rol van sintuiglike evaluering en verbruikersnavorsing in hierdie proses.

Metodes

Keiappels is in twee areas in Suid-Afrika versamel, naamlik tydens die 2002/2003 seisoen , in Bloemhof, Noordwes Provinsie, en tydens die 2004/2005 seisoen in die Oos- en Weskaap. Die eerste Keiappels is gebruik om 'n prototipe funksionele drankie te ontwikkel waaruit verdere ontwikkeling sou spruit. Die tweede oes van Keiappels is op industriële skaal verpulp, waarna daar op kleinskaal 'n kommersiële Keiappelsap vir verbruikerstoetse ontwikkel is. Verskeie geure is gebruik om die sterk kenmerkende smaak en aroma van die Keiappel te masker, waarna die moontlike opsies tot appel, vanielje en kruisement/vanielje verminder is. Verbruikerspaneeltoeste is deur 152 verbruikers, met behulp van 'n vooraf-getoetsde vraelys en hedoniese skaal vir algemene aanvaarbaarheid, volgorde van voorkeur, sowel as 'n reaksieskattingskaal vir voorneme om te verbruik en 'n rangorde toets vir voorneme om aan te koop, gedoen. Alle evaluerings is onder streng gekontrolleerde sintuiglike evalueringsvereistes / omstandighede uitgevoer en respondente van die Potchefstroom kampus, Noordwes-Universiteit, is gevra om anonieme vraelyste te voltooi.

Resultate en Bespreking

Statistiese ontledings is gedoen deur gebruik te maak van Statistica®, sewende weergawe. Ter samevatting van al die verbruikerstoetse, is daar bevind dat verbruikers die appelgeur statisties beduidend meer aanvaarbaar as die ander geure beskou het. Die appelgeur het in terme van smaak, algemene aanvaarbaarheid en voorneme om aan te koop en te verbruik, ook prakties betekenisvol beter gevaar as die ander twee geure. Daar was egter geen betekenisvolle verskille ten opsigte van enige van die veranderlikes, of geëvalueerde voorneme van verbruik of aankope tussen die vanielje en vanielje/kruisement geure nie. Die konsentrasie van totale polifenole is spektrofotometries gemeet en uitgedruk as galaatsuur ekwivalente per liter (GAE/L). Die konsentrasie van totale polifenole was effens laer as wat verwag is, waar die appel- en vanielje/kruisementgeur soortgelyke vlakke gehad het (101.7 en 106.1 GAE/L, onderskeidelik), terwyl die vanieljegeur 'n konsentrasie van 143.0 GAE/L gehad het. Hierdie verskil kan moontlik toegeskryf word aan die struktuur van die vanieljegeur wat ook 'n fenolstruktuur het. Die effe laer as verwagte polifenolvlakke sou ook die resultaat van afbraak gedurende prosessering kon wees. Daar was 'n duidelike verskil in die polifenolinhoud tussen die twee oeste uit verskillende areas. Die eerste oes was suurder gemeet in vergelyking met die tweede, soos bepaal deur die totale sitroensuurinhoud (4.81%w/w teenoor 2.54%w/w) en het 'n hoër suikerinhoud gehad (16.3°B teenoor 8.4°B). Hierdie verskil mag toegeskryf word aan verskeie faktore soos klimaat, grondtoestande en rypheid. 'n Kommersiële produk van die VSA, Ocean Spray® Cranberry, wat vir vergelykende doeleindes gebruik is, het meer polifenole en vry- en gebonde askorbiensuur as die ontwikkelde Keiappel produk. Redes vir die verlies aan vitamien C inhoud van die Keiappelsap is moontlik die hitte behandeling tydens pasteurisasie, asook die blootstelling aan oksidasie tydens die verpulpingsproses.

Gevolgtrekking en Aanbevelings

Die ontwikkeling van 'n funksionele drankie uit Keiappels is moontlik. Soos met meeste nuwe produkte word verdere, onoorkoomlike formule aanpassings benodig. Die verbruikerspaneel was positief oor die appelgeur drankie en verdere ontwikkeling behoort dus op hierdie geur te konsentreer. Die grootste struikelblok in die weg van sukses in hierdie projek is die afwesigheid van 'n effektiewe organiserende liggaam wat met die verbouers kan skakel ten einde 'n koöperasie te vorm en dus 'n volgehoue voorraad van Keiappels kan lewer. Die effek van variasies in oestyd, grondtoestande en klimaat moet ook beoordeel word. Die toename in inkomste vir boere kan slegs 'n moontlikheid word indien alle rolspelers saamwerk en 'n Keiappel-verbouerskoöperasie gevestig kan word.

Sleutelterme: Keiappel (*Dovyalis caffra*), funksionele drankie, polifenole, produkontwikkeling, sintuiglike evaluering deur verbruikers

SUMMARY

Background and Motivation

Interest has grown over the last two decades in the health benefits of polyphenols, with particular relation to degenerative diseases. The Kei apple (*Dovyalis caffra*) is an indigenous plant to South Africa, thought to be rich in polyphenols. This study aimed to produce a functional beverage from the Kei apple, with demonstrable nutritional benefits, which should also be found acceptable by consumers due to its sensory attributes. A long term aim of the study is to assist in improving income of farmers in rural and urban areas by encouraging the participation of small holders in growing the Kei apple for subsequent economic benefit. A thorough literature review was conducted on functional foods, trends for beverages, legislation and on polyphenols as a class of functional ingredients, specifically focusing on fruits and the fate of polyphenols in the body. A review of fruit processing procedures, preservation techniques and legislation, followed by new product development (NPD) and the types of consumers desiring functional foods were investigated as well as the role of sensory evaluation and consumer research were examined.

Methods

Kei apples were collected from two areas of South Africa, namely Bloemhof in the North West Province in the 2002/2003 growing season and from the Eastern and Western Cape in the 2004/2005 growing season. The first set of Kei apples were used to produce a prototype functional beverage, from which future development would take place. The second set of Kei apples were used for pulping on an industrial scale and then a small-scale commercial production batch of the Kei apple beverages for consumer panel testing was produced. Various flavours were used to mask the strong characteristic taste and aroma of the Kei apple for the beverage and these were narrowed down to apple, vanilla and mint & vanilla. Consumer panel testing was undertaken with 152 consumers with a pre-tested questionnaire using hedonic scales for overall acceptance, ranking for preference, a Food Action Rating Scale (FACT) for consumption intent, and a ranking test for purchase intent. All evaluations were carried out under strictly controlled sensory evaluation requirement and respondents were asked to fill in anonymous questionnaires at North West University, Potchefstroom Campus.

Results and Discussion

Statistical analysis of the results were determined by the Statistica® programme, version 7. In summary for all of the consumer testing, it was found that consumers regarded the apple flavoured Kei apple beverage statistically significantly more acceptable than the other flavours. The apple flavoured beverage was found to be rated practically significantly higher for the attributes of taste and overall acceptance, as well as for consumption intent, purchase intent and preference. There was, however, no significant differences in any of the attributes or evaluated consumption and purchase intent between the vanilla and mint & vanilla. The total polyphenols, determined by UV spectophotometry as gallic acid equivalents per litre (GAE/l), were found to be slightly less then expected, with the apple and mint & vanilla showing similar results (as GAE/l) 101.7 versus 106.1 for the latter two, whilst vanilla was 143.0 which may have been attributed to the vanillic acid molecule itself which also has a phenolic structure. This lower-than-expected level of total polyphenols may have been due to degradation during processing. There was a clear difference in the polyphenol content between the different harvests from the two regions. The former showed greater acidity as determined by total citric acid 4.81% w/w and sugar 16.3°B, versus 2.54% w/w and 8.4°B. This may be due to various factors of climate, soil and ripeness. For comparison purposes, a commercial product from the USA, Ocean Spray® Cranberry, was also found to have more polyphenols present than the Kei apple beverage. This was also demonstrated by the amount of ascorbic acid, free and bound. The Kei apple beverage subjected to pasteurization probably lost most of its vitamin C content due to the heat treatment and the exposure to oxygen during pulping.

Conclusion and Recommendations

The development of a functional beverage from the Kei apple is feasible. As with most new product developments, further formulation modifications which are not insurmountable are required. The consumer panel was positive towards the apple flavoured beverage and this flavour should be taken forward for future development. The key stumbling block to the success of this project is the lack of an effective organizing body that could liaise with the growers to form a cooperative and provide a consistent supply of Kei apples. The effect of variations in harvesting time, soil conditions and climate must also be evaluated. The improvement of income of farmers may only be achieved by the involvement of other stake holders and the formation of a Kei apple grower's co-operative

Key words: Kei apple (*Dovyalis caffra*), functional beverage, polyphenols, product development, consumer sensory testing.

TABLE OF CONTENTS

ABBREVIATIONS

ACF	Aberrant crypt foci
ANOVA	Analysis of variance
AOM	Azoxymethane
ASC	Ascorbate
BI	Bioactivity index
CAPE	Caffeic acid phenyl ester
CBG	Cytosolic β-glucosidase
CDC	Centre for Disease Control
CFSAN	Centre for Food Safety and Applied Nutrition
cfu/mL	Colony-forming units per millilitre
CHD	Coronary heart disease
CMC	Carboxymethyl cellulose
CML	Consulting Microbiological Laboratories (Pty) Ltd
COMT	Catechol-O-methyltransferase
CVD	Cardiovascular disease
DHA	Dehydroascorbate
DNA	Deoxyribonucleic acid
DPPH	Diphenyl-2-Picrylhydrazyl Radical
DSHEA	Dietary Supplement Health and Education Act
ECGC	Epigallocatechingallate
FACT	Food Action Rating Scale
FAO	Food and Agricultural Organisation
FAS	Fatty acid synthase
FDA	Food and Drug Administration
FFDCA	Federal Food, Drug and Cosmetic Act
FFE	Fuzzy front end
FMC	Fruit Machinery Corporation
FOSHU	Food for Scientific Health Use
FRAC	Ferric reducing antioxidant power
FRAP	Ferric Reducing Ability of Plasma
FUFOSE	Functional Food Science in Europe
GAE	Gallic acid equivalent
GJIC	Gap-junctional intracellular communication
GSPE	Grape seed proanthocyandin extract
HACCP	Hazard analysis critical control point
HDL	High density lipoprotein
HDPE	High density polyethylene
HIV	Human immuno-deficiency virus
HUT	Home usage trial

IFAVA	International Fruit and Vegetable Alliance
ILSI	International Life Sciences Institute
LDL	Low density lipoprotein
LPH	Lactase phlorizin hydrolase
MMP	Matrix methalloproteinase
NLEA	Nutrition Labeling and Education Act
NO	Nitric oxide
NPD	New Product Development
NRF	National Research Fund
NRF	National Research Fund
NW	North West
NWU	North West University
ORAC	Oxygen Radical Absorbance Capacity
OTC	Over the counter
PAA	Dihydroxy-phenylacetic acid
PASSCLAIM	Process for the Assessments of Scientific Support for Claims on Foods
PET	Polyethylene terephthalate
PROP	Propylthiouracil
PRP	Proline-rich proteins
PVC	Polyvinyl chloride
RDI	Recommended dietary intake
ROI	Return on investment
ROS	Reactive Oxygen Species
SA	South Africa
SULT	Phenol sulfotransferases
TA	Total acidity
TAA	Total antioxidant activity
TEAC	Trolex Equivalent Antioxidant Capaicty
TOSC	Total oxyradical scavenging capacity
UDPGT	Glucuronosyltransferase
USA	United Stated of America
UV	Ultraviolet
w/w	Weight for weight
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

DEVELOPMENT OF A FUNCTIONAL BEVERAGE FROM THE KEI APPLE FRUIT (*DOVYALIS CAFFRA*)

CHAPTER 1

INTRODUCTION AND AIMS

Currently, there are searches for indigenous fruits that have previously been ignored by the food industry and nutritionists as a whole. These include sources that have not necessarily previously been thought of outside their local community. For example, mocan seeds in the Canary Islands of Spain (Dueñas *et al.*, 2003); seabuckthorn in Europe and Asia (Geetha *et al.*, 2003); winery by-products (Gonzalez-Paramas *et al.*, 2004); pomegranate pith (which is considered a holy fruit in the Quran) (Kulkarni *et al.*, 2004); sea algae in Japan (Nagai & Yukimoto, 2003); oak acorn in Spain and Italy (Rakic *et al.*, 2005); prickly pear in Mexico, Latin America, South Africa and the Mediterranean (Ramadan & Mörsel, 2003); peanut skins in the USA (Yu *et al.*, 2004).

South Africa is considered to be a "hotspot" for biodiversity and more than 22,000 plants species occur within its boundaries. This represents 10% of the world's species, although the land surface of South Africa is less than 1% of the Earth. Despite the enormous richness in plant species, relatively few of these plants are economically utilized. Business ventures that have developed from the use of indigenous plants is the trade in medicinal and cultural plants, food crops and ornamental plants (Coetzee *et al.*, 1999). The Kei apple falls into the category of a food crop, although until now this has been limited to jams, jellies or eaten with large amounts of sugar. This has all been focused in small areas, close to where the Kei apple grows. When under-ripe, pickles may be made.

The Kei apple is one of eight species of *Dovyalis* found in Southern Africa, the others being *Dovyalis hispadula* (Bristly Sourberry) found in northern Mozambique, *Dovyalis longispina* (Coastal Kei Apple) found in northern Natal and Mozambique, *Dovyalis lucida* (Glossy Sourberry) found at medium altitudes in evergreen forests and at sea level in the Eastern Cape, *Dovyalis macrocalyx* (Shaggy Sourberry) found in Mozambique and Zimbabwean borders, *Dovyalis rhamnoides* (Sourberry) found near the coast in forested areas and inland in mountain evergreen forests, *Dovyalis rotundifolia* (Dune Sourberry) found in coastal scrub and dune forests, *Dovyalis zeyheri* (Apricot Sourberry) found in open woodland bushveld or

at the margins of evergreen forests. The fruits from all of the species are edible (Coates Palgrave, 2002:761-766).

The shrub or small tree may grow to a height of 9m with a spread of 7.5m, usually with many sharp spines. The leaves, often clustered on short spurs, are oblong-obviate, 2.5cm – 7.5cm long, glossy and short petioled. Pale yellow male and female flowers are usually borne on separate trees. The aromatic fruit is nearly round, bright yellow with a slightly downy, extremely tough skin. The flesh is mealy apricot textured, juicy and highly acidic, with 5 to 15 seeds, arranged in double rings in the centre. The flesh also has an astringent mouthfeel and taste, causing mouth puckering which, according to Miller & Ruiz-Larrea (2002), potentially means that the fruit is high in polyphenols.

Very little is known about the nutritional value of this fruit, but it has been reported to be rich in ascorbic acid 8.3mg/100g and consists of 3.7% pectin (Morton, 1987).

The Kei apple is native to the Kei River of South Africa and occurs abundantly, growing wild in the eastern regions of South Africa (Morton, 1987). Additionally, trees have been propagated in other areas of South Africa, namely Western Cape, Potchefstroom, Rustenberg and Bloemhof. The Kei apple grows well in almost any soil that does not have a high water table, is drought-resistant and tolerates saline soil and salt spray. It is also known to be highly resistant to pests. Generally, the plants bloom in spring and the fruits ripen from August to October (Morton, 1987), although it is thought, and has been seen in the field, that there may be a second crop that occurs.

With the above taken into consideration, the current project originated as part of an initiative from the Potchefstroom municipality and the FLAGH-project of the North-West University to plant trees in township areas, in an effort to improve the ecosystem of the environment, improve environmental awareness between residents, and as an effort to improve income for township dwellers, although at the point of tree planting, no final product had been developed.

As part of a previous project funded by the Innovation Fund of the NRF, orchards of the Kei apple were also established in the Western Cape. However, funding ran out as a successful project with Marula fruits took over (Cori Ham personal communication). The yield of the female trees (after about 2 years) is high and therefore could provide an additional income to

a family, or even income for a family currently obtaining no income, if the fruit could be made into a commercially viable product.

There is currently no literature or research about the Kei apple consumed for specific health reasons or for its inherent health benefits. This also encouraged the present study.

The aims of this study are therefore:

1. Develop (and confirm by panel testing) a product that is preferred by consumers and will be purchased due to its sensory attributes.
2. Develop a product that could increase consumption of fruit juice as part of improving overall diet and thereby increasing consumption of fruit.
3. Identify and quantify the constituents that are key to the success of the developed product's functionality by measuring the polyphenols and as ascorbic acid, using gas chromatography / mass spectophotometry.
4. Develop a product that has provable functional benefits that will assist in alleviating degenerative diseases.

CHAPTER 2

LITERATURE REVIEW

CHAPTER 2

LITERATURE REVIEW

INTRODUCTION

The scope of this project is wide, as it is concerned with the development of a functional beverage from the Kei apple (*Dovyalis caffra*). In order for the development of this product to be successful, it is necessary to have knowledge of several areas which are not necessarily seemingly connected, in a pure academic, nutritional and/or scientific arena.

A basic knowledge of functional foods and beverages is required and an understanding of where the consumer market is going. What are the future trends for beverages in particular? What are the types of consumers that desire these products and what are their needs? If the developed product is to be successful, it must sell. To sell, it must be desired by the consumer and taste acceptable. Additionally, the consumer must not be "duped". The product claim must be valid and legislation is therefore important, not just within South Africa but globally, as perhaps the product developed has potential outside these borders. Sensory and consumer testing therefore form a major role in the development of this proposed product.

Additionally, when formulating a functional product, it is imperative that the active ingredients that perform a clinical function and possess the nutritional value are known, thus a review of the literature has been performed, pertaining specifically to polyphenols in fruits and related products. The product development process and how it fits into bringing a product into the market place is an integral part of the development of products and will thus be discussed. Furthermore, the fruit juice manufacturing process and packaging thereof will also receive some attention in this review.

(NB: throughout this document, the use of page numbers in the references have been made when text books only have been used.)

The outline of the literature review is therefore as follows in the index.

Index

FUNCTIONAL FOODS

The primary role of diet is to provide sufficient nutrients to meet the metabolic requirements of an individual and to give the consumer a feeling of satisfaction and well being through hedonic attributes such as taste (Diplock *et al.*, 1999). However, nutritional science in the developed world is changing significantly, where there is a progression from adequate nutrition to optimal nutrition. As a result of this, functional foods (also sometimes termed nutraceuticals, medical foods or nutritional foods) are one of the major trends in the food industry in this new millennium (Sloan, 2000).

Whilst the East has been using foods to maintain the correct functioning of the body, the latest trend in Western nutrition is that of functional foods. Medical costs are rising globally and now the consumer is looking towards food (and nutritional supplements) to assist them in maintaining health and in self medication (Sloan, 2000). A similar philosophy was adopted by the Greek philosopher Hippocrates, who said 2,500 years ago "Let food be thy medicine and medicine be thy food" (Milner, 1998). People living in the East have always believed certain foods to be beneficial to health. Malaspina (1996) states that these beliefs are primarily anecdotal, based on centuries of tradition and largely under-documented by solid scientific research.

Now the West is catching up with these beliefs, and the key to success for food manufacturers is to develop products that are accepted by consumers and are consistent with the consumer's understanding and appreciation of functional foods within the existing culture. Because the state of the person's health may range from optimal to a state of disease, it is believed that functional foods have a major role to play in all states of health, including maintaining health and preventing disease (Verschuren, 2002).

Aruoma's model, as shown in figure 2.1 suggests that increasing the role of functional foods in the state between disease and health, may reduce the role of prescription and OTC (over the counter) medication drugs.

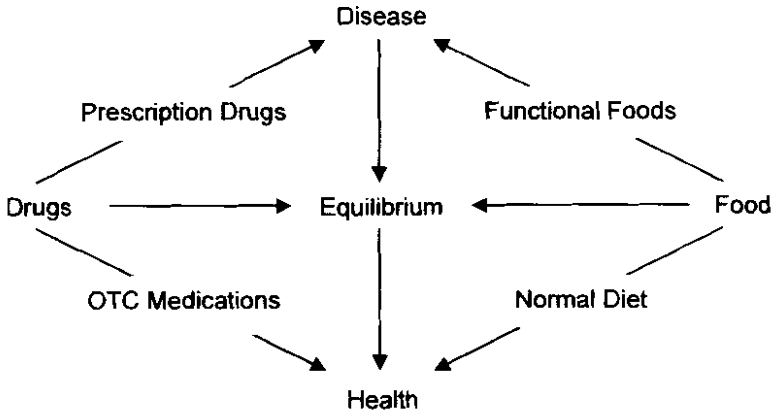


Figure 2.1: Functional relationships between healthy state and disease state and the role that food and drugs might play in the management of health (Aruoma, 1999).

Definitions of Functional Foods

All foods are functional to some extent because all foods provide taste, aroma and nutritive value. However, foods are now being examined intensively for added physiological benefits which may reduce chronic disease risk or optimum health – that is, functional foods (Hasler, 2002). The concept was developed in Japan in the 1980's, when escalating health costs caused the government to introduce a regulatory system to approve certain foods with documented health benefits, in the hopes of improving the health of the nation's aging population. These foods bear a special seal, FOSHU (Food for Scientific Health Use), so that consumers may recognise them as a food for a specific use (Hasler, 2002). However, there is no universally accepted definition of functional foods (Hasler, 2002; Katan & De Roos, 2003).

Perhaps the best definition has come from the work by Diplock *et al.* (1999), who state that a food can be regarded as functional if it has satisfactorily demonstrated to effect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease. Functional foods must remain foods and they must demonstrate their effects in amounts that can normally be expected to be consumed in the diet: they are not pills or capsules, but part of a normal food pattern. The work carried out by Diplock *et al.* (1999) was part of the International Life Sciences Institute (ILSI, Europe) which co-ordinated the European Commission Concerted Action on Functional Food Science in Europe (FUFOSE).

Taking into account the many definitions (not mentioned here for simplicity), there may be many misunderstandings regarding whether a food is functional or not, and can lead to an increasingly permeable interface between food and drugs (Andlauer & Furst, 2002).

For the purposes of this review, a functional food will be accepted as being, according to Diplock *et al.* (1999), a natural food, a food to which a component has been added or removed by technological or biotechnological means. It can also be a food where the nature of one or more components has been modified or in which the bioavailability of one or more components has been modified, or a combination of all of these. A functional food might be functional for all members of a population or for particular groups of the population, which might be defined, for example, by age or genetic constitution.

Legislation

Three US legislative acts of the 1990's have had a significant effect on the promotion of the concept of functional foods. These are firstly the Nutritional Labelling and Education Act passed in 1990, which mandated that the Food and Drug Administration establish regulations requiring most foods to have a uniform nutrition label and also established circumstances under which claims about content and disease prevention could be made about nutrients in foods. Secondly, the Dietary Supplement Health and Education Act (DSHEA) of 1994 defined dietary supplements as any products that contain one or more dietary ingredients and it created a mechanism for dealing with safety issues, regulation and health claims, labelling of dietary supplements, provided for good manufacturing practices and encouraged research on dietary supplements. Lastly, the FDA (Food & Drugs Association) Modernisation Act of 1997 amended the Federal Food, Drug and Cosmetics Act by allowing health claims that are not pre-authorised by the FDA if the claims are based on "authoritative statements" of government agencies such as the Nutritional Academy of Sciences or the Nutritional Institute of Health (Milner, 2000).

The Federal Food, Drug and Cosmetics Act as amended, does not provide a statutory definition of functional foods. Thus, the FDA has no authority to establish a formal regulatory category for such foods. In marketing such foods, manufacturers are responsible for safety requirements, labelling requirements and the appropriate petition or notification procedures for ingredients in their products (Ross, 2000). The onus, therefore, is on the manufacturer to be responsible, which opens many loopholes for misleading claims from manufacturers and

adds no value to the consumer. Table 2.1, extracted from the FDA website and Centre for Food Safety and Applied Nutrition, shows the allowable health claims passed by the FDA.

Table 2.1: Summary of health claims in labelling a food (or dietary supplement) in the United States (http://www.cfsan.fda.gov/cgi, visited 07/07/05).

Approved Claims	
Calcium and osteoporosis Sodium and hypertension Dietary fat and cancer Dietary saturated fat and cholesterol and risk of coronary heart disease (CHD) Fibre-containing grain products, fruits and vegetables and cancer Fruits, vegetables and grain products that contain fibre, particularly soluble fibre and risk of CHD Fruits and vegetables and cancer Folate and neural tube defects Dietary sugar alcohol and dental caries Soluble fibre and certain foods and risk of CHD Soy protein and risk of CHD Plant sterol/stanol esters and risk of CHD	
Claims Authorised Based On Authoritative Statements By Federal Scientific Bodies	
Whole grain foods and risk of heart disease and certain cancers Potassium and the risk of high blood pressure and stroke	
Qualified Claims	
Cancer:	Selenium Antioxidant vitamins
Cardiovascular disease:	Nuts and heart disease Walnuts and heart disease Omega-3 fatty acids and CHD B vitamins and vascular health Mono-unsaturated fatty acids from olive oil and CHD
Cognitive function:	Phosphatidylserine and cognitive dysfunction and dementia
Neural tube defects:	0.8mg folic acid per day

A health claim is an explicit or implied characterisation of a relationship between a substance and a disease or a health related condition. This type of claim requires significant scientific evidence and must be authorised by the FDA. A health claim differs from a structure/ function claim, which does not require pre-approval by the FDA. A structure/function claim describes the role of a substance intended to maintain the structure or function of the body (FDA, 2005). A qualified health claim is supported by less scientific evidence than an authorised health claim. FDA requires that qualified claims be accompanied by a disclaimer that explains the level of the scientific evidence supporting the relationship. Unlike authorised health claims, no regulations for qualified health claims are issued by the FDA.

The legislation for functional foods and foods in general is not globally consistent. Several countries, including the USA (United States of America) and Europe, have developed a coherent body of food related legislation. They have also attempted to agree on common rules at an international level. Codex Committees are established by the Food and Agricultural Organisation (FAO) and World Health Organisation (WHO), for example, the Codex Committee on Food Labelling of Pre-packaged Foods. The commonly defined rules can then be implemented in the participating countries on a voluntary basis. The main mission of Codex Alimentarius is to protect consumer's health and to ensure fair practices in international food trade. Overall, the Codex activities have strongly influenced the patterns of national food laws (Cheftel, 2005). With regard to health claims, the General Guidelines on Claims of the Codex Alimentarius establish the principle that food should not be presented in a manner that is false, misleading or deceptive; nutritional claims have guidelines, but health claims guidelines remain in draft (Hawkes, 2004:v).

In Europe (as opposed to the United States of America), labeling requiring the nutritional content of the product is not compulsory unless a nutrition (or health) claim is made on the label or in the presentation or advertising material of a food (Cheftel, 2005). One of the most general rules of European (and other) legislations can be stated as "no misleading of the consumer" – the protection of the consumer's interests being one of the principles in food laws. The PASSCLAIM project (Process for the Assessments of Scientific Support for Claims on Foods) is intended to provide industry, academics, consumer groups and regulators with the means to evaluate the scientific basis for health claims (Verschuren, 2002). In Japan, a scientific regulatory framework concerning FOSHU made it possible to make limited health claims after receiving approval from the Ministry of Health in 1991. Legislation is lacking in Latin America, but this is done on an ad hoc basis (Verschuren, 2002).

Currently, legislation in South Africa is sketchy, falling under the Department of Health's Foodstuffs, Cosmetics and Disinfectants Act 1972 (Act No. 54 of 1972), Regulations Relating to Labelling and Advertising of Foodstuffs. These regulations are in the process of being updated and revised to take into account global trends and ensure that claims are correctly substantiated and nutrition labelling is correct. The South African draft regulations have been published for comment. However, the final regulations are eagerly awaited. It is thought that these will be modeled on the USA and European guidelines, with strict requirements for efficacy data to avoid loopholes that have been seen in the USA. Table 2.2 outlines the global overview of national regulation and health claims.

Table 2.2: Health claims regulations in 74 countries and areas, by category (Hawkes, 2004:v).

Claims making reference to disease are specifically prohibited	Specified disease risk-reduction claims permitted	Nutrient function and/or other function claims are permitted	Specific framework to permit product-specific health claims	No regulations specific to health claims
Australia (a) Austria (b) Belgium (c,h,q) Brunei Darussalam Costa Rica (c,p) Denmark Ecuador (c) Finland (d) France (h) Germany (e) Greece Honduras (c) Israel (a) Italy Japan (f) Luxembourg Lithuania Malaysia Morocco Netherlands (c,h,q) New Zealand (a) Nigeria (c,p) Portugal Republic of Korea Singapore (c) Spain (h) Switzerland Thailand United Kingdom (h,n) Vietnam (e,k)	Brazil Canada (g) China Indonesia Philippines Sweden (h) United States	Brazil Canada (g) China Belgium (h) Denmark Finland France (h) Germany Greece India (i) Italy Japan (f) Malaysia Poland (j) Netherlands (h) Republic of Korea Spain (h) Singapore Sweden (h) Thailand United Kingdom (h,n) United States Vietnam (k)	Japan (f) Netherlands (h) Sweden (h)	Argentina Bahamas Bahrain Bangladesh Barbados (a) Belize Bermuda Bosnia and Herzegovina Botswana Dominican Republic Chile Croatia (l) Egypt El Salvador Guatemala Hong Kong, SAR (o) Hungary Jordan Kenya Kuwait Mauritius (m) Mexico Nepal Netherlands Antilles Oman Pakistan Paraguay Peru Qatar Saudi Arabia South Africa (a) Turkmenistan United Arab Emirates Uruguay Venezuela

Key
(a) regulations on health claims currently under development
(b) unless pre-approved by the government
(c) only health claims referring to the preventative and/or curative and/or therapeutic nature of foods are prohibited
(d) three permissible function claims allow reference to disease risk-factor reduction
(e) except for dietetic foods
(f) function claims are allowed to mention an improved effect on preliminary stage of disease
(g) policy is currently being developed on product-specific health claims
(h) some form of self-regulatory system for health claims is in place
(i) all foods with false claims are prohibited, but implied nutritional information and health claims are allowed
(j) must be pre-approved
(k) all implied claims must be truthful
(l) health claims are not regulated but are not desired
(m) all false claims on foods are prohibited
(n) the self-regulatory organization has approved claims that refer to disease, but these are not permitted to be used on food products
(o) regulations on nutrient function claims are currently under development
(p) foods with health claims referring to diseases are regulated as medicines
(q) the self-regulatory codes would allow reference to disease risk reduction but no claims have been approved

Consequently, when formulating a functional food that may also have global potential, many legislative considerations have to be taken into account, which at this stage are far from consistent. What is clear is that legislation bodies are tightening up on claims made to

consumers, to ensure that they are truly valid. Therefore, when formulating a new beverage, this must all be accounted for.

Trends In Functional Foods and Beverages

Development of functional food products will continue throughout the 21st century, as consumer demand for healthy products grows (Milner, 2000). Factors contributing to the increase of functional foods in the supply chain include: 1) an aging population; 2) increasing health costs; 3) self-efficacy, autonomy in healthcare and an awareness and desire to enhance personal health; 4) advancing scientific evidence that diet can alter disease prevalence and progression and 5) changes in food regulations.

Sloan (2004) summarises the top 10 functional food trends, including global and US trends. The key points arising from this article (relevant to this review) are purchases of functional foods have increased from 31.0% in 2000 to 57.2% (2001) and 57.7% (2002 and 2003), whilst 'fat-free' and 'low fat' foods have remained stagnant. Fortified food sales have increased. Health consciousness is increasing, for example fruit and vegetable consumption has increased, but not sufficiently. The consumption of carbohydrates has declined with the re-awakened popularity of the Atkins diet (which has impacted on sales of fruit juice, as the carbohydrate content of a glass of fruit juice contains more than is recommended by this regime, although the Atkins Company has recently filed for bankruptcy protection (http//www.nutraingredients-u8sa.com/news/ng.asp?id=61666, 09/08/05). There is an increased focus on healthier foods for kids and the snacks they are consuming are becoming more nutritious and products aimed at reducing chronic lifestyle diseases are being demanded by consumers. The beverage market, especially those products offering functional benefits, is growing on the energy products and especially energy drinks are becoming more popular, with flavour variations becoming more adventurous and innovative.

Restaurants (including fast food chains) are becoming more health conscious and less meat is being consumed, whilst gourmet and organic trends are emerging. Focus on reducing heart disease is increasing, and greater numbers of probiotic products such as Europe's Actimel and Dan Active are being introduced (compared with Woolworths SA introduction of probiotic orange juice with HOWARU™ probiotic cultures, and their range of cultured yoghurts and Danone's Actimel yoghurt).

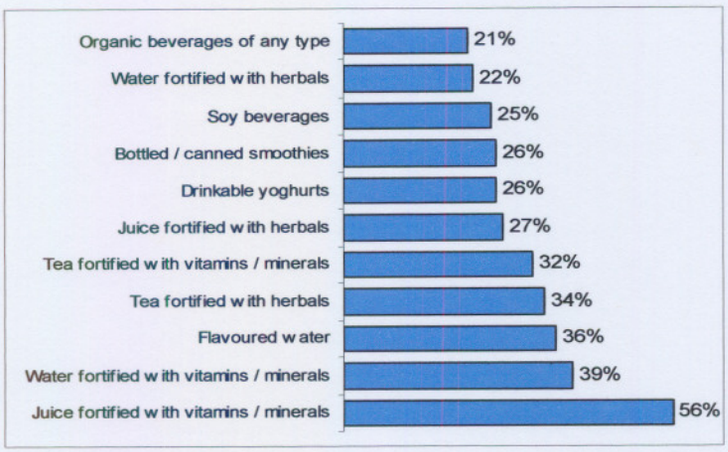


Figure 2.2: Research undertaken with wellness consumers who would consider purchasing various beverages (Hartman, 2003, adapted by Sloan, 2004).

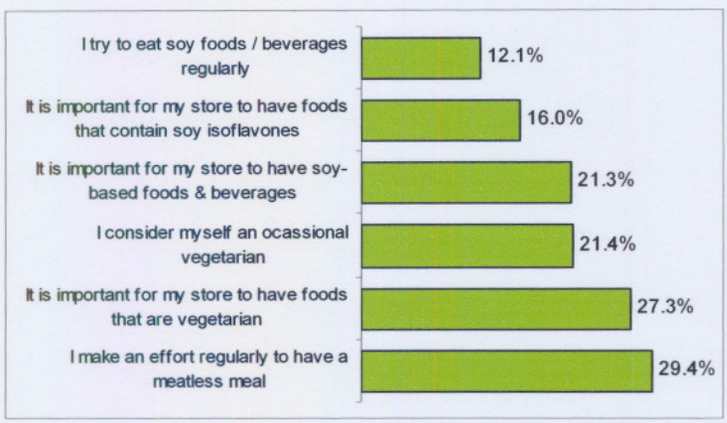


Figure 2.3: Interest in vegetarian products (consumer research undertaken by NMI, 2003, quoted by Sloan, 2004).

Figures 2.2 and 2.3 show examples of research undertaken in 2003 in the USA with consumers. Figure 2.2 clearly indicates that the consumer is ready to adopt more interesting, functional, fortified beverages. Figure 2.3 shows research undertaken with consumers, not retailers, which indicates that consumers are interested in moving away from meat and improve their efforts to eat "meatless" on a more regular basis (Sloan, 2004). It seems as if the consumer is therefore ready to adopt new concepts and is likely to adopt innovative new products. However, it is clear from the aforementioned research and the discussed legislation that the foods / beverages must have a provable nutritional benefit.

Katan and De Roos (2003) point out that if there are clear and strict standards for efficacy and safety of functional foods, then the market has a long-term future. Westrate *et al.* (2002) explain that the following factors need to be taken into consideration in the development of

such functional foods: 1) consumer understanding: what kind of health benefits are required? 2) bioinformatics: what molecules could do the function? 3) *in vitro* and *in vivo* testing; 4) bioavailability; 5) functional food technology: can the ingredients be sourced easily and made attractive food? 6) biomarkers: can relevant effects be measured in man? 7) human intervention studies: does it really work? 8) communication: how do we explain the benefits?

Perhaps, most importantly, it should be remembered that if functional foods are to be successful, they should be desired by the consumer (that is, they should taste good). Drugs will sell, whatever they look and taste like, but foods need taste, convenience and appeal or else they will not be consumed (Katan & De Roos, 2004). They must also provide sufficient consumer value to sustain long-term consumption (Walzem, 2004). In addition, it must be emphasised that these foods are not magic bullets or panaceas for poor health habits. Diet is only one part of a comprehensive approach to good health.

The focus of this project is functional beverages, therefore it is worthwhile discussing briefly the current state of the market and the types of products that make up this market.

New Product Trends – Beverages

Mellentin in 2003 estimated that US sales of functional foods exceeded $18.2 billion in 2001, growing at more than 8% yearly (representing 3.5% of the total food market). Front-line strategic management predicted sales in excess of $327 billion by 2005. Sales of fortified beverages more than tripled between 1997 and 2001. Functional beverages represent another $7 billion and growth rates are upwards of 12% per annum.

Table 2.3: Non-alcoholic beverage introductions (new products introduced globally) for all categories of non-alcoholic beverages (table combined from several global new products databases dating from 2000 to 2004) (Global New Products Database, www.gnpd.com/sinatra/gnpd&lang=uk//).

	2000	2001	2002	2003	2004	Total
Beverage Concentrations and Mixes	522	563	773	882	809	3549
Carbonated Soft Drinks	243	378	486	736	626	2469
Energy and Sports Drinks *	208	312	407	478	429	1834
Hot Beverages	1149	1637	1876	2398	2180	9240
Other Soft Drinks	120	108	199	198	162	787
RTD Iced Tea and Coffee	263	309	525	697	717	2511
RTD Juices and Juice Drinks	984	1402	2049	2501	2517	9453
Water	231	322	429	551	596	2129
Total	3720	5031	6744	8441	8036	31972

* Sports products claimed specifically through scientific analysis to improve sporting performance or recovery afterwards, energy drinks said to improve energy or stimulation boost.

Beverages are split in consumer marketing terms into several categories. These are water, carbonated soft drinks, energy and sports drinks, beverage concentrates and mixes, ready-to-drink (RTD) juices, and juice drinks. Common trends are apparent within these categories and these include the fortification with vitamins and minerals, flavours and reduced calorie drinks. Ingredients also include ginseng, guarana, echinacea, kava roots and ginko biloba extract. Smoothies (a thickened beverage) are becoming popular globally as a meal replacement, often with the addition of soy. Carbonated soft drinks are becoming less popular and being replaced by water, and their consumption has even been banned in Edinburgh (Anonymous, 2004a) and California (Foreyt, 2005). This is presumably a trend that will spread as the global concern for increased obesity continues and the effort to reduce it. Cranberry is increasing in popularity, and organic products are emerging. Marketing gimmicks and characters such as Winnie the Pooh, are common to attract children's sales by encouraging their parents to purchase these products.

Mellentin (2003) states that if a company is a producer of consumer goods and if the ingredients can't be "formulated" into a beverage, 50% of the potential market share in functional foods may be lost. The big success stories of the functional world (see figure 2.4) have been the new beverage brands Yakult, Actimel, Red Bull, Contrex and Pro Viva, while (with the exception of bars), new functional foods have failed to fizz.

Figure 2.4: Examples of new products (Mellentin, 2003).

The reasons for the success are possibly because most health-oriented drinks are based on carriers which have intrinsically healthy images with consumers – water, juice or yoghurt. They also offer convenience, can be grabbed "on the go", consumed as a mid-morning snack and do not require a change in eating habits.

There is potential for beverages to grow in other directions: 1) liquid breakfasts – providing all nutrients (20-30% of consumers give traditional breakfast a miss – and this figure could be conservative), 2) enhanced waters, and 3) liquid dietary supplements (as discussed previously). The potentials are huge for the food manufacturer. However, the question remains – how many of these beverages are truly functional. How many would pass the necessary PASSCLAIM legislation if they were required to? How many of these products are actually good marketing and advertising? How many provide definitive, evidence-based nutritional benefits? The trends shown by Sloan (2004) are encouraging in that there is a definite steer away from meat (figure 2.3) and a trend to vegetarian products with these consumers who are health conscious, but is the food industry negatively capitalising on this trend?

It is clear that the market is extremely active and has huge potential. However, looming legislation (as previously described) may result in the downscaling or even removal of some of these claims. There is a proliferation of products in the market place and a great deal of confusion is being placed in the mind of the consumer due to indiscriminate marketing. Even the consumer who has generally a greater disposable income is confused.

Therefore, there is a huge opportunity for the development of a truly functional fruit juice, with sustainable health claims, that will be able to fit into this market, which has already been primed to readily accept new product introductions. Key to the long-term success of the product is the truly provable evidence-based functional claims.

In this project, the researchers are dealing with a fruit that is indigenous to South Africa, has potential nutritional benefits and has been underutilized in the past for the small-scale production of jam and jellies. The following section on fruits and vegetables deals with their importance in the diet as a result of its functional ingredient contents, as well as why it is important to include them (and thus potentially the Kei apple) at greater quantities in the diet than we currently consume.

FRUITS AND VEGETABLES AND THEIR IMPORTANCE IN THE DIET

The aim of this section of the review is to show that fruits and vegetables are important in the diet and, though far from exhaustive, studies have revealed that the inclusion of fruits and vegetables in the diet will reduce the risk of chronic disease. It is estimated that, if just

dietary guidelines were met, globally 2.7 million deaths per annum would be avoided (Tohill, 2005).

The effect of fruit and vegetable intake on risk for coronary heart disease in The Nurses Health Study and the Health Professionals Follow-Up Study as reported by Joshipura *et al.* (2001), showed that consumption of fruits and vegetables had a protective effect against coronary heart disease (CHD). This study followed up 84,257 women, 34 to 59 years of age, for 14 years; and 2,148 men, 40 to 75 years of age, for 8 years. Hu and Willett (2002) recommend that a diet containing an abundance of fruit and vegetables (plus non-hydrogenated unsaturated fats, whole grains and adequate omega-3 fatty acids) offers protection against CHD. A diet rich in plant foods has long been recommended to supply a large source of phytochemicals, preventing coronary heart disease (Craig, 1997, Visioli *et al.*, 2000).

In addition to numerous *in vivo* and *in vitro* studies, many organizations such as the British Heart Foundation (2004), the United States Department of Health and Human Services and the United States Department of Agriculture in their jointly published Dietary Guidelines for Americans (Anonymous 2005), British Nutrition Foundation (2004) and Department of Health, Republic of South Africa (2003), supported by Association of Dietetics in South Africa and the Nutrition Society of South Africa, to name a few, recommend the increased intake of fruit and vegetables in the diet (at least 5 portions per day). It is fair to say that most government food policies contain a phrase that relates to the consumption of at least five or more portions of fruit or vegetables per day.

Nel and Steyn, 2001 (http://www.mrc.ac.za.chronic/foodstudies.html) report that the consumption of fruit and vegetables in South Africa is low. These authors analysed data from studies performed from 1983 to 2000 and showed that the only fruit and vegetables consumed were grapes, apples, orange juice, peaches, bananas, carrots, pumpkin, tomatoes and onions, and only at very low levels.

Ruel, M.T., *et al.* (2005) report that fruit and vegetable consumption in Sub-Saharan Africa is low and that this low fruit and vegetable consumption is the main contributor to micro-nutrient deficiencies in the developing world.

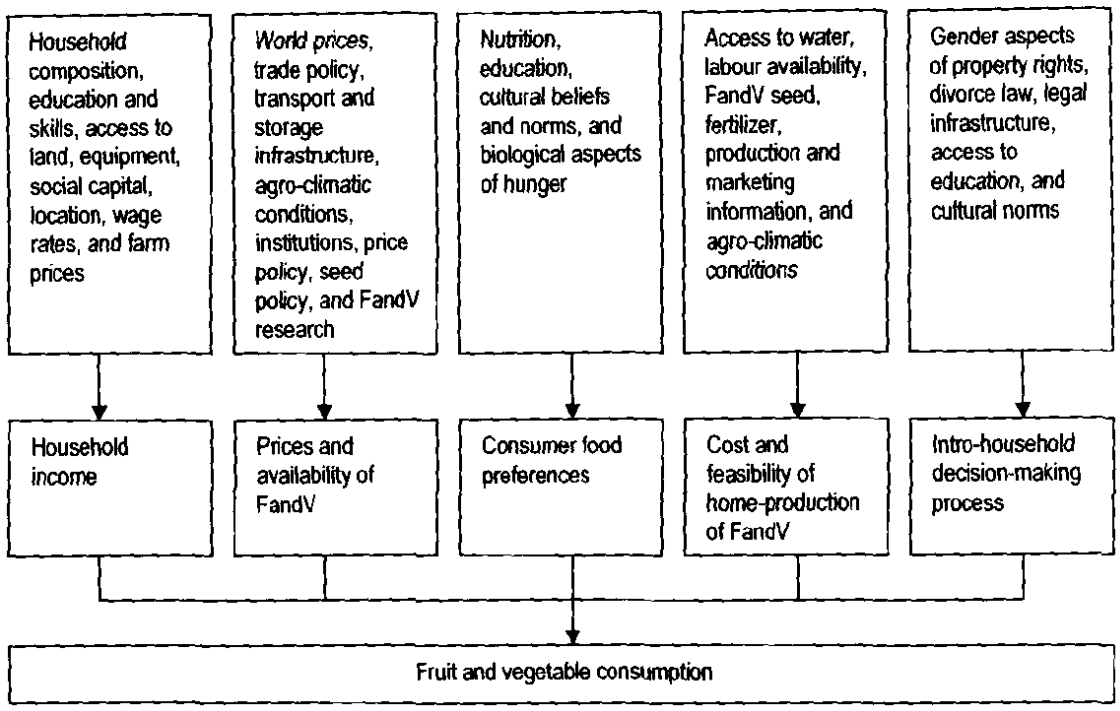


Figure 2.5: Conceptual framework of determinants of fruit and vegetable consumption Ruel, M.T., *et al.* (2005).

Ruel, M.T., *et al.* (2005) also give a good framework that leads to the fruit and vegetable consumption in a given household as demonstrated in figure 2.5. The challenge for any stakeholder in the fight to increase fruit and vegetable consumption is to intervene in the determining factors as outlined in the figure. Thus, for example, if access to water, labour availability, fruit and vegetable seed fertilizer, production and marketing information and agro-climatic conditions were provided or increased, the farmer (or small holder) would be able to produce fruit and vegetables at lower prices and make them available at a lower cost to the market, or have the facilities to produce for themselves. If household income is increased, money becomes available for fruit and vegetables, whereas especially in developing countries and regions like sub-Saharan Africa, the focus for the family is often on less expensive staple starchy foods such as maize meal.

Some other key points arising from the work of Ruel, M.T., *et al.* (2005), relevant to this current project are:

- Home production: promotion of home production of fruit and vegetables is one potential strategy to increase their consumption at the household level. Production interventions need to be complemented by effective education and behaviour-change strategies to achieve a significant impact on consumption, which fits in with the aims of this project.

- The consumption of fruit and vegetables in the 10 sub-Saharan African countries studied ranges from 27kg to 114kg per person per year, far below the recommended 146kg per person per year. Thus, again, the project could increase fruit consumption per capita.
- The demand for fruit and vegetables rises with increasing income, although at a slower rate than income. The income elasticities for fruit and vegetables range from 0.60 to 0.97 and are generally higher for fruit than vegetables. Thus, as people become richer, their demand for fruit increases before vegetables, another motivating factor for this project.
- In most of the countries studied, female-headed households spend significantly more on fruit and vegetables, particularly on vegetables, than male-headed households.

The recent World Health Organisation (WHO) report on Chronic Life style Diseases issued on 5th October 2005 (http://www.who/int/chp/chronic-disease) advocates that one of the ways to decrease the increasing global epidemic of chronic disease deaths is to increase fruit and vegetable intake (http://www.who/int/chp/chronic-disease,report/part4:37-137) through initiatives such as the "5-a-day" programme, including more fruits and vegetables in school meals. At the recent International Nutritional Conference in Durban (September 2005), the International Fruit and Vegetable Alliance (IFAVA) was launched (ifava.org) which aims to encourage and foster efforts to increase the consumption of fruit and vegetables globally for better health by supporting national initiatives, promoting efficiencies, facilitating collaboration and shared aims and providing global leadership, all of which is based on well documented and scientific publication.

The above only emphasizes the importance of producing a beverage that can form part of one of the recommended portions to increase fruit and vegetable intake daily and that will entice its consumption yet add nutritional benefits as a scientifically proven functional beverage.

POLYPHENOLS, THEIR CLASSIFICATION, CHEMISTRY, EFFICACY STUDIES, CONTENT IN FRUITS, HYBRIDS, METABOLISM AND SAFETY

As stated before, fruits and vegetables are very important in the diet, but what is it that makes them so important? This section of the review aims to identify the biologically active components that make fruits and vegetables so vital in the daily diet.

There are many actives that can provide functionality in fruits and vegetables, for example, vitamins, some minerals and fibre. However, of main concern for this thesis are the polyphenols, and for that reason the main area of focus of this review will be the classification of polyphenols, their function and efficacy studies, specifically concentrating on fruits as it is thought that the Kei apple is rich in polyphenols. The advantages and disadvantages of polyphenols are covered, through an assessment of the literature available since the 1980's, which consists of a mixture of reviews and efficacy studies. Although by no means exhaustive, this literature review attempts to assess polyphenols through the results of well designed trials, epidemiology studies or clinical trials and reviews. It aims to show that they are beneficial to human health.

Classification of Polyphenols

Phenol is an important component of all plants. It is synthesized from L-tyrosine or L-phenylalanine via the shikimate acid pathway (Robards & Antolovich, 1997). The enzymes for this pathway are not found in animal cells and, therefore, animals do not manufacture phenol nor break it down. However, they may accumulate phenols in their tissues. Plant-derived phenols in the diet include simple phenols (hydroxybenzoic acids), phenylpropanoids (hydroxycinnamic acids) and flavonoids. Phenols impart an astringent taste to fresh food (Miller & Ruiz-Larrea, 2002). Figure 2.6 demonstrates the biosynthetic pathway leading to various flavanoid classes that occur in plants.

Phenols are produced by plants for a variety of reasons, such as UV protectants, pigments and hormones. They play a significant role in how the plants interact with other organisms in the environment (Miller & Ruiz-Larrea, 2002, Wildman, 2001:23, and Robards & Antolovich, 1997). Phenols produced by a number of plants appear to play a defensive role, providing anti-feeding activity against insect pests, or act as fungicides (Straney *et al.*, 2002, Woo *et al.*, 2002). It has also been hypothesised that some flavonoids may be essential for the symbiosis existing in plant root systems, protecting against root growth deterioration (Vierheilig & Piché, 2002:23). Taylor and Miller, 2002 indicate that specific flavonols are required to induce pollen germination and tube growth in plants. Examples of these include kaempferol and quercetin in petunias. A variety of proteins may be labelled with a flavonol affinity probe including receptors, transport proteins and flavonol biosynthetic enzymes.

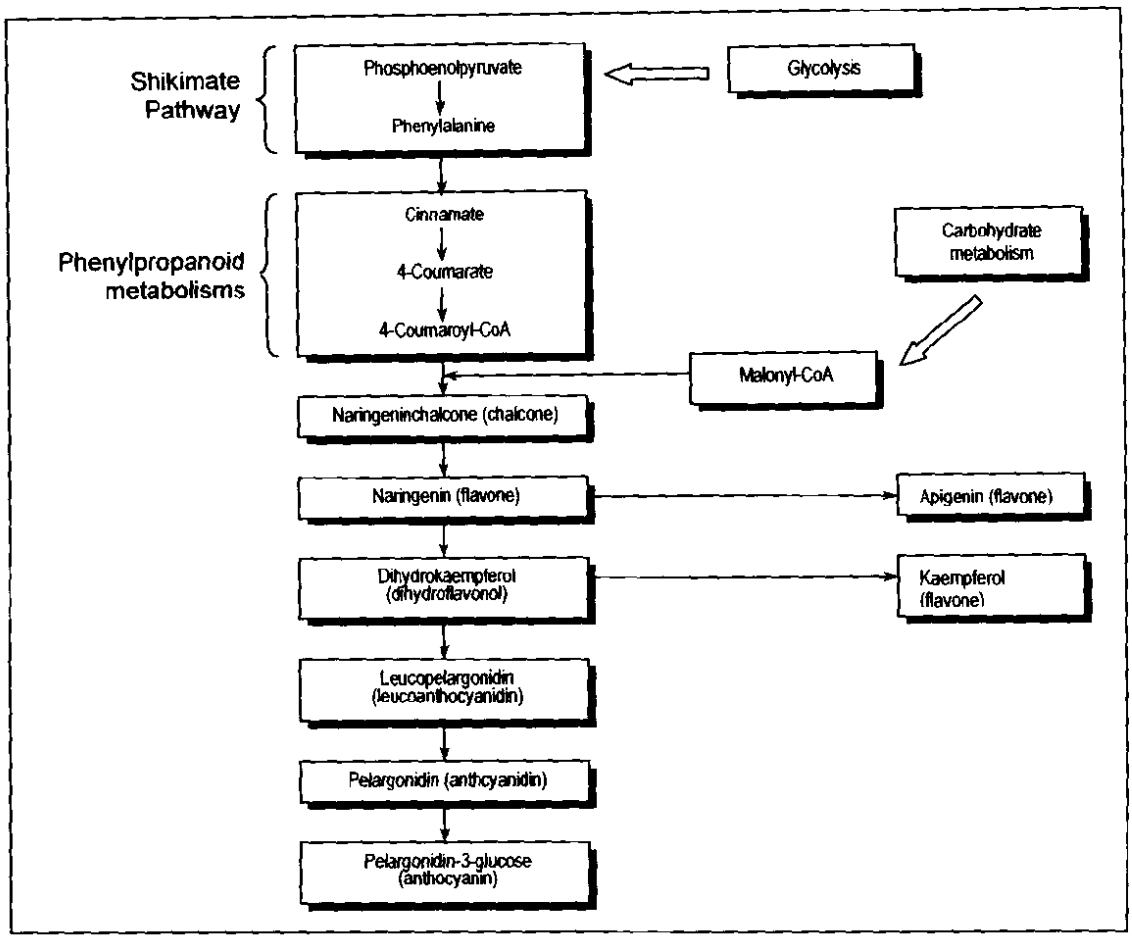


Figure 2.6: Biosynthetic pathway leading to various flavonoid classes (Robards & Antolovich, 1997)

Polyphenols are abundant nutrients in the diet and evidence for their role in the prevention of degenerative diseases has emerged over the last 20 years (Manach *et al.*, 2004, Bravo, 1998).

Polyphenols may be classified into different groups as a function of the number of phenol rings that they contain and of the structural elements that bind these rings to one another. Distinctions are thus made between the phenolic acids, flavonoids and stilbenes (see figure 2.7). There are two classes of phenol acids that can be distinguished, namely benzoic acid and cinnamic acid derivatives. The hydroxybenzoic acid content of edible plants is generally very low, with the exception of certain red fruits, black radish, onion and tea. Due to the fact that the hyroxybenzoic acids (both free and esterified) are found in only a few plants eaten by humans, they have not been extensively studied.

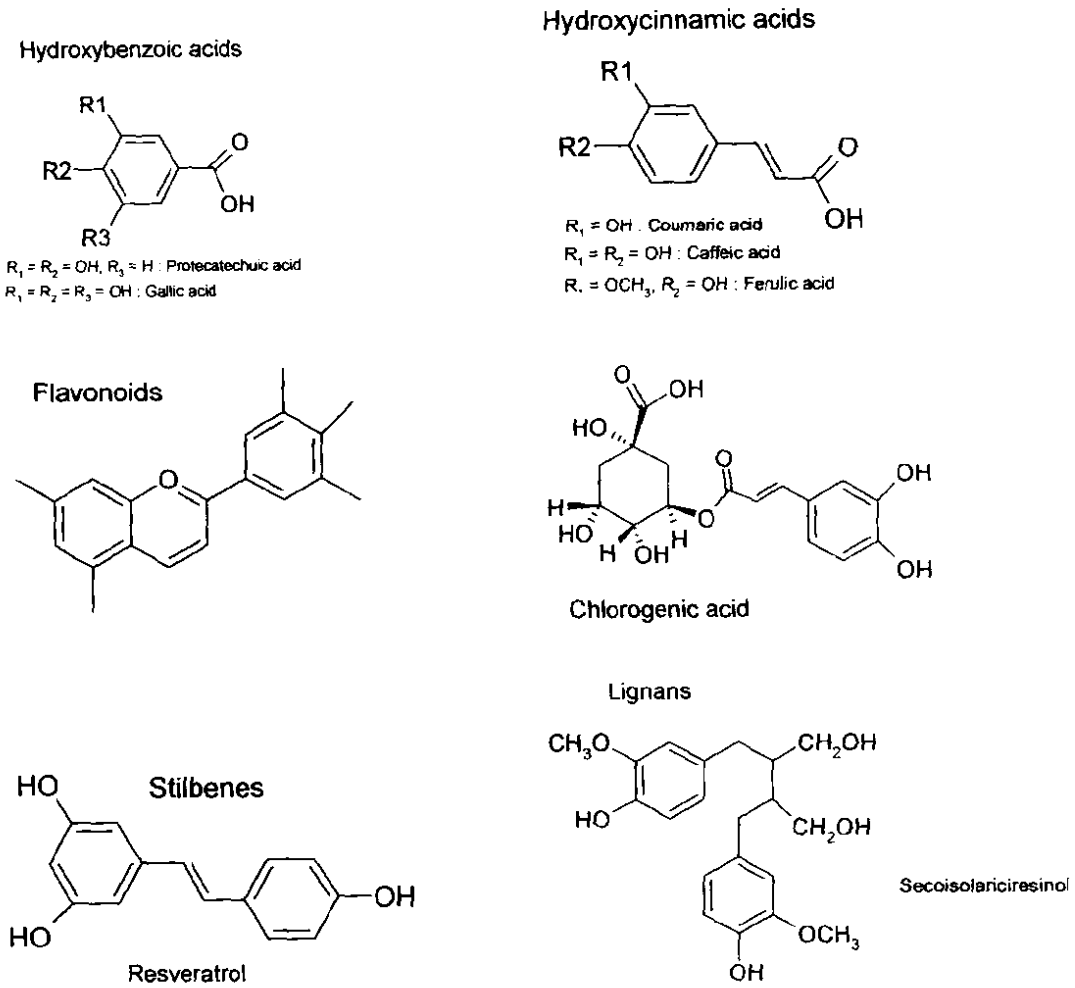


Figure 2.7: Chemical structures of polyphenols (Manach *et al.*, 2004).

The hydroxycinnamic acids are more common than the hyroxybenzoic acids and consist chiefly of p-coumaric, caffeic, ferulic and sinapic acids. Caffeic and quinic combine to form chlorogenic acid found in many types of fruit, coffee and legumes (Manach *et al.*, 2004, Miller & Ruiz-Larrea, 2002).

Figure 2.8 shows the chemical structure of the common dietary hydroxyl benzoic and hydroxycinnamic acids and their typical dietary sources (Miller & Ruiz-Larrea, 2002)

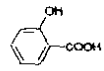
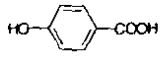
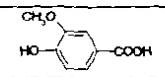
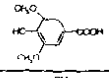
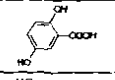
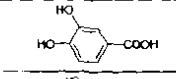
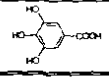
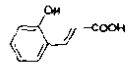
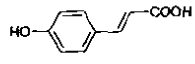
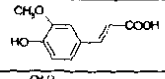
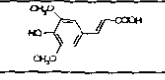
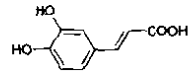
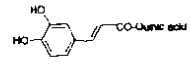
Name	Structure	Typical Dietary Source
Dietary Hydroxybenzoic Acids		
salicylic acid		anise, dill, white mustard, allspice, rosemary, thyme, marjoram
p-hydroxybenzoic acid		raspberry, gooseberry, pecans, anise, fennel
vanillic acid		vanilla, garden cress, paprika
syringic acid		rosemary, basil, thyme, garden cress
gentisic acid		mace, dill, bay leaf, tarragon, clove, absinthe
protocatechuic acid		tarragon, clove, anise, cinnamon, blackberry, blueberry
gallic acid		tea, nuts, olive oil
Dietary Hydroxycinnamic Acids		
o-coumaric acid		cherry, plum
p-coumaric acid		most fruits (esp. blueberry, raspberry and pineapple), apple, tomato, grape, olive
ferulic acid		grains, nuts, turmeric, peppers, citrus fruits, tomato, cabbage, asparagus
sinapic acid		Brussels sprouts, potatoes, rapeseed, trace amounts in citrus, pineapple, tomato
caffeic acid		grape, apple, plum, tomato, eggplant, cabbage, asparagus, endives, potatoes (the most abundant hydroxycinnamic acid)
chlorogenic acid		apple, pear, peach (and most fruits), tomato, coffee

Figure 2.8: Chemical structure of dietary hydroxybenzoic and hydroxycinnamic acids (Miller & Ruiz-Larrea, 2002).

The flavonoids can be further classified into sub classes according to the function of the type of heterocycle involved, that is, flavonols, flavones, isoflavones, flavanones, anthocyanidins and flavanols, *as demonstrated in figure 2.9.*

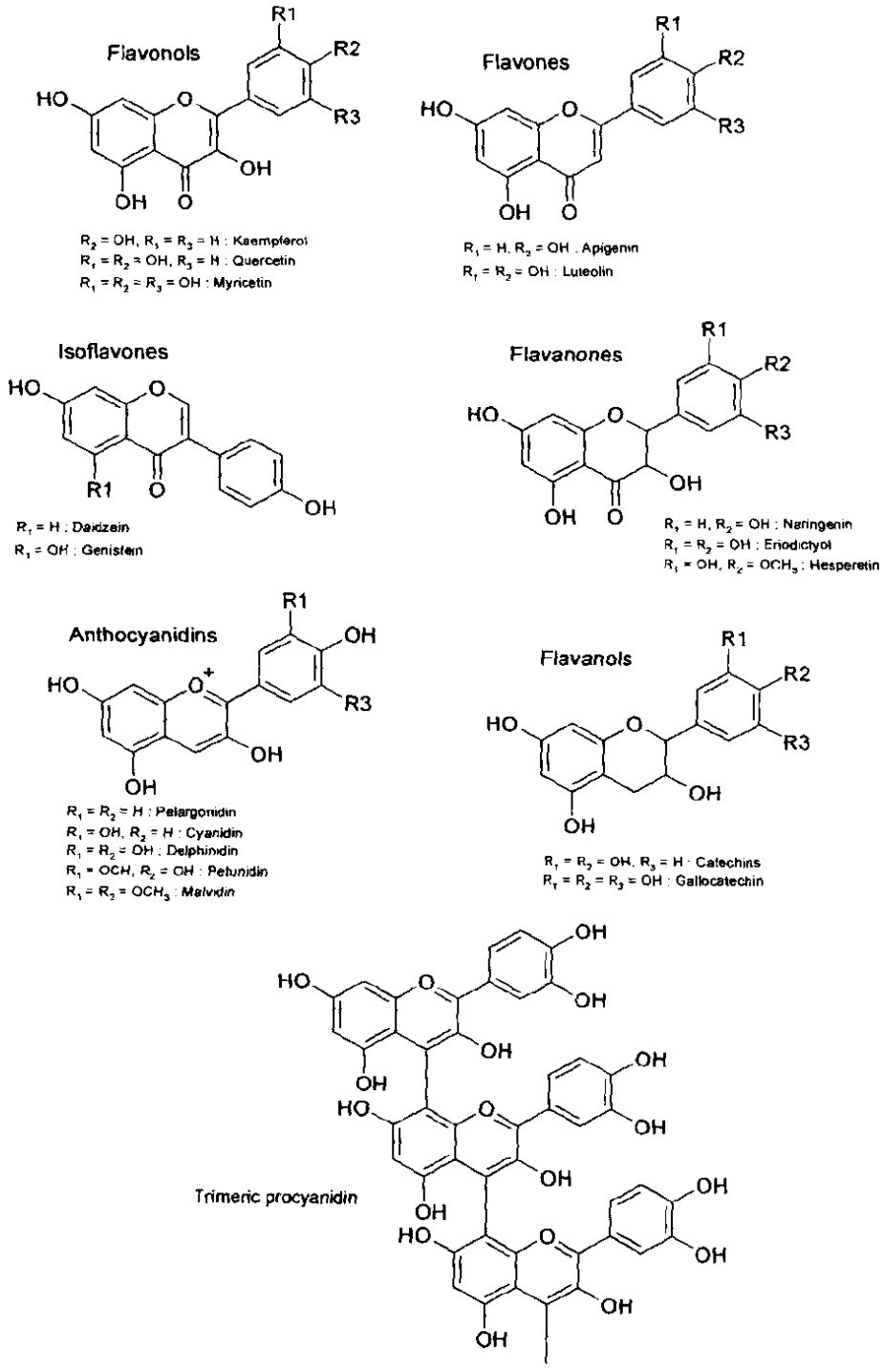


Figure 2.9: Chemical structures of flavonoids (Manach *et al.*, 2004).

Flavonols are the most ubiquitous flavonoids in foods and the main representatives are quercetin and kaempferol, generally present in the glycosylated form. They accumulate in the skin and leaves of the plant. Flavones are less common, and usually exist as glycosides of luteolin and apigenin. Flavones are found mainly in citrus fruits, most commonly as hesperetin and naringenin. Isoflavones have similar structures to estrogen and have pseudohormonal properties, including the ability to bind to estrogens. They are thus classified as phytoestrogens. Flavonoids exist in the monomer form (catechins) and polymer form (proanthocyanidins), with the former being found in many types of fruit, red wines, green tea and chocolate. Proanthocyanidins (also known as tannins) are dimers, oligomers or polymers of catechins, and are known to impart an astringent taste to food. Anthocyanins have pigments imparting colour to a fruit or vegetable (Manach *et al.*, 2004).

Lignans (see figure 2.7) are formed of 2 phenyl-propane units and are found mainly in linseed and to a smaller extent in other foods, primarily pears and prunes (Manach *et al.*, 2004). Stilbenes, another form of polyphenols, are found in low quantities in the diet but resveratrol, found in wine, has shown to have anti-carcinogenic effects and anti-thrombotic effects (Zbikowska *et al.*, 1999, Manach *et al.*, 2004).

Polyphenols and Human Health

Usually, new associations are discovered by epidemiological studies, either cross-sectional or prospective. To establish a causal relationship, further testing is then required, which often includes *in vitro* and *in vivo* testing with human cells and/or animals, based on a specific isolated chemical constituent. Human clinical intervention trials, which may be large (with several hundred individuals) or small (less than 100 individuals), are also undertaken to determine the effect of the active ingredient under investigation and yields important information in terms of the causal relationship between the dietary component and the risk of particular disease.

This experimental process leads to the creation of a body of evidence, which then enables scientists and regulators to draw a conclusion about a dietary component, for example, flavonoid and its ability to affect human health. The conclusion reached must be based on sound scientific evidence that may be duplicated at any time if required, statistically sound and free from bias. If all these criteria are met, then the concluding body of evidence (at that time) may result in a statement, for example "polyphenols are beneficial to human health and

their inclusion in the diet in the form of increased intake of fruit and vegetables should be encouraged".

It was with the aforementioned in mind that a review of the literature relating to polyphenols and their impact on human health was undertaken. A full review of the topic falls outside the scope of this thesis but the evidence provided should be viewed as parts of the picture forming the body of evidence showing the role of polyphenols in human health. The reader is encouraged to refer to the original publication if further details of a particular study are required.

General Efficacy Studies

Recently, there has been an exponential increase in the interest in polyphenols and health and for this reason only a few recent reviews have been studied, which give an overall picture of polyphenols and health.

Hertog (1996) pioneered much of the work on polyphenols in the last decade. In this review, he studied various polyphenols, mainly quercetin, in epidemiological (human) tests and found that the epidemiological evidence on potential health properties of flavonoids in high levels could reduce the risk of cardiovascular disease but was not an important determinant of cancer risk. Arts & Hollman (2005) in their review on polyphenols and disease risk in epidemiological studies suggest that there are beneficial effects of flavonoids on cardiovascular disease but not cancer (with the exception of lung cancer) and there is a need for more research.

Dietary polyphenols and the prevention of disease have been reviewed by Scalbert *et al.* (2005). In summary, these authors conclude that polyphenols support a role in the prevention of cardiovascular disease, cancer, neurodegenerative disease, diabetes or osteoporosis. In cardiovascular disease, they are thought to exert anti-thrombotic effects, inhibit platelet aggregation and modify lipid metabolism by reducing LDL (low density lipoprotein) and improving endothelial dysfunction. Polyphenols have been well documented in animals as having anti-carcinogenic effects, through various mechanisms, and have been muted as useful adjuncts in chemotherapy or radiotherapy treatments. There is an inverse association between the intake of polyphenols and neurodegenerative diseases, although this is not strong, whilst the evidence of the effects of polyphenols on glycaemic or diabetes risk is still very little. Prevention of or lowering of osteoporosis has been found to be

positively associated with polyphenol intakes, especially with those polyphenols related to soy beans, already a high source of calcium. Scalbert *et al.* (2005) go on to conclude that, whilst these health benefits have positive associations, studies have often been undertaken at higher levels than those normally documented in humans and caution should be taken when interpreting the results. More work on further epidemiological and clinical intervention trials is required.

A meta-analysis of tea consumption in relation to stroke, myocardial infarction and all coronary heart diseases based on 10 cohort studies and 7 case-control studies was conducted by Peters *et al.* (2001). Myocardial infarction rates were found to decrease (11%) with an increase in tea consumption of 3 cups per day, but there appeared to be some bias in reporting and variation in geographic areas which may be related to the type of tea and strength brewed. Less conclusive, although positively associated results were achieved with the meta-analysis for stroke and other coronary heart diseases.

Some specific studies, although far from exhaustive, are mentioned below.

Epidemiological Studies

Polyphenols and the prevention of cardiovascular diseases has been reviewed by Manach & Williams (2005). They conclude that a reduction in risk of cardiovascular disease is associated with consumption of polyphenols, which is mainly observed by endothelial function and haemostatic response. However, the exact nature of the most active compounds remains largely unknown. Absorption, metabolism and elimination vary widely between the polyphenols and data on bioavailability must be taken into account to improve experimental design and the interpretation of the observed effects.

Flavonoids in all sources of diet (tea, soy, fruit and vegetables) were studied by Le Marchand (2002) who found that they have a protective effect but more work was required. Biomarker studies were required and more human clinical, not just epidemiological, trials were also required. Peterson *et al.* (2003) studied flavonoids utilising a food frequency questionnaire and found that flavonoid intake and breast cancer risk in a case control study in Greece had a statistically strong significant inverse association with flavone intake for breast cancer, but there was no association for the rest of the classes of flavonoids. The flavonoids quercetin, kaempferol, myricetin, apigenin and luteolin were studied by Sesso *et*

al. (2003) as part of a Women's Health Study. The authors found that there was a non-significant positively reduced risk of cardiovascular disease in women, for broccoli, apples and tea, but no association with any specific flavonols. Knekt *et al.* (2002), as part of a Finnish Mobile Clinic Health Examination Survey conducted during 1966-1972, studied quercetin, kaempferol, myricetin, naringenin and hesperitin. They found that chronic disease risk may be lower at higher dietary flavonoids intakes (for example, ischaemic heart disease, cerebrovascular disease, lung and prostate cancer, type 2 diabetes and asthma). Quercetin was found to be most effective.

Intervention Studies

It is clear that there are insufficient of these, however, Winkler *et al.* (2004) studied German commercially available fruit or fruit/vegetable juices supplemented in the diet for 16 weeks *in vivo* and found lymphocyte proliferation and apoptosis in HIV-seropositive and healthy subjects. The study concludes that the supplementation with fruit and vegetables could be favourable to HIV positive patients. Manach & Williams (2005) reviewed 24 intervention studies and found the antioxidant status of plasma (vitamin E & C, β-carotene) was raised by ingestion of polyphenols over 12 weeks, but contrasting results were found with other studies. Six studies over 1-13 weeks showed improvements in blood lipids (decrease in total cholesterol, increase in high-density lipoprotein (HDL) and apoplipoprotein A-1), with the ingestion of tea, virgin olive oil, cocoa, soybean or red clover, but six studies showed no effects on lipids. These authors conclude that further intervention studies should include a detailed assessment of the bioavailability of polyphenols. Besides clinical trials carried out with polyphenol-rich food, more studies with pure polyphenols are also needed to establish their role in the prevention of disease. Further intervention studies will be discussed under the fruits section of this literature review.

Mechanistic

The examples that follow, whilst showing only a handful of the studies available, can only give an indication of how the specific polyphenol under investigation may act, and can therefore not be taken in isolation but rather as the starting point for future work.

Examples of mechanistic studies include Alexandrakis *et al.* (2003), who studied the flavones kaempferol, morin, myricetin and quercetin *in vitro* with human leukemic mast cells and found that flavones inhibit proliferation and increased mediator content in human leukemic mast cells. The order of highest effect (in decending order) was flavone, kaempferol, quercetin, myricetin, morin, showing that the structure of the flavonoid clearly has an influence. Epigallocatechingallate (ECGC), luteolin, quercetin, kaempferol, apigenin, taxifolin, along with other polyphenols were studied by Brusselmans *et al.* (2005) for their ability to inhibit cancer cell lipogenesis. These polyphenols were found to be particularly effective and it is thought that their ability to inhibit fatty acid synthase (FAS) is the key to induce apoptosis in cancer cells. The flavonoids quercetin, myricetin, fisetin, luteolin were studied *in vitro* by Dajas *et al.* (2003), who found that these flavonoids showed potential to protect against strokes. Virgili *et al.* (2004) studied the flavonoids naringenin and quercetin and found they blocked cancer cell growth by anti-estrogenic activity.

Okuda (2005) attempted to study many traditional medicinal plants and investigated the tannin content of these plants. This researcher found that the tannin structures were often very specific in their function and health benefit. This vast piece of research covered medicinal plants globally and re-emphasises the continuing interest in polyphenols globally. Structurally related flavonoids were studied *in vitro* by Rusak *et al.* (2005). These authors found that cell cycle progressional apoptosis of human acute leukemia cells was affected by the structure of flavonoids C2-C3 double bond and 6-hyroxyl group important structural requirements for cystostatic effects 6-hyroxyl group unfavourable.

Overall, the body of evidence suggests that there are positive health benefits to consuming polyphenols in the diet. However, what was clear from reviewing this part of the literature was that there was a lack of integration of results and few intervention trials. Due to the complex nature of foods itself, it is difficult to isolate any one component from another. Then the question must be asked – does one component act on its own or in synergy with others? What was the base health state of the population being studied? *In vitro* and *in vivo* experimentation cannot always be compared (Haenen *et al.*, 2005), due to the metabolism products produced during *in vivo* and the unnatural situation of *in vitro* experimentation. Both types of experimentation, however, have their role to play.

Caffeic Acid

Caffeic acid is the most common hydroxycinnamic acid in fruits (chapter 2:24). This constituent has been the subject of several studies that have been found to be positively associated with chronic disease improvement. The studies reviewed below are examples of mechanistic studies which give indications as to how caffeic acid may work in the body. Most of the studies are lacking *in vivo*, and there is a clear need for well designed clinical intervention studies. However, it is critical to mention these studies here as the main constituent of Kei apple is thought to be caffeic acid, and caffeic acid, if present in sufficient quantities and with or without the presence of other flavonoids may have potential health benefits, which was one of the motivating factors for investigating the Kei apple.

Caffeic acid was studied *in vitro* by Ahn *et al.* (1997), who concluded that it may be effective as a chemosensitizing agent in multi-drug resistant MCF-7/Dox human breast carcinoma cells. Caffeic acid was studied *in vitro* by Bassil *et al.* (2004) who found that caffeic acid, in the presence of L-cysteine, potentiates antiradical activity but that the reducing capacity is lowered. The effect of caffeic acid and caffeic acid phenyl ester (CAPE) on hepatocarcinoma cells was investigated by Chung *et al.* (2004). Caffeic acid and caffeic acid phenyl ester were found to successfully suppress the growth of HepG2 tumour xenographs in nude mice *in vivo*. Subcutaneous and oral administrations of caffeic acid and caffeic acid phenyl ester significantly reduced liver metastasis, and it is postulated that this is through the selective suppression of the angiogenic enzyme matrix methalloproteinase MMP-2/9.

Frank *et al.* (2003) studied hydroxycinnamic caffeic acid and chlorogenic acid *in vivo* and found that vitamin E and cholesterol concentrations were elevated in Sprague-Dawley rats. Protocatechuic, p-coumaric, caffeic, ferulic, sinapic, vanillic, methoxycinnamic acids and tricin were studied *in vitro* by Hudson *et al.* (2000), who concluded that extracts of brown rice were shown to inhibit the growth of human breast and colon cancer cells. Tricin was found to be most effective. Kampa *et al.* (2004) studied caffeic, ferulic, protocatechuic, 3,4-dihydroxy-phenylacetic acid (PAA), sinapic acid and syringic acid *in vitro*. Inhibition of T47D human cancer cells was found with acids studied in the order effect listed. Caffeic and 3,4-dihydroxy-phenylacetic acid induced apoptosis, 3,4-dihydroxy-phenylacetic acid induced an inhibition of nitric oxide synthase. Caffeic acid competes for binding and results in an inhibition of aryl hydrocarbon-induced CYP1A1 enzyme. There was a dose dependant result – the higher the dose, the higher the level of inhibition.

Park *et al.* (2005) studied caffeic acid *in vitro* and found that MMP-9 was inhibited by caffeic acid – one of the enzymes known to degrade type IV collagen, the major constituent of the basement membrane in cancer invasion and metastasis. Sodium caffeate was studied *in vitro* by Xu *et al.* (2004), who concluded that it inhibited tumour monostasis and had anti-angiogenic effects.

POLYPHENOL CONTENT AND EFFICACY STUDIES OF FRUITS AND VEGETABLES

Efficacy Studies of Fruits Specifically

This project is aimed specifically at the formulation of the Kei apple into a beverage. Therefore, it was essential that research concerning the polyphenol content and efficacy of the polyphenol constituents of fruits specifically be investigated and included in this review. Wherever possible, literature was sought that focused on a specific named fruit. A short historical background has been given on the origin of the fruit, indicating how it was cultivated and spread globally from an agricultural perspective, which is possible if the ecosystem and climate is correct for any species, providing that it does not become an invasive species. If the Kei apple project were to be successful, potential for cultivation outside South Africa may be possible.

Citrus

With the exception of the grapefruit (*Citrus paradisi*), citrus fruits originated from Asia. Orange (*Citrus sinensis*) and tangerine (*Citrus reticulata*) originated in China and were brought to Rome by Arab traders. The Romans and Greeks only knew of the bitter orange (*Citrus aurantium*). Sweet oranges were brought to Europe from India in the 17th century. Lemons (*Citrus limon*) originated in Malaysia or India. They were introduced into Assyria where they were discovered by the soldiers of Alexander the Great, who took them back to Greece. The crusaders introduced them into Europe. Limes (*Citrus aurantifolia*) originated in India and were introduced as a crop into the West Indies. The original grapefruit was discovered in Polynesia and introduced into the West Indies where it was developed and brought to Europe in the 17th century (Saltmarsh, *et al.*, 2003:125).

Breinholt *et al.* (2004) studied naringenin *in vivo* and concluded that citrus-derived naringenin exerts uterotropic effects in female mice. Narigenin in citrus fruit was found by Frydoonfar *et al.* (2003) to significantly inhibit cell proliferation in HT29 colon cancer cells exposed to naringenin at levels greater than 0.71 mmol (*in vitro*). This research suggests that citrus fruits may reduce the risk of colon cancer in humans. Kurowska *et al.* (2000) fed orange juice to 25 healthy volunteers and found that 750ml orange juice improved blood lipid profiles in hypercholesterolemic subjects. Citrus flavonoids were studied *in vivo* and *in vitro* by Manthey *et al.* (2001), who found that they reduce inflammatory response and have anti-cancer properties, influencing hepatic phase I and II enzymes. They postulated in their review that citrus flavonoids impact on blood leukocytes and microvascular endothelial cells, and stated that the pharmacological actions of citrus flavonoids may be linked to the abilities of these compounds to inhibit enzymes involved in cell activation, interacting with the nucleotide binding sites of phosphodiesterases, kinases, topoisomerases and other regulatory enzymes. They are also potent antioxidants and thus interfere with, and suppress, many of the events of cancer.

Fresh orange juices were examined for their antioxidant effectiveness as influenced by their phenolic content by Rapisarda *et al.* (1999). Several tests were used to assess this "*in vitro*" - bleaching of DPPH (Diphenyl-2-Picrylhydrazyl Radical), linoleate peroxidation, scavenging against nitric oxide (NO), total antioxidant and antioxidant profile, and total antioxidant status (TAA). The findings of this study indicated that the antioxidant efficiency of orange juices may be attributed to their total phenol content, with ascorbic acid playing a minor role. The antioxidant activity is related not only to structural features of the phytochemicals in them, but to their ability to bind to biomembranes and is influenced by their anthocyanin level. Antioxidant activity is not related to a single phytochemical compound but widely distributed among the phenolic constituents. These researchers also postulate that the supplementation of natural antioxidants contained in the orange juice through a balanced diet could be more effective and economical than the supplementation of an individual antioxidant against oxidative damage under different conditions. The anti-cancer and health properties of citrus fruit components were reviewed by Silalahi (2002), who concluded that the citrus flavonoids nobiletin and tangretin, in conjunction with other polyphenols, posses anti-cancer activity *in vivo* and *in vitro*. It was stated that it was not just the consumption of the flavonoids in isolation that have health benefits, but rather the combination of other nutrients in citrus fruits such as vitamin C, β-carotene, limonoids, folic acid and dietary fibre, and that flavonoids should not be judged in isolation.

Proteggente *et al.* (2003) measured the phenolic compositions, the ascorbic acid contents and the antioxidant activities of fresh Sicilian orange juices from pigmented and non-pigmented orange varieties. The pigmented variety *Tarocco* showed higher levels of hesperitin. All pigmented varieties showed higher levels of cyaniden-3-glucoside and cyanidin-3-(6"malonyl)-glycoside, especially *Moro*. The authors conclude that the oranges are a food source of flavonoids, but pigmented oranges are better.

Berries

A wide range of berries are consumed by humans globally. Most are cultivated but some are picked from the wild. The range includes strawberry (*Fragaria x ananassa*), raspberry (*Rubus idaeus*), blackberry (*Rubus* spp.), blueberry (*Vaccinium corymbosum*), elderberry (*Sambucus nigra*), cranberry (*Vaccinium oxycoccus*), gooseberry (*Ribes grossularia*), black currants (*Ribes nigrum*), red currants (*Ribes rubrum*) and white currants. The modern strawberry is the descendent of the tiny woodland strawberry that was grown by the Romans. Modern cultivated strawberries derive from cross between an American and a Chilean variety that occurred around 1750. Raspberries are native to Europe and have been cultivated since the Middle Ages. Blackberries have been eaten since Neolithic times and the Greeks prized them for the medicinal value of their leaves. Cranberries grow wild in both northern Europe and northern USA. Native Americans prized them for both their nutritional and medicinal properties and are said to have introduced the first Europeans to cranberries to help them prevent scurvy. The cranberry is *Vaccinium oxycoccus*, while *Vaccinium macrocarpon* is the large or American cranberry which is grown commercially in both America and Europe (Saltmarsh *et al.*, 2003:128).

Strawberries have been shown to have high antioxidative properties due to their flavonoid content rather than their vitamin C content (Aaby *et al.*, 2005). These authors focused on the by-products of strawberry producing juice and puree and found that there was merit in using the waste (chelenes or insides of the strawberry) for isolating and extracting flavonoids that may be useful in the neutraceutical industry. Wild blueberry and berry mix (OptiBerry) were found *in vivo* testing with mice to possess anti-antiogenic properties (Atalay *et al.*, 2003). Endothelioma cells pre-treated with berry powders showed diminished ability to form hemangiomas, common in premature infants (these lesions occur 1:100 in normal newborns, but 1:5 in premature babies with <1kg weight) and can be life threatening. It is concluded that the berries inhibit chemokine monoycte chemotactic protein 1 expression, responsible

for recruiting macrophages to sites of infection or inflammation. Hannum (2004) examined the impact of strawberries on the diet and concluded that strawberries are rich in a variety of phytochemicals that have been shown to be bioactive, namely ellagic acids, anthocyanin, catechin, quercetin and kaempferol. Thus, a diet rich in strawberries may have anti-cancer, anti-inflammatory (lower heart disease) and anti-aging properties, although no clear consumption level is indicated.

Whole black raspberries (lycophilized or finely pulverized) were found to inhibit multiple parameters of azoxymethane-induced colon cancer in rats, a tumour process similar to human colon cancer in which aberrant crypt foci progress to adenomas and then to adenocarcinoma. The aberrant crypt foci reduction across all categories after the administration of the carcinogen indicated that the black raspberries may inhibit proliferation of induce apoptosis in the rat's colon even after the process of carcinogenesis had been stated (Harris *et al.*, 2001).

Henig and Leahy (2000) reviewed the science supporting the folklore around cranberries and urinary-tract health and concluded that results from intervention, epidemiological and mechanistic studies support a beneficial effect of cranberry juice in maintaining urinary-tract health. The studies indicate that cranberry juice inhibits bacteria adhering to mucosal surfaces, mainly due to the presence of fructose and proanthocyanins (the authors make note that not all cranberry juices marketed are 100% juice). Hu *et al.* (2005) found potential for Saskatoon berries to possess free radical scavenging powers and inhibit intracellular oxidation. Supplementation with blueberries (150g in a milkshake) was found to be marginally better than supplementation with 1250mg Vitamin C owing to F_2-isoprostanes, lipid hydroperoxides, FRAC (ferric reducing antioxidant power) potential and urate in males subjected to exercise in hot (35°C 70% relative humidity) conditions (McAnulty *et al.*, 2004). A small study was carried out by Netzel et al (2005) on elderberries in 8 healthy human volunteers. It was found that their total antioxidant status improved after ingestion of 400ml of elderberry juice. Pedersen *et al.* (2000) examined the effects of blueberry and cranberry juice consumption (500ml) on plasma antioxidant capacity of healthy female volunteers. The blueberry juice was an organic product and the cranberry juice was a vitamin C-fortified, more widely available commercial product. These researchers found that the cranberry juice, but not the blueberry, resulted in an increase in antioxidant capacity of plasma as measured by its ability to reduce Fe^{3+} and Fremy's salt (a synthetic free radical, potassium nitrodisulphonate). This was mainly attributable to an increase in plasma concentrations of

vitamin C and not total phenolics. Furthermore, it was suggested that the phenolics in the blueberry juice were not totally bioavailable.

Reed (2002) reviewed cranberry flavonoids and explained that cranberries contain both hydroxycinnamic acids and flavonoids – the latter belonging to three groups – anthocyanins, flavonols and proanthocyanidins. The review postulated that monomeric and oligomeric flavonoids may be absorbed and metabolised and have post absorptive effects on the development of atherosclerosis and cardiovascular disease (CVD), such as 1) protection of LDL from oxidation by arterial endothelial cells, arterial smooth muscle cells and intimal macrophages, 2) inhibition of the inflammatory response of these cells to modified LDL and direct effects on immune cells involved in the inflammatory process, 3) vasodilation and improved blood flow, 4) inhibition of platelet aggregation and thrombosis, and 5) reverse cholesterol transport and clearance of cholesterol. It was concluded that cranberries have health benefits as a function of food, due to its complex mixture of polyphenols, but that more research on potential synergistic effects among flavonoids and other food constituents was required.

Roy *et al.* (2002) studied six berry extracts, namely wild blueberry, bilberry, cranberry, elderberry, raspberry seed and strawberry (plus a grape seed proanthocyanidin extract). The findings of this paper were that edible berries may assist in decreasing the risk of angiogenesis-related diseases such as cancer and inflammation. Interestingly, using oxygen radical absorbance capacity (ORAC) as a measurement, strawberry powder and grape seed proanthocyandin extract (GSPE) were higher than cranberry, elderberry or raspberry, and wild bilberry and blueberry extracts possessed the highest ORAC values. Ruel, G., *et al.* (2005) studied the effects of cranberry consumption with one 14-day intervention trial in 21 men. It was found that cranberry juice reduced LDL levels in circulating blood. It was concluded that antioxidant-rich food had a potential role in maintaining health and preventing CVD. Seeram *et al.* (2004) showed that enhanced antiproliferative effects against human tumours were obtained when cranberry extract was enriched with polyphenolic content by removing fruit sugars and organic and phenolic acids. There were also additive or synergistic antiproliferative effects resulting from the combination of anthocyanins, proanthocyanins, and flavonol glycosides compared to individual purified phytochemicals. These results suggested that there was some potential for cranberry extracts to be used in appropriate animal models of cancer studies and human cancer prevention trials.

Pomegranate

The pomegranate tree is native from Iran to the Himalayas in northern India and has been cultivated since ancient times throughout the Mediterranean regions of Asia, Africa and Europe. The fruit was used in many ways as it is today, and was featured in Egyptian mythology and art, praised in the Old Testament of the Bible and in the Babylonian Talmud, and it was carried by desert caravans for the sake of its thirst-quenching juice. The most important growing regions are Egypt, China, Afghanistan, Pakistan, Bangladesh, Iran, Iraq, India, Burma and Saudi Arabia. There are some commercial orchards in Israel on the coastal plain and in the Jordan Valley. Production declined from lack of demand in the 1930's, but new plantings were made when demand increased in the 1960's (Morton, 1987:352).

A small study of 10 people was carried out by Aviram *et al.* (2004) over 3 years with pomegranate juice, which indicated that 50ml of juice consumed over 3 years, daily, reduced systolic blood pressure and serum LDL. Maximum effects were seen after 1 year of pomegranate juice consumption. Li *et al.* (2005) stated that the pomegranate is well-known to contain high levels of phenolics but that the peels were particularly rich in antioxidants as determined by the FRAP (ferric reducing antioxidant power) assay, confirming the results of a previous study undertaken by the authors. Pomegranate peel extracts were studied by Negi *et al.* (2003), who found that the pomegranate peel extracts contained ellagic tannins, ellagic acid and gallic acid and that there was potential for this fruit by-product to be used commercially, as a biopreservative in food applications and neutraceuticals. The Ames test for mutagenicity showed that there were anti-mutagenic properties, and the study indicated that antioxidant properties were evident.

Mango

Mango (*Mangifera indica*) has been eaten for over 6000 years in Indian and Malaysia and was introduced to South America and the West Indies in the 18th century. Mango is a good source of β-carotene and vitamin C and is a major contributor of these nutrients, especially in developing countries (Saltmarsh *et al.*, 2003:126).

In vivo testing (rats) with mango kernel *Mangifera indica* and *Embica officinalis* (gooseberry) showed reductions in the activities of oxidative free radical scavenging enzymes superoxide

dismutase and catalase were significantly elevated, and levels of lipid peroxides were reduced, although the specific flavonoids responsible were not identified (Anila & Vijayalakshmi, 2003). Singh *et al.* (2004) studied the different phenolics in Indian mango cultivars. What was interesting in this study was that different cultivars, although from the same species, varied in their absolute content phenolics and their make up of phenolics. The main phenolics identified were tannic, gallic, vanillic, caffeic, ferulic, chlorogenic and cinnamic acids. In raw fruits, the tannic acid was fairly low compared with ripe fruits.

Schieber *et al.* (2000) examined mango puree concentrate (although it is not stated which variety or if it is mixture of varieties) and found a broad pattern of polyphenols including quercetin, mangiferin, and phenolic acid gallic acid. Coupled with its high carotenoid content, the researchers concluded that mango is a rich source of antioxidants.

Apples

Small, bitter crab apples are very widely distributed throughout the world and have been eaten since prehistoric times. However, the first apples resembling modern apples (*Malus x domestica)* probably grew on the slopes of the Tien Shan between China and Kazakhstan. The Romans first cultivated the fruit, grew at least a dozen varieties and are believed to have introduced it to northern Europe including Britain. The Pilgrim fathers took pips to America. Cox's Orange Pippin was first grown in England in 1826 and later that century Granny Smith was grown in Australia. More than 7000 named varieties are know known worldwide (Saltmarsh *et al.*, 2003:121-122).

Apples are well known for their quercetin content (refer to table 2.8 on flavonoid content of common foods). Hertog *et al.* (1993) and Hertog *et al.* (1994) noted that apples were also high in quercetin (as were onions), and thus would potentially be beneficial to health. This research stimulated much further work on the health benefits of flavonoids. These groups were amongst the first to describe the relationship between flavonoids or flavonoid-containing foods and disease.

Kang *et al.* (2004) studied apples (Empire) and found that these apples showed protective effects on hydrogen peroxide (H_2O_2) induced inhibition of gap-junctional intracellular communication (GJIC). The promotional phase of carcinogens is closely linked to epigenetic

events involving inhibition of gap-junctional intracellular communication, which could be mediated by reactive oxygen species (ROS).

Grapes and Grape Extracts

Grapes were among the earliest cultivated crops. The Egyptians, Greeks and Romans all made wine and the Romans bred many new varieties. Concord grapes (*Vitis labrusca*) are characterized by a red-coloured flesh and skin. They are grown in America and are a different species from the European grape *Vitis vinifera*. Fresh red *Vitis vinifera* grapes contain in the region of 4mg/kg of phenolic material, mainly in the skin and seeds, including gallic acid, caftaric acid, anthocyanins, monomeric flavan-3-ols and oligomeric proanthocyanidins. Some of this phenolic material is carried into the grape juice, which is either sold as such or fermented. However, red wine fermented over skins and seeds tend to have a higher phenolic content, as additional phenolics are extracted in the presence of alcohol. Table grapes are picked earlier and do not ripen to the same extent as grapes used to make wines. They are therefore likely to contain much lower levels of polyphenols. Nowadays, red grapes for table use are usually seedless varieties and so will contain much lower levels of flavan-3-ols and their oligomers than grapes used to make red wine (Saltmarsh *et al.*, 2003:130-131).

Bagchi *et al.* (2000) assessed grape seed proanthocyandin extract (GSPE) for its importance in human health and disease prevention. It was found that GSPE was superior to vitamins C, E and β-carotene in its protection against free radicals and free radical-induced lipid peroxidation and DNA (deoxyribonucleic acid) damage. GSPE also showed cytotoxicity towards human breast, lung and gastric adenocarcinoma cells, whilst enhancing the growth and viability of normal human gastric mucosal cells. GSPE was subjected to a complex study by Bagchi *et al.* (2003) in which its cardioprotective properties were examined in rat, mice, hamsters and humans for a variety of mechanisms. These researchers concluded that GSPE had potential as a therapeutic tool to reduce cardiovascular disease.

In vitro testing with grape juice by Chen *et al.* (1998) showed that red and white grape juice was most effective in modulating the action of aromatase and estrogen receptors in breast cancer cells, leading to a suppression of the breast cancer cell growth. *In vivo* testing with mice showed a reduction in the size of chemically-induced tumours. Grape juice and red wine were shown to have anti-thrombotic effects by Folts (1998), confirming the French

paradox although it seems unclear which polyphenols were responsible and red wine and red grape juice were more effective than white wine. The French paradox was discovered when it was realized that, despite a higher intake of fat in their diet, the French nation had lower CVD events than countries with similar diets. This was attributed to their intake of red wine (Ferrières, 2004, Gueguen, 1998, Kopp, 1998).

The phenolic compounds in grapes and grape juices and their antioxidant effects were reviewed by Frankel and Meyer (1998). It was concluded that LDL oxidation was reduced by consumption of grapes, juices and their extracts, and offer protection against atherosclerosis and coronary heart disease (CHD). Dried grape seeds obtained as a by-product in the process of wine-making after colour oxidation and alcohol distillation were shown to possess a high source of flavonols, even if submitted to high temperatures, thus provided a potential, cheap dietary supplement (González-Paramás *et al.*, 2004). The antioxidant effects of Concord grape juice flavonoids were found to be more effective than α-tocopherol on markers of oxidative stress in healthy adults by O'Byrne *et al.* (2002).

Mixed Juices

Arendt *et al.* (2001) studied German commercially available fruit or fruit/vegetable juices supplemented in the diet for 16 weeks *in vivo* and found that the plasma antioxidant capacity of HIV (human immuno-deficiency virus)-seropositive and healthy subjects can be increased by the consumption of these juices. Breinholt *et al.* (2003) examined apple juice and blackcurrant juice on the redox status and anti-cancer biomarkers in female rats. Grapefruit juice and orange juice were included in the study as they contained the highest flavonoids in comparison with other juices analyzed. It was found that the total flavonoid content varied from brand to brand, and within brand and type of juice (and thus batch of juice). The authors hypothesised that the observed differences reflected variations in the quality of the fruits, cultivars, manufacturing processes, storage, duration as well as the presence of other antioxidants in the juice that may function to stabilize the flavonoids. The results of the study were inconclusive as to whether fruit juice has anti-cancer properties in humans, and further work was required. Fruit juices (varying in their composition and not commercially available) were given to 27 male volunteers, and a randomized cross-over study was carried out by Bub *et al.* (2003). Both juices contained approximately the same amount of polyphenols but varied in their contents, but both showed enhanced antioxidant status, reduced oxidant DNA damage and stimulated immune cell functions.

A review of polyphenols would not be complete if it did not briefly mention tea and cocoa, two of the most widely consumed beverages in the world.

Teas

Tea (*Camellia sinensis*) is the most consumed beverage in the world (Rietveld & Wiseman, 2003). Tea is consumed daily by individuals worldwide. Tea arrived in Europe in the 18th century, having been drunk elsewhere for centuries, for example by the Chinese for probably 5000 years. Tea was initially sold in coffee houses, but became more popular than coffee. The popularity of tea was such that from the latter part of the 18th to the beginning of the 19th century, a transition took place in the drink of the labouring class, as tea replaced beer and gin (Duthie & Crozier, 2003:150).

Several reviews and studies have shown that tea consumption leads to a significant increase in the antioxidant capacity of the blood (with either oolong black or green tea) (Anonymous, 2004(b), Lambert & Yang, 2003, Rietveld & Wiseman, 2003, Anderson & Polanksy, 2002, Dufresne & Farnworth, 2001, Langley-Evans, 2000). A review by Trevisanato and Kim (2000) links the consumption of tea with moderate protective effects against cancer, cardiovascular disease, formation of kidney stones, bacterial infections and dental caries, and suggests that this is due to the rich source of dietary flavonoids in the tea. According to Vita (2003), human tea consumption has a beneficial effect on the vascular endothelium and thus the author concludes that tea consumption is positively associated with reduced cardiovascular disease risk.

A considerable amount of investigative work has been done on tea, and less on fruit and vegetables (Buttriss, 2004). Green tea is derived from drying and steaming the fresh tea leaves directly after plucking, when no fermentation occurs. Oxidation (for fermentation) occurs naturally when tea polyphenols are complexed by contact with oxygen. Oolong tea is derived by partial oxidation stage before drying and steaming. Black tea undergoes a full oxidation stage before drying and steaming. Tea extract has anti-bacterial, anti-fungal, antioxidative, anti-tumour and anti-mutative activities which are largely due to the powerful scavenging and antioxidative properties of high concentrations of unpolymerised catechins and their gallates (Geetha *et al.*, 2004). Tea flavonols in green tea, black tea and green tea

extract were studied *in vivo* human tests by Henning *et al.* (2004), who found that all showed strong antioxidant activity.

Interest has also been shown in herbal teas. Joubert *et al.* (2003) have shown rooibos tea *Aspalanthus linearis* to possess significant levels of flavonoids. Ramirez-Mares *et al.* (2004) studied 2 herbal teas and green tea and found the herbal teas ardisia (*Ardisia compressia*) from Mexico and mate tea (*Ilex paraguariensis*) from Argentina to potentially possess similar *in vitro* properties to traditional tea.

Cocoa

Cocoa was consumed by the Mayas before 1000 AD and the pods were used for their sweet pulp as much as 2000 years before that. The tree was cultivated in central America for over 2000 years and cocoa was a major item of commerce for the Aztecs and their predecessors. The origin of the wild cocoa tree is believed to be the upper regions of the Orinoco and Amazon River systems but here the crop was never valued as it was by the Aztecs (Duthie & Crozier, 2003:162).

The impact of cocoa in the diet was first noticed with the Kuna Amerinds in Panama who, despite their high intake of salt, showed lower levels of hypertension for their age compared to similar populations who consumed a westernised diet. This was due to their large consumption of homemade cocoa, the tropical fruit *Thesbroma cacoa*, which is consumed in large quantities and is a similar paradox to the well known French wine paradox (Ferrières, 2004, Gueguen, 1998, Kopp, 1998). Cocoa has subsequently been found to contain large quantities of polyphenols especially flavonols (Pearson *et al.*, 2005). Several studies have been performed and these include, though far from exhaustive, a study by Mursu *et al.*, in 2004. It was found that cocoa polyphenols may increase the concentration of high-density lipoprotein (HDL) cholesterol, whereas chocolate fatty acids may modify the fatty acid and composition of low-density lipoprotein (LDL) and make it more resistant to oxidative damage. However, these researchers were skeptical about the long-term effect of chocolate due to its negative impact on weight gain. The effects of flavanols and procyanidins from cocoa extracts were examined on colon cancer cells by Carnésecchi *et al.* (2002). The effects of cocoa powder and extract on Caco-2 cell growth, a human colon cancer, and on polyamine biosynthesis was examined. It was found that there was a potent anti-proliferative effect of the cocoa flavanols and procyanidins on human colon cancer cells. They also cause non

apoptopic cell death and affected the cell cycle by causing a blocked G2/M phase of the cell cycle. Inhibition of polyamine biosynthesis may be one of the several targets of the anti-proliferative effect of the cocoa flavanols and procyanidins.

Keen *et al.* (2005) reviewed cocoa antioxidants and cardiovascular health and found that there is now a large body of information that supports the idea that cocoa flavonols and procyanidins have the ability to act as *in vivo* antioxidants. Several *in vivo* studies have provided strong support for the hypothesis that the consumption of flavonol-rich foods such as certain cocoas and chocolates may be associated with reduced risk for vascular disease. However, as with all the flavonoids, there is little information on the extent to which flavonoids interact with other nutrients in the diet before and after absorption, on the intracellular metabolism of these compounds and on the bioactivity of the different metabolites.

Content of Polyphenols in Fruits and Vegetables

To understand how fruit and vegetables may be useful in the diet and potential benefits of one fruit or vegetable over another, the actual content of the phenolics is useful. This section of the review attempts to give an indication of the work done recently in quantifying the content of polyphenols of fruit and vegetables consumed.

Sun *et al.* (2002) explain that the total phenolic content of fruits and their antioxidant activities were underestimated in the literature because bound phenolics were not included. Thus, these researchers set about to determine 1) the profiles of total phenolics including both soluble, free and bound forms in common fruits, 2) total antioxidant activities of common fruits, 3) anti-proliferative activities of these fruits on liver cancer call growth, and 4) to estimate the bioactivity index (BI) of these fruits for preventing cancer. Tables 2.5 and 2.6 show the distribution profile of phenolics and bioactivity index of selected fruits.

Table 2.5: Percentage distribution profile of phenolics in fruit (Sun *et al.*, 2002) – free, bound and total percentage for a selection of fruit.

		Bound (%)		
Fruit	**Free (%)** [a]	**Bound-E** [b]	**Bound-W** [c]	**Total** [d]
Apple	91.8	1.7	6.5	8.2
Banana	62.1	4.8	33.1	37.9
Red grape	90.5	3.0	6.5	9.5
Grapefruit	61.9	14.5	23.6	38.1
Lemon	80.9	3.7	15.4	19.1
Orange	70.0	5.7	24.3	30.0
Peach	77.2	3.8	19.0	22.8
Pear	76.0	9.7	14.3	24.0
Pineapple	42.9	45.8	11.3	57.1
Strawberry	92.3	2.6	5.1	7.7
Cranberry	96.2	2.2	1.6	3.8
Average	*76.5*	*8.9*	*14.6*	*23.5*

a = freely available b = bound, only extracted by ethanol c = bound with water d = total of bound ethanol + water

This study revealed that cranberry followed by apple, red grape, strawberry, peach, lemon, pear, banana, orange, grapefruit and lastly pineapple had the highest to lowest bioactivity index values as demonstrated in table 2.6.

The intake of polyphenols per individual or country is an unknown factor, as intakes vary widely. However, efforts are being made to quantify this (Prior & Gu, 2005). A proposal for a recommended dietary intakes (RDI) has been discussed by Dreosti (2000). The author argues that phytochemicals (including polyphenols) are not established nutrients, although they seem to contribute significantly to protection against degenerative diseases.

To be regarded as an essential nutrient, a dietary component must be a single identified compound or a close derivative. It should have a demonstrated key biological role and characteristic deficiency syndrome. Therefore, setting an RDI is difficult due in part to the large number of chemically different phytochemicals and lack of deficiency syndrome or inherent physiological role in almost all cases. It is more likely that they would form part of a set of dietary guidelines (Love & Sayed, 2001), as in the South African Dietary Guidelines for all people above seven years of age.

Table 2.6: Bioactivity Index (BI) of selected fruits for dietary cancer prevention (adapted from Sun *et al.*, 2002).

	Total Antioxidant Activity		
	TOSC (μmol of vit C equiv/g)	Score	Rank
Cranberry	176.98	1.00	1
Apple	98.56	0.55	2
Lemon	42.75	0.24	6
Strawberry	64.37	0.36	4
Red grape	64.70	0.36	3
Peach	49.45	0.28	5
Banana	32.80	0.19	8
Grapefruit	24.66	0.14	10
Pear	34.24	0.19	7
Orange	31.48	0.18	9
Pineapple	16.93	0.10	11

Key:
TOSC = total oxyradical scavenging capacity
[a] BI = 1/2 (score of total antioxidant activity + score of antiproliferative activity

The total phenol and vitamin C contents of a wide range of fruit and vegetables were determined by Proteggente *et al.* (2002). Results are summarised in table 2.7 below. The antioxidant capacities of aqueous / methanolic extracts were comparatively assessed, using the trolex equivalent antioxidant capacity (TEAC), ferric reducing ability of plasma (FRAP) and oxygen radical absorbance capacity (ORAC) assays, which comprise contributions from polyphenols, simple phenols and ascorbate component (Proteggente *et al.*, 2002). The order of antioxidant activity is shown in the table. Additionally, the specific phenols for each fruit or vegetable were determined but, for simplicity, are not reported here.

Table 2.7: Total vitamin C (mean of two samples), total phenolics (GAE = gallic acid equivalents), TEAC, FRAP and ORAC (mean ± SEM of a minimum of three samples) in fruit and vegetable extracts (adapted from Proteggente *et al.*, 2002).

Fruit / Vegetable	Total Phenolics (mg GAE/100g FW)	Total Vitamin C (mg/100g FW)	TEAC (μmol Trolox/100g FW) ①	FRAP (μmol Fe^{2+}/100g FW) ②	ORAC (μmol Trolox/100g FW) ③
Strawberry [a]	330±4	61	2591±68	3352±38	2437±95
Raspberry [a]	228±6	26	1846±10	2325±53	1849±232
Red plum [a]	320±12	5	1825±28	2057±25	2564±185
Grapefruit [b]	150±4	52	861±53	829±6	1447±67
Orange [b]	126±6	46	849±25	1181±6	1904±259
Red cabbage	158±4	37	1377±49	1870±18	2124±68
Broccoli [c]	128±4	45	648±25	833±16	1335±62
Onion [c]	88±1	6	532±29	369±13	988±30
Green grape [c]	80±4	2	594±72	519±48	872±48
Spinach [c]	72±1	7	757±54	1009±35	1655±115
Green cabbage [c]	58±1	28	492±18	694±14	1180±68
Pea	32±1	22	440±18	251±9	704±62
Cauliflower	30±1	15	295±16	259±5	425±44
Leek [c]	22±1	16	240±11	160±1	413±15
Lettuce [c]	14±1	<2	171±12	124±7	319±37
Pear [d]	60±3	3	282±19	315±24	587±50
Apple [d]	48±1	6	343±13	394±8	560±18
Peach [d]	38±1	6	244±9	336±4	764±49
Banana	38±4	10	181±39	164±32	331±59
Tomato [d]	30±1	18	255±14	344±7	420±39

[a] – anthocyanin-rich
[b] – flavone-rich
[c] – flavonol-rich
[d] – hydroxycinnamate-rich

① TEAC = Trolex Equivalent Antioxidant Capacity
② FRAP = Ferric Reducing Ability of Plasma
③ ORAC = Oxygen Radical Absorbance Capacity

The following figures 2.10 to 2.13 (inclusive) have been extracted from table 2.7. Figure 2.10 shows the anthocyanin-rich fruits, figure 2.11 shows the flavone-rich fruits, figure 2.12 shows the flavanol-rich vegetables and fruits, figure 2.13 shows the hydroxycinnamate-rich fruits. Each figure gives the TEAC, FRAP, ORAC and total phenolics for each of the respective fruits and vegetables. An overall picture is clear, that the order of activity for trolex equivalent antioxidant capacity, ferric reducing ability of plasma, oxygen radical absorbance capacity and total phenols is anthocyanidins, flavones, flavanols and finally hydroxycinnamics. However, it must be kept in mind that actual contents of the relevant polyphenols in the fruits and vegetables and their relevant activities are not necessarily related to their specific action in the body, although it must be postulated that there is a strong relationship.

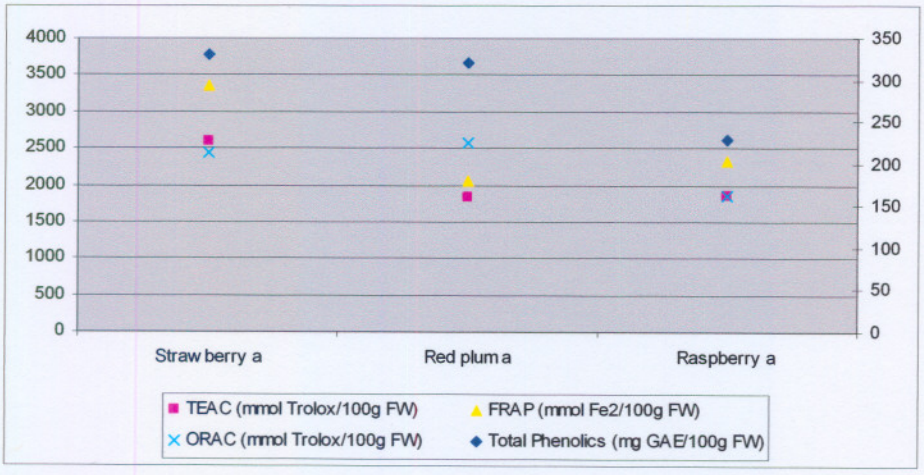


Figure 2.10: Figure indicating TEAC, FRAP, ORAC and total phenolic for the anthocyanin-rich fruits strawberry, red plum and raspberry (figure adapted from Proteggente *et al.*, 2002).

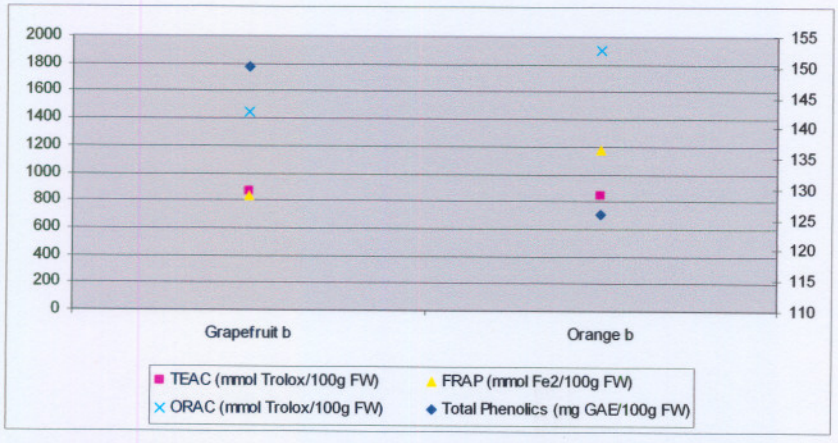


Figure 2.11: Figure indicating TEAC, FRAP, ORAC and total phenolic for the flavone-rich fruits grapefruit and orange (figure adapted from Proteggente *et al.*, 2002).

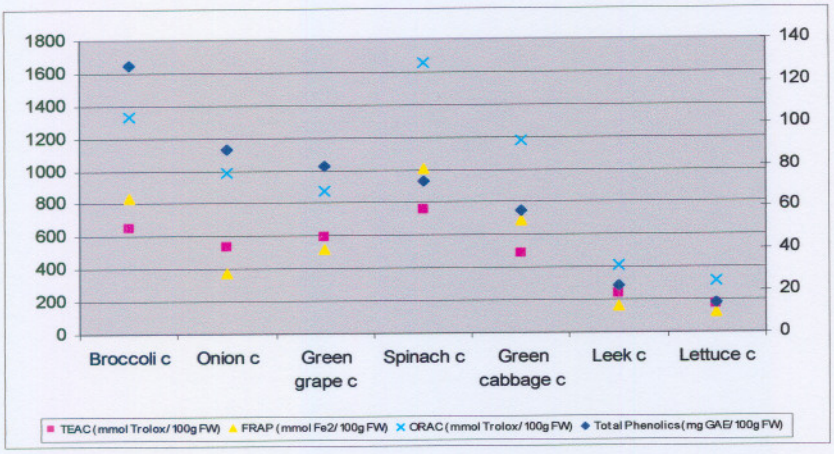


Figure 2.12: Figure indicating TEAC, FRAP, ORAC and total phenolic for the flavanol-rich vegetables and fruits broccoli, onion, green grape, spinach, green cabbage, leek and lettuce (figure adapted from Proteggente *et al.*, 2002).

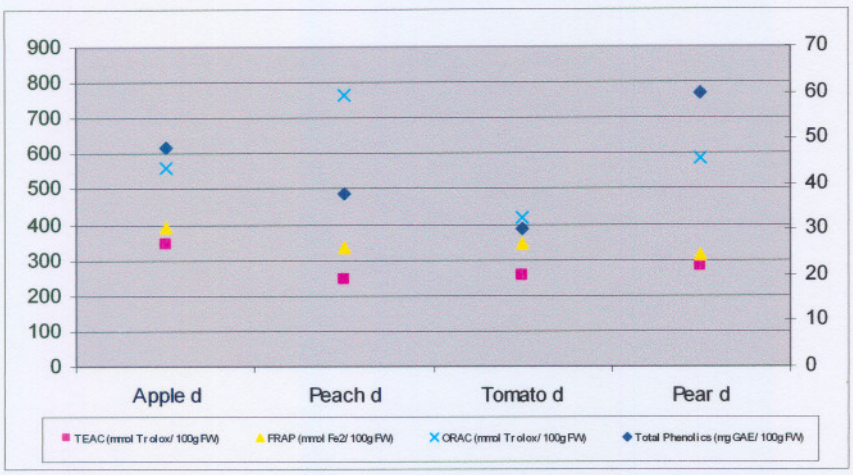


Figure 2.13: Figure indicating TEAC, FRAP, ORAC and total phenolic for the hydroxycinnamate-rich fruits apple, peach, tomato and pear (figure adapted from Proteggente *et al.*, 2002).

The United States Department of Agriculture has been collecting data from various studies since 2003 and has formed a database for the flavonoid content of selected foods (Mayer & Stern, 2003). This is being continually updated. The actual document contains flavonoid values for 225 foods, from a list of 97 data sources. A table which has relevance for this project has been extracted and used for this project and is shown in table 2.8. It has been greatly simplified. Only foods that are of relevance to this project have been reported, mean values only have been recorded and data sources have not been noted as these can be obtained from the original document and would make the key to the table too onerous to read. Note that this table has been included here to give the reader an idea of the complexity of the analysis.

Table 2.8: Flavonoid content of foods (relevant to this project) extracted from the USDA database for flavonoid content of selected foods as prepared by the Nutrient Data Laboratory, Food Composition Laboratory, Beltsville Human Nutrition Research Centre, Agricultural Research Centre and US Department of Agriculture (Mayer & Stern, 2003).

Description	Flavonoid	Mean mg/100gs	Sources of Data *
Apples, raw with skin	Quercetin	4.42	38, 69
Apples, raw without skin	Quercetin	1.50	16, 14
Apricots, raw	(-)-Epicatechin	6.06	5
Blackcurrant juice	Myricetin	1.86	34
	Quercetin	1.15	34
Blood orange, raw	Hesperetin	13.12	8, 55, 57
	Naringenin	1.68	8, 55, 57
Blueberries, raw	Cyanidin	15.02	29
	Delphinidin	29.54	29
	Malvidin	49.21	29
	Peonidin	7.05	29
	Petunidin	11.05	29
	(-)-Epicatechin	1.11	5
	Myrecetin	0.82	12, 33
	Quercetin	3.11	12, 33, 44
Cherries, sour, red, raw	Cyanidin	6.64	96
Cocoa dry powder	Quercetin	20.13	43
	Myrecetin	0.05	39
Cocoa brewed with tap water	Myrecetin	0.05	39
Cranberries	Myrecetin	4.33	12, 33, 40, 44
	Quercetin	14.02	12, 33, 40, 44
Cranberry juice	(+)-Catechin	0.92	18
	Myrecetin	0.41	18
	Quercetin	16.41	18
Fennel	Isorhamnetin	9.30	89
	Kaempferol	6.50	89
	Myrecetin	19.80	89
	Quercetin	48.80	89
	Kaempferol	2.12	11
	Quercetin	0.12	11
Grape juice	Myrecetin	0.58	39
	Quercetin	0.41	39
Grapefruit juice	Hesperitin	0.52	78
	Naringenin	18.11	24, 78, 79
Grapefruit juice concentrate	Naringenin	31.18	14
Lemon juice, canned or bottled	Eriodictyol	11.64	32
	Hesperitin	14.36	32, 44
Logonberries, raw	Quercetin	12.16	33, 34, 54
Logonberry juice	Quercetin	1.02	34
Mangos, raw	(+)-Catechin	1.72	5
Milk chocolate, reduced fat	(-) Epicatechin	0.26	6
	(+)-Catechin	0.82	6
Onions, raw	Isorhamnetin	1.91	90
	Kaempferol	0.18	10, 25, 38, 52
	Quercetin	13.27	10, 25, 38, 44, 52, 54, 64, 65, 90
Onions, red, raw	Cyanidin	13.14	26
	Isorhamnetin	17.94	26, 90
	Kaempferol	0.89	10, 40, 52
	Quercetin	19.93	10, 40, 52
Orange juice (from concentrate)	Hesperetin	3.61	28
	Naringenin	1.47	28
Orange juice, raw	Myrecetin	0.05	39
	Quercetin	0.19	8, 17, 39
	Hesperetin	12.54	8, 17, 53, 55, 28, 44, 73
	Naringenin	2.27	8, 17, 28, 44, 53, 55, 73

Description	Flavonoid	Mean mg/100gs	Sources of Data *
	Eriodictyol	0.17	8, 17, 28, 44
Oranges, raw	Hespertin	32.73	44, 54
	Naringenin	11.15	44, 54
Peaches, raw	(+)-Catechin	2.33	5
Pears, without skin, raw	(-)-Epicatechin	1.74	5
	(+)-Catechin	0.14	5
Pears with skin, raw	(-)-Epicatechin	3.17	1, 5, 80
	(+)-Catechin	0.26	1, 5
	Isorhamnetin	0.30	1, 5
Strawberries, raw	(+)-Catechin	4.47	5
	Kaempferol	0.79	33, 34, 38, 44
	Quercetin	0.65	33, 34, 38, 44
Tea, black	Thearubigins	15.82	91
Tea, ready to drink	Kaempferol	0.33	91
	Myrecetin	0.12	91
	Quercetin	0.72	91
Tea, oolong, brewed	(-)-Epicatechin	2.59	47, 50, 51
	(-)-Epicatechin 3-gallate	6.73	47, 50, 51
	(-)-Epigallocatechin	6.00	47, 50, 51
	(-)-Epigallocatechin 3-gallate	36.01	47, 50, 51
	(+)-Catechin	0.23	47, 51
	Kaempferol	0.90	39
	Myricetin	0.49	39
	Quercetin	1.30	39

* refer to original database, not quoted here for simplicity

In summary, measuring the actual polyphenol content of fruit and vegetables is an onerous task as is seen by varying levels within table 2.8, according to the reference source, and from similar work undertaken by the researchers Sun *et al.*, 2002 and Proteggente *et al.*, 2002. It is dependant on the source of product, method of extraction and climatic conditions as will be explained in the Results and Discussion. However, an attempt has been made here to give an idea of the activity of the polyphenols, the vast amount of work required in quantifying them and the ongoing task that is being pursued by the United States Department of Agriculture (USDA) in their database. Table 2.8 is a small extraction of the USDA database and has been included here only so that the reader may observe how difficult it is to quantify the polyphenols and obtain consistent results without actual reference to the database in this Literature Review.

Hybrids – Modification of Existing Species of Fruits and Vegetables by Agriculturists

As the polyphenol content has been shown to be important in human health, hybrids are being developed that may be claimed to be more "healthy". For example:

Gorinstein *et al.* (2004) demonstrated that the hybrid of Jaffa sweeties (white grapefruit) showed more antioxidant activity due to its greater content of trans-hydroxycinnamic acids (caffeic, p-coumaric, ferulic and sinapic). Rapisarda *et al.* (2003) studied a hybrid Orno-31 and its parents clementine cv. Oroval and found the hybrid to be higher in total anthocyannins, flavones and hydroxycinnamic acids and thus of greater nutritional benefit. The level of antioxidants in tomatoes has been increased by genetically modifying the flavonoid biosynthetic pathway (up to 78 times the total flavonoid content) (Verhoeyen *et al.*, 2002).

It is interesting to note, therefore, how agriculturalists are trying to increase the value of their crops by modification (not necessarily genetically) to attract consumers as ultimately they will be trying to make a marketing claim along the lines of "with extra antioxidants" or similar.

METABOLISM OF PHENOLIC COMPOUNDS

Once ingested, it is important that the fate of the phenolic compounds is known. The absorption and subsequent distribution, metabolism and excretion thereof in humans has not been well studied. Absorption of flavonoids from the diet was long considered to be negligible, as most flavonoids are present in foods bound to sugars as β-glycoids. The aglycones were considered to be able to pass the gut wall and no enzymes that can uplift these predominantly β-glycosidic bonds are secreted into the gut or present in the intestinal wall (Hollman & Katan, 1997 and 1999).

However, this was not confirmed by Day *et al.* (2000), who studied the enzyme lactase phlorizin hydrolase (LPH), a membrane-bound, family 1 β-glycosidase found on the brush border of the mammalian small intestine. Lactase phlorizin hydrolase was found to be active in deglycosylating dietary isoflavonoid glycosides and thus assisting in their metabolism.

A model (as shown in figure 2.14) has been proposed by Scalbert *et al.* (2005) that shows the prediction of the uptake of phenolics from the diet.

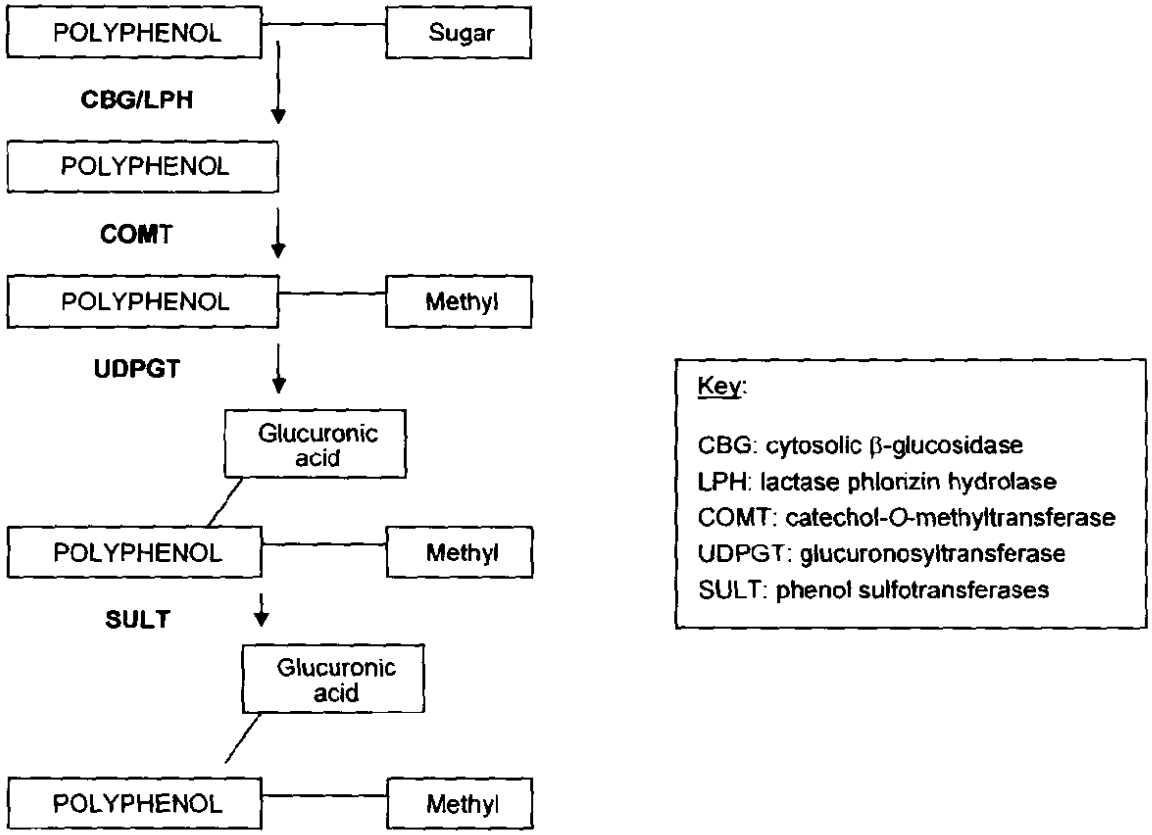


Figure 2.14: possible metabolic pathway of phenolics (Karakaya, 2004, Scalbert *et al.*, 2005).

Karakaya (2004) in his review shows that phenolic compounds are metabolised by deconjugation and reconjugation reactions. Phenolics are hydrolysed to their free aglycones then are conjugated, methylated, sulfated, glucosidated or a combination. It is also thought that colonic bacteria may be influential in the process (Blaut *et al.*, 2003, Gee & Johnson, 2001, Hollman & Katan, 1997).

Manach *et al.* (2004) reviewed the bioavailability of polyphenols and the following is a summary of their findings. The polyphenols that are the most abundant in the human diet are not always the most active or are poorly absorbed, highly metabolized or rapidly eliminated. The metabolites may also differ from the native substances in terms of biological activity. Metabolism of the polyphenols occurs in a common pathway. The aglycones can be absorbed from the small intestine. However, most polyphenols are present in food in the form of esters, glycosides or polymers that cannot be absorbed in their native form, where they are hydrolyzed by intestinal enzymes or colonic micro-organisms. During the course of absorption, polyphenols are conjugated in the small intestine and later in the liver. These

mechanisms are highly efficient and aglycones are generally either absent in blood or present in low concentrations after consumption of nutritional doses. Polyphenols are able to penetrate tissues, particularly those in which they are metabolized, but their ability to accumulate within specific target tissues needs to be further investigated. Polyphenols and their derivatives are eliminated chiefly in urine and bile. They are secreted via the biliary tract into the duodenum, where they are subjected to the action of bacterial enzymes, especially β-glucoronidase, in the distal segments of the intestine, after which they may be re-absorbed. This enteropathic recycling may led to a larger presence of polyphenols within the body and therefore some limited storage of polyphenols is thought possible. The absorption of the simple hydroxycinnamic and hydroxybenzoic acids is fairly simple though – they are rapidly absorbed from the small intestine but, because these compounds are naturally esterified in plant products, their absorption may be impaired and it is thought that colonic bacteria are helpful here in increasing absorption. However, Manach *et al.* (2005), in their review of 97 bioavailabiity studies, indicates that the least well absorbed polyphenols are the proanthocyanidins, the galloylated tea catechins and the anthocyanins. These authors postulate that data is still too limited for assessment of hydroxycinnamic acids and other polyphenols.

Overall, absorption of the polyphenols is not simplistic and cannot be viewed as being attributable to one biochemical pathway. There are many factors involved, which only emphasizes the importance of established strongly-designed human when evaluating the efficacy of any of the polyphenols.

SAFETY OF POLYPHENOLS

Dosage of phenolics and flavonoids has been raised by Galati and O'Brien (2004) and Skibola and Smith (2000) as cause for concern. Whilst there are definite health benefits of these compounds, both these reviews urge caution in over-dosing, for example, by consuming large quantities of fruits and vegetables and taking many supplements. In summary, these reviews state that there is too much that is unknown about the effects of phenolics and flavonoids in the body and, in high doses, their effect may outweigh the benefits in the body. Galati and O'Brien (2004) state further that whilst most flavonoids have been found to be safe, there have been reports of toxic flavonoid-drug interactions, liver failure, contact dermatitis, hemolytic anaemia, male sterility concerns and breast cancer. This is an issue when potential "over-dosing" is reached, either through diet or

supplementation. This could lead to overwhelming of the system, leading to the formation of reactive oxygen species, and ultimately DNA damage. This is further exacerbated during foetal development. Donma and Donma (2005) also raised this issue when dealing with nutrient intakes in children.

Kruger and Mann (2003), in their review of safety evaluation of functional ingredients, state that the latter encompasses elements of drugs, nutrients and food additives and thus the framework to evaluate functional ingredients should encompass their history (that is, are they new ingredients, or additives, or supplements). A full risk / benefit assessment be taken in all cases, taking into account of whether there is a potential for lifetime exposure and unsupervised consumption.

Specifically, grapefruit juice has been reviewed for its extent, probably mechanism and clinical relevance with drug interactions by Fuhr (1998). Grapefruit juice was found to potentiate the effect of calcium antagonists, cyclosporin, some anti-histamines, theophylline and caffeine, some benzodiazepines. The site of influence appears to take place in the gut wall, and the component that causes the main interferences is the psoralens, mainly 6', 7'-dihydroxybergamottin. There are two ways to approach this: 1) to use grapefruit as a drug-sparing agent, and 2) to use warnings on package inserts. Both would be too unreliable due to the many interactions involved and individual variations. It is likely that, if grapefruit juice is consumed, it is done on a regular basis and would not affect ongoing treatment. Drug sparing by grapefruit juice is too risky and has been researched by Brunner *et al.* (2000) and found to be too unpredictable. It must be remembered that this study would have been performed on pure grape fruit juice as opposed to most grapefruit juice marketed which usually contain a mixture of a base fruit such as grape, apple or pear to sweeten the product.

Van der Heide *et al.* (2003), in their review of a study with rats, have shown that flavonoids have a strong interference with many aspects of thyroid hormone synthesis and availability. Of most concern was for the foetus, where normal brain development could be affected. Polyphenols consumed in high amounts could have pro-oxidant effects. They can reduce iron III to iron II and thus form general hydroxyl radicals through the Fenton reaction, although such pro-oxidant effects have not been demonstrated *in vivo*. Conjugating enzymes are likely to play an essential role in detoxifying polyphenols and limiting their mutagenicity *in vivo*. Flavonoids may influence the thyroid function and have genitrogenic effects. The effects of polyphenols on iron absorption have been well demonstrated in clinical studies, but is not yet firmly established. Adverse effects of excess polyphenol

consumption on cardiovascular diseases have been recently suggested due to their effect on raising levels of homocysteine in the blood, a known risk factor for coronary heart disease (Scalbert *et al.*, 2005).

In summary, given the nature of the nutraceutical industry to produce supplements with high levels of polyphenols, and the individual potential to overdose on over-the-counter supplements, care should be taken in recommending supplements at the risk of overdosing.

All in all, the positive evidence for the health benefits of polyphenols seems to outweigh the negative effects, but as in all cases with human health and nutrition, care must be taken and the individual looked at specifically before carteblanche advice is given to include as many polyphenols in the diet as possible. Prudent dietary guidelines are always advisable.

FRUIT JUICE PROCESSING AND PACKAGING

In order to produce a beverage from the Kei apple, it is necessary to understand what fruit juice processing is about. The following provides a summary.

The term "fruit" is the structure that encloses, protects or harbours seeds until they are ripe, a critical stage in the reproduction stage of botanical species, and they can be categorised into two groups according to their condition when ripe: dry fruits, and succulent or fleshy fruits (Taylor, 2005:36).

The conversion of fruits into fruit juice was originally developed as a method for making use of a suppliers surplus to the fresh fruit market (Pollard & Timberlake, 1971:573). Fruits used for juice manufacture are often those rejected because of the high specification for the fresh market, or they may be off-cuts from other fruit processes, or fruits which are specifically grown for juicing (Rutledge, 1996:70). The manufacture of juices from fruits and vegetables is as old as (or older than) agriculture. During the ripening process, most fruits soften to the point where simply holding or transporting them yields more juice than flesh, albeit often partially fermented. Through trial and error, humans learned practical ways of extracting juice from various sources, and most importantly which attractive but toxic fruits to avoid (Bates *et al.*, 2001:4).

A full description of fruit processing will not be dealt with in this review, but a generalised scheme for fruit juice processing is shown in figure 2.15. At each stage of the process, it is critical that relevant quality checks are adhered to in terms of hygiene and safety, for example, Hazard Analysis Critical Control Point (HACCP) (Early, 2002:91), which determines if there are any potential biological, chemical, physical or pest problems that may affect the health of any individual.

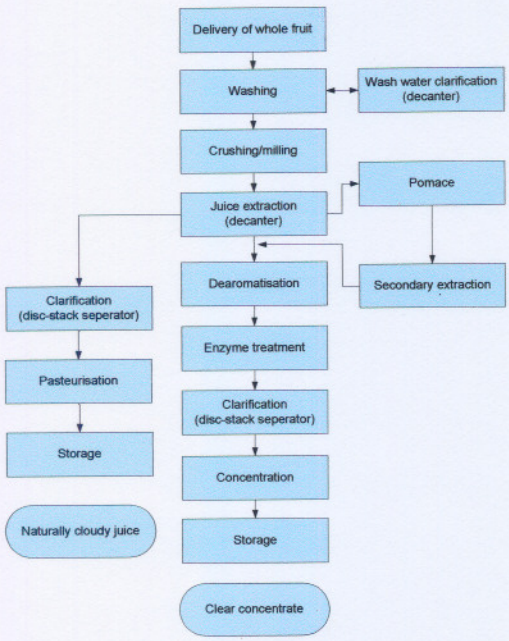


Figure 2.15: Flow diagram of the fruit juice concentration process (Taylor, 2005:53)

There is a key difference between citrus fruit juice processing and other fruit juice processing (stones or seed/pip-containing fruits). Citrus fruits are processed after cleaning by the Fruit Machinery Corporation (FMC) extractor. The extractor unit is designed to process individual fruits in rapid succession. Each extractor is constructed of stainless steel and comprises two cups, one inserted above the other. During operation, fruit is received into the lower cup and the upper cup descends to press down upon it. Simultaneously, a perforated stainless steel tube is forced up through a channel in the lower cup, cutting a plug out of the bottom part of the fruit and the juice is extracted through the tube, which also acts as a sieve. The solids are later ejected at the end of the process cycle in readiness for the next processing operation. As the two cups move together to enclose the fruit, the outside vesicles are ruptured to release oil, which is removed by water to be recovered later. The expressed juice flows into a manifold attached to the line of extractors, where the juice is progressively screened to remove excess pulp and bring it to range of set specifications (pulp may then be recovered for animal feed) (Taylor, 2005:50).

Processing of other fruits are generally simpler, and the usual method is to apply pressure to the pulped or whole fruit to force the liquid portion through a screen or cloth (Taylor, 2005:44). Table 2.9 below indicates the type of pre-treatment required, depending on the type of fruit.

Table 2.9: Fruit characteristics affecting juicing (Bates *et al.*, 2001 and Food & Agricultural Organisation, 6:2).

Type	**Procedure**	**Example**
Soft, all edible	Comminute, grind, press	Berry
Soft, seed inedible	Crush, press	Grape
Firm, seed inedible	Grind coarse, press	Apple
Firm, inedible skin and seeds	Ream, press flesh	Citrus
Soft, inedible skin and seeds	Pre-peel, gently pulp flesh	Mango
Brittle, inedible skin and seeds	Slice, gently press flesh	Passion fruit, lychee
Tough adhering skin and seeds	Hand or contour peeling	Pineapple
Soft, inedible skin	Roller / squeeze peel	Banana

There are various types of presses used and those are: 1) pack press, consisting of a set of frames and the pulp presses over each frame, continuously creating pressure to force juice out. This process is labour-intensive and suitable for smaller operations; and, more common is 2) the horizontal rotary press. A hydraulic piston operates within the cylindrical hollow body of the press. Between the specially designed end-plate and the piston face-plate run a large number of flexible drainage cores with well-defined ribbing along their lengths to act as juice channels. Each line throughout its length is covered with a coarsely woven filter sack; and finally, 3) centrifuges may be used to extract juice from a continuous fruit juice stream (Taylor, 2005:44).

Use of Enzymes

Enzymes may be used in the fruit juice processing stage, depending on the final outcome of the juice required. These enzymes are (Taylor, 2005:48):

- Pectases – to remove pectin and prevent / remove cloudiness of final product.
- Amylases – to break down residual starch which could gelatinise during processing and give rise to precipitation and haze effects.
- Cellulases – removal of colour.

Food Safety and Stability

The importance of safe food for the consumer cannot be overemphasized. There are many cases of food-borne diseases (poisoning) and these can be costly, in terms of discomfort or even lives, to the consumer, that is, days lost from work and lost to a company in terms of loss of sales and good faith. Some specific examples are in 2000, Snow Brand milk, containing *Staphlococcus aureus*, caused an 80% drop in sales volume. An excess of 14,000 cases of illness were caused by *Escherichia Coli* (0157:H7) in 1997. In 1998, the Sara Lee company was forced to withdraw 13.5 million kilograms of contaminated hot dogs and deli meats in the USA, after 82 cases of serious illness were reported and 21 official deaths in 22 states, due to *Listeria monocytogenes*. In South Africa, deaths in 2002 from *Clostridium botulinum* were reported, and in October 1999, there were cases of food poisoning from fruit juice at the All Africa Games (Von Holy & Marais, 2001:14-16).

Foods begin to lose their quality the moment they are harvested through changes resulting from physical, chemical, enzymatic or microbiological reactions. Food preservation prevents these deteriorative reactions, extending a food's shelf life and assuring its safety. Micro-organisms and enzymes are the main agents responsible for food spoilage and therefore the targets of preservation techniques. The main micro-organisms that are problematic within fruit juices are yeasts, *Lactobacillis*, *Clostridium*, *E.coli* (especially *E.coli* O157:H7) and *Salmonella*. Sources of contamination are many – from the fruit itself, to ingredients, processing, operators, distribution, and at the retail outlet. Any method of manufacture must minimise the risk pathogenic bacteria ultimately present, namely the risk to human health (Wareing & Davenport, 2005:279).

Escherichia coli (*E.coli*) are a group of four enterovirulant bacterium that cause gastroenteritis in humans, these being enterotoxigenic *Escherichia coli*, enteropathogenic *E.coli*, enteroinvasive *E.coli* and *Escherichia coli* O157:H7 or enterohemorrhagic *E.coli*. *E.coli* is the dominant species found in faeces. *E.coli* O157:H7 has recently been associated as having a presence in fruit juice – particularly apple juice, in addition to meat. Other strains of *E.coli* present in the intestines normally form a useful function in the body by suppressing the growth of harmful bacterial species and by synthesizing vitamins (FDA, 2005, http://www.cfsan.fda.gov/~mow/chap13-16.html, 27/09/05). As the *E.coli* is indicative of faecal contamination and the strain *E.coli* O157.H7, its presence in fruit juice is concerning and therefore must always be included in any testing methodology.

Spoilage in fruit juices is often due to yeasts (especially *Zygosaccharomyces bailii*) and their resultant fermentation process – resulting in the formation of carbon dioxide and alcohol and off odours from the fruit sugars (Waring & Davenport, 2005:278).

Mould problems can be divided into two types: again due to poor hygiene, or slow growth of the mould within the processed product. The former type can cause tainting, discolouration, and other general problems associated with gross mould growth. The latter type can result in slow growth of the mould with the processed product. There is some overlap between the two groups. Xerophilic (highly sugar-tolerant) fungi are likely contaminants if hygiene is poor (Waring & Davenport, 2005:284).

In addition to the bacteria found on fruit, moulds and yeasts often comprise the majority of microflora on raw fruits largely due to the acidic pH of fruit tissue (Burnett & Beuchat, 2000). Yeasts and moulds are not necessarily pathogenic in nature but are spoilage agents which will affect the taste and quality of the product. Yeasts in general, and *Zygosaccharomyces bailii* in particular, remain the key spoilage organisms because of their overall physiology and resistance to organic acid preservatives (Waring & Davenport, 2005).

Another spoilage organism (bacteria) that has been isolated over the last 23 years is *Alicylobacillus*, first isolated in 1982, which is of particular interest in the fruit juice industry, as it has been identified as a major spoilage organism in the fruit juice industry (especially pasteurised apple juice) as the organism can tolerate acidic conditions. The organism results in undesirable off odours, which are off putting to consumers (Chang & Kang, 2004). As time goes on, more and more bacteria are being discovered that are responsible for food poisoning and therefore the issue of food safety cannot be overemphasised.

Contamination typically occurs early in the production process rather than just before consumption (Tauxe, 1997) and thus it is only under unusual conditions that the consumer is at fault. It is the responsibility of the harvester, manufacturer, distributor and retailer to ensure that the product arrives in good condition, that is, fit for human consumption.

The US Centre for Disease Control and Prevention (CDC) has linked a variety of pathogens to fresh fruits and vegetables harvested in the United States and elsewhere. Those specifically applicable to the production of fruit juice are shown in table 2.10.

Table 2.10: Examples of pathogens implicated in causing outbreaks of diseases associated with raw produce and produce products (extracted from Burnett & Beuchat, 2000).

Produce	Pathogen
Juice	
Apple	*C. parvum*
Apple	*E. coli O157:H7*
Apple, orange	Salmonellae
Coconut milk	*Vibrio cholerae*

The presence of pathogenic bacteria, viruses and parasites on fresh fruits and vegetables can be attributed to the following sources as listed in table 2.11 (Beauchat & Ryu, 1997).

Table 2.11: Sources of pathogens on fruit and vegetables (Beauchat & Ryu, 1997).

Pre-Harvest

- Faeces
- Soil
- Irrigation water
- Water used to apply fungicides, insecticides, etc
- Green or inadequately composted manure
- Air (dust)
- Human handling
- Wild and domestic animals (including fowl and reptile)

Post-Harvest

- Faeces
- Human handling
- Harvest equipment
- Transport containers
- Wild and domestic animals
- Insects
- Air
- Wash and rinse water
- Processing equipment
- Transport vehicles
- Improper storage
- Improper packaging
- Cross contamination
- Improper display temperatures
- Improper handling after wholesale or retail purchase

Once the product has been produced, its microbial stability must be maintained before consumption. The fruit juice is a fairly good medium for food spoilage and pathogenic growth. Microbial problems within fruit juices can be divided into two groups (Wareing & Davenport, 2005:279): 1) growth in, and deterioration of, the product by general organisms to produce spoilage; and 2) growth in, or contamination of, the product by pathogens to produce food poisoning.

Preservation Techniques

An ideal method of food preservation has the following characteristics: 1) it improves shelf life and safety by inactivating spoilage and pathogenic organisms; 2) it does not change

organolepic and nutritional attributes; 3) it does not leave residues; 4) it is cheap and convenient to apply; 5) it encounters no objections from consumers and legislations (Raso & Barbosa-Cánovas, 2003).

The principle of maintaining product integrity of the fruit juice is either by heat preservation, chemical preservation or a combination of both. Briefly, there are five main processes for juice: 1) flash pasteurisation; 2) hot filling; 3) in pack pasteurisation; 4) aseptic filling; 5) chilled distribution. Each process either involves heating the product to destroy microbes (1-4) or ensuring that microbial growth cannot occur (5) (Lea, 2005:185-190).

There are four preservatives currently used by the soft drinks and fruit juice industry. These are: 1) Sulphur dioxide, which acts as an antioxidant, preventing browning. It is a most effective and wide-acting preservative. However, it can be detected by some tasters. 2) Benzoic acid or sodium benzoate, which is effective against most yeasts and moulds. It can be difficult to dissolve and has a tendency to precipitate out and can impart a slight flavour at higher levels. 3) Sorbic acid or potassium sorbate, which is more effective at lower acidities than Sodium Benzoate. It is less effective against some bacteria, less soluble in cold temperatures and can precipitate out. 4) Dimethyl dicarbonate, which acts as a sterilant, killing microorganisms. It hydrolyses in contact with water and so is not present when the consumer opens the container (http://www.britishsoftdrinks.com/htm/qua/Additives Ingredients/preservatives/...htm, 15/10/05). The first three preservatives are often used in combination with one another to make them more effective, whilst the latter preservative is used on its own. Due to the total breakdown of dimethyl dicarbonate, a preservative-free claim may be made, as there is no residue left after 24 hours. This latter preservative, although being widely used in Europe, has only recently been introduced into South Africa under the trade name of Velcorin (Bayer personal communication).

Packaging

There are several forms of packing for fruit juices and, far from being mere containers, they must be considered in many lights. They are the carriers of branding, manufacturer's information, legal and nutritional information, and must have product / pack integrity, must not leak, match up to manufacturing processes, protect the product in the distribution channel and yet be cheap enough for the consumer to purchase the product.

Different forms of packing include glass (still thought by many traditionalists to offer the best taste profile for juice) (Siegmund *et al.*, 2004), PET or polyethylene terephthalate (which has gained considerable ground since the 1970s), PVC (polyvinyl chloride), HDPE (high density polyethylene) and polypropylene. Other containers include cans (mainly aluminium) and cartons (Tetra pak and Tetrabrik, the latter consisting of layers of paper, polyethylene and aluminium to maintain barrier properties and strength).

Legislation

In Europe, for fruit juice it should be clearly indicated when a product is a mixture of fruit juice and fruit juice from concentrate and, for fruit nectar, when it is obtained entirely or partly from more concentrated products. The terms "made with concentrate(s)" or "partially made with concentrate(s)" should be used, clearly visible on the packing and near the product name. For products from 2 or more fruits, a list of the fruits should be listed after the product name, in descending order of volume. The addition of sugar or an extra pulp or cells must be indicated. For fruit nectars, the minimum content of fruit juice and/or fruit purée must be indicated (Cheftel, 2005). The USA legislates fruit juice under the auspices of the FDA.

In South Africa, all food labels and health claims fall under the Foodstuffs, Cosmetic and Disinfectants Act 54/1972 (Department of Health, South Africa, 2001). The legislation of fruit juice itself falls under Department of Agriculture, 1980, Agricultural Products Standards Act. Regulations relating to the classification, packaging and marketing of fruit juice and drinks intended for sale in the Republic of South Africa and falls under GN R2286, 7th November 1980. Common definitions for fruit juices are presented in table 2.12.

Table 2.12: Some common fruit juice designations (Bates *et al.*, 2001, 1:4).

Term	Criteria	Remarks
Pure juice 100%	All juice	No adjustment, not from concentrate
Fresh squeezed	Not pasteurized	Held refrigerated, food safety concerns
Chilled, ready to serve	All juice	Held refrigerated, made from concentrate or pasteurized juice
Not from concentrate	Single strength	Pasteurized after extraction
From concentrate	Made from concentrate	Reconstituted and pasteurized
Fresh frozen	Unpasteurized	Single strength, frozen after extraction
Juice blend	All juice	A mixture of pure juices
Puree	Pulp-containing	More viscous than juices, totally fruit
Nectar	Pulpy or clear	Sugar, water and acid added, 25-50% juice *
Nectar base	Requires reconstitution	Possesses sufficient flavour, acid and sugar to require water dilution for consumption *
Juice drink	Low in juice	Contains 10-20% juice *
Juice beverage	Low in juice	Contains 10-20% juice *
Juice cocktail	Low in juice	Contains 10-20% juice *
Fruit + ade	Lemonade	Contains >10% fruit juice, sugar and water *
Juice extract	Water extract	Fruit extracted by water, then concentrated *
Fruit punch	Token juice	~1% juice, + natural flavours
Natural flavoured	Token juice	Usually >1% juice

* differing country standards for juice solids minimum

Thus again the consumer must look carefully at the label of the juice to ensure that they are actually buying what they think they are buying. It is easy for clever marketing claims to override legislation and explains why (chapter 2:10) such a proliferation of beverages with spurious claims exist.

NEW PRODUCT DEVELOPMENT (NPD)

This project is concerned with the development of a new product, that is, the Kei apple product. In order for the project to be successful, it must be developed as any other new product would be developed. Therefore, a short review of New Product Development is given.

The developing and marketing of successful new food products is recognised as an important competitive (although risky) strategy of a modern food company. Markets cannot be sustained without product initiatives and revitalised with new products. Four factors stand out in recognising the need for new product development: 1) market places in turmoil – consumers' changing needs and expectations for new products (and therefore functional foods), 2) technological progress, 3) competitive pressure, and 4) shortening product life cycles (Buissan, 1995).

It is worthwhile describing briefly what classifies "New Products", summarised from Cooper (2001:14). These are 1) new to the world: first of their kind and create a new market, for example, functional probiotic foods; 2) new product lines: not new to market but new to company and health claims; 3) additions to new product lines: flavours; 4) improvements and revisions to existing products: better taste; 5) repositions: for example appeal to younger consumers as well; 6) cost reductions.

The new product development or innovation process may be divided into three areas: the fuzzy front end (FFE), the new product development portion (NPD) and commercialization part (Koen, 2005:82). The subject of this thesis is concerned with developing a product from the FFE into a situation that it may be taken into the NPD process and finally commercialization, as shown in figure 2.16.

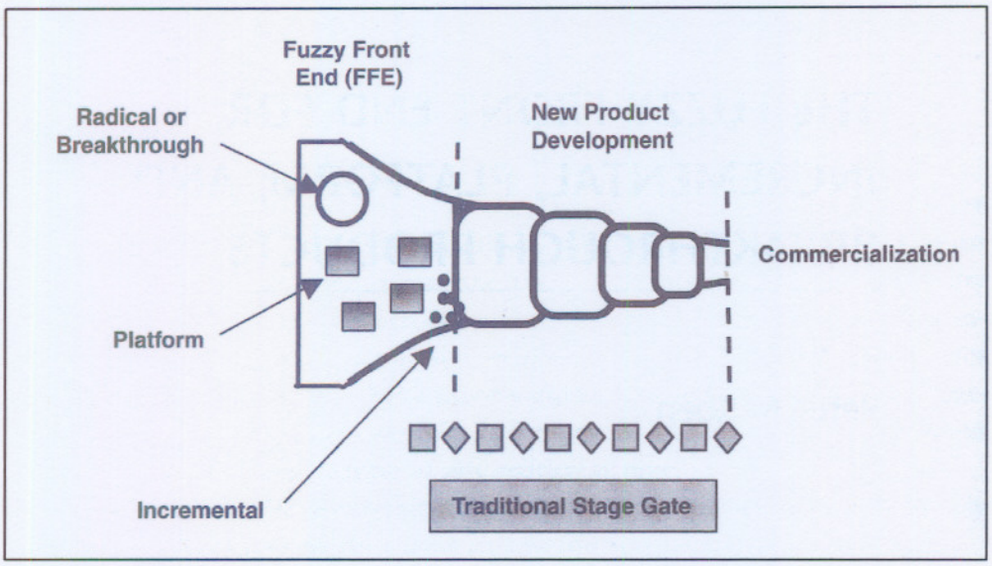


Figure 2.16: Three parts of the innovation process: fuzzy front end, new product development and commercialization (Koen, 2005)

The FFE is the terminology used when a product is being developed when it is not certain what is going to be developed, and is often performed outside an institution, either outsourced or at a university. In a company or organisation, the development of a new product involves a stage-gate process (Annacchino, 2001, Cooper, 2001:130,147 and 148), as demonstrated in figure 2.17.

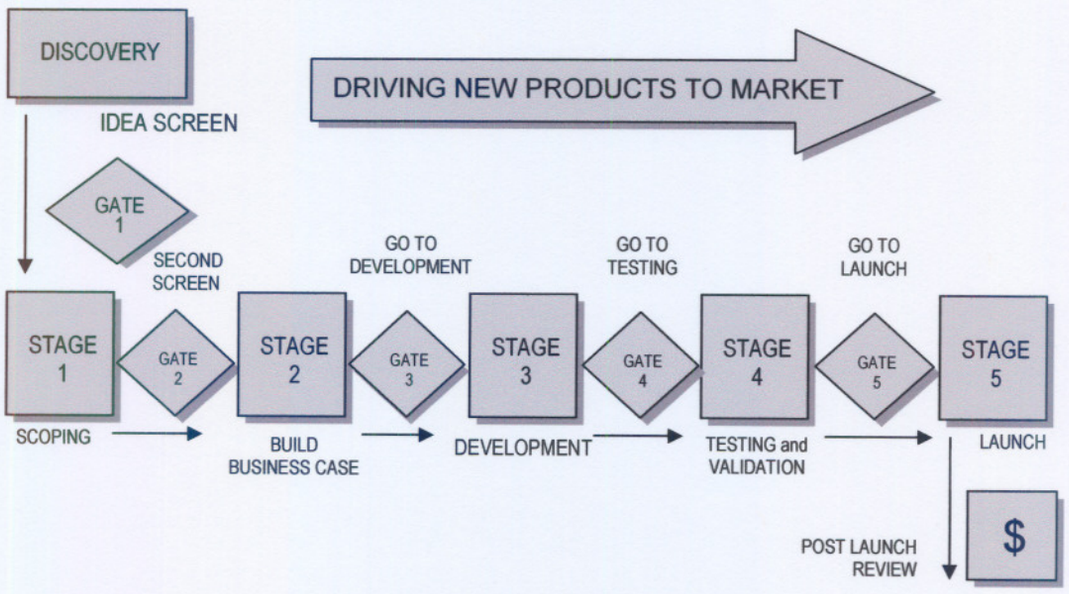


Figure 2.17: The typical stage-gate model – from discovery to launch (Cooper, 2001:130).

Each square in the stage marks areas of activities where the product is progressed further. At the diamond, a decision is taken whether to progress the project based on if the product fits the company's criteria for new products, which could be based on finance, strategy, superiority, timing of launch, depending on the company (Annacchino, 2001:144).

The process is a multi-disciplinary approach, taking into account all aspects of a business from finance, supply chain, marketing, sales and research and development. Information and processes are free-flowing, dynamic and flexible (Holman *et al.*, 2003) to increase the speed of development. A full review of new product development is beyond the scope of this literature review, but a summary of the critical success factors, according to Cooper (2005:84) is set out in table 2.13. It should be noted that the language style of this table is that of the marketing expert and not the scientist and this table has been extracted verbatim from Cooper's work, who is considered by many to be an expert in the field of new product development.

Table 2.13: Critical success factors in a product innovation (Cooper, 2005:84).

1. The number one success factor is a unique, superior product: a differentiated product that delivers unique benefits and superior value to the customer.
2. A strong market orientation: a market-driven and customer-focussed new product process is critical to success
3. Look to the world product: an international orientation in product design, development and target marketing provides the edge in product innovation.
4. More pre-development work: the homework – must be done before product development gets under way.
5. Sharp and early product and project definition is one of the key differences between winning and losing at new products.
6. A well-conceived, properly executed launch is central to new produce success. And a sold marketing plan is at the heart of the launch.
7. The right organizational structure, design and climate are key factors in success.
8. Top management support doesn't guarantee success, but it sure helps. But many senior managers get it wrong.
9. Leveraging core competencies is vital to success – "step out" projects tend to fail.
10. Products aimed at attractive markets do better: market attractiveness is key project-selection criterion.
11. Successful businesses get tough. Go/kill decision points into their new product process, where projects really do get killed: better focus is the result.
12. New product success is controllable: more emphasis is needed on completeness, consistency and quality of execution of key tasks from beginning to end of project.
13. The resources must be in place – there is no free lunch in product innovation.
14. Speed is everything! But not at the expense of qualify of execution.
15. Companies that follow a multi-stage, disciplined new product process – a Stage Gate™ process – fare much better.

Typically, a company takes a standard procedure and adopts and evolves it to suit their own organisation (Rudder *et al.*, 2001).

All NPD must be consumer-driven (Jaeger *et al.*, 2003) and it is important that customer needs are taken into account (Griffin, 2005:213). The main reasons why new products fail have been identified by Cooper (2001:23-26) as being inadequate marketing analysis: 24%; product problems or defects: 16%; lack of effective marketing effort: 14%; higher costs than anticipated: 10%; competitive strength or reaction: 9%; poor timing of introduction, technical or production problems: 6%; all other causes 13%. Other reasons for failure include poor planning, which incorporates issues such as developing a product that doesn't fit a company's strategy, competencies and/or distribution strength; poor management, which does not support product development, is a primary cause of new product development failure; as is a poor product concept which lacks a compelling consumer belief (a "me too" product for example). To be successful, in essence, a new product must deliver on the concept promises, have advantages in terms of quantity and quality matters, competitive advantages, correct distribution to drive sales, and long-term support (Lord, 2000:52-65).

It is critical to develop a product with the end consumer in mind, and that consumer research is critical at many stages of the FFE and NPD stages (van Kleef *et al.*, 2005(a), van Kleef *et al.*, 2005(b)). As a result, whilst developing food products either at the FFE or during the NPD stage, if the product passes into commercialization, sensory evaluation is a necessity to determine if it is acceptable to consumers and whether they would consider purchasing the product (Hollingsworth, 1998). Stewart-Knox and Mitchell (2003) summarise that in addition to the aforementioned processes and procedures, key issues to successful product development are market, consumer and retailer involvement. This is especially important for development of functional product where neophobia (fear of trying new products) may hinder product trials, and it is urged that more emphasis be placed on a consumer-driven product development process.

Various studies have been performed on consumer perceptions of functional foods, as shown in table 2.14. These perceptions and insights must be taken into consideration when formulating new products on functional foods. In essence, the end consumer must always be front of mind when developing a product.

Table 2.14: Consumer perceptions of functional foods (in chronological order), as summarized by consumer research in several countries from 1997 to 2005 inclusive.

Consumers	Conclusion	Study
1005 adults telephonically interviewed, conducted in the USA using a ten-point ranking scale.	55% of the adult population believed naturally-occurring substances in fruit, vegetables and cereal grains can prevent disease: females > males, older > younger. 40% showed an interest in regularly purchasing a food or food product to prevent disease: females > males. These figures increased since the last survey and showed females to be the most important purchaser. There was a strong anti-science prejudice held by many consumers.	Childs and Poryzees, 1997 Foods that help prevent disease, consumer attitudes and public policy implications.
5 focus groups conducted – in total 35 Australian participants included.	Popular brands of functional margarine, cereal and yoghurt were discussed. High cholesterol health issues stimulated food choice, price was a negative. Most respondents did not trust manufacturer's claims but were influenced and there was a clear indication for the need for legislation. Price of functional food was an issue (especially with younger participants) and they were unlikely to switch at this age, unless concerted efforts by all stakeholders were made.	Bhaskaran and Hardley, 2002 Buyer beliefs, attitudes and behaviour: foods with therapeutic claims.
1552 members of Dutch Health Care Consumer Panel (representative of Dutch demographics) – questionnaire used. Panel established in 1991 by Netherlands Institute for Health Services Research.	Consumption of cholesterol-lowering margarines more likely to have poorer subjective health. Dutch consumers more sceptical than US consumers, but in favour of concept – supplement users more likely to be female, better educated, affluent, non-smokers, light drinkers.	De Jong *et al.*, 2003 Demographic and lifestyle characteristics of functional food consumers and dietary supplement users.
6 product categories examined: yoghurt, spread, juice, carbonated soft drink, sweets, ice cream (50 Finnish: 19 men and 31 women participants). One-to-one interviews conducted.	Choice factors found to be quality, price and taste, but no family preference. Healthiness was seen to be a multi-dimensional choice factor. Health and food related to improved performance and overall well-being. Yoghurt and juice – probiotics and stomach; general well-being – yoghurt, spread, juice, carbonated soft drinks and ice cream; improved performance – juice and carbonated soft drink; functional sweets associated with preventing disease; Xylitol associated with teeth. In summary, varied with product category.	Urala and Lähteenmäki, 2003 Reasons behind consumers' functional food choices.
Finnish, Danish and US respondents (500 households) were interviewed on their perceptions of juice, yoghurt and spreads. Face-to-face interviews concucted in each household.	All 3 countries showed common patterns. However, Finnish more positive than Americans and Danes, but consumer's perception of the healthiness of functional foods is more dependant on their perception of the nutritional qualities of the base product than any type of health claim – juice and yoghurt being perceived as more healthy than spreads.	Bech-Larsen and Grunert, 2003

Consumers	Conclusion	Study
Review of several studies.	Various beliefs affect consumer's responses to acceptance of functional foods. Main findings were cultural; women more knowledgeable than men; if less at risk from health, may be less likely to be concerned; food must be palatable; lack of belief in other people's opinions; genetically modified foods are an issue; price should not be an issue (consumers not willing to pay a premium). Overall, consumer's needs must be well understood if food is to be successful.	Frewer *et al.*, 2003 Consumer acceptance of functional foods: issues for the future.
1158 Finnish consumers studied for perceptions against probiotic juice, juice and milk with added calcium, cholesterol-lowering spread, meat and fibre, blood pressure-reducing milk, energy drink, sugar-free gum, plus: low-fat cheese, low salt food, organic bread, rye bread – reference foods. 7-point hedonic scale questionnaire.	Consumers do not perceive functional foods as one homogenous ground – their willingness to use food depends on their confidence and attitude, feelings of well-being and control and life and health. Functional foods may offer a way of offering consumers part of healthy lifestyle – achieving modern and positive self images. Task was seen as important. Bad neophobia had slightly negative affect because functional foods may be perceived as new products.	Urala and Lähteenmäki, 2004 Attitudes behind consumer's willingness to use functional foods.
350 Finnish respondents: probiotic yoghurt, omega-3 eggs and fruit juice with added fibre and calcium with non-conventional items. (5-point hedonic scale questionnaire).	Consumers interested in healthy foods; generally better educated, older and women; buyers more disciplined but more innovative and less gentle (selfish). Women more familiar. In summary, must be careful with consumer messages to be successful.	Saher *et al.*, 2004 Impressions of functional food consumers.
215 consumers in Belgium in 2001. 5-point hedonic scale questionnaire; face-to-face interviews.	46.5% accepted concept of functional food, likelihood believing increased with the presence of an ill family member, and consumer age.	Verbeke, 2005 Consumer acceptance of functional foods: socio demographic, cognitive and attitudinal determinants.

These studies have been undertaken mainly in Europe and the United States with consumers of a higher income level than would be found in less developed countries. However, it is these consumers that companies generally target their "functional" products to. Several general comments can be made from the studies. It appears that consumers that are health-conscious are generally older and are more likely to purchase functional

foods. Once a member of the family is ill, or the person themselves falls ill, they are more likely to become more health conscious. Taste must not be compromised at the expense of a product "good for you". There was some skepticism of the claims made by companies, as to whether they were really believable, and therefore a need for legislation. Women were seen as being the major role players in the choice of food consumed and were therefore more knowledgeable about the science / nutrition regarding the food.

Astringency in Relation to Polyphenols and Beverages

Before proceeding with the rest of this review, it is relevant to comment on the astringency related to some fruits as mentioned by Miller and Ruiz-Larrea (2002). The word astringency, from the Latin *ad stringere*, meaning "to bind" reflects what is believed to be the primary chemical process that gives rise to the sensation. Simply put, compounds that have the ability to bind with and cross-link proteins, are considered to be astringents. The most common of these are the polyphenols, which, because of their use in the tanning processing, are also known as "tannins".

Astringency and high levels of acidity make it difficult to formulate a palatable product (Drewnowski & Gomez-Carneros, 2000), as the human palate is very sensitive to low levels of acidity and astringency, compared to preference for sweet and salty products (Jellinek, 1985:40-50). Thus high levels of acidity and astringency are difficult to mask. The perceptions of the four basic tastes, that is, sweet, salty, sour and bitter, are explained in the textbooks Jellinek (1985), Meilgaard *et al.* (1999), Stone and Sidel (1985) and others mentioned previously. It is known that tannins have a profound astringent effect, that is a puckering or a drying effect on the palate (Prinz & Lucas, 2000). Fukui *et al.* (2002) state that the wine components responsible for bitterness and astringency are primarily phenolic compounds, especially flavonoid phenols which originate from grape seeds, skins and stems.

Vidal *et al.* (2003) state that bitterness and astringency are two sensory terms of critical importance for describing the sensory properties of wine. Whereas bitterness is a taste mediated by sensory receptors, astringency is considered to be a tactile sensation resulting from the precipitation of salivary proteins and leading to a loss of mouth lubrication. However, the same definitions may be applied to fruits. It can be deduced that if a product is too astringent, it may therefore not be acceptable to the consumer and purchased

(Drewnowski & Gomez-Carneros, 2000). However, the astringency of the product gives a clear indication that the product or food is potentially rich in polyphenols (it was for this reason that it was decided to further study the Kei apple).

Valentová *et al.*, (2002) studied astringency on a time-intensity basis, as the astringency of a product lasts long after swallowing. It was concluded that astringency can be counteracted in wine and orange juice more than a in control water solution when tannic acid and catechin were tested alone, and sugar decreased the astringency of wine. However, the D-fructose and D-glucose already present in orange juice contributed to decreased perception of astringency. Prinz and Lucas (2000) studied saliva-tannin interactions and concluded that tannic acid significantly reduces the lubricating qualities of human saliva, both by decreasing its viscosity and increasing friction, and its effect depends on the presence of salivary proline-rich proteins (PRP). This may explain cross cultural preferences for tannin-rich beverages such as tea, coffee and red wine and may be explained by reduction in adhesion of food particles to the oral mucosa, allowing their rapid oral clearance (that is, the particles of food left behind in the mouth and oral cavities are loosened by the tannin-PRP reaction).

Certain individuals have the ability to taste the chemical 6-n-propylthiouracil (PROP), which is an extremely bitter compound (Fox, 1931 as quoted by Pickering *et al.*, 2004). The researchers Ishikawa and Noble, 1995, found that PROP tastes are more sensitive to astringency sensations in red wines. This obviously would affect consumer's perceptions of a product, depending on the proportion of PROP tastes in the consumer palate. Mennella *et al.*, 2005, discovered that variations in a taste receptor gene accounted for a major proportion of individual differences in PROP bitterness perception in both children and adults, as well as a portion of individual differences in preferences for sweet flavours in children but not in adults.

Thus, for some individuals, perception of bitterness and astringency is lower than for others, making astringent products more acceptable to them. However, for very astringent and bitter products, the challenge for a new product is to formulate it in such a way that the majority of the population accepts it.

SENSORY EVALUATION AND CONSUMER RESEARCH

During and after a product or prototype has been developed, it is necessary to evaluate it with the target consumers, and this is where the realms of sensory evaluation and consumer research prove to be of utmost importance.

There is great debate as to when sensory evaluation turns into market research or consumer testing but, in essence, market research is concerned with consumers and sensory evaluation is applicable inside a company or institution. Currently there are several definitions for sensory evaluation, which will subsequently be given.

Sensory evaluation is a science that concerns itself with measuring the responses of people to products in terms of its appearance, aroma, taste, texture and aftertaste as a reaction to the different senses, without benefit of a label, pricing or either imagery (Stone, 1999, Stone & Sidel, 1995). Thus evaluation of products without the label, price and branding differs from market research, where the latter is performed using branded products. It is usually used to answer three broad categories of questions: "What is the product in terms of its perceived characteristics?", "Is the product different from another?" and "How acceptable or preferred is the product?"

The methods employed in sensory evaluation vary from simple (difference testing) to complex panel testing, where individuals are trained in terms of the language of the consumer. It is important that, when performing sensory evaluation, the method applied fits the end objective of the test (O'Mahony, 1995).

This is demonstrated by a supplier's failure to perform adequate sensory testing when it launched its new beverage product in Japan. The product had a sediment which, in the rest of the world, was perceived as a "natural" product but was disliked by the Japanese (Hollingsworth, 1998).

Testing Methodology

There are two basic types of sensory methods tests – analytical and affective. The aforementioned (analytical method) encompasses discriminative tests (a few examples are shown in table 2.15) to evaluate whether the products are perceived as being different; and

descriptive tests to evaluate the type of differences and their magnitudes. Analytical tests are performed by trained panels. Examples of difference tests are paired difference, triangle, duo-trio tests (Jellinek, 1985:189) and ranking tests (Jellinek, 1985:252). Descriptive analysis represents the most sophisticated of the available sensory methodology compared with discrimination and acceptance tests. These include Flavour Profile®, Texture Profile® and Quantitative Descriptive Analysis® and generics, therefore as the other methods are registered, fees must be paid. The methods include a complete sensory description of test products and provide a basis for determining the sensory characteristics that are important to acceptance (Stone & Sidel, 1985:165). However, they are extremely time-consuming and lengthy and are often not used commercially.

Table 2.15: Examples of different sensory methods and its purpose (adapted from Meilgaard, *et al.*, 1999:61 and Stone & Sidel, 1985:230-233)

Test	***Purpose***
Paired comparison	Participants are requested to indicate which of two coded products is preferred, or indicate if there is no preference. Easy to set up, as there are only 2 ways of presenting the samples.
Triangle test	Three samples are presented to each individual.
Multiple paired test	Here, more than one sample is evaluated. The test is more complicated as the samples must be presented in all possible combinations to the consumer.

It is important to differentiate between trained and consumer panels. Once the panelists of an analytical panel have been trained, they tend to become more aware of the various attributes of a product, and weigh attributes differently from the way a normal consumer would, in terms of the contribution of each attribute to the overall quality, blend or balance (Meilgaard *et al.*, 1999:165).

Affective tests, which are usually performed by untrained consumer panels, are concerned with how well the product is liked (and here the use of scales, such as hedonic, may be utilized) and preference (which product is preferred). In larger scale consumer testing, other types of methods also will be used, such as appropriateness, purchase, intent, agree / disagree (Stone, 2005). Affective testing thus refers to the acceptance of a product, and may generally be referred to as acceptance testing. In the sensory testing sequence, acceptance testing usually, but not always, follows discrimination and descriptive tests which have reduced the number of product alternatives (Stone & Sidel, 1985:227). This former testing can be performed with the trained panellists in-house to reduce costs if the

development of a product is being undertaken by a company. Often, companies have their own trained panels for such purposes before the consumer research (more costly) is performed.

Preference can be measured directly by the comparison of two or more products with each other, that is, which of two or more products is preferred (Stone & Sidel, 1985:227). Preference testing is extremely important in determining the success of a product, especially food. This is borne out by Hess (1997) who states, "From a nutritional perspective, if a food does not taste good, neither health professionals nor those they counsel are likely to eat it. Medications, chronic disorders and radiation therapy can alter taste perception, resulting in loss of appetite. Individualising nutritional advice with consideration to taste, health needs, and personal preference, is a 'signature dish' of quality dietetics practice". Each consumer has their own specific likes and dislikes which, for a company selling food products, is ultimately related to sales. These preferences are determined by cultural influences and psychological factors. There is evidence that these likes/dislikes start to develop in the womb and are further influenced by breast feeding – directly related to those foods consumed by the mother (Mennella, 1998).

The primary purpose of affective tests is to assess the personal response (preference and/or acceptance) by current or potential consumers of a product, a product idea or specific product characteristics. For the average company's products, the amount of testing generated by intended and unavoidable variations in process and raw materials far exceeds the demand of consumer panel testing and thus there is little choice but to use in-house panels for the latter (Meilgaard *et al.*, 1999:233).

For the purpose of this study, affective tests were used and will thus be discussed in more detail.

Methods Employed in Affective Testing

Paired Comparison Tests

These are generally less informative than the direct scaling methods such as the hedonic scale. This is because the former test gives no magnitude estimation of the level of preference. For example, both products may be disliked. This may be overcome by the use

of the multiple paired test, but these are generally cumbersome, for example, for four products, there are six pairs, ten pairs for five products, 15 pairs for six products, etc. However, the paired comparison is useful in situations where the chemical senses (taste and smell) are not involved (Stone & Sidel, 1985:233-234). Generally, discrimination tests, although having their place in consumer panels, are not used and are left to the trained panels.

Hedonic Scales

The hedonic scale is generally the most preferred for affective testing, as it is easily comprehended by consumers who are inexperienced in evaluating products with minimal instruction and is reproducible with different groups of subjects. As with all scales, there are criticisms, that is, use of parametric methods for analysis with a scale that is bipolar, the lack of definitive evidence of the equality of the intervals and avoidance of the neutral category (Resurreccion, 1998:21).

The hedonic scale measures the degree or strength of liking for each product, and responses can be converted to ranks (indirect measurement of preference, for example). It is the most useful method of measuring liking for a product. For two or more products, results include for each product: mean and measure of variability by order and independent of order, frequency distribution by order and independent of order, magnitude of difference (product vs. 1 vs. 2 vs. 3, for example), and conversion to preferences (Stone, 2005).

The hedonic scale can be used on a vertical basis, as show in table 2.16, and figure 2.18 is an example of how it can be converted into a line scale on a horizontal axis.

Table 2.16: Hedonic score card (Meilgaard *et al.*, 1999:244 and adapted from Stone, 2005)

Name:	Code:	Date:
Circle the statement that best reflects your opinion about this product:		
9-point scale	*7-point scale*	*5-point scale*
Like extremely	Excellent	Excellent
Like very much	Very good	Good
Like moderately	Good	Fair *
Like slightly	Fair *	Poor
Neither like nor dislike *	Poor	Terrible
Dislike slightly	Very poor	
Dislike moderately	Terrible	
Dislike very much		
Dislike extremely		
* Indicates mid-point of scale		

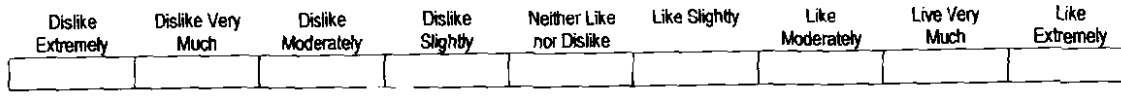


Figure 2.18 Hedonic scale for evaluation of overall acceptance (Resurreccion, 1998:21)

The hedonic scale has been used for many years in sensory evaluation to determine the acceptance of a food and provide a benchmark to compare results. The scale can be three, five, seven or nine points, but three is not used for adults, as they tend to avoid using the ends of the scales (Resurreccion, 1998:21). The hedonic scale may also be used with children if smiley faces are used (Resurreccion, 1998:22).

It is important to note that, when these scales are developed for the purpose of the project (be they nine-, seven- or five-point scales) they must be balanced, evenly spaced and contain a mid-point which is considered neutral for consumers to evaluate from and that encourages them to rate from this point. Examples of scales that are unclear in balance or spacing are shown in table 2.17.

Table 2.17: *Examples of hedonic scales that are unclear in balance or spacing* (Meilgaard *et al.*, 1999:244)

9-point quartermaster (unbal.)	*6-point wonderful (unbal.)*	*6-point (unbal.)*
Like extremely	Wonderful, think its greats	Excellent
Like strongly	I like it very much	Extremely good
Like very well	I like it somewhat	Very good
Like fairly well	So-so, *its just fair*	Good
Like moderately	I don't particularly like it	Fair
Like slightly	I don't like it at all	Poor
Dislike slightly		
Dislike moderately		
Dislike intensely		

Another type of scale often used in affective tests, namely the FACT rating scale, as shown in table 2.18, was devised by Schultz in 1965 (as quoted by Resurreccion 1998:21) to measure the acceptance of a product by a population. It is a measure of general attitude towards a product. The rating scale includes action as well as affective type statements. The table shows an example of a nine-point scale. However, as with hedonic sales, the scale can be adapted to suit the purpose of the project and the ratings can be converted to numerical scores to facilitate statistical analysis of data, or scaled downwards. Therefore, a seven-point or five-point scale can be produced. It is a measure of general attitude towards

a product, and the panellist is asked to decide which of the statements on the scale best represents his or her attitude (Resurreccion, 1998:21).

Table 2.18: Descriptors used in the Food Action Rating Scale (FACT), as devised by Schultz (1965) (Resurreccion, 1998:22).

I would eat this food every opportunity I had.
I would eat this very often.
I would frequently eat this.
I like this and would eat it now and then.
I would eat this if available but would not go out of my way.
I don't like it but would eat it on occasion.
I would hardly ever eat this.
I would eat this only if there were no other food choices.
I would eat this only if I were forced to.

The Subjects / Consumers in Affective Tests

With consumer sensory testing, subjects are selected as a sample of some larger population about which a conclusion is drawn. In analytical discrimination tests (difference and descriptive), people with above-average or average abilities are used to detect difference. It is then assumed that if those individuals cannot perceive a difference, the larger population will not. This is not the case for affective testing. Here, the population or target market for which the product is intended is first determined (Meilgaard *et al.*, 1999:235). Consumer testing is then performed on a representative sample of the target market group. It is critical that the sample of individuals used is of a large enough size and of the correct demographics to predict accurately how the target market will behave towards the product.

The demographics typically used in determining the sample subjects are: user group – light, moderate or heavy users, for example tea or coffee, determined as cups per day; age, for example, adolescents for snack market; sex; income; geographical location; nationality, ethnic group, religion, level of education and pet ownership.

Location of Testing

There are varying locations that consumer testing may take place. The characteristic of these tests are summarized in table 2.19. As can be observed in the table, each location

has their own disadvantages and advantages. Generally, as the new product development process is followed, the progression of testing proceeds from the laboratory to a central location (such as a supermarket) and finally to a home-use situation. This occurs as the product becomes closer and closer to the actual product that will be launched. Every time a sensory test is performed, usually (but not always) a modification is made to the product as input from the respondents or consumers is received, until it matches the exact profile of that which is required for the market place.

Table 2.19: *Different types of sensory acceptance tests* (Stone & Sidel, 1985:240)

	Laboratory	Central Location	Home Use
Consumer type	Employee	Public (general or selected)	Employee or public
Responses per product	25-50	100+	50-100
Product number	2-5	2-4	1-2
Test type	Preference, acceptance, but *not* quality	Same as Laboratory	Preference, acceptance, performance (intensity and marketing information)
Advantages	Controlled conditions. Rapid data feedback. "Test-wise" subjects. Low cost.	Large number of subjects. No company employees.	Product tested under actual use conditions. Obtain all family's opinion. Marketing information (pricing, frequency of use, etc).
Disadvantages	Familiarity with product. Limited information.	Lack of control. Limited information. No lengthy or distasteful tasks. Limited instructions. Large number of subjects required.	Little or no control. Time consuming. Expensive.

Qualitative and Quantitative Testing

Two types of results can be obtained from affective testing, either qualitative or quantitative. The former gives an idea of the product attributes, whereas the latter measures the product attributes for preference (Meilgaard *et al.*, 1999:239-241). The former indicates subjective preference for product attributes, but shows no level of measurement – just an indication – whereas the latter indicates the degree of preference.

Qualitative

Qualitative tests are those used to measure subjective responses of consumer to the sensory properties of products and are used to: 1) identify trends in consumer behaviour and product use; 2) assess initial response to a product / concept; 3) learn consumer

terminology to describe the sensory attributes of a concept, prototype or product category. This is critical if a larger questionnaire for quantitative evaluation is to be developed at a later stage, where it is empirical that consumer language is used. Thus, product attributes in qualitative tests are discussed in the consumers own words; 4) consumer behaviour regarding a particular product. These types of tests are usually group-based discussion "focus groups" (Boike *et al.*, 2005:197) or questionnaires that are undertaken by face-to-face interviews (Alam, 2005:252), telephone or e-mail (Resurreccion 1998:27).

Quantitative

Quantitative tests are used to determine: 1) overall preference or liking of a specific product, 2) to determine preference or liking for broad aspects, 3) to measure consumer responses to specific sensory attributes. Hedonic scales are often utilised here.

These are then classified into two main categories:

		Questions
Preference	Choice	Which sample do you prefer?
		Which sample do you like better?
Acceptance	Rating	How much do you like the product?
		How acceptable is the product?

Popper *et al.* (2004) studied the effect of asking attribute questions about a product first, that is before questioning participants about which product is preferred, because attribute questions may make a respondent more critical and aware of the several ways a product falls short of perfection. Obviously, there needs to be careful consideration to be adhered to when considering drawing up of questionnaires.

Whilst sensory evaluation has its place in new product development, it is important to recognise that this must be performed with consumers in mind, otherwise taste tests on food to predict the market performance of the products, such as Burger King's new french fries and the classic example of new Coke. For food scientists to perform tests that accurately predict consumer behaviour at the point of purchase, it is necessary to include in the experimental design, marketing personnel and insights to assure that consumers are responding to the product as they would in an actual food setting. Suggestions to improve

predictions are: 1) define target market correctly; 2) choice of subjects; 3) identification of a subset of trends when the choice is wide, for example, soft drinks; 4) correct choice of evaluation method (as discussed previously); 5) possible inclusion of competitor brands; 6) inclusion of total marketing mix – price, promotion, advertising, product design and branding, claims (Garber et al, 2003).

In summary, Garber *et al.* (2003) recommend that, due to the heightened sensitivity of consumers to food products in particular and to the complex nature of such food characteristics as taste, smell, flavour, texture, colour, packaging and other visual elements, more sensory research integrating these variables is needed. Wansink (2003) continues the above recommendations and states that in testing with brand names with consumers, extra care must be taken when considering setting up questionnaires and sensory consumer testing. It is critical to include a control in the experimentation, and the use of expanded questions is useful, with probing questions for consumers that ask them to compare against similar products. Marshall (2003) states that the world of the consumer is far from the laboratory and there must remain serious questions about the reliability of sensory tests to predict market place performance. It is therefore recommended that sensory scientists should consider and include marketing variables (as in their consumer response experience) and thus obtain more relevance for performance in the market place.

Grunert (2003) is clear in his thinking. Food choice is an area which has attracted researchers from different disciplines and two major camps (the sensory scientist and consumer marketer) have developed considerably over the last 12 years. Both disciplines should learn more about each other's fields. If it is required to increase the external validity of testing studies, they need to be approximated to real world environment and not just their own disciplines. This is often where the new product development process can fail, if there is a lack of communication between the sensory scientist and that of the other functions involved in the development and commercialization of the product. Moskowitz (2003) backs up the opinion of Grunart, 2003, and goes on to say that for a product to succeed from a sensory science and therefore consumer science perspective, the following are critical success factors: 1) market researchers and sensory researchers should work together (that is, the whole team must work together effectively in order to product a produce that will be successful); 2) everyone must take responsibility for their roles; 3) the team must be constant and not suffer changes in personnel; 4) must have top management commitment.

The above fits in with the discussion on NPD in this review, and only emphasizes the fact that the success of this project to develop a Kei apple functional beverage, involves commitment from all stakeholders in the project.

CONCLUSION

This literature review attempts to relay the complexity of the project. Functional foods have been discussed, as well as their legislation, trends and emphasis on beverages. The key active ingredients that are found in fruit were discussed as well as the importance of fruit and vegetables in the diet. New product development and the role of the consumer was discussed. Subsequently, a discussion on fruit juice processing followed.

The purpose of this project is to produce a new product. This chapter, therefore, also covers what is needed as background for the development of similar products, the chemistry behind them, the constituents (the polyphenols), how they may benefit health and the levels of polyphenols expected from the product.

None of this can help with the actual development of the product. Subsequently, a discussion on new product development, consumers and sensory evaluation took place. Without knowledge of these, it is not possible to bring such products to the marketplace, nor get to the position where a product may be evaluated for its potential health benefit.

CHAPTER 3

METHODS AND MATERIALS

CHAPTER 3

METHODS AND MATERIALS

Granor Passi (Pty) Ltd was involved as the commercial partner in the formulatory work. This company is the largest producer of fruit juice concentrates and fruit juice blends in South Africa, and regularly exports fruit juice to Europe. The storage facilities, laboratories, and processing plant were used throughout the formulatory work of this project, apart from the pilot feasibility study which was not undertaken under formal laboratory conditions.

Granor Passi (Pty) Ltd became interested in the project as they recognized its potential to promote the idea of indigenous fruit and the potential health benefits of the Kei apple fruit. Their plant in Polokwane specialises in processing citrus fruit and exotic fruits such as guava, peach, apricot and litchi. A plant in the Eastern Cape specialises in apples, pears and grapes which often form the basis of 100% fruit juice, as the natural sweetener, as discussed in the literature review. Granor Passi (Pty) Ltd is also a partner in the Marula project mentioned in the introduction of this study. Therefore, by using the company as partners, access to all equipment required and processing equipment was facilitated.

3.1 Collection of Kei Apples

The first batch of Kei apples were obtained from a farm in Bloemhof in the North West (NW) region of South Africa. These were used for the preliminary investigations in formulating a Kei apple beverage. Whilst Bloemhof is situated in Gauteng, it is on the border of the Orange Free State and thus is subjected to a climate of hot summer months with moderate rainfall and winters that experience frost.

The second batch of Kei apples were collected from the Eastern and Western Cape and amalgamated into one batch. Harvesting took place from the third week of January until the first week of February 2005. Approximately two hundred kilograms (200kg) of Kei apples were obtained from the N1 highway between Cape Town and Stellenbosch, and one hundred and twenty kilograms (120kgs) from outlying districts approximately 20 kilometers west of East London towards the Kei river. Both sets of

apples were frozen and transported to Granor Passi (Pty) Ltd in Polokwane for further work.

Figures 3.1 and 3.2 show examples of the apples harvested from East London and the Kei apple bushes that these were harvested from.

Figure 3.1 Kei Apples Harvested From East London

Figure 3.2 Kei Apple Bushes and Trees

3.1.1 Product Development – Pilot Feasibility Study

The aim of this experimental work was to determine if a beverage from the Kei apple was feasible at all. Previously, it had been reported that only jam and pickles had ever been formulated. A small-scale study had been undertaken by the University of Stellenbosch (Cori Ham personal communication) with Kei apple juice but this had not been progressed. However, several orchards of the Kei apples had been planted in the Western Cape during the course of the previous work.

Kei apple juice was obtained from the North West University, Potchefstroom campus. The Kei apple juice was prepared from Kei apples harvested in the 2002/2003 season from the Bloemhof area. Small-scale juice extraction was performed using a steam extraction process, utilising a double-jacketed steam kettle at Potchefstroom by the team examining the content and extraction methods of polyphenols from the Kei apple. Fruits were cut into fragments and placed into the kettle and the resultant juice (which was fairly clear and pinkish / blood grapefruit colour in appearance) was used in a very small scale sensory evaluation test.

The Kei apple juice was mixed with Hanepoot white grape juice, purchased from Woolworths (pasteurised in one litre tetra pak containers) at levels of 25% (M), 15% (L) and 10% (K) of Kei apple juice. The codes M, L and K were used to distinguish each beverage from one another, yet have some anonymity for the individual tasters. Nineteen individuals were then recruited and asked to evaluate the juices according to a nine point hedonic scale, where 1 = dislike extremely and 9 = like extremely. The evaluation form was described to each individual and then participants were asked to rinse out their mouths with tap water between each tasting. All samples were presented in white plastic cups and the participants could not see any difference in colour between the blends. Samples were presented in increasing concentration. Participants were not told what they were tasting and were asked not to discuss anything with other participants until all evaluations were complete. The evaluation form is as shown in Appendix 1. It is important to note that this was a very rough evaluation, to steer the thinking in what level of Kei apple juice it would be possible to start formulating a beverage.

3.2.2 Industrial Formulation of fruit juice

Brix and Acidity and their Relevance for Fruit Juice Production

Brix is a measure of the concentration of soluble solids in a solution and is based upon the relationship between the specific granularity % w/w (weight for weight) soluble solids of a pure sucrose solution, that is, 1° Brix = 1% sugar w/w. Whilst this relationship is only strictly applicable to sucrose solutions, the Brix provides a useful indication of the soluble solids of a fruit juice (http://www.britishsoftdrinks.com/htm/qua/FruitJuices.htm, 15/10/05). The

concentration of most fruit juices is conveniently measured in degrees Brix (°Brix, °Bx, °B), although the strict interpretation of this measure refers to pure solutions of sucrose in water. For juices with a high proportion of sugars to acids such as orange, pineapple and apple, this is a useful and convenient means of measuring concentration. In some instances, a correction factor may be introduced to take account of the acidity (although in this project, the acidity was not taken into account). Brix measurement is simply related to refractive index and there is a slightly different relationship between the refractive index and concentration of citric or malic acids and that of simple sugars (Ashurst, 2005:131). The Department of Agriculture of South Africa, in the Agricultural Products Standards Act GN R2286, laid down set Brix standards for fruit juices according to their type, to protect the consumer from being sold diluted products or adulterated products, for example, by substituting non-nutritive sweeteners for sugar in a fruit juice nectar, which by law may contain part fruit juice and part sugar. Set Brix values are laid down for apple (10.5 ° B), apricot (11.0 ° B), grape (12.5 ° B), granadilla (9.0 ° B), guava (8.0 ° B), orange (8.6 ° B), naartjie (8.0 ° B), pear (12.0 ° B), peach (12.0 ° B), grapefruit (8.0 ° B), pineapple (9.5 ° B), lemon (7.5 ° B) and mixed juice (° B is equivalent to weighted average of the juices).

Generally, the ° B is determined by the use of a refractometer. The sample to be measured is placed on the viewing plate and the amount or degree to which light is refracted through the sample measured and is directly proportional to the concentration of sugar in the sample. The temperature at which the reading is taken must be constant, or taken into account in the test method. As with all instrumentation, Brix refractometers vary in their make and sophistication. If the juice or concentrate being measured does have a high acidity level, this must be measured and compensated for. A set of calibration tables accompanies instrumentation to assist in this.

The acid character of a juice contributes to its flavour type and is taken into consideration when assessing the value of the juice for inclusion into a new beverage formulation. Acid content % w/w (weight for weight) is determined using a pH meter by direct titration against standarised alkali solution (for example, 0.1m sodium hydroxide) to an end point of pH 8.1 (Taylor, 2005:62). The use of a pH meter, phenolphthalein, an indicator or an auto-titrator incorporating the pH meter may be used to determine the end point.

Generally, acidity is recorded in terms of citric acid both for citrus fruits and most other fruits, although other acids (for example oxalic, iso-citric, tartaric) may be present. Where apple and other pome fruits are concerned, the major organic acids are malic and citric acid and thus acid is usually quoted as % w/w malic acid. As a general rule, the acidity of juices will decrease with increasing maturity or with increasing levels of sugars in the resulting juice. The Brix / acid ratio is frequently used to establish sensory or taste qualities for incoming juice supplies to minimize the effects of seasonal variations. The higher the Brix value in relation to the acid content of the juice, the higher the ratio, and the "sweeter" the taste (Taylor, 2005:62).

The pilot feasibility work undertaken in 3.2.1 indicated that the Kei apple juice could be added at levels between 10-15% to give a palatable drink. Thus, the aim of this part of the experimental work was to determine if a more acceptable drink using a method that was more precise, likened to an industrial process could be formulated. The quantity of Kei apples available from Bloemhof were limited as none had been harvested in the 2003/2004 season, and thus it was critical at this stage that a formulation be developed that could be used once the 2004/2005 harvest be obtained, otherwise the project would cease.

Samples of the frozen Kei apples, harvested at Bloemhof in the 2002/2003 season, were transported to the Granor Passi (Pty) Ltd plant at Polokwane. The fruit was defrosted, rinsed and skinned by hand. It was then placed in an industrial strength (Waring) blender. The resultant juice was sieved through a normal kitchen sieve (20 mesh size). In order to deactivate the enzymes, the juice was microwaved (LG Multiwave), 4 litres at a time, until such a time that it was just bubbling.

The resultant total acidity of this juice was 4.81% w/w (weight for weight), calculated as citric acid (Granor Passi (Pty) Ltd laboratory method, 2003a) and the Brix was 16.3° (Granor Passi (Pty) Ltd method, 2003b). The juice yield of the fruit was 87%. This was determined by weighing the fruit before juiced and weighing the amount of juice obtained afterwards, and determining the resultant percentage.

As a fruit juice was being formulated to give a final Brix of 12°, the formulation:

$\frac{a-12}{12} \times b = c$ was utilised, where a = current Brix of product, b = weight of pulp required to bring juice to 12° and c = weight of water required to add to juice. During any formulatory work, the Kei pulp was reduced to a Brix of 12° Brix (°B), as is required by law (chapter 3:85). This brought the acidity of the juice to 3.54% w/w (as citric acid). Apple juice (12°B) was then added to the Kei juice as a base sweetener, in addition to a variety of other juices, e.g. litchi, banana purée, peach, apricot, marula and pear. Each juice was added separately to the Kei / Apple juice blend and a simple taste test performed in the laboratory indicated that all these blends were far too acidic to the palate, as evaluated by the author, Rikie van der Merwe, Theresa Els and Niel van Rensburg. These three individuals are employees of Granor Passi (Pty) Ltd who are involved in the production of formulations of the company's own juices. They are involved in evaluating the taste profiles of the company's own products before sale to their customers, and gave the author assistance in this capacity when selecting flavours.

Consequently, it was decided to focus only on formulating with Kei apple juice and a de-ionized apple juice, which had no acidity, for the rest of the development work. Two formulations were arrived at, which are shown in table 3.1.

Table 3.1 Final formulations for Kei apple juice for the Kei apples harvested at Bloemhof

	A* (12%) % w/w *(weight for weight)*	**B* (15%) % w/w *(weight for weight)***
Kei apple juice 12° B [a]	12	15
De-ionized apple 12°B [b]	88	85
Sodium benzoate (preservative) [c]	0.01	0.01
Potassium sorbate (preservative) [d]	0.01	0.01
Sodium carboxymethyl cellulose (stabilizer) [e]	0.04	0.04
Guar gum (thickener) [f]	0.10	0.10
Sodium citrate (buffer) [g]	0.04	0.04

Key

* percentage of Kei apple juice
a = pulp Kei apple juice
b = de-ionized apple juice (Southern Canned Products, Cape Town)
c and d = sodium benzoate and potassium sorbate (Savannah Fine Chemicals, Germany)
e = sodium carboxymethyl cellulose (Emulsion b.v. Holland)
f = guar gum (C.J. Petrow, Cape Town)
g = sodium citrate (Savannah Fine Chemicals, Germany)

Throughout the formulatory work, the following additives were used: de-ionized apple juice, sodium benzoate, potassium sorbate, sodium carboxymethyl cellulose, guar gum and sodium citrate. De-ionized apple is a concentrated apple juice that has been through an ion-exchanger, reduced in acidity and therefore contains very little malic acid. It is supplied by Southern Canned Products, Cape Town, South Africa. Sodium benzoate and potassium sorbate are preservatives (chapter 2:59), supplied by Savannah Fine Chemicals, Germany. Sodium carboxymethyl cellulose is used as a stabilizer to prevent the juice from separating and is supplied by Emulsion b.v. Holland. Guar gum is a thickener obtained from C.J. Petrow, Cape Town, South Africa. Sodium citrate is a buffer obtained from Savannah Fine Chemicals, Germany. Therefore, for simplicity's sake, the use of the chemical name only will be made in the rest of this section of the formulatory work.

Two litres of product were made up at a time in the laboratory. De-ionized apple juice was added to a 5 litre plastic beaker (with a small amount retained for later use) and the guar gum thickener (pre-mixed with a small amount of sucrose) was added into the vortex of a high speed stirrer (Heidolph 048H, Germany) to prevent the formation of lumps. Sodium carboxymethyl cellulose, sodium citrate, potassium sorbate and sodium benzoate were pre-mixed in the retained de-ionized apple juice, added to the beaker and stirred until homogenous. Finally, the Kei apple juice was added. A final Brix level of 12.0° was achieved. A Mettler Toledo PB3002 (Switzerland) laboratory balance was used to perform any weighings through the experiment.

The natural flavour and aroma of the Kei apple juice was palatable but was improved by the addition of manufactured flavours (artificial and natural). All these flavours were from internationally recognized flavour houses, globally represented, who have expertise in flavouring all types of foods. However, they were not approached individually at this stage in the development work. The flavours were those used in Granor Passi (Pty) Ltd formulations or were flavours that had been presented to Granor Passi (Pty) Ltd for potential use in the development work. For proprietary reasons, it is not possible to disclose which of these flavours, if any, are currently being used commercially by Granor Passi (Pty) Ltd in their commercial formulations.

Several flavours were tried in the laboratory, i.e. kiwi, grapefruit, passion fruit, violet, plum, mixed fruit and pear. Finally, three flavours were chosen – Apple (mixture of

Apple Red 52675C and Apple Green 52681C, Firminich, Switzerland), Vanilla (INN44301, IFF, England) and Vanilla and Mint (Vanilla as before and Mint MHO360, Duckworths, Cape Town).

As the flow of the formulatory work with two different harvest of Kei apples may be difficult to follow, figure 3.3 assists in outlining the process. On the middle (horizontal) line of the figure, the source of Kei apples is indicated; the extremes of the figure shows at what stage in the product development they were used in this project and for what; and when chronologically were these activities took place.

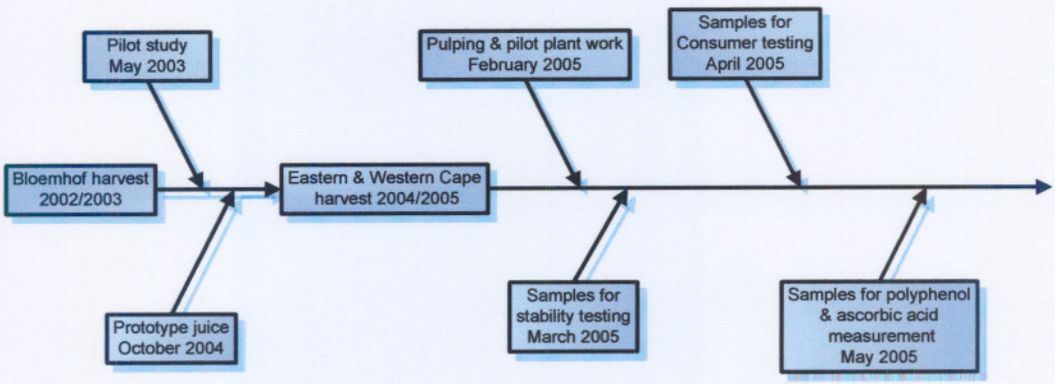


Figure 3.3: Illustrated diagram, integrating collection of Kei apples with pilot study and development work.

These prototype formulations in table 3.1 were used as a basis for the next stage in the product development, which was to prepare samples for sensory evaluation.

3.2.2.1 Formulation of Kei apple fruit juice in preparation for sensory evaluation tests

The aim of this part of the formulatory work was to determine if the Kei apple beverage could be developed further, utilizing an existing plant at Granor Passi (Pty) Ltd – that of the guava processing plant (based on Granor Passi, 2003c – processing guava, Work Instruction FP WI 1.20). In addition, once the resultant pulp had been obtained, could it be mixed using the basic formulation obtained in 3.2.2 on a small industrial scale?

The work was carried out in two parts:-

1) Pulping through the guava plant and processing of 100kgs of unflavoured juice, and

2) Production of 4 x 200kgs of juice, unflavoured apple, mint & vanilla and vanilla respectively approximately 4 weeks later in preparation for the sensory evaluation tests to assess the potential of the formulated beverage with consumers.

3.2.2.2 Pulping of apples and processing of 100kg of unflavoured juice

The Kei apples obtained from Bloemhof were insufficient in quantity for the preparation of product for the consumer testing. The Bloemhof Kei apple season proved unreliable in 2004 and subsequently ripe Kei apples were sourced from the Eastern Cape and Western Cape in January 2005 – at the end of the Kei apple season. These apples were frozen and transported via frozen storage to the Granor Passi (Pty) Ltd facility in Polokwane. In total, 311kg Kei apples were obtained. The apples were allowed to defrost and then put through the Granor Passi (Pty) Ltd custom-made guava processing plant (as shown in figures 3.4 and 3.5) in order to obtain the Kei apple pulp to be used for later blending.

Figure 3.4 Granor Passi (Pty) Ltd custom-made guava processing plant

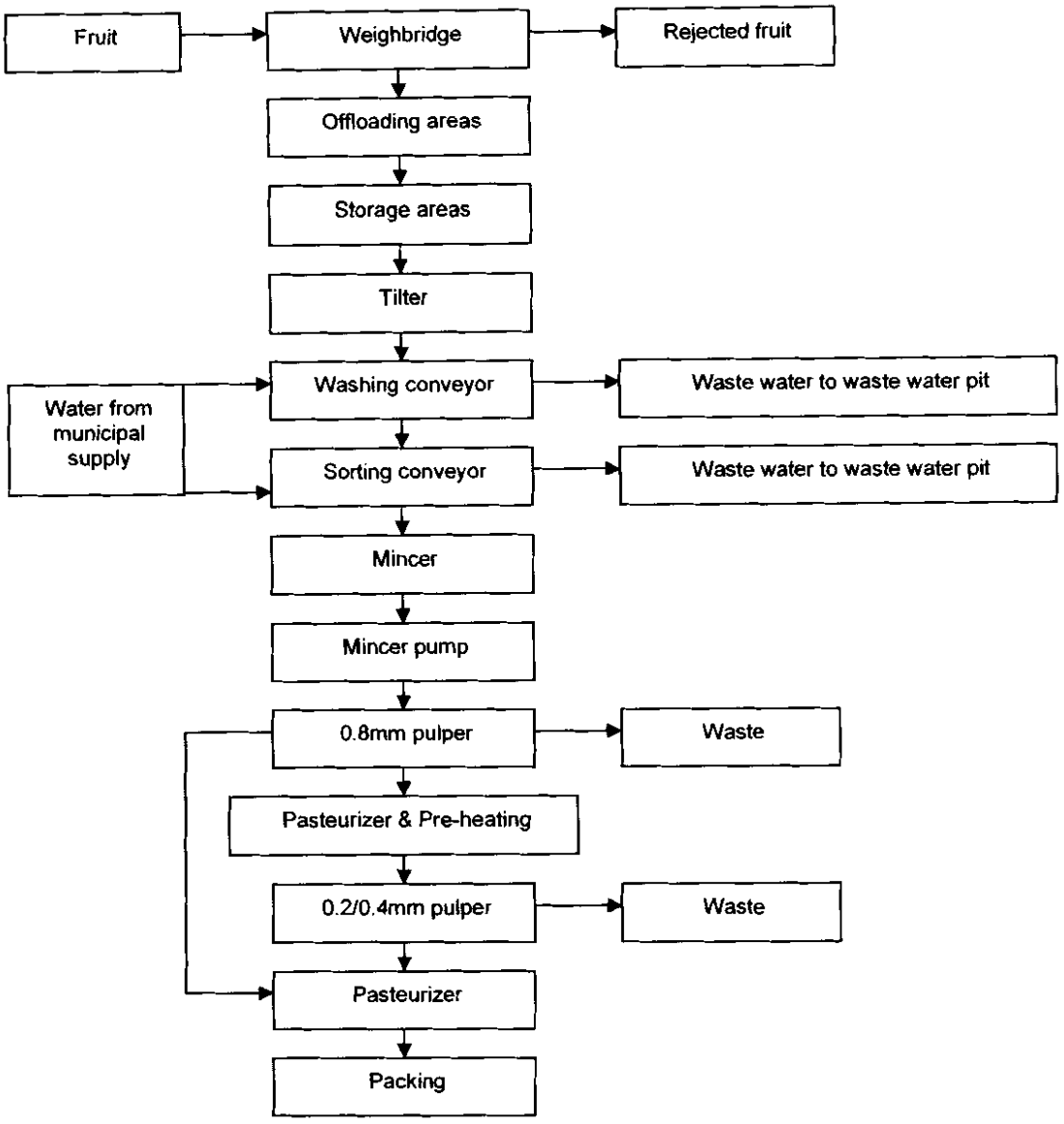


Figure 3.5: Flow diagram of guava plant

The resultant pulp gave a Brix of 8.4°B and a total acidity of 2.54% (calculated as citric acid w/w). One trial batch of 100kg was attempted after the production of the Kei apple juice. As the acidity and Brix of the Kei apple pulp from this harvest were lower than that of the Bloemhof Kei apples (which did not appear to be as ripe as the Cape apples), Kei apple juice was added at 22.5%. This is in contrast to the 12-15% estimated from the pilot study, and was added at a higher level to compensate for the lower level of Brix (and therefore sugar) obtained with the Cape Kei apples. The Brix of the de-ionized apple juice was adjusted to 13.2°B, to compensate for the lower Brix level of the Kei apple juice. The resultant formulation, as follows and shown in table 3.2 (Brix 11.6°B), and Kei juice was added at a level of 22.5% w/w.

Table 3.2 Formulation of trial manufacture of 100kg of unflavoured Kei apple juice.

	100kg of product
Kei apple juice 8.4° B [a]	22.5kg
De-ionized apple 13.2°B [b]	to make up 100kg
Sodium benzoate [c]	12g
Potassium sorbate [d]	12g
Sodium carboxymethyl cellulose [e]	40g
Guar gum [f]	100g
Sodium citrate (buffer) [g]	40g
Key a = pulp Kei apple juice b = de-ionized apple juice (Southern Canned Products, Cape Town) c and d = sodium benzoate and potassium sorbate (Savannah Fine Chemicals, Germany) e = sodium carboxymethyl cellulose (Emulsion b.v. Holland) f = guar gum (C.J. Petrow, Cape Town) g = sodium citrate (Savannah Fine Chemicals, Germany)	

The guar gum was mixed in the laboratory using some of the de-ionized apple (c. 10 litres) in 3 parts, to avoid the formation of lumps. The guar gum was mixed in the vortex of the high speed stirrer (Heidolph 048H, Germany) as before. The rest of the de-ionized apple juice was added to the steam jacketed steel vessel (figure 3.6), fitted with a paddle-stirrer, and brought to a temperature of 85°C (the equivalent process scale as performed in the microwave), after which time it was allowed to cool naturally. The weighing of the dry ingredients was performed in the laboratory using the balance as before.

Figure 3.6 Granor Passi (Pty) Ltd steam jacketed steel vessel

As the scale-up exercise from the laboratory to the pilot plant at Granor Passi (Pty) Ltd was successful, the bulk fruit juice from the guava plant was frozen in 50 litre drums, until such time that the fruit juice for sensory testing could be prepared (approximately 4 weeks later).

3.2.2.3 Preparation of juice for sensory evaluation testing

Two hundred litres of the Kei beverage was prepared in Polokwane in one day, using the same procedure described in 3.2.2.1 as with the 100 litres. With the larger volume of guar gum in these batches, it was found difficult to use the stirrer as previously used – lump formation was excessive. Therefore, the Waring Commercial Laboratory Blender (South Africa) was used to mix guar gum and de-ionized apple for one minute at high speed, approximately 15-20g at a time, utilizing approximately half of the de-ionized apple juice. The final formulations are as shown in table 3.3. These were packed into 25 litre plastic lined drums, and 50 litres of each variant was sent to Potchefstroom for sensory evaluation. The final Brix (°B), acidity (citric acid w/w) and pH of all these formulations were determined according to the Granor Passi (Pty) Ltd standardized methods.

Table 3.3 Final formulations for sensory testing.

Ingredient	Unflavoured Juice	Apple	Mint & vanilla	Vanilla
Kei apple juice 8.4°B [a]	45kg	45kg	45kg	45kg
De-ionized apple 13.2°B [b]	Balance	Balance	Balance	Balance
Sodium benzoate [c]	24g	24g	24g	24g
Potassium sorbate [d]	24g	24g	24g	24g
Sodium carboxymethyl cellulose [e]	80g	80g	80g	80g
Guar gum [f]	200g	200g	200g	200g
Sodium citrate [g]	80g	80g	80g	80g
Flavour – Apple Red (52675C, Firminich, Switzerland)	-	40g	-	-
Flavour – Apple Green (52681C, Firminich, Switzerland)	-	60g	-	-
Vanilla (INN44301, IFF, England)	-	-	40g	60g
Mint (MHO360, Duckworths, Cape Town)	-	-	10g	-
Brix	12.0°	12.4°	12.4°	11.9°
Acidity as % citric acid w/w	0.52	0.59	0.54	0.51
pH	3.04	3.02	3.03	2.98

Key
a = pulp Kei apple juice
b = de-ionized apple juice (Southern Canned Products, Cape Town)
c and d = sodium benzoate and potassium sorbate (Savannah Fine Chemicals, Germany)
e = sodium carboxymethyl cellulose (Emulsion b.v. Holland)
f = guar gum (C.J. Petrow, Cape Town)
g = sodium citrate (Savannah Fine Chemicals, Germany)

3.3. Stability Testing of Kei Fruit Beverage

The aim of this part of the experimental work was to determine if the formulated product would be able to attain a shelf life of four weeks at chilled storage 4-8°C.

Samples of product were taken from the first factory scale up, as described in 3.2.2.1. Three separate litres of product were flavoured with 1) apple flavours, 2) vanilla, 3) mint & vanilla respectively, at the levels given in the formulations in table 3.3. One litre of the beverage was left unflavoured. These were placed into 4 glass bottles and subjected to pasteurization at 85°C for 1 minute. The bottles were sealed well, chilled to between 4°C and 8°C and then sent to Consulting Microbiological Laboratories (Pty) Ltd (CML) for microbiological analysis, after 1, 7, 14, 21 and 28 days for total plate count, lactic acid bacteria, total coliforms, *E.coli*, moulds and yeasts, using established microbiological tests.

All test methods used to determine the presence of and the quantity of micro-organisms present as nationally and internally accepted test methods to determine the presence of total microbial load and potential pathogenic and fruit juice spoilage organisms. Discussion with CML and the author's previous experience resulted in the test methods being adopted for a large food retail outlet in South Africa for 100% fruit juice, namely Pick 'n Pay.

3.4. Consumer Testing of Kei Apple Beverage

The aim of the consumer testing of the beverage was to determine the overall acceptability of the product, preference, consumption intent and purchase intent. If the results of this testing proved favourable, the beverage would have potential to be taken further down the product development process.

3.4.1 Samples

The juice prepared at Granor Passi (Pty) Ltd in Polokwane as described in section 3.2.2.2. was transported via chilled transport to the Granor Passi (Pty) Ltd depot in Kempton Park, in 25 litre containers. Fifty (50) litres of each flavour (8 containers)

were then transported by the author from Kempton Park to Potchefstroom for consumer testing. It was decided, prior to the final consumer testing, that the unflavoured juice would be excluded as this would increase the complexity of the testing, and it was unlikely that the unflavoured juice would be progressed as a commercial product as by itself as it was not thought to be palatable. At this stage, a benchmark commercial product was also excluded, as there was very little on the market locally or internationally that was similar, and the inclusion of another type of functional drink like a probiotic orange juice, for example, would totally skew the results.

3.4.2 Recruitment of Participants

The participants for the consumer testing were recruited from the Potchefstroom campus of the North West University. A notice was placed on the bulletin board of the university's intranet, requesting volunteers. Volunteers were also sought from the Administration, Physics, Consumer Science and Nutrition departments. Participants were also asked to bring along as many individuals as possible. Participation was on a strictly voluntary basis and it was requested that no smokers be involved. One hundred and fifty volunteers took part in the test, of which forty one were men and one hundred and eleven were women.

3.4.3 Demographics

The participants were asked to fill in the Form A (Appendix 3) which gave anonymous details of their age, gender, occupation, nutritional awareness and fruit juice consumption. Fruit juice consumption figures, including nectars and blends, were estimated at an average of 14 litres per person per annum (BMI Foodpack cc, 2002). This is equivalent to 270mls per week. Thus, a definition of a heavy user (consumer) of fruit juice was taken as being light 2-3 months, moderate 1-2 weeks, and heavy >3 times per week, given that a portion size is 250ml and fruit juice consumption figures have risen since 2002 (chapter 2:16).

3.4.4 Questionnaire

An adapted version of a pre-tested sensory evaluation questionnaire (Bosman, 1998) was used, as shown in Form B of Appendix 3. This consisted of four questions. Question 1 dealt with overall acceptability, asking participants to rate, using a seven point hedonic scale, the appearance, taste, texture and overall acceptance of the beverages, where 1 = totally unacceptable and 7 = very acceptable.

Question 2 dealt with consumer preference regarding the samples and the respondents were asked to make a choice indicating their order of preference.

Question 3 dealt with consumption intent for all three beverages and here a modified Food Action Rating Scale (FACT) was used.

Question 4 was the final question which dealt with propensity to purchase these beverages. Again respondents were asked to ensure that they made a choice between either yes or no.

3.4.5 Preparation of Samples

All procedures were standardized and pre-tested on preparation, handling and serving. In total, 12 tasting sessions were held for 5 days over a period of 2 weeks, ranging from 1 participant to a maximum of 20 participants at any one time. One hundred milliliters of each coded beverage was served chilled at between 4°C and 8°C, in a glass. Tap water at room temperature was served with each set of beverages for participants to rinse their palates before and during the tasting sessions.

3.4.6 Presentation of Samples

The evaluation form (Appendix 3) was also explained in detail to each individual. Participants were asked not to confer with one another during the evaluation or afterwards, and to remain silent during the evaluations to avoid influencing other's opinions. Participants were screened off from one another and were unable to view

anyone else participating in the evaluation. The samples were presented to subjects in balanced order so as to minimize order effects, being either 1) PTN, MLQ, then VSH; 2) MLQ, VSH, then PTN; or 3) VSH, PTN, then MLQ; where PTN = apple; MLQ = mint & vanilla; VSH = vanilla. Evaluation sessions lasted approximately 30 minutes each and were conducted under controlled conditions in the taste panel room and NWU, Potchefstroom. Each sample was evaluated once by each participant. Each participant was thanked for their involvement and rewarded with two small chocolates afterwards.

3.4.7 Testing

All consumers were welcomed upon arrival, seated and informed about the purpose of the study. They were given the same instructions verbally (Appendix 2) before commencing with the tasting. Before the evaluations commenced, all participants were given background information on the beverage they were about to consume, namely that it had been newly developed and may have some added nutritional benefits. The beverages may aid in the reduction of degenerative diseases such as heart disease and cancer and boost the immune system. They were asked to fill in section A (Appendix 3), giving anonymous demographic details, and then complete section B (Appendix 3) and evaluate the acceptability, preference, consumption and purchase intent regarding the three samples. Participants were able to fill in sections A and B in either Afrikaans or English, but all instructions were given in English. Five individuals chose to complete the forms in English and 147 in Afrikaans.

3.4.8 Statistical Analysis

Statistical analyses were performed with the Statistica® programme, version 7, (www.statsoft.com). Demographic data of subjects were analysed using descriptive statistics. Repeated measures ANOVA was used to determine if there were any statistical differences between taste, appearance, texture and overall acceptance of the different beverages for the different age, gender, occupation and fruit juice consumption groups.

Chi-square tests were done to determine if there was a statistical significant relationship between preference and age, gender, occupation and fruit juice consumption. The same was done for purchase intent and age, gender, occupation and fruit juice consumption.

Non-parametric tests were used for the variable consumption intent as evaluated on a food action rating scale, with unequal distances between categories. Mann-Whitney and Kruskall-Wallis tests were used to determine if age, gender, occupation and fruit juice consumption had a statistical significant effect on consumption intent.

Practical significance was used to determine whether differences were large enough to have an effect in practice. The practical significance (effect size) for the following questions was determined according to the following formulae.

Questions 1, 2 and 3 for the difference in average overall acceptance, preference and consumption intent:

$$d = \frac{|\bar{x}_1 - \bar{x}_2|}{s_{max}}$$ S_{max}

where $\bar{x}_1 - \bar{x}_2$ = difference in averages of respective samples and S_{max} is the maximum standard deviation between the two samples.

Question 4 – for the difference in proportions of consumers who intended to purchase beverages:

$d = \frac{|p_1 - p_2|}{\sqrt{p(1-p)}}$, where p_1 and p_2 are the respective proportions and

$p = \frac{X_1 + X_2}{n_1 + n_2}$, the average proportion with X_1 and X_2 the number of respondees that intended to buy each beverage.

The following guidelines for the interpretation of the effect sizes are given (Ellis & Steyn, 2003):

d = 0.2 = small effect; 0.5 = medium effect and 0.8 = large effect. Data with $d \geq 0.8$ are considered as practically significant, since it is the result of a difference having a large effect.

When it is important to know whether a relationship between two variables are practically significant, the effect size is given by $w = \sqrt{\frac{X^2}{n}}$, where X^2 is the usual Chi-square statistic for the contingency table and n is the sample size, see Ellis and Steyn (2003). The following are guidelines for the interpretation of the above in the current case:

(a) small effect: $w = 0.1$, (b) medium effect: $w = 0.3$, (c) large effect: $w = 0.5$.

A relationship with $w \geq 0.5$ is considered as practically significant.

3.5 Determination of Total Polyphenols

The concentration of total polyphenols in the Kei apple juice was measured by UV spectrophotometry, based on a colorimetric oxidation/reduction reaction. The oxidizing agent used was Folin-Ciocalteu (Singleton & Rossi, 1965). The following were analyzed: apple, mint plus vanilla and vanilla flavoured Kei apple juices, plus unflavoured and unpasteurized beverages. The Bloemhof pulp from 2003 and the combined Eastern and Western Cape pulp from 2005 and a commercial product from the United States, Cranberry Ocean Spray®, were also analyzed.

Ocean Spray® is North America's leading producer of canned and bottled juices and juice drinks, and has been the best selling brand name in this category since 1981 (http://www.oceanspray.com, 24/10/05). A product from Ocean Spray® was chosen as a benchmark to evaluate total polyphenols and ascorbic acid as this product is the brand leader in "functional beverages" as currently defined in the USA market. The product is packed in a 500ml PET bottle and it is claimed by the manufacturer that "A daily dose of Premium 100% Cranberry blend helps maintain urinary tract health. There is no sugar added and one glass of Premium 100% juice counts as full serving of fruit and provides 100% of the antioxidant vitamin C." (http://www.oceanspray.com/ healthy_living/cranberry_health.asp, 23/10/05). The

product is clear, astringent to the taste and was included in the evaluations for comparison purposes. All the Kei apple products were centrifuged for 10 minutes at 1500rpm in a Hettich Universal 16R centrifuge and the supernatant used for analysis. The Bloemhof pulp was diluted 200 times and the Cape 2005 pulp diluted 100 times and the Ocean Spray® product was diluted twice.

Prior to analysis, a standard calibration curve was produced using gallic acid as a standard to determine the concentration of total polyphenol (as gallic acid equivalent GAE/l) in the samples.

Samples (200 microlitres) were introduced into test tubes followed by 1ml Folin-Ciocalteu's reagent (Sigma, USA). This was allowed to stand for 8 minutes at room temperature. Next, 0.8ml sodium carbonate (7,5%) was added, mixed and allowed to stand for 30 minutes. Absorption was measured at 765nm (Shimadzu UV – 1601 Spectrophotometer, Japan). Total phenolic content was expressed as gallic acid (Aldrich, USA) equivalents (GAE) in milligrams per litre (mg/l). As total ascorbic acid and sugars continue to respond to the Folin-Ciocalteu assay, corrections for these were done (as described by Asami *et al.*, 2003 and Slinkard & Singleton, 1977) and total Brix analysis (Granor Passi (Pty) Ltd 2003). Mean values of polyphenol content were expressed as gallic acid equivalents (GAE) per litre ± standard deviation (n=3).

3.6 Determination of Ascorbic Acid

Ascorbate (ASC), dehydroascorbate (DHA), and total ascorbate (ASC + DHA) concentrations were determined in the fruit juices spectrophotometrically at 578 nm (UVIKON XX double beam spectrophotometer) using a method described by Beutler (1984). These measurements are necessary for not only the nutritional evaluation of the juice but the correction of the polyphenol concentrations determined by the Folin –Coicalteu assay. Mean values of ascorbate content were expressed as milligrams per litre mg/l ± standard deviation (n=3), Sugar analyses were determined by Brix (Granor Passi (Pty) Ltd, 2003b).

The methods and materials used to obtain or harvest the Kei apples from Bloemhof in 2002/2003 and subsequently from the Eastern and Western Cape in 2004/2005 were described. The development of a pilot formulation was described, bulk pulping of the Cape

apples and the development of three flavoured beverages for consumer testing and stability testing were also described. Methods and materials utilized to analyse these final products and the different harvests for total polyphenols as Gallic acid equivalents per litre (GAE/l), and ascorbic acid were finally described.

The results of these methods are now presented and discussed in chapter 4.

CHAPTER 4

RESULTS AND DISCUSSION

CHAPTER 4

RESULTS AND DISCUSSION

The results of the experimental work undertaken in chapter 3 will now be discussed and interpreted.

4.1 Collection and Harvesting of Apples

Kei apples were collected in 2002/2003 from Bloemhof and used in the first half of the formulatory work, whilst those collected from the Western and Eastern Cape in 2004/2005 were used in the final formulatory work, bulk manufacturing, consumer testing and chemical analysis for total polyphenols and ascorbic acid. The Kei apples from the Western and Eastern Cape were found to be much riper than those from Bloemhof and could even be tolerated on their own, although still extremely tart and astringent, which was not the case for the Bloemhof apples. The Brix and percentage acidity, calculated as citric acid, of the pulped Kei apples from Bloemhof were found to be 16.3°B and 4.81% weight to weight respectively. The Brix and percentage acidity, calculated as citric acid, of the combined Kei apples from the Western and Eastern Cape were 8.4°B and 2.54%mm respectively.

This may possibly be due to several reasons. The Bloemhof apples harvested in 2002/2003 were smaller and less ripe than the Cape apples and thus may have had less sugar and water content, leading to a greater respective Brix and acidity content. Many changes take place when fruits ripen (Ozawa *et al.*, 1987), leading to a loss in astringency and potentially polyphenols. These authors also state that variety, maturity and climate are known to influence the astringency of fruit and present evidence suggesting that each fruit must be considered as a separate case rather than there being an all-embracing explanation for the loss of astringency on ripening. Monomeric flavonoid phenols are primarily bitter, but as the molecular weight increases upon polymerisation, astringency increases more rapidly than bitterness (this is why a young red wine is often very bitter and astringent, while bitterness and then astringency decreases in older wines) (Noble, 1994). Thus, it may be postulated that the astringency that may have been experienced with the Bloemhof apples could have been attributable to bitterness, whilst the riper Cape apples were

not perceived as so bitter and astringent. This requires further investigation. The pH of orange juice, for example, is known to increase during the harvesting season (Bull *et al.*, 2004). This increase in pH is coupled with a decrease in titratable acid content and total soluble solids.

The collection of the Kei apples at the beginning of 2005 involved a field trip to East London (20km west towards the Kei river mouth) into the outlying districts, and many observations were made.

It was found that the Kei apple shrubs and bushes were not as abundant as originally thought. There was also some confusion between the Kei apple and Star apple (*Diospyros lyciodes*) (figure 4.1) – of which there are 26 in this genus (Coates Palgrave, 2002:895). Care must therefore be taken in harvesting the Kei apple so that no confusion takes place. Some of the species of the Star apple family are poisonous. It appeared that, wherever modern agriculture had been practiced, there seemed to be a decline in the number of Kei apple shrubs, although this can only be postulated and not proven.

However, it was encouraging to see that the local communities were extremely knowledgeable about the Kei apple, and were eager to help pick the fruit for this study at a fee of R5/kg. This shows potential to introduce a similar scheme as operates successfully in Marula harvesting, where the chief of a community organises the picking of the Marula fruits to a central point where it is either washed and frozen or pulped and frozen for later processing.

Figure 4.1 Star Apple (*Diospyros lyciodes*)

It was pointed out to the author by the locals that most of the Kei apples had already passed their harvest time and that it was late to harvest fruit. Normally the shrubs were abundant in their yield. It was also mentioned that there may be a second (smaller) harvest of fruit after January, which has still to be established, although Coates Palgrave (2002:763) indicate that fruit appears from November to January only. It is possible that the exact timing of the harvest is unclear according to the traditional text books and this would require further investigation.

The farming community seemed very optimistic towards planting Kei apple trees in this, their natural habitat, if it were to provide profit for them in the long run.

4.2 Product Development

Table 4.1: Results of pilot preliminary tasting with Hanepoot white grape juice

	Average * Extent of Acceptability [a]	Average * Consumption Intent [b]	Average * Propensity to Purchase [c]
K	6.7	6.7	4.6
L	4.2	4.2	6.1
M	3.0	3.4	7.2

Key

K = 10% Kei apple juice; L = 15% Kei apple juice; M = 25% Kei apple juice

* averages are quoted only as questionnaires were misplaced and Standard Deviations could not be calculated

a 1 = dislike extremely; 9 = like extremely (refer to Appendix 1 for complete description)

b 1 = would drink this every opportunity I had; 9 = would drink this only if I were forced to

c choice between definitely would purchase this product and definitely would not purchase this product (1 = would and 9 = would not)

The pilot sensory testing with the Kei apple juice as described in section 3.2, indicated that levels of 15% juice using a steam distillation method could be tolerated by consumers. However, the level of acidity in the Hanepoot white grape juice was not taken into consideration and thus merely provided a simple stake in the ground to start any formulatory work. After this, the idea of using the whole of the Kei apple (pulped) was investigated and Granor Passi (Pty) Ltd became involved with the project.

The reason that pulped fruit was used as opposed to a steam distillation process was that it would be much easier to utilise existing plant equipment. The distillation process is more suitable for citrus fruit, whereas a pulping process is more suitable for stoned fruits. Additionally, any polyphenols in the seeds of the Kei apple are captured during the pulping process and are thus included in the fruit pulp.

The only Kei apples available were those from Bloemhof, harvested in 2002/2003 and these had been kept frozen and used in other experimental work at the North West University (Potchefstroom Campus). As stated in section 3.2.2, various other juices were evaluated in combination with the Kei apple pulp. The % w/w (weight for weight) acidity of these juice concentrates by themselves is respectively (acidity as citric acid w/w) peach pulp <4.0, banana pulp 0.3 – 0.5, apricot <4.5, marula 0.4 – 0.8, and litchi 0.15 – 0.25. Apple juice concentrate has a maximum acidity of 1.4% w/w as determined as malic acid (Granor Passi (Pty) Ltd, Polokwane). The South African palate tolerates an acidity level of not more than 0.5% w/w in a fruit juice (Granor Passi (Pty) Ltd personal communication) if there is insufficient sugar. Therefore it was felt, to gain the maximum benefit from any nutritional and economic aspects of the Kei apple, that no other fruits should be included in the formulation as the level of Kei apple added would have to be reduced to accommodate the acidity of any other fruit added.

Further restrictions in formulating a fruit juice are placed by the South African regulations relating to the classification, packing and marking of fruit juices and drinks intended for sale in the Republic of South Africa (GN R2286, 7 November 1980). These regulations are very specific for the common fruits, for example, apple, apricot, grape, granadilla, guava, pear, peach, pineapple and citrus. The contents of the drink, be it a fresh juice, unsweetened, sweetened, a nectar or, if it is to be blended with other fruits, should be specified precisely and must be declared on the label.

The goal of this formulatory work was to make this beverage as natural as feasible, including as much of the Kei apple fruit as possible and achieving consumer acceptance. Therefore, because the Kei apple is not a named fruit and falls under the auspices of "other", the legislation was followed as tightly as possible to enable the claim of a 100% fruit juice to be made. As stated, the acidity levels of the Kei apple pulp and therefore the resultant ready-to-drink acidity levels in the juice are high. It was necessary to sweeten the Kei apple with de-ionized apple juice. This is

a product where most of the acidity (mainly malic) has been removed. However, the claim that all the ingredients of the drink from fruit may be made, although it may be argued that the formulated product could have been sweetened with sucrose. In alignment with the regulations and the South African taste for sweeter products (Givaudan presentation, 2004), the Brix of the final product was always taken to 12°B. The de-ionized apple is commonly used in the fruit processing industry when the acidity of the citrus fruits, e.g. orange, grapefruit, and naartjie / tangerine and lemon is too high to be tolerated. This occurs when the acidity of the soil where the citrus fruit is grown is high, or a shortage of water is experienced during the growing seasons, and is a commonly accepted practice in the fruit juice industry globally (Granor Passi (Pty) Ltd personal communication). It must be remembered that 100% fruit juice rarely means 100% of the named fruits, as the base sweetener is usually either deflavoured apple, pear or grape juice, and this has been accommodated for in most global regulations including the South African ones.

To improve the acidity of the juice further, the addition of tri-sodium citrate at a level of 0.1% w/w as a buffer was added. Informal taste testing in the laboratory (4 people, as mentioned in section 3.2.2, Methods and Materials) comparing the product with and without tri-sodium citrate showed that the acidity levels were reduced with the addition of the buffer. It was felt that because three of the tasters (excluding the author) were experts in their fields, formulatory work could proceed with the tri-sodium citrate. Expert tasters are often used in their specific fields, for example, wine (Solomon 1991:273), and tea, where an individual or groups of individuals are so specialized in their field of expertise and have been trained in the analytical tests and descriptive tests as described in chapter 2:71. Expert tasters are used to eliminate (or narrow down the number of) products when there are too many available for consumer testing.

To improve the stability of the formulation, sodium carboxymethyl cellulose was added at 0.4% w/w. This assisted in reducing the separation of the Kei apple pulp from the resultant drink. The fruit juice was also thickened slightly by the addition of guar gum. The idea of thickening the fruit juice was to give the consumer the feeling of "fullness", improving their satiety. Mattes and Rothacker (2001) showed that humans perceived drinks that were thicker to satisfy hunger better than drinks which were thinner but of equal calories. This developed beverage is also aimed at attracting consumers to drink one portion per day (no more) in an attempt to increase

their daily consumption of fruit and vegetables. It is therefore not necessarily aimed at being "thirst quenching". McEwan and Colwill (1996) found that viscosity was one of the factors that were not associated with thirst quenching. Increasing the level of viscosity of a solution has also been shown to decrease flavour and taste intensity, and thus acidity (Delwiche, 2004).

The additives used to improve the formulation have been confirmed by other researchers. Peleg and Noble (1999) investigated the effect of viscosity, temperature and pH on astringency in cranberry juice. Increasing pH of cranberry juice had the largest effect of lowering astringency regardless of temperature or viscosity. The role of viscosity in decreasing astringency has been speculated to arise in part from the lubrication it contributes. As tannins precipitate salivary proteins, lubrication effectiveness of saliva is diminished, resulting in the perception of friction in the oral cavity. Consistent with this, viscosity of saliva has been shown to decrease upon reaction with tannins. Perceived astringency is also reduced with temperature, being due to either the decreased flow rate of saliva to cold solutions, or the tannin protein interaction.

Lowering the level of acidity in the beverage also assists in consumer acceptability. This was found by Jaeger *et al.* (2003) with research on the kiwifruit, who concluded that consumer preference was determined primarily between the sugar-acid balance. Siebert and Chassy (2003) found that the presence of acid in beverages affects the perception of astringency as it was found that when acid was not present, the interaction between salivary protein and salivary polyphenols is less intense and therefore astringency was perceived to be less.

The aforementioned additives to the Kei juice pulp were made to give the resultant ready-to-drink juice a perceived lower level of acidity. However, the Kei apple itself has a distinct smell of its own which requires either the consumer getting used to and liking it for its uniqueness or masking it, using a masking agent such as a flavour / aroma. The observations made during the trials with the Kei apple indicate that most potential consumers found the aroma of the Kei apple strange and the researcher felt that, if this product was to be marketed, initial purchase might be made but because of the uniqueness of the aroma, repeat purchases may be difficult. It is well documented that smell and taste are linked closely (Delwiche, 2004 and Meilgaard *et al.*, 1999).

Several flavours were considered for use, as discussed in section 3.2.2, but only the apple, vanilla and mint & vanilla were found acceptable to be taken forward for final testing with the consumer. Again, the tasting was performed in the laboratory with the expert tasters to determine the flavours that were not found to be acceptable, or mask the natural aroma of the Kei apple. Flavours play essential roles in the production of a wider range of food products versatile in aroma to allow consumer choices and to meet consumer needs. Therefore, flavour manufacturers require expertise in flavour formulation, research and technical services, while the flavour user need fundamental knowledge of flavour applications (Wu *et al.*, 2002:233). Consequently, at this stage of the development of the beverage (the fuzzy front end of the new product development process), the use of existing flavours at Granor Passi (Pty) Ltd were used and no help was sought from an external flavour house. The next stage of the product development process is to fine-tune the flavour of the beverage and would involve briefing a flavour company for assistance.

The fruit juice must also be fit for human consumption throughout its declared shelf life. The product that was aimed for should be stable for 4 weeks under chilled conditions. As mentioned in chapter 2:59, the principle of maintaining product integrity is by heat preservation, chemical preservation or by a combination of both. As this was a product of which there was no prior knowledge, it was decided to use a mild pasteurization process (heat to 85°C and allow to cool slowly), combined with the addition of the preservatives sodium benzoate and potassium sorbate at levels of 0.01% w/w, which is equivalent to 100ppm were made. Legislation – Food, Drugs and Cosmetics Act of 1972 (Department of Health, South Africa, 2001) – allows these preservatives to be present at levels not exceeding 300ppm.

4.3 Stability of Kei Fruit Beverage

Foods begin to lose their quality the moment they are harvested through changes resulting from physical, chemical, enzymatic or microbiological reactions. Food preservation prevents these deteriorative reactions, extending a food's shelf life and assuring its safety. Micro-organisms and enzymes are the main agents responsible for food spoilage and therefore the targets of preservation techniques.

The choice of using a combination of pasteurisation and chemical preservatives was the most convenient, and cheapest, option available at this stage of the development process. There are alternatives that are being investigated within the fruit juice industry which would assist in keeping the freshness of the product intact as some organoleptic changes may take place in heat processing (San Martin *et al.*, 2002). However, these procedures are fairly new and costly at this stage to be considered for the scope of the project and this is why it was decided that the more commonly adopted preservation methods be adopted. Other technologies that are being investigated are high pressure processing (Baxter *et al.*, 2005), high hydrostatic pressure (San Martin *et al.*, 2002), pulsed electric field (Jaya *et al.*, 2004) and ultraviolet treatment (Tran & Farid, 2004).

As stated before, microbial problems within soft drinks and fruit juices can be divided into two groups or scenarios 1) growth in, and deterioration of, the product by general organisms to produce spoilage and 2) growth in, or contamination of, the product by pathogens to produce spoilage (Wareing & Davenport, 2005:279)

Therefore, the implication is, that in the first scenario, the product itself is contaminated and has not been correctly handled during harvesting or processing and the micro-organisms have remained in the product have been allowed to multiply. In the second scenario, the implication is that the product has been contaminated by poor processing, usually by human contamination (improper hygiene) or in the distribution process.

Given the complex nature of the micro-organisms that may or may not be present in the fruit and the resultant beverage, it is challenging to determine whether the pasteurization process was adequate and whether the level of preservatives were adequate to give a four week shelf life at chilled conditions?

It is also impossible to test for every possible micro-organism that could be present in the final product. Therefore, compromise must be reached. The tests carried out were indicative of whether the juice was likely to contain any pathogenic bacteria and, if not, the risk of spoilage from yeasts and moulds was minimal.

Table 4.2: Summary of microbiological testing of Kei apple beverages, from day one to day twenty-eight.

Test	Method ①	Specification ②	Microbial load of variant: – Unflavoured (cfu/ml) Number of days from start of test					Microbial load of variant: – Apple (cfu/ml) Number of days from start of test				
			1	7	14	21	28	1	7	14	21	28
Total Plate Count	AOAC 966.23	<1000 cfu/ml	2	1	1	7	3	9	13	10	30	3
Lactic Acid Bacteria Count	SABS ISO 15214	<1000 cfu/ml	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Coliform Count	Biorad 07/1-07/93	Absent / ml	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
E.coli Count	Biorad 07/1-07/93	Absent / ml	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Mould Count	SABS ISO 7954	<100 cfu/ml	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Yeast Count	SABS ISO 7954	<10 cfu/ml	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1

Test	Method	Specification *	Microbial load of variant: – Vanilla (cfu/ml) Number of days from start of test					Microbial load of variant: – Mint & vanilla (cfu/ml) Number of days from start of test				
			1	7	14	21	28	1	7	14	21	28
Total Plate Count	AOAC 966.23	<1000 cfu/ml	10	11	60	50	27	1	<1	1	2	70
Lactic Acid Bacteria Count	SABS ISO 15214	<1000 cfu/ml	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Coliform Count	Biorad 07/1-07/93	Absent / ml	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
E.coli Count	Biorad 07/1-07/93	Absent / ml	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Mould Count	SABS ISO 7954	<100 cfu/ml	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Yeast Count	SABS ISO 7954	<10 cfu/ml	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1

Key
cfu/ml colony forming units per milliliter
① Recognised international test methods
② Specification adopted by Pick 'n Pay, South Africa, for 100% fresh fruit juices

Samples were stored between 4°C and 8°C for the duration of the test to simulate cold-chain conditions

The total plate count for all of the products tested (unflavoured, apple, vanilla and mint & vanilla) was lower than the specification given of 1000 colony-forming units per mL (cfu/mL), showing that the total microbial load was low. This is also true for lactic acid bacteria – a spoilage organism – where, in all cases, no lactic acid was found. No pathogens, coliforms or *E.coli* were found in any of the samples, which may indicate faecal contamination or poor hygiene in areas of the production process. The mould and yeast counts were less than 1cfu/mL, showing that the product was unlikely to spoil over the four week period. However, the pH of the beverages, ranging from 2.98 to 3.04 (as shown in table 3.3) would not be conducive to most microbial and bacterial growth (Wareing & Davenport, 2005:280).

4.4 Consumer Testing of Kei Apple Beverage

When using a food panel, HUT (home usage trial), large-scale consumer testing with complete target demographics or a trained panel or expert panel (the latter being far less expensive after training has been completed, but the initial training is expensive in terms of time and finance), all factors influencing food choice cannot be eliminated. Figure 4.2 from Gains (1996) summarises very effectively all the factors influencing an individual's food choice. Thus, when using a panel to evaluate foods, at least one variable is constant and, allowing for consumer variation and environmental factors to alter, the context in which the food was consumed remains constant. This is the same for all products evaluated and must be taken into consideration. However, again it only reiterates the importance of consumer panels to form a standard test in consumer research.

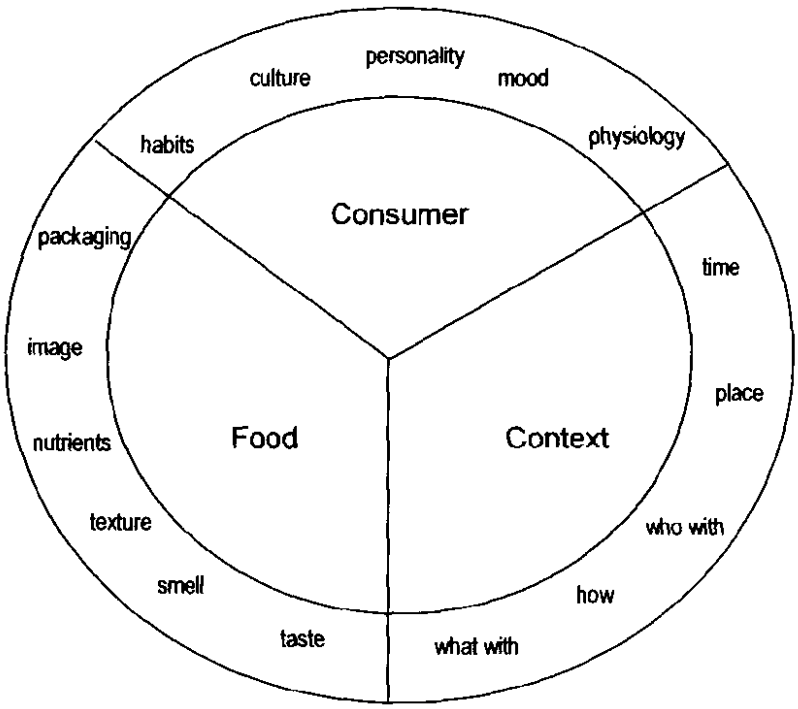


Figure 4.2: factors influencing food choice (adapted from Gains, 1996)

Again, the validity of a consumer testing panel is always debatable. Kozlowska *et al.* (2003) found that post consumption ratings (similar to a home test) are more reliable than ratings briefed on a brief exposure, for example, one taste or drink not in the

context of a full meal, or familiar surroundings. These authors also stress that there is limited value in one day exposure tests in the development process. However, consumer panels still provide a base from which to work. They can indicate if a product is violently disliked, and comparisons can easily be made. Thus, a position from where to go on, and if and what modifications must be made to the product can be determined, and therefore panel testing is always necessary. It is relatively cheap and easier than HUT testing.

4.4.1 Demographics of the consumer population in the Kei apple panel testing

Demographics refers to a basic objective descriptive classification of consumers, such as their age, gender, income, education, size of household, ownership of home, and so on. This does not include classification by subjective attitudes or opinions of consumers (hppt://advertising.utexus.edu/research/terms/#D). In the context of this project, demographics would refer to the types of consumers that would be potentially consuming and purchasing this product.

The demographics of the respondents are shown in table 4.3.

Table 4.3: Demographic data of respondents in consumer testing, according to age, gender, occupation, awareness of role of nutrition in health, and fruit juice consumption

PARAMETER	GENDER		TOTAL	% OF TOTAL
	MALE	FEMALE		
Age				
18 – 24	22	74	96	63.2%
25+	19	37	56	36.8%
Total (n)	41	111	152	100%
Occupation				
Student	22	73	95	62.5%
Educational (Academic)	6	13	19	12.5%
Administration	3	17	20	13.2%
Total (n)	41	111	152	100%
Awareness of Role of Nutrition in Health				
Yes	39	110	149	98.0%
No	2	1	3	2.0%
Total (n)	41	111	152	100%
Fruit Juice Consumption				
Light (2-3/month)	4	20	24	15.8%
Medium (1-2/week)	24	49	73	48.0%
Heavy (>3/week)	13	41	54	35.5%
Total (n)	41	110	151	98.34%

Whilst there are a large proportion of students (62.5%) included in the total and of a lower age category 18-24 (63.2%), the population total for a consumer test was 152, which is large enough to give meaningful results (Stone & Sidel, 1995:240). Ideally, the split of age and occupation should have been less skewed towards students and those of a lower age group, and included more of the male sex. However, the goal of this project should be remembered – the development of a functional beverage from the Kei apple. Given the lower level of Kei apples harvested in 2005, none in 2004 and only a few in 2003 from limited areas, the product developed could only be expected to be taken into the feasibility stage of the product development process. There is sufficient science that back up the consumer testing but budget restraints deny full scale consumer testing where all preferred demographics may be met. The product is aimed at a consumer with an awareness of nutrition. It was encouraging that, within these demographics, 98% of the consumers were aware of the role of nutrition in disease prevention, although this is likely that the respondents are "nutritionally aware", given the academic environment that the consumer testing was carried out in.

4.4.2 Order of presentation of samples

The effect of order of presentation of the samples was examined. It was found that, in most cases, there was no statistical significant effect on the order of presentation for appearance. For taste, vanilla was statistically significantly preferred over mint when it was tasted after mint, but this was not practically significant (d=0.28 and 0.29). In all other cases, there was no statistical significant order effect therefore order effect was not reported in a table format.

4.4.3 Overall acceptance of the beverages

Consumers acceptance rating and the statistics of the different product attributes are given in table 4.4.

Table 4.4: Acceptability# evaluation of product attributes

Attribute	Variable	*n*	Mean	SD	p-value
Mint & vanilla	Appearance	151	5.32	1.17	
Vanilla		152	5.22 *	1.19	
Apple		152	5.36 *	1.14	=0.03
Mint & vanilla	Taste	150	4.26 *	1.69	<0.001
Vanilla		150	4.46 #	1.68	<0.001
Apple		150	5.48 * #	1.48	
Mint & vanilla	Texture	150	5.33 *	1.27	=0.003
Vanilla		150	5.26 #	1.27	=0.0002
Apple		149	5.60 * #	1.17	
Mint & vanilla	Overall Acceptance	150	4.50 *	1.58	<0.0001
Vanilla		149	4.60 #	1.62	< 0.0001
Apple		149	5.77 * #	1.39	

n = number of participants SD = Standard Deviation p = statistical significance

1 = totally unacceptable; 2 = unacceptable; 3 = slightly unacceptable; 4 = neutral; 5 = slightly acceptable; 6 = acceptable; 7 = exceptionally acceptable

Means of a particular variable with a symbol in common differ significantly from one another.

Neither gender, occupation nor fruit juice consumption had a statistical or practical significant effect on any of the attributes measured for acceptability. Only age had a statistically significant effect on appearance (p=0.04) and taste (p=0.03), but it was not of practical significance as d values were between 0.5 and 0.6 and thus a medium effect. This is why only one table of acceptability has been reported.

Table 4.5: Level of practical significance (d-values) of differences in acceptability scores between the three beverages

	Vanilla vs. Mint & vanilla	Apple vs. Vanilla	Mint & vanilla vs. Apple
Appearance	0.08	0.13	0.04
Taste	0.12	0.61	0.72
Texture	0.06	0.20	0.16
Overall Acceptance	0.06	0.67	0.74

d = 0.2 small effect; d = 0.5 medium effect; d = 0.8 large effect

In the discussion of the results of question 1 for appearance, taste, texture and overall acceptance, reference will be made to table 4.4 for statistical significant differences in acceptability (p), and to table 4.5 for practical significance (d) in this regard.

For appearance, there was a statistically significant difference in acceptability between the apple and vanilla beverages (mean=5.36 and mean=5.22 respectively) (p=0.03). However, this was not of practical significance (d=0.13).

For taste, there was a statistically significant difference in acceptability (p<0.001) between mint & vanilla (mean=4.26) and apple (mean=5.48). This difference was also of a medium to large practical significance (d=0.72). There was also a statistically significant difference (p<0.001) between vanilla (mean=4.46) and apple (mean=5.48). This difference was of a medium practical significance (d=0.61). There was no statistically significant difference between mint & vanilla and vanilla.

For texture, there was a statistically significant difference in acceptability (p=0.003) between mint & vanilla (mean=5.33) and apple (mean 5.60), which was not of a practical significance (d=0.16). There was a statistically significant difference (p=0.0002) between vanilla (mean 5.26) and apple (mean=5.60), which was again not of a practical significance (d=0.06). There was no statistically significant difference between mint & vanilla and vanilla.

For overall acceptance, there was a statistically significant difference (p<0.0001) between mint & vanilla (mean=4.50) and apple (mean=5.77). This difference was of a medium to large practical significance (d=0.74). There was a statistically significant difference (p<0.0001) between vanilla (mean=4.60) and apple (mean=5.77). This difference was of a medium practical significance (d=0.67). There was no statistically significant difference in overall acceptance between mint & vanilla and vanilla.

Overall, it can be concluded that there is no statistically significant difference between the acceptability or appearance or texture of the beverages, which is to be expected as the only difference (unknown to the participants) was the flavour. The difference in acceptability of taste and overall acceptance between apple and vanilla was of statistically significant difference and of medium practical significance, while the difference in acceptability of taste and overall acceptance between mint & vanilla and apple, was of statistically significant difference, as well as of medium to large practical significance.

Question 1 on acceptability demonstrates how the hedonic scales discussed in chapter 2:74, can be used to translate consumers' perceptions about the product into

tangible results which can clearly discriminate one product from another. It demonstrates here that the apple beverage was clearly more liked by consumers in terms of taste and overall acceptance.

4.4.4 Preference of the beverages

There was no statistical or practical significant effect of gender, age, occupation or fruit juice consumption on preference of the samples.

When the respondents were asked to rate their preference for the samples, apple flavour (mean=1.45) was significantly preferred over both the vanilla (mean=2.29) and mint & vanilla (mean=2.29) (see table 4.6). Furthermore, there was no statistically significant difference in preference for vanilla and mint & vanilla.

Table 4.6 Preference rating# of the three beverages.

	Preference #				
	1 n	2 n	3 n	Mean Mean	SD s
Mint & vanilla	26	56	9	2.29	0.74
Vanilla	24	64	62	2.29	0.71
Apple	102	31	19	1.45	0.71

n = number of respondents SD = Standard Deviation
# 1 = most preferred; 2 = second preferred; 3 = least preferred

The difference in preference between apple and vanilla (d=1.18) and apple and mint & vanilla (d=1.14) were both of practical significance. There was no practical significant difference between preference for vanilla and mint & vanilla. The effect sizes 1.18 and 1.14 are large, indicating that in practice the consumers considerably preferred the apple beverage over the other two flavours. Question 2 on preference can be classified as a quantitative affective test which is commonly used with consumers, where the product is ranked as being better than another (Meilgaard *et al.*, 1999:241). Consequently, the results of this question show that the apple beverage is far preferred over the other two flavours and there is very little difference in preference between the other two. This is also a forced choice question, where the consumer has to make a choice and therefore it is not surprising that the level of practical significance in differences is high.

4.4.5 Consumption intent for the beverages

Table 4.7 Consumption intent# for all three beverages.

	Consumption Intent #		
	Mint & vanilla	**Vanilla**	**Apple**
Median	4	4	2
Lower Quartile	3	3	2
Upper Quartile	4	4	3

1 = I will drink it very often; 2 = I will drink it often; 3 = I will drink it occasionally; 4 = I will drink it only when nothing else is available; 5 = I will never drink it

There was no statistically significant difference nor practical significant difference in gender, age, occupation or fruit juice consumption on consumption intent overall. Age had a statistically significant effect (p=>0.0001) on consumption intent for vanilla (p=0.003), but this was not of practical significance (d=0.16). Thus, the statistics have been reported simplistically as in table 4.7 and figure 4.2.

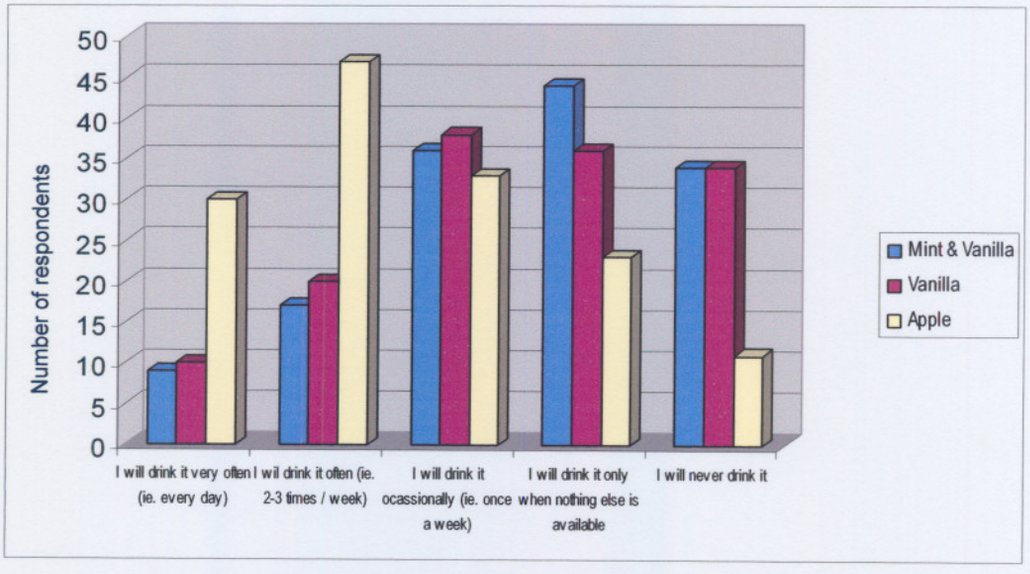


Figure 4.2 Consumption intent for all three beverages

The respondents were asked to indicate their consumption intent for all three beverages and from table 4.7, it is clear that the respondents are more likely to consume the apple flavoured beverage than the others. The difference in medians ± [lower and upper quartile] for consumption intent for apple flavour as opposed to mint

& vanilla (2 ± [2-3] vs. 4 ± [3-4]) was of medium practical significance (d=0.55); while the difference in consumption intent for apple as opposed to vanilla (2 ± [2-3] vs. 4 ± [3-4]) was of medium to large practical significance (d=0.74). The difference between consumption intent for the mint & vanilla and vanilla flavours was, however, not of practical significance. Observing figure 4.2, where the frequencies for consumption intent of the different beverages are given, it appears that there was not great enthusiasm about the vanilla and mint & vanilla drinks and therefore further modifications may be required, although there is no branding, pricing or health benefits shown which may further influence the consumer's consumption intent.

Consumption intent, as determined by question 3, is an adapted FACT scale as discussed in chapter 2:75. This measured the general attitude of the consumers regarding their consumption intent (how often) for the beverage (Resurreccion, 1998:23). It was clear that consumers were of the intention to consume the apple beverage most often, compared to either vanilla or mint & vanilla.

4.4.6 Purchase intent for the beverages

Finally, the participants were asked what their likelihood of purchase intent was, as reported in table 4.8.

Table 4.8: Frequency of purchase intent for all three beverages.

	Definitely would purchase this product		**Definitely would not purchase this product**	
	n	**%**	**n**	**%**
Mint & vanilla	49	30.2	93	61.2
Vanilla	48	32.6	96	63.2
Apple	111	73.0	37	24.3

There was no statistically significant effect of gender, age, occupation or fruit juice consumption on purchase intent. Thus, for simplicity, table 4.8 has been reported without taking into account the aforementioned.

Significantly more consumers were of the intention to purchase the apple flavour (73.0%) beverage as opposed to the vanilla (32.6%) and mint & vanilla (30.2%). The

consumer's purchase intention was practically significantly higher for the apple when compared to vanilla (d=0.81) and for the apple when compared to mint & vanilla (d=0.86). The differences in purchase intent between vanilla and mint & vanilla was very small (2.4%) and thus of no practical significance.

As with question 2 on preference for the beverages, this question is a forced choice question which forces the respondent to make a choice. It is clear that there is an overall skew towards the apple flavour and that this flavour has the most potential for the future. Here, a clear indication from the respondents has been given that they would purchase the apple beverage, and thus a correlation with liking the product can obviously be made, although no other factors such as pricing and branding were given, apart from the nutritional benefits stated in the instructions in the panel testing.

The respondents were also requested to give their written opinions on the fruit juices. These are shown in Appendix 4. Overall, the comments concur that the apple flavour is preferred. However, there are some preferences for the mint & vanilla, and the vanilla as first choice. The possibility of a polarized choice may exist, i.e. some people show a real preference for the apple and intensely dislike the other two flavours, whilst others show the reverse. Whilst formulating the product, it was noticed that, if the product was served with ice, the preference for vanilla and mint & vanilla increased. However, this was on a very informal basis. What is clear is that future work on the flavour and the flavour / product interactions would be necessary, although at this stage it still seems that the apple flavour would be the direction to follow. As with most commercial products, it may be possible to introduce several flavours to the market at one time to see if one product flavour or "line" is successful and then introduce other flavours as the product starts to be successful in the market place.

4.5 Determination of Total Polyphenols

The total polyphenol content of the samples were analysed as gallic acid equivalents and the results are summarised in table 4.9.

Table 4.9: Total polyphenol content of analysed samples measured as total mg GAE/l in Kei apple beverages, pulps and Ocean Spray® Cranberry.

	Total Polyphenols (uncorrected)	Dilution Factor*	Total Polyphenol - Corrected for Dilution Factor	Total Polyphenol - Corrected for Ascorbic Acid	Brix °	Brix Factor % for Correction	Concentration Corrected After Brix	Average	Standard Deviation
Mint & vanilla [a]	116.4	1	116.4	109.5	11.9	2.5	106.8		
	111.6	1	111.6	104.7	11.9	2.5	102.1	106.1	3.68
	119.1	1	119.1	112.2	11.9	2.5	109.4		
Vanilla [b]	154.3	1	154.3	146.1	12.4	2.5	142.5		
	155.2	1	155.2	147.1	12.4	2.5	143.4	144.00	0.42
	154.7	1	154.7	147.6	12.4	2.5	143.0		
Apple [c]	112.2	1	112.2	105.2	12.4	2.5	102.3		
	112.8	1	112.8	105.8	12.4	2.5	103.2	101.7	1.99
	109.0	1	109.0	102.0	12.4	2.5	99.4		
Unpasteurised [d]	120.6	1	120.6	107.1	12	2.5	104.4		
	120.9	1	120.9	107.5	12	2.5	104.8	103.1	2.71
	115.9	1	115.9	102.5	12	2.5	99.9		
Pulp 05 [e]	10.58	100	1058	976.5	8.4	1.5	952.1		
	9.02	100	901.6	820.4	8.4	1.5	799.9	864.1	78.85
	9.43	100	943.0	861.8	8.4	1.5	840.2		
Pulp 03 [f]	13.12	200	2625	2461	16.3	3.0	2387		
	13.20	200	2639	2475	16.3	3.0	2401	2423	52.08
	13.62	200	2724	2560	16.3	3.0	2483		
Cranberry Ocean Spray® [g]	173.5	2	347.1	306.5	13.8	2.9	298.8		
	174.6	2	349.2	308.7	13.8	2.9	300.9	302.2	3.99
	177.5	2	355.0	314.4	13.8	2.9	306.5		

a = mint & vanilla Kei apple beverage
b = vanilla Kei apple beverage
c = apple beverage
d = unpasteurized, unflavoured Kei apple beverage
e = pulp from 2004/2005 growing season from Eastern and Western Cape
f = pulp from 2002/2003 growing season from Bloemhof
g = Cranberry Ocean Spray® commercial product
* indicates how much the sample was diluted before analysis was undertaken

The apple flavour was reported as one of the triplicates of the first analyses and was found to differ markedly from the other two (therefore, the analysis was repeated to rule out experimental error). In summary, the average values for Gallic acid equivalent (mg GAE/l) were mint & vanilla 106, vanilla 144 and apple 102. The Cranberry Ocean Spray® contained 302mg GAE/l. The pulp of the Kei apple

harvested in 2004/2005 contained 864mg GAE/l and the pulp of the 2002/2003 apples contained 2423mg GAE/l.

It appears that the polyphenol content of the formulated Kei apple juice was greater with the vanilla flavour. It is possible that the structure of the vanilla molecule (vanillic acid) (Miller & Ruiz-Larrea, 2002) which contains a benzene ring, may have interfered with the analysis causing an increase in the amount, although it is strange that a pro-rata increase was not seen with the mint & vanilla (chapter 2:25). This would need to be discussed with a flavour house if the development were to be taken further. However, at this stage, as the vanilla was not liked by most of the respondents in the consumer test, it would not be pursued as a flavour for the beverage. The level of total polyphenols in the unpasteurized sample were 103mg GAE/l, indicating that pasteurisation of the juice did not appear to affect the level of polyphenols.

There was a very large difference between the levels of polyphenols in the two pulps which may be applicable to a variety of reasons. The fruits were of a different ripeness, and this may have affected the astringency (and therefore total phenolics). The fruits were grown in two different geographical areas, namely Bloemhof and the Cape. The Eastern and Western Cape apples were combined before processing so unfortunately no difference could be measured between these for total polyphenols. However, both sets appeared ripe and juicier than those from Bloemhof. However, it should be noted that this was an incidental observation and not the main aim of the project, although at some stage variations in harvest should be investigated. Manach *et al.* (2004) state that environmental factors have a major effect on polyphenol content. These factors may be pedro climatic (soil type, sun exposure, rainfall) or agronomic (culture in greenhouses or fields, fruit yield per tree). They also state that the degree of ripeness has a considerable effect on the flavonoids, and phenolic acids generally decrease during ripening which ties in with the theory that the astringency also decreases with ripening. Manach *et al.* (2004) also state that there can be marked differences in concentration of flavonoids between pieces of fruit on the same tree because their biosynthesis is stimulated by sunlight.

Another key factor that would influence the content of the polyphenols in the pulp is the processing of the fruit. In our particular situation, the Bloemhof apples were

processed in the laboratory and sieved manually, whereas the Cape apples were processed down the actual plant.

Wu *et al.* (2004) indicate that sampling time during the year significantly influenced lipophilic and/or hydrophilic oxygen radical absorbance capacity (ORAC) values in some foods, and that to get an accurate total antioxidant capacity of a given food both these values need to be taken into account. Food processing, such as cooking and peeling also affects the values. Then again, it can be postulated that for total polyphenol and ascorbic acid content of the Kei apple juices, a similar conclusion may be reached. Yu *et al.* (2004) compared wheat flours grown at different locations for their antioxidant properties and found that the variety and growing location had a significant influence on their free radical scavenging abilities against the stable 2,2-diphenyl-l-picrylhyrazyl radical (DPPH) and 2,2-anino-di [3-ethyl-benz thiazoline sulfonate] radical cation ($ABTS^{+}$), and for Fe^{2+} chelating capacities and total phenolic contents. If reference is made to table 2.8 (chapter2:49), the USDA database of flavonoid content of foods, variations are apparent according to methodology, for example, apples with and without skin. This is another factor leading to variability.

Studies with other fruits have demonstrated that processing has a huge influence on the content of polyphenol and the total antioxidant activity (TAA) of the juice. The use of an integrated membrane process to clarify and concentrate citrus and carrot juices compared to the traditional thermal treatment by concentration via distillation was investigated by Cassano *et al.* (2003). It was found that TAA was lower with thermal concentration than with membrane concentration technology (reverse osmosis and ultra filtration) performed at room temperature. The juices concentrated this way also retained their colour and a large part of their aroma.

Fruit juices on the market are generally constituted by two types of products: fresh juices, obtained by simple squeezing and then submitted to mild pasteurization (with the addition of preservatives), or juices reconstituted from concentrate. It is known that the thermal treatment by pasteurization and/or thermal concentration produces modifications of some components with consequent degeneration of taste and chemical characteristics (Cassano *et al.*, 2003).

Eisele and Drake (2004) examined the partial compositional characteristics of apple juice from 175 apple varieties, covering 12 countries and several USA geographical

areas. Distribution of phenolics between the various varieties was highly variable, with some juices containing little if any phenolic compounds. The mean values for some of the attributes did not match existing compositional database value means (malic acid and phenolics in particular), but some of the overall minimum and maximum value for the various attributes (°B, pH, ash, total acidity (TA), sugars, sodium and calcium) fitted in well with existing databases. This was a study carried out with well known juice, namely apple, yet variability in the phenolics was found to be either due to variety and/or climate, soil and growing conditions.

Shimoda *et al.* (2003) investigated the influence of the thermal treatment on the odours of apple juice by sensory evaluation. The odours of the apple juice changed dramatically with the temperature and residence time during thermal treatment. Thus it can be postulated that there are also chemical changes that are caused due to changes in processing. Beecher (2003) states that the quercetin in onion is lost in storage and this may be due to evaporation and the hydrophilic quercetin being lost as the onion dries out. Marin *et al.* (2002) showed that the variety of lemon fruit affects the level of flavonoids, while these researchers also examined different extraction methods and found that an industrial extraction method with greater pressure resulted in juices with higher levels of TAA.

It is not surprising that there are variabilities with the two harvests, given all the variables of climate, time of harvesting, area grown, storage and processing.

The levels of polyphenols in the Ocean Spray® Cranberry are nearly three times that of the formulated Kei apple juice (302mg GAE/l). However, in future formulatory work, it may be possible to increase the level of the Kei apple in the base juice as the resultant juice was not perceived by the respondents to be overly sour or astringent. The briefing of an appropriate flavour house may also assist in helping mask the aroma of the Kei apple more effectively. The viscosity of the beverage could further be increased which may decrease any perceived increase in acidity further. However, variations in harvest conditions must be ruled out in the long term.

4.6 Determination of Ascorbic Acid

The levels of ascorbic acid as free ascorbic and bound dehydroascorbic acid in the formulated Kei apple beverages, the Bloemhof and Cape pulps and Ocean Spray® Cranberry were analysed and are as reported in table 4.10.

Table 4.10: Total ascorbate, as measured by dehydroascorbate (DHA mg/l) and total ascorbate (TA mg/l) in Kei apple beverages, pulps and Ocean Spray® Cranberry.

	Total	Dilution Factor*	Concentration	Standard Deviation		Ascorbate	Average	Standard Deviation	Dehyro Ascorbate	Average	Standard Deviation	Ascorbic Acid Effect
				AV	SD							
Mint & vanilla [a]	32.3	1	32.26	31.4	0.93	3.65	3.65	0.61	28.60	27.79	0.70	6.91
	30.4	1	30.43			3.04			27.39			
	31.7	1	31.65			4.26			27.39			
Vanilla [b]	34.4	1	34.39	36.8	2.60	8.82	8.62	0.35	25.56	28.10	2.92	8.10
	39.6	1	39.60			8.21			31.34			
	36.5	1	36.52			8.82			27.69			
Apple [c]	30.7	1	30.73	32.0	1.22	4.26	3.80	0.46	26.47	28.15	1.67	7.03
	33.2	1	33.17			3.34			29.82			
	32.0	1	31.95			3.80			28.15			
Unpasteurized [d]	62.4	1	62.38	61.2	6.15	35.60	34.08	1.52	26.78	27.10	5.31	13.5
	66.6	1	66.64			34.08			32.56			
	54.5	1	54.51			32.56			21.95			
Pulp 05 [e]	7.46	100	745.54	745.5	45.65	530.0	532.0	46.06	215.5	213.58	1.72	164.0
	7.91	100	791.18			579.0			212.2			
	7.00	100	699.89			486.9			213.0			
Pulp 03 [f]	4.43	200	886.88	941.9	47.82	699.9	699.9	91.29	187.0	241.97	106.89	207.2
	4.83	200	964.94			791.2			173.8			
	4.87	200	973.76			608.6			365.2			
Cranberry Ocean Spray® [g]	181.4	2	362.72	369.1	6.39	339.8	338.8	1.46	22.9	30.26	6.64	81.20
	187.8	2	375.51			339.6			35.9			
	184.5	2	369.09			337.2			31.9			

a = mint & vanilla Kei apple beverage
b = vanilla Kei apple beverage
c = apple beverage
d = unpasteurized, unflavoured Kei apple beverage
e = pulp from 2004/2005 growing season from Eastern and Western Cape
f = pulp from 2002/2003 growing season from Bloemhof
g = Cranberry Ocean Spray® commercial product
* indicates how much the sample was diluted before analysis was undertaken

In order for the beverages full potential functionality to be evaluated the level of total polyphenols present in the beverage needed to be measured. Ocean Spray® was included as a control, as no other equivalent products exist on the South African market and this is a leading product in the USA market. The body of scientific evidence on 100% cranberry juice consumption may also support a legal function claim in the future.

The total levels of ascorbic acid (mg/l) for mint & vanilla, vanilla and apple were 31.4, 36.8 and 32.0 respectively; levels of free ascorbic acid (mg/l) were 3.7, 8.6 and 3.8 respectively and levels of dehydroascorbic acid (mg/l) were 27.8, 28.1 and 28.2 respectively. Little free ascorbic acid was available. The level of total ascorbic acid in the unpasteurized sample was 62mg/l, free ascorbic acid was 34mg/l and dehydroascorbic acid was 27mg/l. It appears that the pasteurisation process had destroyed some of the available ascorbic acid in the formulated beverage. The total ascorbic acid in the Ocean Spray® Cranberry product was 369mg/l, free ascorbic acid was 339mg/l and dehydroascorbic acid was 30mg/l. It appears that there is added free ascorbic acid in this commercial product which is a commonly adopted practice in the food industry to prolong the shelf life of fruit juices (Taylor, 2005:103).

The total ascorbic acid of the pulps from the Cape and the Bloemhof apples were 746mg/l and 942mg/l respectively, free ascorbic acid 532mg/l and 698mg/l, dehydroascorbic acid 314mg/l and 242mg/l. The differences in vitamin C levels are not as apparent as the polyphenol levels, which suggest that the climate, ripeness and other factors have had a larger influence on the levels of polyphenols. Ascorbic acid loss as a result of blending and pasteurizing has been well documented (Kaur & Kapoor, 2001), whilst the same authors state that it remains unclear whether processing affects polyphenols or not. Sahari *et al.* (2004) studied the effect of freezing on the ascorbic acid content of frozen strawberries and found that the ascorbic acid losses were greater after 15 days of storage and did not differ dramatically with slow or quick freezing. Thus storage time appears to have an effect on the ascorbic acid content of the product.

It has been found that, whilst titratable acidity levels in Hayward kiwifruit appeared quite stable during storage at 0°C, it is known that citric acid levels decline but malic acid levels are maintained, and even increase with storage at 4°C, indicating that the storage conditions of the kiwi storage are critical to maintain consistent quality of the product (Marsh *et al.*, 2004). Thus, storage conditions are critical in maintaining the level of actives in the product such as ascorbic acid and polyphenols, as well as processing. This needs to be borne in mind for future work.

This loss of ascorbic acid is not surprising and when formulating the Kei apple beverage again, must be taken into consideration as with all other fruit juices, and ascorbic acid added back, as is common industrial practice.

The results and discussion and all the formulatory, consumer panel testing work and analytical work will now be evaluated in chapter 5 (Conclusions and Recommendations) in an attempt to evaluate whether the original aims of the project were met or not. If these aims were not met, what would be required in the future in terms of further work or resources to meet them?

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

CONCLUSION AND RECOMMENDATIONS

Kei apples were taken from two areas of South Africa in two different seasons. The first batch of apples were used to develop a prototype formulation and the second batch of Kei apples were used to assess if this prototype formulation could be scaled up to an industrial size plant and then subjected to evaluation by consumers and analytical chemistry for total polyphenols and ascorbic acid.

At this point, the aims of the project must be revisited and assessed as to whether they have been met, giving specific reasons if they have not been met and what needs to be done to ensure that they can be met in the future?

1) **Develop (and confirm by panel testing) a product that is preferred by consumers and will be purchased due to its sensory attributes.**

The Kei apple beverage was developed as described. It was noted that the original pilot study beverage was extremely acidic and astringent. In order to decrease this perceived acidity, a buffer (tri-sodium citrate) was added, and a thickener (carboxymethyl cellulose) was added. Increasing viscosity with CMC (carboxymethyl cellulose) also reduced perceived acidity and astringency.

From the consumer panel testing, it was clear that a product had been developed which met this aim of the project. For further product improvements, the same guidelines as were used in the developmentary work, namely alteration of acid/sugar ratio, addition of a buffering agent and the using of a thickening agent are the correct guidelines to continue with.

2) **Develop a product that could increase consumption of fruit juice as part of improving overall diet and thereby increasing consumption of fruit.**

Provided that the developed product can be marketed correctly, there is no reason why this aim of the project cannot be met. In order to achieve this, a commercial partner must be identified to market the product once it has been produced. This product could easily form one of the portions of fruit and vegetables as

recommended by the International Fruit and Vegetable Alliance (IFAVA), in the quest to increase consumption of fruit and vegetables globally. Some of the fibre may be lost during processing but this can be minimized if processing (especially with pulp technology) is utilized, as was here.

The stakeholders involved in the Kei apple project must work together in order to make it successful or provide more reliable fruit. Elstein (2005) states the example of some of the "minor fruits" (to the United States) that are being researched by Agricultural Research Services of the United States for potential as major crops. These researchers identified the potential of these crops such as elderberries, black raspberries and then work to introduce them as potential for future growth into major crops.

The same is true for the Kei apple fruit consumption to be increased – it must be organised in a similar fashion to the Marula Project, where everyone from the picker, grower, processer, seller, producer to the marketer is involved. This project has identified opportunities and has proved that there is a feasible product that is desired by consumers. However, a leader in the community must be identified to form a co-operative of Kei apple growers in order to take this forward in a similar fashion to the Marula project.

Adding weight to the formation of co-operative and obtain financial backing, Schieber *et al.* (2001) examined the by-products of plant food processing as sources of functional compounds, many common fruits such as apples, grapes, peaches, apricots, citrus fruits, pineapples, bananas, guavas, papaya, passion fruit and kiwi fruit offered valuable by-products in terms of their seeds or skins that may be useful in terms of their value as potential food ingredients as antioxidants due to their polyphenol content. This is encouraging as it means that there is a shift in towards thinking about what may be healthy for the individual and a more interdisciplinary approach is being taken, in the fields of food technology, chemistry, nutrition and toxicology. If a beverage is not feasible, perhaps polyphenol extraction from the Kei apple should be examined, which could motivate increased production of the product, and thus could contribute in meeting this objective.

Overall, there is no reason why this aim of the project cannot be met. The project work clearly defines a consumer-preferred product. It is up to the other stakeholders to play their part.

3) **Identify and quantify the constituents that are key to the success of the developed product's functionality by measuring the polyphenols and ascorbic acid mg/l, using gas chromatography / mass spectophotometry.**

The active ingredients that were identified as being present in the Kei apple were polyphenols GAE/l – thought to be caffeic acid and its derivatives, oligimers and polymers (Du Toit Loots personal communication). A lower level of total polyphenols was found in the 2004/2005 season's harvest of Kei apples, which may have been due to a variety of reasons already discussed. The levels of total polyphenols as GAE/l in Ocean Spray® Cranberry juice, used as a benchmark for comparison, were three times that of the formulated beverage and a functional claim allowable under current USA legislations is being made on the first-mentioned product. If this would be allowed in the future under SA regulations, is dubious. The level of polyphenols in the final beverage may easily be increased by increasing the level of the Kei apple pulp in the beverage and using the modifications suggested on the previous page to ensure that acceptance to the consumer is still maintained. Alternatively, the effects of processing as well as the effects of different harvests, conditions of harvesting, areas and growing conditions must be investigated.

The levels of ascorbic and dehydroascorbic acids found in the final beverage were very low, and this was attributable to the processing. However, as seen with the Ocean Spray® Cranberry, ascorbic acid is added after processing and thus levels of free ascorbic acid would be easily increased.

Overall, the aims of this part of the project have been met, but require further investigation, which are largely related to agriculture. It must also be remembered that the definition of a functional food is very vague globally and thus no finite measures exist for functional beverages as to what the actual legal level of active ingredient present needs to be, as with a drug.

4) **Develop a product that has proven functional benefits that will assist in alleviating degenerative diseases.**

A product has been developed that is liked and accepted by consumers. However, unless clinical human intervention trials are undertaken, it cannot be demonstrated

that the product does have proven functional benefits and thus this aim of the project has not been met. It is also recommended that, before this happens, the formulation be increased to include more Kei apple and therefore more resultant polyphenols. It is suggested that after all variables have been ironed out, a consistency in supply of Kei apples is obtained and all stakeholders, growers, cooperative farm organizations, processers, marketers and distributors have been identified and the correct financing has been obtained, full-scale clinical testing be initiated.

In summary, the time is ripe for such a project to be funded and followed through. The identification of the suitable stakeholders / partners is required for all phases of the project – planting, harvesting, processing, formulation / research and marketing of the project. If the question is asked "was the project a success?", the answer would be given as affirmative, provided that the following recommendations for further development work and research are met:

a) Investigation of the variations in harvest and subsequent effects on product characteristics.

b) Quantification of the amount of Kei apples shrubs and the yield nationally, and potential resurrection of the Kei apple orchards planted in the Western Cape when the NRF Innovation Project commenced, should be investigated.

c) Absolute definition of harvest time.

d) The establishment of a Kei apple cooperative that will address the concern of the growers and organize transport to a central location for storage before processing.

e) Identification and commitment of the key stakeholders involved. In order for any functional food to be successful, all the stakeholders involved must work together efficiently. Those stakeholders are the agriculturists, the food industry, consumers, health sectors and government (McConnon *et al.*, 2002): 1) the food industry providing the product through research, either through its own research or through outsourcing, 2) consumers by providing insights into their needs and showing concerns about their health – taking responsibility for their own health, especially with rising medical costs, 3) the health sector, meaning all professionals, must carry health messages forward, and 4) governments, being responsible for ensuring that fair

legislation exists and that public health messages are supported from a financial and priority basis.

f) Analysis of polyphenol content versus varying agricultural and climatic conditions.

g) Identification of the precise actives involved – are they caffeic acids and their derivatives and others, and how biologically active are these actives in the body?

h) Fine tuning of the formulation to increase the level of total polyphenols as GAE/l, increase of ascorbic acid and improvement of aesthetic qualities to capitalize further on the positive consumer panel results, with the official involvement of flavour houses.

i) Key to all of this is to identify an industrial partner that would take interest and further the project financially, as it passes along the NPD process and to eventual market launch, where the initial return on investment (ROI) may be paid back due to the success of the project, not forgetting what the actual project has potentially achieved for the impoverished communities around the country, especially in the Eastern Cape, the very origin of the Kei apple (*Dovyalis caffra*).

CHAPTER 6

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APPENDICES

APPENDIX 1

Sensory evaluation of the acceptability of a newly developed beverage

1. Evaluation the ACCEPTABILITY of the following beverages according to the given criteria by indicating your choice with a cross (×) in the applicable block.

		EXTENT OF ACCEPTABILITY								
Criteria	Sample Code	Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Live Very Much	Like Extremely
Taste	K									
	L									
	M									
Overall Acceptance	K									
	L									
	M									

2. Please indicate your propensity to CONSUME this product. Mark only one block against each sample code.

	CONSUMPTION INTENT								
Sample Code	Would drink this every opportunity I had	Would drink this very often	Would drink this frequently	Would drink this now and then	Would drink this if available, but would not go out of my way	Would drink this on occasion	Would hardly every drink this	Would drink this only if there were no other choices	Would drink this only if I were forced to
K									
L									
M									

3. Please indicate your propensity to PURCHASE this product. Choose only one in each column and indicate your choice with a cross (×).

	PURCHASE INTENT	
Sample Code	Definitely would purchase this product	Definitely would not purchase this product
K		
L		
M		

APPENDIX 2

INSTRUCTIONS!

Thank you for coming here today.

We have developed fruit juices that have some added nutritional benefits. They may aid in the reduction of degenerative diseases such as heart disease & cancer and boost the immune system. We would like you to evaluate the sensory acceptability of these juices by completing Forms A and B.

Form A is a **simple questionnaire** which gives some anonymous details as to your age & occupation and as to how often you normally drink fruit juice.

Form B is the **sensory form** which evaluates acceptability, preference, consumption intent and purchase intent.

We'll go over the forms to ensure you understand them completely. Please do not confer with anyone during the evaluation or make noises that would influence other people's opinion, nor discuss the results afterwards as we have several other sessions still to take place.

We are indebted for your co-operation. Thanks, once again.

APPENDIX 3

CONSUMER QUESTIONNAIRE: STRICTLY CONFIDENTIAL

QUESTIONNAIRE NO.: ☐☐☐

SECTION A: DEMOGRAPHIC INFORMATION

Mark the appropriate square with a cross **X** :

1.	Gender	Male		1	☐
		Female		2	☐
2.	Age	Between:	18 & 25	1	☐
			26 & 39	2	☐
			Over 40	3	☐
3.	Occupation	Student		1	☐
		Educational (Academic)		2	☐
		Professional		3	☐
		Administrative		4	☐
4.	Are you aware of the role of nutrition in health?	Yes		1	☐
		No		2	☐
5.	How often do you drink fruit juice?	Light user	2 – 3 / month	1	☐
		Moderate user	1 – 2 / week	2	☐
		Heavy user	> 3 times a week	3	☐

APPENDIX 3 (continued)

VERBRUIKERSVRAELYS: STRENG KONFIDENSIEEL

VRAELYS NO.: ☐☐☐

AFDELING A

DEMOGRAFIESE INLIGTING

Merk die toepaslike blokkie met 'n kruisie X :

1	Geslag	Manlik		1	☐
		Vroulik		2	☐
2	Ouderdom	Tussen:	18 & 25	1	☐
			26 & 39	2	☐
			Ouer as 40	3	☐
3	Beroep	Student		1	☐
		Opvoedkundig (Akademies)		2	☐
		Professioneel		3	☐
		Administratief		4	☐
4	Is u bewus van die rol van voeding in gesondheid?	Ja		1	☐
		Nee		2	☐
5	Hoe dikwels drink u vrugtesap?	Ligte gebruiker	2 – 3 / mnd	1	☐
		Matige gebruiker	1 – 2 / week	2	☐
		Swaar gebruiker	> 3 keer / week	3	☐

APPENDIX 3 (continued)

SECTION B: SENSORY EVALUATION OF THE ACCEPTABILITY OF NEWLY DEVELOPED FRUIT JUICES WITH AN ADDED HEALTH BENEFIT

You are presented with 3 samples of juice. Please evaluate them in the specified order. You may cleanse your palate with water in between tasting. You must finish all of the samples by the end of the tasting session, however, you may re-taste during the evaluations.

1. Acceptability

Evaluate the acceptability of the following beverages according to the given criteria by indicating your choice with a cross in the appropriate block:

Criteria	Sample Code	Totally unacceptable 1	Unacceptable 2	Slightly unacceptable 3	Neutral 4	Slightly acceptable 5	Acceptable 6	Exceptionally acceptable 7
Appearance								
Taste								
Texture								
Overall Acceptance								

2. Preference

You have now evaluated all the samples for acceptability according to the above criteria. Please rank the samples in order of preference, starting with your most preferred sample first:

Sample Code	Order of Preference
	1
	2
	3

Please comment on the reasons for your choices: _____

_____

_____

_____

APPENDIX 3 (continued)

3. Consumption Intent

Mark only **one** option against each sample code:

		Sample Code		
		MLQ	VSH	PTN
I will drink it very often (ie. every day)	1			
I will drink it often (ie. 2 – 3 times / week)	2			
I will drink it occasionally (ie. 1 x / week)	3			
I will drink it only when nothing else is available	4			
I will never drink it	5			

4. Purchase Intent

Please indicate your propensity to purchase this product. Only choose one in each column and indicate your choice with a cross:

Sample Code	Definitely would purchase this product	Definitely would not purchase this product
MLQ		
VSH		
PTN		

Thank you for your co-operation.

Mary-Jane Gore

APPENDIX 3 (continued)

AFDELING B: SINTUIGLIKE EVALUERING VAN DIE AANVAARBAARHEID VAN 'N NUUT ONTWIKKELDE VRUTESAP MET 'N TOEGEVOEGDE GESONDHEIDSVOORDEEL

U word van 3 vrugtesap monsters voorsien. Evalueer dit asseblief in die gespesifiseerde volgorde. Spoel u mond tussen die evaluering van die verskillende vrugtesap monsters met water uit. Teen die einde van die evalueringsessie moet elk van die monsters opgedrink wees, maar u hoef nie elkeen met een slag op te drink nie.

1.Aanvaarbaarheid

Evalueer die aanvaarbaarheid van die volgende drankies volgens die gegewe kriteria deur u keuse met 'n kruisie in die toepaslike blokkie aan te dui:

Kriteria	Monster Kode	Totaal onaanvaar-baar 1	Onaanvaar-baar 2	Effens onaanvaar-baar 3	Neutraal 4	Effens aanvaar-baar 5	Aanvaar-baar 6	Besonders aanvaar-baar 7
Voorkoms								
Smaak								
Tekstuur								
Algemene Aanvaarbaar-heid								

2 Voorkeur

U het nou al die monsters volgens bogenoemde kriteria vir aanvaarbaarheid geëvalueer. Rangskik nou die monsters in volgorde van u voorkeur, beginnende by die monster wat u grootste voorkeur geniet:

Monster Kode	Volgorde van voorkeur
	1
	2
	3

Lewer asseblief kommentaar aangaande die redes vir u keuse:

_____

_____

APPENDIX 3 (continued)

3 Voorneme t.o.v. verbruik

Merk slegs **een** opsie met 'n kruisie teenoor elke monster kode:

		Monster Kode		
		MLQ	**VSH**	**PTN**
Ek sal dit baie dikwels drink (elke dag)	1			
Ek sal dit dikwels drink (2 – 3x / week)	2			
Ek sal dit af en toe drink (1x / week)	3			
Ek sal dit slegs drink indien geen ander drankies beskikbaar is nie.	4			
Ek sal dit nooit drink nie.	5			

4 Voorneme t.o.v aankope

Dui asseblief u voorneme aan om hierdie produk te koop. Kies slegs een opsie in elke kolom en dui dit met 'n kruisie aan:

Monster kode	**Sal beslis hierdie produk koop**	**Sal beslis nie hierdie produk koop nie**
MLQ		
VSH		
PTN		

Baie dankie vir u samewerking.

Mary-Jane Gore

APPENDIX 4

COMMENTS FROM PARTICIPANTS

1st Choice	Comments
VSH	MLQ had a sort of chemical taste that I did not like. I liked the other two, but liked the flavour of VSH more. Differences in texture and appearance did not play a role for me – only taste.
VSH	VSH & PTN are just as good – I had difficulty choosing an order between them. MLQ has a bit of a grassy taste.
PTN	PTN: full, 'round' flavour. VSH: in-between the others. MLQ: still like flavours – slightly sour.
VSH	VSH taste and texture is very good and good flavour. VSH taste and texture is very good and good flavour.
PTN	PTN taste is great – tastes like fruit juice. Had very nice aroma. Colour is also good.
PTN	PTN has best texture. VSH: after-taste.
~~MLQ~~ PTN	PTN has an 'apple' after-taste – can be sharp, but no bad. MLQ seems to taste 'easier' on palate. VSH: I don't like taste much – nearly like carrot and pear combination. *Added comment on last page*: PTN: the more I got used to this taste, it tasted better than my initial 'best' choice of MLQ; PTN would be my favourite.
MLQ	MLQ's taste is better, not so sharp. Also, MLQ's texture is better than PTN or VSH.
PTN	I like PTN's taste; MLQ & VSH have an slightly 'ground' after-taste. The texture of all three is acceptable.
PTN	PTN smells and tastes the nicest. MLQ doesn't taste or smell as nice as PTN. VSH doens't smell very nice.
PTN	PTN is better than the other two samples. VSH is really not nice, smells sour and tastes bad. MLQ is neutral – bit sour but sweeter than VSH.
PTN	The after-taste is not very nice, but PTN has a thick consistency which is nice.
VSH	I would really like it if you sold it at the University.
PTN	PTN has a nice, fresh & refreshing apple aroma and a slightly 'wild' taste.
PTN	MLQ has a bit of a sour taste that I like. But PTN is more of a taste that I would drink more frequently. VSH is too sweet.
VSH	MLQ has a strong (waaneembare) strange after-taste and aroma. PTN has a sour after-taste. VSH has an offensive after-taste.
PTN	PTN is tasty and refreshing. I didn't finish the samples – fruit juice makes me feel unwell, especially if I don't like it.
MLQ	I would be happy if this were sold at the University.
PTN	PTN has got a sour, fruity taste which I like very much. The only funny thing about all 3 juices is the tiny, white pieces in the juice. MLQ has got a blunt taste which I dislike. As for appearance and texture, they're all the same. VSH is reasonable ... a little bit of both I guess (blunt and sour/fruity). Good luck!

1st Choice	Comments
PTN	PTN: tastes nice. MLQ: bit too strong and too 'smelly'. VSH: too sweet and too 'smelly'.
PTN	(couldn't read this candidate's comments – 018)
MLQ	MLQ has a natural taste, which is delicate. PTN has more of a 'wild' taste, a stronger taste. VSH is even stronger.
MLQ	VSH and PTN have very much the same taste. MLQ has a better, more acceptable taste.
PTN	MLQ tastes like mint. VSH is really not nice.
PTN	I like the slightly sweet taste / aroma.
PTN	MLQ: slightly sweet/sour. VSH: slightly sour.
PTN	VSH has somewhat of a nice after-taste. Both MLQ and PTN have a slightly sweet taste, but MLQ is more 'strong' on the tongue than PTN. My personal favourite is PTN.
PTN	PTN was drinkable, the other two were really not very nice. Both MLQ and VSH are on the same 'level' – both were not nice.
MLQ	Appearance and texture are both not important criteria, but taste is definitely important, which is why I prefer MLQ.
PTN	PTN: more 'known' taste – not so sharp or sour. VSH: neutral-type taste, slightly (poeierige) texture. MLQ: sharp taste, sharp smell.
PTN	MLQ and VSH have a unfamiliar taste and a very sharp after-taste (almost burning or strong) on the tongue.
PTN	PTN: like this the most; like smell and taste; tastes like juice. MLQ: don't like smell or taste – tastes strange. VSH: doesn't taste 100% natural – artificial taste.
VSH	Has a 'grass' taste.
PTN	PTN has a nice aroma and also tastes nice.
PTN	PTN and VSH don't taste too bad. MLQ isn't very nice, especially the after-taste. PNT is the only one that smells ok.
PTN	MLQ has a very strange after-taste which I can't identify with fruit juice (mint). VSH is very sweet. PTN has a very strong apple taste – nice.
PTN	I like the apple flavour a lot. Can distinctly taste the apple flavour and it is not thick. MLQ's taste is unknown, with a sour after-taste and is a bit too thick for fruit juice. VSH's taste is unfamiliar – I can't associate it with a fruit and it is the thickest of the three. Fruit juice should be juice and not thick like (melkskommel).
VSH	Taste is important.
VSH	Smooth.
MLQ	MLQ has a nice, sweet taste which tastes like fruit juice. PTN is a bit sour, but not too bad – is acceptable. VSH is sour – a strange, unfamiliar taste.
MLQ	PTN is not a nice taste. MLQ is nice. VSH is acceptable.
PTN	VSH has a slightly pear taste – I don't like pear juice. MLQ is nice but has a slightly citric after-taste. PTN has almost a creamy taste, but not too (oordompelend) – really a special, nice taste.
PTN	MLQ is very sour. PTN is sweeter, a better taste, more acceptable. VSH is too

1st Choice	Comments
	sweet, horrible after-taste.
VSH	VSH has good taste and fresh after-taste / smell. MLQ also has good, fresh taste. PTN – did not like the after-taste. *Added comment on last page*: would purchase MLQ & VSH, although it would depend on the ingredients – I'd only buy it if it is unsweetened, unenriched and 100% pure juice.
PTN	The MLQ sample had a very distinct taste and smell that was not altogether pleasant. MLQ's texture was also not that pleasant. The VSH sample was less distinct (overpowering). PTN was more like the fruit juices I've drunk before.
PTN	The tastes of all three are ones which I am not used to. The PTN sample had an apple aroma which made it nicer.
PTN	PTN had a nice, fresh aroma (smell) and tasted like it smelt – not too sweet and didn't feel 'thick' in the mouth. VSH & MLQ had a strange aroma which I didn't like.
PTN	Had a strange taste, but PTN is acceptable.
PTN	It seems that the taste got sharper (from PTN to VSH).
PTN	MLQ smells a bit offensive and tastes 'wild'. VSH is a bit too sour. I couldn't drink a full glass of MLQ or VSH. PTN is very acceptable.
PTN	PTN smells and tastes like fruit juice. VSH and MLQ have a strange smell.
PTN	I liked all three, but MLQ had a taste which I couldn't really describe, slightly sour, but is something else as well. PTN is a little bit too sweet.
PTN	PTN has a sweet taste; has better texture as VSH.
MLQ	VSH has a very strong, 'negative' smell. PTN less.
VSH	VSH: no (bysmake), nice sour taste. MLQ: slightly 'herby' taste. PTN: slightly (frank) taste.
MLQ	Because it is very nice and strong.
PTN	PTN had a lovely taste. MLQ and VSH have a sharp smell which you smell first. Further, the tastes are not as nice as PTN.
PTN	VSH and MLQ have a slightly (afkeurende) after-taste, while PTN has a taste of apple juice, which I love. VSH and MLQ taste 'foreign', (maar ek raak die smaak gewoond).
MLQ	Has the most acceptable taste of the three samples – slightly softer on my palate.
VSH	PTN has a (frank) taste.
PTN	PTN – soft, apple taste.
PTN	MLQ tastes like medicine (as if it has gone off). VSH is better than MLQ. PTN is definitely the nicest.
MLQ	MLQ is more rich and it ? are more balanced.
PTN	PTN is smooth, odourless and tastier than the others. VSH can make people cough – I am experiencing that now.
VSH	The VSH sample is best for me; the taste is good.
PTN	PTN definitely tastes the best and also smells better than MLQ or VSH.
MLQ	PTN is also nice; has a slightly sour taste than MLQ. VSH did not have an (onderskeibare) taste – I would drink it, but feel that I can't identify it – tastes less

1st Choice	Comments
	'fresh', if I can use that word!
VSH	The taste of MLQ is unacceptable – definitely not nice. PTN is slightly tasteless. VSH is acceptable, but not one of the three juices is really nice.
PTN	Taste is acceptable; texture is smooth enough – not too (krummelig). Appearance is normal.
PTN	Don't like the (lemmetjie) taste in MLQ. VSH is too sour. PTN is sweeter than the other two.
VSH	I don't like the strong, 'mint' taste in MLQ. PTN has a very nice taste, but VSH has a new, unique and unfamiliar taste. VSH is one of the tastiest juices that I've ever tried.
PTN	PTN's aroma and taste is nice. MLQ and VSH had a horrible after-taste.
PTN	Taste is nice.
MLQ	PTN had the nicest texture. MLQ had the nicest taste.
MLQ	For appearance and texture, all three samples are very similar. For taste, MLQ is by far the nicest. PTN tastes a bit funny.
PTN	VSH tastes slightly sour. MLQ is neutral. PTN is acceptable / sweet – nice! Drinks have a funny smell.s
PTN	VSH tastes like peanuts, or peanut oil has been added. MLQ is very nice, little too sweet. PTN has nice texture, not too sweet.
PTN	I based my choices totally on the taste of the juices.
PTN	It tastes a lot like the fruit juice I'm used to.
MLQ	MLQ's taste is more 'exotic' than the other two. The after-taste of MLQ is not as sour as the other two.
PTN	PTN has a sweeter taste, so it is more acceptable to me. MLQ is a bit less sweet but still acceptable and VSH has more bitter taste for me.
PTN	MLQ definitely has a medicine taste. VSH is very sweet. PTN's smell is the best of the three – nice and fruity.
PTN	MLQ and VSH taste the same for me. Texture, smell are all the same but PTN tastes very good for me – also like the citric smell of it.
PTN	PTN has a good balance between sweet and sour. VSH tastes like vegetables, not fruit juice. MLQ is something between PTN and VSH.s
PTN	PTN has a nice smell and taste; the texture is slightly (growwer) with more (vesel) bits. MLQ is totally too smooth and has a smell which puts me off.
PTN	PTN is slightly sweeter than the other two and therefore nicer.
VSH	VSH has a 'prickly' sour taste which MLQ and PTN do not, but in a sweeter way. VSH is also not as syrupy-thick in texture as the other two.
PTN	PTN tastes better and has a better texture.
MLQ	MLQ has a better taste. PTN is slightly (mellerig). VSH is (mellerig).
PTN	PTN has an (aanloklike) smell and a sour (prik).
PTN	PTN: appearance and texture is good; taste is known and outstanding. MLQ: appearance and texture is not too bad; smell is nice but unknown. VSH: taste is bad; appearance and texture are good. My overall (aanvaarbaarheid) is PTN is

1st Choice	Comments
	the best and VSH is not good.
PTN	Both VSH and MLQ have a smell which is unnatural. PTN has a nice, fruit-juice smell with a nice taste.
VSH	VSH has a nice taste which stays in your mouth – interesting taste. PTN has a taste which I can't really identify. I don't like MLQ.
PTN	PTN tastes sweeter than the others. I don't like the taste of MLQ.
PTN	Tasty.
PTN	PTN is sweet and nice, but MLQ has a very sour taste. My taste may have been influenced by the fact that I brushed my teeth before I came here. VSH is also too sour. VSH and MLQ have a strange, slightly nice after-taste.
MLQ	PTN has an unnatural after-taste and a sharp smell. VSH is nice, but has a slightly burny after-taste. MLQ smells nice and is a bit more fruity; but also has funny after-taste.
PTN	PTN's taste is more natural.
PTN	MLQ has an after-taste which is not nice.
VSH	The smell of the juice may have an influence on certain people, otherwise the product is standard and tastes like normal fruit juice. Very nice – it's different.
VSH	PTN tastes slightly sour. MLQ has a funny smell and is sweet. VSH tastes the most like natural fruit juice.
VSH	All the fruit juices have the same strange taste, but it is weakest in VSH.
PTN	I like the sour taste as well as the apple taste.
PTN	PTN is nice and sweet, while the others have a slightly sweet taste.
VSH	VSH: the smell is not as sharp; tastes like fruit juice – has better fruit smell. PTN: the taste is too sweet – tastes like it was made too strong. MLQ: smell is too overwhelming; sour taste.
MLQ	VSH tastes half bitter. PTN is slightly sweeter without the bitterness. MLQ tastes like a nice combination.
PTN	VSH smells terrible. PTN smells fresh and a lot of apple. All the juices have too many 'strange bits' in.
PTN	VSH does not smell nice to me. MLQ tastes too much of mint.
PTN	PTN tastes very nice – fresh apple taste; oh, yes, and it also smells nice – yum! VSH has an interesting taste, slightly vanilla which is ok. MLQ made me think of toothpaste – yuk!
PTN	PTN is very tasty. MLQ is also nice. But VSH is really not nice for me – it's very sweet.
PTN	PTN has a very nice taste and the texture is also good. In comparison with the other two, I would choose PTN over the others.
VSH	MLQ tastes strange, almost (meulerig). VSH and PTN taste better – nicer to drink.
PTN	MLQ has a horrible after-taste – makes me think of ?. VSH also has a bad after-taste – very bitter. PTN was semi-? – the taste of apples came through strongly. PS: the water was very nice!

1st Choice	Comments
MLQ	It's really bad, it looks horrible, it smells bad.
PTN	The smell of VSH puts me off. MLQ is ok but I would never buy it. PTN has the best sweet taste and smell.
PTN	PTN: very nice juice. VSH: it is sweet. MLQ: doesn't taste nice.
VSH	MLQ has a very sour taste. VSH is (vleiend) for tastebuds. PTN is nice, but a little bit too sour.
PTN	PTN has a better taste. You can taste that all three are healthy, but VSH and MLQ taste too healthy! VSH and MLQ did not have a very nice taste.
MLQ	VSH is too sweet for me, but MLQ's taste is very nice.
MLQ	VSH tastes too much like medicine – the juice is acceptable as a health remedy, but definitely not as a nice fruit juice. Anyway, better than most medicines and other health remedies.
PTN	PTN and MLQ are both nice, but the slightly sour after-taste of PTN is nicer for me and therefore my favourite.
PTN	MLQ has a very strong taste, which works your tongue. VSH is very thick juice. But all the juices are really nice ("flippen lekker!").
PTN	PTN has a nice taste and the after-taste is not bad.
PTN	PTN has the nicest taste. I don't like the taste of MLQ at all. All the textures are the same for me, so that didn't influence my choices. PTN is just the nicest.
PTN	MLQ has a 'mint' taste which I didn't like. PTN has a 'pear' taste which, for me, is a good combination.
PTN	PTN has a nicer fruit taste. The aroma of the fruit comes through stronger than the other two.
PTN	PTN has a nicer taste. The other two taste pretty much the same – VSH is more sharp and more interesting than MLQ. The texture of PTN is better, and there were less bits left behind in the glass; it is also not too thick.
PTN	MLQ and VSH had a sour after-taste.
PTN	MLQ had a bad, 'mealy' taste. PTN and VSH both tasted nice – the tastes actually don't differ much.
PTN	MLQ had a vinegar taste. The rest of the juices are really nice.
MLQ	Is nicer.
PTN	All three are acceptable. The sweet-sour taste makes it special. The difference in tastes between the samples is small. PTN tastes the most like a fruit juice (or at least fruit juices that I am used to). The grainy texture is less acceptable.
MLQ	MLQ: has a clean taste of 'mint'. VSH: is sweet and has a taste of 'caramel'. PTN: tastes like home-made fruit juice.
VSH	The VSH sample has a nicer taste than the others.
PTN	PTN's tastes is slightly better than the other two samples.
PTN	PTN and VSH are very similar, just a bit of a texture difference. I prefer a smooth texture above little pieces of fruit. MLQ had a very sour after-taste.
VSH	MLQ's taste was too sour, but VSH had an acceptable taste.
PTN	VSH's texture is nice but it has a very strange taste – I wouldn't recommend it.

1st Choice	Comments
	PTN's taste comes through much better and is not such a strange taste.
PTN	PTN has a special taste. MLQ is very nice. VSH: the smell put me off a bit.
PTN	I must say that the taste is something else – PTN is a more acceptable taste. VSH is slightly sour tasting. MLQ tastes minty to me. All in all, it is acceptable and something new to try. Tastes also more (suiwer).
PTN	PTN has a lovely taste. VSH is acceptable for an 'every day' fruit juice. MLQ is a bit bitter.
PTN	PTN has a very nice taste. VSH had too many bits in. MLQ has an after-taste.