PINEAPPLE DIETARY FIBRE

AND

THE EFFECTS OF PROCESSING

ON ITS

FUNCTIONAL PROPERTIES

by

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by

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SUMMARY

The waste streams from the pineapple canning industry are a valuable source of carbohydrates, in particular dietary fibre (DF) and neutral sugars. In addition, the waste is a suitable source of antioxidants, enzymes such as bromelin and flavours. Since this waste (core and peel) is currently sold at minimal cost to cattle farmers for use as cattle feed or compost, alternative uses of the material with commercial potential was investigated.

Biochemical profiling of the raw waste from South African grown Smooth Cayenne species (*Ananas comosus)* of pineapple confirmed that it contains high levels of fibre with the core containing relatively more soluble fibre than the peel. Unlike other sources of fibre, such as those derived from cereals, pineapple DF is associated with valuable antioxidants and flavour compounds, vitamins and minerals as well as the enzyme bromelin. High levels of general antioxidant activity have been recorded for both the core and peel fractions. Work done to identify the individual active components such as phenols and vitamins C and E has shown that the phenolic compounds are most prevalent, and are likely to be responsible for the high antioxidant activity in the pineapple material.

Micro-elements present include sodium, calcium, magnesium, iron, phosphorus and zinc and a 100 g portion of the dried fibre will provide from 10% to 50% of the recommended daily allowance (RDA) for the various microelements. In addition, it was found that certain growing conditions result in favourable low glycaemic index values

that correlates to the sugar profile of the soluble DF portion that comprises mostly glucose, galactose, xylose and arabinose. In the pineapple fibre production process, a washing step is required for the removal of the soluble neutral sugars to prevent the discoloration of the final DF product; it also reduces the microbial load and increases the total DF content of the final product. A high temperature drying process is also necessary for the production of a microbiologically stable DF product.

An evaluation of thermal, mechanical and chemical modified processes showed that low temperature drying could effectively retain the bioactives but was ineffective in controlling the microbial growth. It was also found that the alkali peroxide treatment improved the water binding capacity (WBC) of the DF. The WBC was also influenced by the particle size of the DF and the decrease in particle size decreased the WBC of the DF. Further modification may be required to produce a pineapple DF that can be commercially competitive.

The aim of this project was to develop a DF product, targeting the food and health-care markets; and to assess the carbohydrate content and structure of the product in relation to its chemical and physical reaction to various processing treatments. The pineapple waste contains a suitable DF profile to make this a viable market opportunity.

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ABBREVIATIONS

AOAC:	American Association of Analytical Chemists
AODF:	Antioxidant Dietary Fibre
BCR:	Bureau Communautaure de reference
CHO:	Carbohydrates
CODEX ALIMENTARIUS	Food Codes
CCK:	Cholescytokinin
CFU/G:	Colony Forming Units per gram
DMSO:	Dimethylsulfoxide
DF:	Dietary Fibre
EPIC:	European Programme Investigation on Cancer
EPS:	Exopolysaccharides
FDA:	Food and Drug Administration
FOS:	Fructo-oligosaccharides
FF:	Functional Fibre
FID:	Flame Ionization Detector
FTC:	Ferric thiocynate
GAGS:	Glycosaminoglycans
GLC:	Gas Liquid Chromatography
GIT:	Gastrointestinal tract
GC:	Gas Chromatography
g:	Grams
HDL:	High Density Lipoprotein
HPLC:	High Performance Liquid Chromatography
HPAEC:	High Performance Anion Exchange Chromatography
HPSEC:	High Performance Size Exclusion Chromatography
IDF:	Insoluble Dietary Fibre
kg:	Kilogram
LAB:	Lactobacteria and Bifidiobacterial species
LDL:	Low Density Lipoprotein
MALLS:	Multi-angle laser light scattering
mg/ℓ:	Milligrams per litre
mg/kg:	Milligrams per kilogram
mℓ :	Millilitre
NaOH:	Sodium hydroxide
nm:	Nanometers
NEC:	Necrotising Enterocolitus
NDO:	Non-digestible Oligosaccharides
NIDDM:	Non-insulin Dependent Diabetes Mellitus
NSP:	Non-starch Polysaccharides
NFU/100 g:	National Fine Units per 100 g
PSIG:	Pressure KPa
SCFA:	Short Chain Fatty Acids
SDF:	Soluble Dietary Fibre
TCA:	Trichloroacetic acid

TIM:	TNO Intestinal Model
TDF:	Total Dietary Fibre
TFA:	Trifluoroacetic acid
TMA:	Total Microbial Assay
TNO:	Technology Nutritional Organisation
US:	United States
UV:	Ultraviolet
WHC:	Water Holding Capacity
WBC:	Water Binding Capacity
WHO:	World Health Organisation

CHAPTER 1

1. INTRODUCTION

1.1. Background to the Pineapple Canneries

Pineapples are grown throughout the year in the Eastern Cape Province of South Africa. Of the two varieties found in the Eastern Cape Province, the smooth leaf Cayenne and the Queen, the Cayenne is the larger fruit and is the only variety suitable for canning. The canneries receive the pineapples from 50 farms throughout the Eastern Cape Province, from Komga to Alexandria. Summerpride processes about 100 000 tons of fruit a year, while Collondale pineapple factory, which is only a third of the size of Summerpride cannery, processes up to 30 000 tons a year.

Pineapple plants grow from the beginning of February to the end of November, producing 3 cycles of fruit during its lifespan of three years (Hugo, 2001a). The size and the sweetness of the fruit are dependent on the number of reproductive cycles the plant has experienced. The first harvest produces the biggest and least sweet fruit, while the third cycle produces the smallest, but sweetest fruit (Hugo and Tinley, 2001).

During the 1980`s five canneries in the Eastern Cape Province received 200 000 tons of fresh pineapples annually from the farmers in this region. From 1983 to 1993 the South East Asian pineapple producers increased their output threefold, thereby flooding the world market, which resulted in a depression in prices and in the industry in general. The added pressure from sanctions and the fact that the compulsory purchase by government of large areas of pineapple growing lands for incorporation into the old

Ciskei homeland, contributed to the collapse of the pineapple industry in the Eastern Cape Province.

1.1.1. Constraints on World Competitiveness

The South African pineapple industry is not affected by inhibiting factors such as variety type, soil quality or pests and diseases that can have a negative impact on the global market.

Smooth Cayenne, which is the internationally accepted cultivar, is the only one grown in South Africa for canning. South African farmers experience fewer pest and disease problems than in other countries.

The major constraint on South African competitiveness on the world market is the fact that the industry is located further south than any other major pineapple producing country and the pineapples are grown at lower air and soil temperatures which results in altered sugar profiles.

1.1.2. Pineapple Farms

- *Climatic Conditions*

A temperate climate with a high humidity enhances the sugar content in the fruit. This is evident in the pineapples grown in Thailand and the Philippines, which are two of the world's leading pineapple-producing countries, as they have a comparably sweeter fruit than that grown in South Africa. The optimum temperature for pineapple growth is between 20°C and 28°C with an average of 24°C. For the Bathurst region in the Eastern

Cape Province, the average monthly minimum temperature ranges from 10°C in July to 17°C in February. The average minimum temperature is only above 15°C in two months of the year (Hugo 2001b).

- *Improvement Process for the Soil and the Fruit*

After the pineapple plant has completed its third cycle of fruit production it is comminuted and ploughed back into the soil. This process aids in the soil fertilisation. The soil is also treated with nitrogen based fertilisers to provide the necessary supplements for growth.

A hormone-based chemical called *Ethrel* controls the growth of the fruit. This hormone causes the crop to ripen at an even pace, which makes the harvesting process less labour intensive and cheaper. *Ethrel* is sprayed onto the crowns (normally *via* aeroplanes) when they are first planted, and this should result in the plant producing its first fruit 18 months later. Once the fruit is picked, the plant is sprayed with *Ethrel* for the second time and nine months later the second fruit is ready for picking. After the picking process is complete, the plant is sprayed for the third time so that nine months later the third cycle of fruit is ready for picking. As mentioned earlier, there is a decrease in size and an increase in sugar content of the fruit as the number of crop cycles increases from the first to the third cycle. There are legal constraints to the maximum residual amounts of hormone found in the fruit. These are 1.0 part per million in South Africa and 0.05 parts per million in Europe. The sprays also contain pesticides and chemicals that protect the plant from various diseases (Hugo and Tinley, 2001).

1.1.3. Pineapple Fruit Waste

The East London pineapple canning factories produce in excess of 60 000 tons of waste per annum. This consists of the outer pineapple skin, the centre core, and trimmings and off-cuts obtained during the sorting process. The waste is pressed to extract as much juice as possible. Filtering of the juice results in production of waste sludge, which, together with press cake, forms the final waste material. The waste material contains very small amounts of juice since most of it is removed during the pressing process in the canneries. An efficient pressing process will remove between 75 and 85% of the juice, which is 5.0 to 8.7% of the tonnage that enters the cannery. Dry presscake results in reduced transportation and storage costs.

The waste is commonly used as cattle feed and can be ensilaged, used fresh or as dehydrated pineapple material. The silage making process entails loading the fruit waste into a silo and leaving it there for a week or two without adding any preservatives. The advantage of pineapple silage is that it can be made throughout the year and it has a pleasant smell, making it suitable for cattle feed (Dalldorf 1996).

However, the cattle feed is not a financially viable option because the profit made barely covers the transportation cost of the waste material to the farms. Therefore other applications of the press cake need to be explored in order to make profitable use of this waste material. The press cake can be returned back into the soil as a green fertiliser or as compost. Despite the fact that it is not very rich, it does help to improve soil fertility, but profitability also depends on the transportation costs.

1.1.4. Cannery Production Process

The Canneries receive their pineapples from the local farms in the Eastern Cape Province. The pineapples entering the processing line in the canneries are peeled, sliced into rings, cooked in a can, and then packaged for distribution to the market.

Approximately a third of the fruit entering the cannery leaves as waste material (Table 1.1).

Table 1.1: Pineapple Waste Produced in the Canneries

MATERIAL	MASS
Fresh pineapple	1 ton
Waste Material	353 kg
Peel **Cores/Trimmings** **Sludge**	298 kg 18 kg 37 kg
Canned Product	166 to 199 kg
Juice at 60° Brix of canning fruit	35 kg
Juice at 60°Brix of total fruit	75 to 85 kg

This waste material consists largely of plant cell walls and therefore contains a high amount of dietary fibre (DF) that consists of cellulose and hemicellulosic components as well as lignin. These components will be described in more detail in the following section.

1.2. Some Molecular Structural Components of Pineapple Waste

Plant cell walls contain many different types of non-starch polysaccharides (NSP) in the primary or secondary cell walls. The polymers are linked together by covalent or non-covalent linkages or *via* non-carbohydrate compounds such as phenolic acids, lignin or protein (Dusterhoft 1993).

Total food carbohydrates consist of available and unavailable carbohydrates. The former comprises digestible sugars, dextrins and starches whereas the unavailable carbohydrate consists of indigestible polyols, oligosaccharides, resistant starch, cellulose, hemicellulose, gums, pectins and lignin (Dusterhoft 1993).

1.2.1. Free Sugars

Sugars are white crystalline carbohydrates that are soluble in water. Their monosaccharide classifications are based on the number of carbons in the structure. Glyceraldehydes are the simplest with three carbon atoms while glucose the most common sugar in the plant and animal kingdom is more complicated with six carbons in its structure. The monosaccharide structures differ in orientation of the hydroxyl groups. Slight changes in the structure can influence the biochemical properties and the physical properties of this sugar. The simple sugars can exist in a chain form or a ring form, the ring form is favoured in aqueous solutions, and they can form a five member ring called furanose, or they can form a six-membered ring called pyranose (Greenwood and Munro 1979).

1.2.2. Polysaccharides

Polysaccharides are divided into three groups termed: reserve, algal and structural polysaccharides (Dusterhoft 1993).

Reserve Polysaccharides

Consist mostly of starch that comprises of α-(1→4) linked amylose or α-(1→4) and α-(1→6) branched polymer amylopectin.

- *Oligofructan and Inulin*

Fructans are not structural polysaccharides. They consist of one or more fructose linkages and are generally classified as inulin. Inulin consists of linear chains of fructosyl units linked with a glucosyl unit. These molecules resist hydrolysis by human digestive enzymes but are fermented in the large intestine.

Recent research has shown an important physiological action for inulin, as inulin is a preferred food for the lactobacilli in the intestine and can improve the balance of "friendly" bacteria in the bowel (Hidaka *et al.* 2001). Inulin is sometimes recommended for diabetics because it is not absorbed and does not affect the blood sugar levels. It is also used as a bulking agent in starchy foods.

- *β-glucan*

Lipids, protein and low molecular weight material that do not precipitate in 80% ethanol are components that are not generally considered as DF. β-glucan, commonly found in cereals such as barley and oats, is a soluble DF component that has gained much attention over the past few years in connection with its association with the alleviation of coronary heart disease. The Food and Drug Administration (FDA) approved a health claim that oat products in a daily diet could reduce the risk of coronary heart disease. However, this claim is valid only if more than 3 g of β-glucans are consumed (Jenkins 2003). (Wood 2001) could not establish the mechanism responsible for the cholesterol lowering effect but suggests that viscosity plays an important role.

- *Resistant Starch*

Resistant starch is another non structural polysaccharide described as starch molecules that resist digestion in the small intestine and therefore pass through the small intestine and are available for fermentation in the large intestine. Resistant starch can be determined in conjunction with the total DF determination as the sample is hydrolysed with starch-degrading enzymes. The non-resistant starches are removed and the residue is hydrolysed and quantified for resistant starch (McCleary and Monaghan 2001).

Structural Polysaccharides

These consist of the following wide range of different polysaccharides:

- *Cellulose*

Cellulose consists of the β-(1→4)-D-glucopyranosyl units, which are joined by intra molecular bonding to form two anti-parallel chains along an axis. Multiple chains can be formed through intermolecular hydrogen bonding to form microfibrils of cellulose that can occur in a variety of conformations. The proportion of amorphous and crystalline regions may vary depending on their origin and tissue type (Dusterhoft 1993). Cellulose is found in plants as microfibrils (2-20 nm diameter and 100-40 000 nm long). These form the structurally strong framework in the cell walls its structure is illustrated in Figure 1.1.

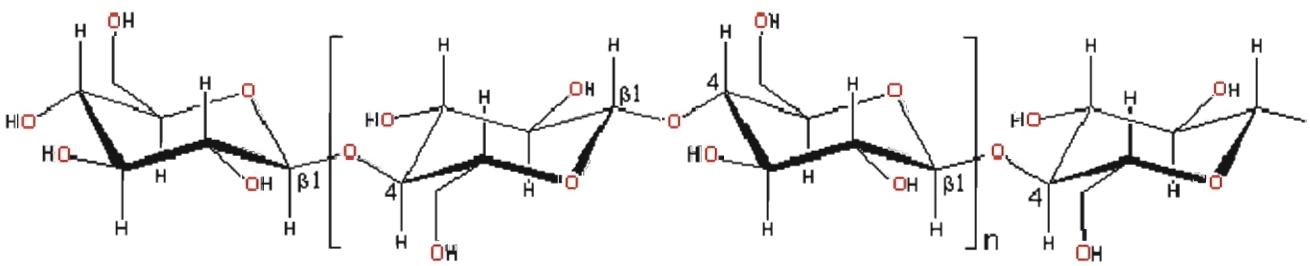


Figure 1.1: Structural Unit of Cellulose

The overall structure is of aggregated particles that have extensive pores capable of holding relatively large amounts of water by capillary action (Dusterhoft 1993).

Function and Functionality

Cellulose has many applied uses: as an anti-cake agent, emulsifier, stabilizer, dispersing agent, thickener, and gelling agent but these are generally subsidiary to its most important function of water retention. Dry amorphous cellulose absorbs water, becoming soft and flexible. Less water is bound by direct hydrogen bonding if the cellulose has high crystallinity but some fibrous cellulose products can hold on to a considerable amount of water in the pores and cavities. Cellulose can give improved volume and texture particularly as a fat replacer in sauces and dressings but its insolubility means that products will be cloudy. About a third of the world's production of purified cellulose is used as the base material for a number of water-soluble derivatives with pre-designed and wide-ranging properties dependent on groups involved and the degree of derivatization (Delmer 1983).

- *Hemicellulose*

Hemicelluloses comprise a large group of short chain partially soluble polymers consisting of xylosyl-, glucosyl-, galactosyl-, arabinosyl- or mannosyl-residues (Dusterhoft 1993). They are soluble in dilute alkali and bind to cellulose by multiple hydrogen bonds and to lignin by means of covalent bonds. They comprise about 20-30% of all plant cell walls and some of the hemicelluloses are described below.

- *Xylans*

These polymers all consist of a common backbone of β-(1→4) linked xylopyranosyl residues.

- *Mannans*

This consists of a group of polymers composed of β-(1→4) linked manopyranosyl residues. Gluco-mannans can have both glucosyl and mannosyl residues. In galacto- and gluco-mannans, single unit side chains of α-D–galacto-pyranose are attached to the mannosyl backbone units. The increasing attachment of pyranose units to the backbone is associated with increased solubility of these polysaccharides (Dusterhoft 1993).

- *Xyloglucans*

Xyloglucans are hemicelluloses and are therefore not soluble in water but in aqueous alkali (e.g. 1 and 4M KOH). Other hemicellulosic polysaccharides include xylan, glucuronoxylan, arabinoxylan, mannan, glucomannan and galactoglucomannan. Xylo-glucans consist of β-(1→4) linked glucopyranosyl residues (Dusterhoft 1993).

- *Mixed Linked β-glucans*

These consist of glucan β-(1→3) and β-(1→4) linkages in ratios of 1:2 or 1:3 in which continuous β-(1→4) linked pyranosyl units are interrupted by β-(1→3) linkages. This results in polymers having a less ordered structure that exhibits greater water solubility than cellulose.

- *Pectic Polysaccharides*

Pectins are complexed carbohydrates that play an important functional role in plant cell walls. The three major pectins normally found are homogalacturonan, rhamnogalacuronans and substituted galacturonans. Pectin comprises of several different neutral and acidic polysaccharides that consist of rhamnogalacturonans chains of α-(1→4) linked galacturonosyl residues. They have varying degrees of D-galactosyl, L-arabinosyl, or L-rhamnosyl residues and are predominant in the middle lamella, the layer between neighbouring cells.

1.2.3. Non-Carbohydrate Components

- *Lignin*

Lignins are complexed networks of aromatic compounds composed of polymers of three phenylpropanoid alcohols: coniferyl, sinapyl and p-coumyl alcohol. Ester-ester bonds and carbon-carbon bonds link these units together thereby producing a very hydrophobic polymer.

There are a number of monomers of lignin and this depends on the source in nature. These structures are often associated with cellulose and other structures within the cell wall. Lignin, together with hemicelluloses, acts as cement to exploit the strength of cellulose while conferring flexibility (Dusterhoft 1993).

Figure 1.2 shows the structures of lignin constituents.

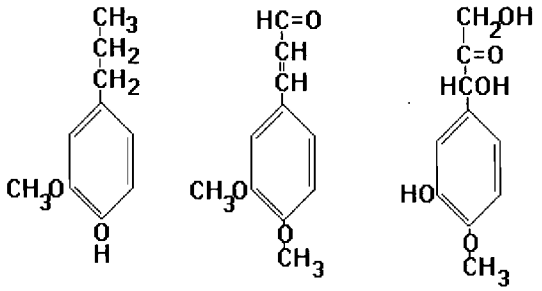


Figure 1.2: Molecular Constituents of Klason lignin

The aliphatic or the aromatic hydroxyl groups can react with each other or with aldehyde or ketone groups to form ether linkages. These large lignin molecules fill three dimensions and are heavily cross-linked.

- *Extensins*

Extensins are found in all plant cell walls. They are hydroxyproline-rich glycoproteins that form major structural wall proteins. Furanosyl and galactopyranosyl residues can attach to the hydroxyproline and serine residues (Dusterhoft 1993). Extensins are essential and integral components of the macromolecular cell wall. They seem to have an influence on the wall's flexibility. Hydrogen bonds between the molecules of the different classes help stabilize the cell wall.

The polysaccharides associate by non-covalent interactions in solution to form cell wall polymers that can vary in rigidity and porosity.

Sims et al. (1988) have shown that polymers can form the following covalent linkages between different cell wall polymers:

- *Phenolic cross linkages that join polysaccharide chains;*
- *Polysaccharides that bind to lignin via ether or ester linkages of uronosyl residues; and*
- *Protein-polysaccharide linkages and lignin-protein linkages.*

DF comprises mostly of plant cell wall components and associated non-starch polysaccharides. Most of these components were briefly described in section 1.2.2. Although DF has been recognized as an essential food ingredient to aid in the prevention of certain health disorders, the issues of its definition and measurement remain undecided. This is discussed in the next section.

1.3. DF

Pineapples consist mostly of insoluble dietary fibre (IDF) (Larrauri *et al.* 1997). This highly insoluble DF of pineapple is an important factor for a potential DF product as it plays a significant role in the absorption of glucose, glucose diffusion and the release of glucose from starch through the inhibition of α-amylase activity. The insoluble DF retards the utilisation and absorption of carbohydrates and helps control the postprandial serum glucose level. However, the effectiveness is dependent upon the source, preparation and composition of the IDF fractions - alcohol insoluble solid (AIS) and water insoluble solid (WIS). Chau *et al.* (2003) have explained that this mechanism is either through blocking the glucose molecules or through the entrapment of the glucose through DF. In addition, IDF also has a superior glucose retardation index to soluble dietary fibre (SDF) as the IDF is capable of reducing the α-amylase activity.

Even though high DF content is associated with several health benefits, it is also important to consider the mineral absorption capacities of DF as this could impact negatively on the DF product. DF can also be associated with reduced bioavailability of nutritive minerals such as calcium, iron and zinc (Jenkins 2003). This property is often associated with the SDF components such as pectin and oligofructans rather than the IDF component. The DF profile shows that the peel and the core consist predominantly of IDF and that the pineapple SDF component is low as it comprises less than 10% of the total DF component. SDF components with high-esterified pectin and oligofructose levels would be able to bind to certain metals and prevent its bioavailability (Caers 2003).

Fruit DF sources with associated bioactives are linked to the amelioration of cardiovascular diseases and cancer which is a useful property associated with DF. Most DFs are cereal based (Saura-Calixto 2003), even though fruit fibres are generally better than cereal fibres due to their associated bioactive compounds and the well balanced composition (IDF: SDF ratio).

1.3.1. DF Definition Methodology and Interpretation

Over the last 25 years a number of methods have been developed for the determination of DF (Asp 2003). The principle of these methods is to mimic the digestion process in the small intestine by removing the digestible portion of the food using enzymes. The indigestible portion is left and is designated as the fibre content.

In 1967, van Soest and Wine introduced the method for measurement of "neutral detergent fibre" (so called due to the methodology used) also referred to as crude fibre (Asp 1996). Englyst and Cummings (1986) defined DF as the non-starch polysaccharides, but this definition was later expanded by Asp (1996) to include phenolic polymers, enzymatic resistant proteins and proanthocyanidin polymers.

In 1985, Trowell *et al.* described DF as the remnants of edible plant cell polysaccharides, lignin and associated substances resistant to digestion by the enzymes in humans. It included material such as celluloses, hemicelluloses, oligosaccharides, pectins and gums. The method developed by Asp (1995) was accepted by the AOAC 1995 as the official method for analysis in 1995 (Reference No 985.29). The CODEX

Committee of WHO/FAO also adopted this method as the official method of the determination of DF for nutritional labelling of foods by the US and the US Food and Drug Administration (FDA) and US Department of Agriculture (USDA). Prosky *et al.* (1994) initiated further developments into the methodology, by introducing the measurement of soluble and insoluble DF. They found that the accuracy of this method was subject to various parameters and the distinctions between the soluble and the insoluble portion depended largely on the solubility of the DF in a pH-controlled environment.

The "gold standard method" for the measurement of DF is AOAC method 985.29 (AOAC 1995) (McCleary 2003). This method has been modified to allow for the measurement of soluble and insoluble DF.

One of the main concerns regarding the DF measurement is the relationship between measured values and digestibility and the fact that the absorption of nutrients is dependent upon various factors and conditions in the gastrointestinal tract (GIT) after ingestion.

The current AOAC method that determines the insoluble and soluble DF measurement has not been effective in the determining the relationship between GIT effects of fibre and metabolic activities. The characteristics of fibre that affect the GIT function include viscosity, water-binding capacity (WBC), bulk and binding of bile acids and fermentability. According to Carr *et al.* (2003), the understanding of these physical characteristics is useful in predicting the physiological response to new sources of DF.

McCleary and Monaghan (2001) revealed that certain components of DF, fructo-oligosaccharides and resistant starch, were not hydrolysed by the enzymes nor were they soluble in 78% ethanol. In the current AOAC method (991.43) these components are measured as indigestible DF. However, in the human body these components are broken down by the micro-organisms of the lower intestine under fermentative processes that produce short chain fatty acids such as propionic, butyric and acetic acids. Over the last decade these acids have been associated with a variety of health benefits. This finding has urged scientists to re-evaluate the AOAC 985.29 DF method and to develop AOAC methods for the measurement of these components.

McCleary and Monaghan (2001) have developed specific methods for the measurement of β-glucans (AOAC 995.16), fructans (AOAC Method 999.03) and resistant starch (AOAC Method 2002.2). The new resistant starch method was published in 2005.

However, quantification of DF by the AOAC method does not take into account the microbial fermentative processes that take place in the intestine. Instead, these components are recorded as either insoluble or soluble DF thereby elevating the actual DF measurement. Presently there is no consensus as to which components of carbohydrate should be included in the definition of DF (Asp 2003). Some include non-starch polysaccharides and resistant starch, whilst others suggest that non-starch oligosaccharide should be included in the definition. In general, the agreement that non-starch polysaccharides are the principle part of DF persists, but it remains undecided as to whether the other components will be included in the definition. Chemical analysts and nutritionalists do not share the same views regarding the measurement of DF. From

a nutritionalist perspective individual components that comprise DF need to be fractionated and measured. In addition, the types of glycosidic bond between polymers need to be identified as this determines the digestibility status of the polymer. According to the analytical definition, DFs are substances that completely or partially resist hydrolysis by digestive enzymes, are not absorbed in the small intestine, and reach the colon.

1.4. DF Components

DF is considered a food carbohydrate which is usually defined according to its degree of polymerisation into three main classes:

- *Sugars: subdivided into monosaccharide, disaccharides and polyols;*
- *Oligosaccharides: subdivided into malto-oligosaccharides and other oligosaccharides*
- *Polysaccharides i.e. starch and non-starch polysaccharides.*

Polysaccharides are described as having ten or more monomeric residues. Sugars are often defined as monosaccharides and disaccharides. Glucose, fructose and the disaccharides sucrose and lactose, are important sugars in diets. The polysaccharides are usually divided into the starches, which are linear (amylose) or branched (amylopectin) homopolymers of glucose and non-starch polysaccharides. Cellulose is a linear β-glucan, and hemicellulose, pectin and hydrocolloids include a range of heteropolysaccharides with a variable degree of polymerisation, branching and monomeric composition. The monomeric composition is the basis for the classification of these NSP.

1.5. Associated Health Benefits

DF is generally accepted as having protective effects against a range of diseases predominant in developed western countries including colorectal cancer, coronary heart disease, diabetes, obesity, and diverticular disease. However, many of these diseases are becoming more prominent in Africa and developing countries throughout the world. This means that alternative sources of DF, particular from fruit waste streams, may be added to staple foods in the future.

McIntosh's (2003) biological definition of DF, which has been accepted by the American Association of Cereal Chemists, states: "*DF is the edible portion of plants and analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. The fibre includes polysaccharides, oligosaccharides, lignin and associated plant substances, and effects laxation, blood cholesterol attenuation and glucose attenuation, or one or more of these.*" The three types of non-digestible carbohydrates include NSP, resistant starch and oligofructans, such as inulin from chicory, which have a degree of polymerization of less than 50.

A literature survey revealed that the major sources of carbohydrate and DF in the human diet are (in descending order): cereals, root crops, sugar crops, vegetables, fruits and milk products. Trends over the last 20-30 years have shown a growth in the production of cereals, sugar cane, vegetables and fruit, and a decrease in root crop

production in countries such as Asia and Europe. This suggests a change in food preference away from roots towards cereals, fruit and vegetables. Roots are an excellent source of carbohydrates and there is a growing concern regarding the decline in their cultivation. It is uncertain whether current carbohydrate production will suffice for the world's population in the future. The projections for future growth in Africa and developing countries of the world seem bleak unless efficient waste beneficiation programmes are developed. A solution could be the use of fruit and vegetable waste streams to produce suitable products rich in carbohydrates that could be recycled back into the food chain. These products will often have bioactive components associated with the high DF component (Miller-Jones 2003).

A review of the health benefits associated with high DF consumption revealed that it could be of use in the prevention of several life threatening diseases. These health benefits are described in detail in the following sections.

1.5.1. Epidemiological Studies

Governments and health promotion organizations in various countries have recommended a DF intake of between 25-37 g/day. Over 50% of the American population fails to reach 20 g DF per day and 90% fails to reach 25 g of fibre per day (Miller-Jones 2003).

It was found that fibre consumption was affected by racial and ethnic eating patterns, and that people living in rural areas eat more traditional foods that are generally higher

in fibre than processed foods found in urban areas. Miller–Jones (2003) found that fibre consumption was generally higher in older but more physically active groups of the population than the younger and less active groups.

Socio-economic factors may also influence the fibre intake as the use of food prepared away from home has decreased amounts of fibre in the diet and increased caloric intake. Miller-Jones (2003) also found that groups selecting more fibre in their diets often exercise more frequently as well, and this makes interpretation of the epidemiological data difficult as their good health could be attributed to positive lifestyle choices rather than a high fibre diet.

Epidemiological studies have shown that ingestion of cereal fibres was inversely related to risk of type-II diabetes, and that coronary heart disease was reduced by all types of fibre, e.g. soluble, insoluble, cellulose, fruit and vegetable fibre. A Spanish study has shown that fruit fibre was more effective in reducing coronary heart disease than cereal fibres (Miller-Jones 2003).

An area of controversy is the role that fibre plays in prevention of cancers, particularly colon cancer. A recent epidemiological intervention study did not support earlier work indicating that diets high in DF could reduce colon cancer. However, a study called European Prospective Investigation of Cancer (also referred to as the EPIC study) with over 519 000 persons in Europe showed that high fibre diets were associated with reduced colon cancer risk.

The role of DF in the prevention of certain diseases remains controversial, and whilst some studies suggest that DF diets reduce the risk of cancer, type-II diabetes and coronary heart disease, others prove that it is not effective.

1.5.2. Colon Cancer

According to McIntosh (2003), DF in foods provides a useful marker of the presence of some associated components potentially beneficial to health. Case studies have shown that insoluble DF from cereal grains contains components such as phytate, lignin, polyphenols, phytosterols and resorcinols that offer protection against colon cancers. As most of these data come from controlled animal experiments, there is a need for further investigation.

It is known that insoluble DF plays an important role in prevention of colon cancers, but the mechanism has not yet been identified. According to McIntosh (2003), there is a possibility that insoluble DF increases faecal bulking and binding of mutagenic toxic agents that cause cancers. In addition, the antioxidant components play a role in cancer prevention alongside butyrate, which is a product of microbial fermentation in the large intestine. This theory has been supported by a study that shows the reduction in the development of adenocarcinomas in the colon in several case studies with rats.

Studies by Rowland (2001) have shown that DNA damage in the cells of the mucosa is an early event in the process of carcinogenesis. Case studies comparing sucrose with lactulose diets (synthetic sugar) in rats that were injected with colon carcinogen DMH 1,2-dimethyl hydrazine hydrochloride showed that the DNA damage was less in

lactulose fed rats. A similar study comparing resistant starch and lactulose showed that the level of carcinogen induced damage was reduced by 50% with lactulose. This study also showed that non-digestible carbohydrates play an important role in protecting against DNA damage in the colon.

Further work by Rowland (2001) has shown that 10% inclusion of FOS or inulin in the diets of azomethan (carcinogen)-induced rats can decrease the development of aberrant crypts focus by between 25 and 35%. According to Bingham (2003) of the Medical Research Council (Dunn Human Nutrition) in the United Kingdom, recent intervention studies have shown that supplements of bran, soluble fibre or vegetables have not reduced colorectal cancer polyps in patients, and that mortality rates for colorectal cancers in vegetarians are similar to non-vegetarians.

The results of the EPIC study previously mentioned also indicated that DF consumption was inversely related to the incidence of bowel cancers. It was found that most protection against the cancers was in the group that consumed 35 g of total dietary fibre (TDF) per day, of which 12 g consisted of cereal fibre.

Whilst the past epidemiological, clinical and mechanistic studies have shown possible links between dietary habits and the preventative effect on colon cancer, the mechanism underlying this effect is unclear. Stierum (2003) has initiated research to integrate nutritional studies with genomic technologies to gain a better understanding of the potential preventative action of DF. Story and Kritchevsky (1976) identified possible mechanisms of action in the prevention of cancers and have suggested that fibres bind to the bile acids and bile salts that promote carcinogenesis.

Fermentation of fibre by the micro-organisms of the gut into short chains fatty acids (SCFA) such as propionate, butyrate and acetate play a vital role in the prevention of colon cancers. Several case studies have shown the relationship between the reduction of occurrence of colon cancers and SCFA. The study by Rowland (2001) showed that butyrate slows the growth human colorectal cell lines *in vitro* and it induces apoptosis.

- *The role of non-digestible carbohydrates in the prevention of cancer.*

McIntosh (2003) and Rowland (2001) showed that IDF can prevent carcinogenesis by increasing faecal bulk, binding to bile acids and delaying transit time of contact between carcinogens and gut mucosa.

According to Birkbeck (1999), NSP, especially resistant starch, increased faecal bulking and delayed transit time, FOS increased stool weight by 20%, whilst retrograded starch increased stool weight by 30%. The increase in stool weight was due to increased bacterial biomass. The non-digestible carbohydrates are fermented by micro-organisms in the large bowel, a process that affects the quality of bacteria and their metabolic activities in the colon. The micro-organisms metabolic activities include the formation of genotoxins, carcinogens and tumour promoters. Gibson *et al.* (1995) suggested that the non digestible oligosaccharides (NDO) and resistant starch are responsible for the increase in lactobacteria and bifidiobacterial species (LAB) in the gut and decrease the growth of pathogenic bacteria such as *Clostridia* and *Enterobacter.*

As LAB do not have these metabolic activities as compared to the bacteriods, *Eubacteria* and *Clostridia* (Schneeman 2001), the proliferation of the LAB species in the gut could reduce the toxicant producing enzymes. Studies by Rowland *et al.* (2001) have shown that soybean oligosaccharide and inulin can reduce activities of bacterial enzymes and formation of potential carcinogens in the gut. The metabolic activities associated with the colon carcinogenesis are ammonia concentration, β-glucuronidase activity and the formation of 7-OHIQ (genotoxic derivative of quinoline). These are used as indicators to evaluate the effectiveness of several oligosaccharides and a study of this kind that compared sucrose with resistant starch showed that resistant starch prompted the lowest activities associated with carcinogenesis.

Although many case studies have shown that fibres are generally able to prevent the occurrence of colon cancers there are some case studies that show that increased DF consumption does not play a role in the prevention of colon cancers. One of the reasons for this controversial result between population studies and case controlled studies is that epidemiologists focused on diets rich in plant foods, whilst the reductionist model of food scientists is based upon the extraction of DF from the diet. A diet rich in plant foods contains a large amount of possible anticarcinogenic phytochemicals which may augment fibre action.

1.5.3. Cardiovascular Disease

There are differences between the DF components of oats, bran, rice and barley bran, yet they all seem to have cholesterol-lowering properties (Kahlon *et al.* 1993).

Rowland (2001) investigated the effect that rolled oats bran has on the serum cholesterol levels in male and female rats. His study concluded that there was a 73% reduction in serum cholesterol with a 25% rolled oat diet compared to wheat starch. This is supported by the work of Wood (2001) who concluded that both the fat and non-fat fractions of oat bran had cholesterol-lowering ability. Studies by Rowland (2001) have also shown that the inclusion of oats in the diet can result in lowering the total cholesterol and low density lipoproteins (LDL) cholesterol.

The mechanism behind the DF cholesterol-lowering ability remains theoretical but work completed by Illman *et al.* (1991) suggests that the cholesterol-lowering components of oat bran are pentane-soluble and not related to tocopherols and tocotrienols.

Rice bran also has the ability to reduce the total cholesterol and LDL cholesterol in hyperchloesterolaemic subjects. Kahlon *et al.* (1993) conducted a series of tests demonstrating the effect of processing on the cholesterol-lowering properties of rice bran by comparing the effects of defatted rice, fat stabilized rice and par boiled rice bran. The result showed that fat-stabilized rice bran was more effective in lowering total cholesterol levels than defatted rice bran, that par-boiled rice bran was ineffective

in both total cholesterol and liver cholesterol reduction, and that the cholesterol-lowering ability was not due to β-glucans but was related to a mechanism that conjugates fibre with lipids and bile acids. Sharma and Rukmini (1987) have also shown a relationship between the unsaponifiable matter and cholesterol-lowering ability as unsaponifiable matter reduces the reabsorbtion and absorption of cholesterol fat and bile acids of the intestinal tract.

Psyllium husk, another DF that has cholesterol-lowering abilities, has been used in India for centuries as a digestive aid and laxative. It is extracted from *Plantago psyllium* and is composed primarily of heteroxylans with β-(1→3) and β-(1→4) linkages with short side chains containing arabinose, galactose and rhamnose.

More than 60 published studies have been conducted that explore psyllium's cholesterol-lowering properties and it has been noted that psyllium can reduce elevated cholesterol levels of 2.6% to 2.0%. Fulgoni (2001) assessed the effect of psyllium in the diet and this study showed that 10.2 g of psyllium per day significantly lowered LDL cholesterol. The cholesterol-lowering properties were independent of age or gender.

These data convinced the US Food and Drug Administration to approve a health claim for foods containing psyllium. Foods and supplements with at least 1.7 g of soluble fibre of psyllium per serving can claim that "Soluble fibre of psyllium as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease."

Lairon (2003) is currently conducting his research in lipid metabolism and its relation to cardiovascular risks. He suggests that recent epidemiological data show that whole grain intake reduces the risk of coronary heart disease (CHD).

The studied risk factors for CHD were plasma and LDL cholesterol, and numerous clinical trials suggest that soluble fibres have the greatest cholesterol and LDL cholesterol-lowering effects. The mechanisms of action are still under verification and remain theories at this stage. It is suggested that DF alters the secretion of pancreatic enzymes and reduce the activity of the gut lipases, which in turn reduce the absorption of fatty acids and cholesterol. DF also binds to the bile salts in the small intestine resulting in the excretion of fat, cholesterol and bile salts, and changes the lipid metabolism by altering the chylomicron secretion. His research has also shown that the bioactive compounds such as phytosterols can inhibit cholesterol absorption and could also play an active role in reducing the risk of CHD.

Jenkins (2003) suggested that introducing a combination of cholesterol-lowering foods in the diet might reduce the risk of CHD. Since the National Educational Programme of USA recommended 2 g/day of plant sterols and 10-25 g/day of viscous fibre in the diet to reduce the risk of CHD, the FDA has permitted health claims for CHD reduction in foods that deliver adequate amounts of plant sterols, viscous fibre such as β-glucans, psyllium and soya. In view of the possible mechanisms of action and the fact that each agent in acceptable doses may reduce the serum cholesterol by 5-10%, he assumed that their combined effects were likely to be additive and that in combination, a reduction in cholesterol could be achieved similar to the starting dose of lipid lowering drugs.

In another study by Zunft (2003) of the German Institute of Nutrition, it was shown that the insoluble DF of carob pods is capable of achieving the same cholesterol-lowering effects as viscous soluble fibres. The carob pods contain a high content of insoluble DF and polyphenols (tannins). In an eight-week pilot study using volunteers suffering from elevated total serum cholesterol levels, 15 g of carob preparation was given daily in conjunction with the normal diets. This was presented in the form of muesli bars, powdered drinks and a breakfast cereal. After the eight-week period there was a 12% reduction in cholesterol levels in the subjects. In a similar controlled blind study, a 10% reduction in the cholesterol levels was recorded.

One of the proposed mechanisms of this cholesterol-lowering effect involves the binding of the DF to cholesterol and bile acids in the intestinal lumen causing a decrease in the absorption of cholesterol and fat. It is also likely that the water-soluble polyphenols such as flavonols and glycosides are important in lowering cholesterol absorption.

Kahlon (2001) has shown that wheat bran appears to be protective against the development of cardiovascular disease. This is contrary to the Miller-Jones (2003) case study that showed that the fruit fibres are more beneficial than the cereal fibres. These studies have been complemented by work by Jenkins (2003) who has also shown correlations between low glycaemic diets and the risk of cardiovascular disease. According to Jenkins (2003), viscous fibres have the ability to lower lipids in both normal and hyperlipidaemic subjects to the same extent as currently used drug therapies.

Processing such as cooking does not affect the cholesterol-lowering properties of most DFs, whilst extrusion cooking under certain conditions could enhance its potential to lower cholesterol.

These studies have all shown that oat DF reduces total blood serum cholesterol levels and improves lipoprotein ratios to reduce the risk of heart disease in hypercholesterolemia subjects. The active components in oat DF fractions are reported to include β-glucan soluble fibre, phytosterols and soluble fibre lipid interactions. It is understood that SCFA production, increased faecal cholesterol and bile acid excretion are all possible mechanisms that have resulted in the cholesterol-lowering properties of DF.

1.5.4. DF Effects as Prebiotics

Prebiotics are defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth or activity of one or a limited number of bacteria in the colon.

Caers (2003) has conducted several studies on the role that prebiotic fibres, such as chicory and inulin, play on the process of calcium absorption. His current research has shown that these compounds are fermented in the human colon leading to the increase in bifidobacterial levels in the lower intestine that are associated with an increase in calcium absorption. More recent studies have shown that 40 g of inulin per day resulted in an increase in calcium absorption of more than 60%. In addition, this study also proved that the calcium was deposited in the bones.

Caers (2003) explained that the chain length and the fermentation profile of oligosaccharides were critical factors that affect the calcium absorption rate. By manipulation of the chain length of the oligosaccharide, he was able to optimize the calcium absorption. This led to the development of a modified enriched inulin oligosaccharide that outperformed the traditional inulin and oligofructose fibres.

Meyer (2003), showed how fermentable fibre such as inulin can shift the metabolic activity of the human colonic microbiota in a direction that results in the production of less toxic metabolites, both under normal conditions but especially under conditions with disturbed microbial community. This information can be used to develop clinical foods that support general gut growth but are able to prevent diarrhoea.

Work completed by Geel-Schutten (2003) at the Centre for Carbohydrate Bioengineering at the TNO (Nutrition and Food Research) and at the University of Groningen in the Netherlands has resulted in the ability to isolate lactic acid bacteria, e.g. *Lactobacillus reciteri 121* (food grade micro-organisms) capable of producing two different soluble homopolysaccharides, fructan and glucan. These exopolysaccharides (EPS) find many applications in the food and non-food industries where they can be used as viscosifying, thickening, gelling and water binding agents. In addition, they also promote health effects such as cholesterol-lowering, immunomodulating, anti-tumoural and prebiotic activities.

1.5.5. DF Role in Obesity

According to Blom (2003), the gastric hormone ghrelin could be used in the regulation of food intake and meal initiation. As research has shown that ghrelin levels decline shortly before a meal. EPS such as Reuteran were used and its effects on satiation and satiety were established by monitoring the ghrelin activity.

DF is known to play an effective role in the satiation and weight reduction and Burley and Blundel (1995) have reported that increased fibre intake can lead to the increased satiety and reduced food intake. Its exact mechanism is unknown at this stage, but possible mechanisms of action include:

- *Displacement of available nutrients by fibre, which demands chewing that slows the intake and induces satiety.*

- *Reduction of the absorptive efficiency of the intestine. According to Schneeman (1990), soluble DFs can change various metabolic processes of dietary lipids and alter several physiological processes such as carbohydrate and fat digestion and absorption as well as fermentation processes in the colon and liver metabolism.*

- *Lairon (2003) has shown that fibres from sugar beet, bran and psyllium affect pancreatic lipase activity. It is believed that the enzyme binds to the fibre resulting in decreased enzyme activity. Schneeman (1990) has shown that an addition of five to 15 g of pectin to a meal can lower pancreatic output by 40%.*

- *Fat digestion is determined by emulsification that is dependent upon the activity of the gastric and pancreatic lipases. These enzyme activities are dependent upon the size of the fat globule and studies have shown how high viscosity fibres have reduced tryglyceride lipolysis of emulsions by gastric lipases. This can be attributed to the positive effect that viscous fibres have on the size of the emulsified droplets.*

- *Studies by Mathers and Daly (2001) have also shown that viscous fibres increase and thicken the intestinal membrane that forms an aqueous phase which blocks out cholesterol or reduces the rate of diffusion or absorption of the cholesterol and free fatty acids.*

1.5.6. Effects of DF on Glycaemic Index

Low glycaemic foods are slowly absorbed and therefore result in a flattened glucose peak. These foods have fibres that reduce both the availability of starch and enzymatic access to it. This allows starch to enter the colon where it is slowly absorbed.

Brennan (2003) conducted several studies on the effect the diet can have on the hyperglycemia and hyperinsulinaemia in the control of type-II-diabetes. He mentioned that high carbohydrate/low fat diets have been widely recommended as healthy options. More recent research indicates that these raise plasma glucose insulin and tricylglycerol levels and reduce HDL cholesterol. In addition, patients with type-II-diabetes experience undesirable side effects such as attenuation of hyperinsulinemia. This new

development has prompted new efforts to reduce glycaemic index in foods in an attempt to control carbohydrate metabolism. Research has focused on comparing the effects of low and high carbohydrate diets in subjects, without taking into account the source of their dietary carbohydrates.

Digestibility of carbohydrate rich foods is greatly reduced by using non-starch polysaccharides. Other factors influencing the digestibility of the food are: physical form of the food, physical integrity, rate of starch gelatinisation, type of starch (high amylose *vs.* low amylose content) and starch interaction with food components such as lipids, proteins and DF.

There are many factors that could affect the absorption of glucose from the gut to the peripheral tissues. Work by Mathers and Daly (2001) showed that the ratio of amylose to amylopectin in starches affected the postprandial glycaemia, because amylopectin has a much faster rate of hydrolysis than alpha amylose.

Mathers and Daly (2001) showed that glycaemic response can be affected by chewing of food. Unchewed food produced a flatter glycaemic response than the same food chewed well, indicating that particle size, surface area and accessibility of hydrolytic enzymes in the gut can have an affect. Mathers and Daly (2001) also demonstrated a change in the glycaemic response of various cereals through the change in the particle size. This is commonly known as the 'milling effect'.

The viscosity of polysaccharides also plays a role in slowing the rate of digestion of starch and glucose and its absorption. The glycaemic index was used to compare the physiological effects of food carbohydrates with respect to postprandial glycaemia (Jenkins 2003). Certain studies have shown the effect that processing has on glycaemic response. An example is in the cooking of potatoes, where studies have demonstrated a change in glycaemic response of cooked potatoes as compared to potatoes that have undergone cycles of cooking and cooling. The latter procedure increases the amount of resistant starch in the potato and that ultimately reduces the available glucose in the small intestine, as it is resistant to digestion.

Wheat bran plays a minor role in glucose tolerance and postprandial insulin response. The effects of viscous fibres on glucose intolerance are largely dependent on viscosity and studies by Jenkins (2003) have shown that there is a slower rate of absorption with the inclusion of more viscous fibres. These mechanisms have been described by Mathers and Daly (2001) and appear to relate to the impedance of movement of the products of digestion from the lumen to the active pathways in the cell. Jenkins (2003) states that viscous fibres can improve the metabolism by blunting the glucose rise and the endocrine responses by influencing the insulin and endocrine gut hormone and gastric inhibitory polypeptides.

1.5.7. Effects of DF in Infant Nutrition

Non-starch oligosaccharides commonly found in human milk provide a prebiotic function in breast fed babies as they selectively promote the growth of bifidobacterial species.

A more diverse intestinal flora containing more bacteria and clostridia are found in formula fed babies. In addition some oligosaccharides and glycoconjugates in human milk can protect the infant against infections as neutral oligosaccharides inhibit bacterial binding to the epithelia.

Prebiotics in the form of non-digestible oligosaccharides such inulin or galacto-oligosaccharides, can be added to infant formula in place of probiotics. Research has shown that this improves the growth of *bifidiobacteria* and *lactobacilli*, improves the stool consistency and protects infants and children from infection. *Bifidobacteria* protect against infections in the gut such as NEC by inhibiting the growth of *Clostridium butyricum* or *Clostridium perfringens* (Catala *et al.* 1999; Danan *et al.* 2000). Studies by Saavedra *et al.* (1999) examined the effects of paediatric weaning and compared foods supplemented with oligofructose in breast-fed infants. They concluded that consumption of a prebiotic-supplemented cereal was associated with a decrease in the severity of diarrhoea disease.

Another study conducted by Firmansyah *et al.* (2000), involved the examination of the effects an infant cereal with milk supplemented with a prebiotic (consisting of

oligofructose and inulin) had on the immune response of infants that had received a measles vaccination. This study showed that the supplemented group had a higher immunoglobulin G antibody levels (96%) than the unsupplemented group.

Bindels (2003) has compared the intestinal flora of breast-fed infants to bottle-fed infants. She has found that in breast-fed infants, *Bifidobacteria* become the more dominant constituent of the flora, while in bottle-fed infants a more diverse flora develops.

Past research has associated bifidogenic bacteria with lower incidence of GIT infections and an optimal development of the immune system in breast-fed babies. The exact mechanism for this is unknown, but it is believed that the oligosaccharides in human milk are responsible for the prebiotic function, and are also capable of binding to several pathogenic microorganisms thereby boosting the immune activity in the infant.

It was also found that 50% of these oligosaccharides are fermentable in the human colon. Isolated human milk oligosaccharide fraction has been shown to selectively stimulate the growth of all relevant bifidiobacteria strains.

Due to the fact that these oligosaccharides could not be reproduced in a baby formula, an alternative concept simulating the prebiotic effect was developed. This consisted of low molecular weight trans galacto-oligosaccharides and high molecular weight inulin.

Extensive *in vitro* studies have shown that there was an increase in the bifidobacterial and lactobacilli intestinal flora of babies, and the short chain fatty acid spectrum from the faeces of the prebiotic supplemented-fed babies was similar to that of the breast-fed babies (Bindels 2003).

1.5.8. DF Effect on Gastrointestinal Function

The measurement of insoluble and soluble DF has not been an effective method to aid in understanding the effect of fibre on gastro intestine and metabolic consequences. The characteristics of fibre that affect GIT function include viscosity, water binding capacity (WBC), binding of bile acids and fermentablility. The physical characteristics are useful in predicting the physiological response to new sources of DF (McCleary 2001).

- *Viscosity*

Viscosity is the ability of certain polysaccharides such as gums, pectins and β-glucans to thicken when mixed with liquid. The degree of thickening will depend on the composition of the polysaccharide. Studies by Marciani *et al.* (2000) have shown that the viscosity of polysaccharides declines when swallowed due to mixing with saliva and gastric secretions. According Mathers and Daly (2001), viscous polysaccharides slow down the rate of absorption of nutrients from the small intestine due to the thickening of the layer between the cell surface and the transport systems in the cells.

- *WBC*

WBC is the ability of a fibre source to swell when mixed with water and to hold water within in its matrix. It is known that viscous polysaccharides have a high WBC. According to Schneeman (1999), these types of polysaccharides increase the volume of aqueous phase of intestinal content slowing down the absorption of nutrients because the volume increases thereby diluting the concentration of nutrients. Even though lipids are not soluble in this aqueous phase, they form micelles that are able to be absorbed thereby slowing down the rate of lipid absorption. High WBC of a fibre allows penetration of water soluble or hydrophilic substances into the fibre. This reduces the absorption of the fibre but makes it more accessible for digestion by the organisms in the large intestine.

- *Bulk*

Bulk refers to the non-digestible nature of the fibre; this is fibre that is not degraded by the microorganisms in the large intestine. The ability for the microorganisms to gain access and degrade the polysaccharide is also dependent upon the water holding capacity.

- *Binding to Bile Acids*

Story and Furomoto (1990) have shown that fibre sources with high WBC are more likely to enhance bile acid secretion. An *in vivo* study from Nishina *et al.* (1991) has shown that fibres increase the activity of enzymes involved in the conversion of cholesterol to bile acids. According to Buhman et *al.* (1998) the interaction between fibre and bile acids is hydrophobic in nature.

- *Fermentation*

Inagaki and Sakata (2001) suggested that an increased consumption of wheat bran and oat bran could result in increased stool weight. They believed that the fibre residue from the wheat bran contributed to the increased stool weight, and the microbial fermentation of the oat bran into SCFA further contributed to the increase of stool weight. There is a relationship between high microbial mass in stool and relative fermentability of polysaccharides.

1.5.9. Fermentative Properties of DF

Polysaccharides such as indigestible oligosaccharides can be fermented by gut bacteria to produce SCFA such as propionate, butyrate and acetate, and gases. Lactic and succinic acids are also produced, but to a lesser extent. Energy produced during fermentation stimulates the bacterial growth and results in the synthesis of proteins from peptides, amino acids and ammonia.

Most of the SCFA are absorbed through passive diffusion or anion exchange. Butyric acid is the main source of energy for the colonic epithelial cells and provides approximately 70% of the energy to the colonocytes (Roediger 1995). The liver metabolizes the butyric and the kidney and the mammary gland metabolize propionic acids while some of the acetic acid is utilized as an energy source and for fat synthesis. Succinic and lactic acids are taken up by the tricarboxylic acid cycle, but at a much slower rate of absorption, which often leads to their accumulation in the lumen. The slow absorption of succinic and lactic acids does not provide enough energy for the host.

Wolwer *et al.* (1995) have shown that SCFA (except succinic and lactic acid) stimulate the absorption of water and sodium from the large intestine, thereby facilitating the prevention of diarrhoea, and that succinic acid can induce diarrhoea, as it stimulates water secretion from the ileum. Trinidad *et al.* (1996) have also shown that SCFA's and fructooligosaccharides increase the absorption of calcium and magnesium in the large intestine.

The literature provides relevant background information on the health benefits associated with DF consumption and suggests target components. These aspects should be considered during the processing of DF where changes to the physico-chemical properties of the fibre could affect the functional heath properties of the end product. An example is β-glucan activity, which is susceptible to mechanical shear forces during processing.

The functionality of fibre will have a direct impact on the marketing and selling of the end product.

1.6. Functional Properties of DF

DF provides foods with texture, firmness and mouth feel. These technological characteristics are determined by the physical characteristics of DF. In addition DF products are suitable for use as water binding agents, thickeners, suspending agents, gelling agents and film formers, emulsifying agents and stabilisers (Meuser 2001).

The WBC, swelling and viscosity are the most important characteristics of DF products. These characteristics can be suppressed or activated by certain processing conditions such as pH, temperature and ionic concentration. It is important to understand the individual molecular components of DF, and how their structure relates to the physicochemical characteristics. This helps to understand the relationship between the structure and function of the DF, thereby facilitating the application of DF for specific purposes.

Manufacturing processes that include extraction, cleaning and drying have a direct effect on the DF composition and the physicochemical characteristics of the end DF product. This is particularly noticeable with the WBC capacity of the fine and the coarse fibre particles. The dry and the wet processes can affect the hydration capacity of the fibre (Cadden 1987).

Most fibres have a yellow to brownish colour and a bitter fruity flavour that limits application in a variety of food products. Highly purified or chemically treated fibres

that are white or slightly yellow, and have no unpleasant taste or odour have a broader application base and are thus more marketable.

1.7. Applications of DF in Food Products

It would seem reasonable to enrich cereal products such as breakfast cereals products, pasta and snacks rather than highly refined foodstuffs because the consumer acceptance threshold is far greater with less refined products. Wheat bran is most commonly used as a DF source in many baked products but there is growing interest in using dehusked barley or oats as an alternative. The dehusked grains are becoming popular due to their high soluble DF content and these products are finding a wider spread of applications in the food industry.

Other fibre sources are apple, orange, carrot, beet and potatoes. However these DFs share the same problems that are associated with cereal based fibres due to the limits to enriching fibre based foodstuffs. High fibre dosages can have a negative effect on the sensory characteristics, appearance, mouth feel, flavour, and taste and chewing characteristics of many processed foods such as processed meat, cereals and baking products

In the UK the amount of disposable income that is spent on food consumption in the home has declined from 30% in 1940 to 8.2% in 1998 (Van der Kamp 2003). It is also interesting to note that bread consumption in the home has declined by 50% during the last 50 years but breakfast cereal consumption has tripled. These constraints have forced the food industry to become more innovative and has lead to the production of

more than 5000 new food products entering the food market from 1999 to 2003 (Van der Kamp 2003).

Through all these changes the public continues to consume less DF, and in order to address this issue the development of new DF products or new marketing strategies should be developed. DF can be considered to be a functional food and although it has a low nutritional value its physiological effects have more importance. A functional food can be described as a processed food containing ingredients that aid specific bodily functions in addition to being nutritious.

There is a growing area of research in investigating the role of how non-nutritive components of the diet affect human health (Larrauri 1999). Functional foods, neutraceuticals, designer foods and pharmafoods are commonly used terms to describe products that are selected to provide increased intake of non-nutrient phytochemicals. These phytochemicals are found in natural plant materials and possess medical and health benefits. DF is classified as a phytochemical as well, and in the US nutritional labelling area, DF has two permitted health claims due to its beneficial effects in reducing the risk of coronary heart disease and cancer.

The growing interest in DF's could be due to its association with good health as well as the influence that DF plays on the sensory quality of foods. One approach to address this is to process DF products from raw vegetable materials or to manufacture a substance that behaves similarly to DF from carbohydrates.

DF is known to perform a sensory function and it is used to improve the shelf life of the food product or to enhance the texture and the taste of food products. The improved shelf life can be attributed to the high WBC of the fibre.

DF should have suitable WBC and high shear stability in order to be suitable for food formulations. This is evident with the wrinkled pea DF that has superior WBC and shear stability due to its non-starch polysaccharide structure, compared to its competitors that contain high amounts of cellulose and hemicelluloses (Meuser 2001).

According to Inglett and Carriere (2001) future work in this area should evaluate the use of DF (particularly fruit DF) in a variety of food products such as processed meats, dairy products and breakfast cereals. The application and benefits of these DF in food are summarised in the following section.

1.7.1. Baked Products

DF can be used for the enrichment of white bread and in the production of gluten free bread and low caloric baked products. Fruit fibres often have a superior WBC to wheat bran fibres and the viscosity enhancement will provide the stabilising effect that guarantees volume and crumb of the bread. Fruit fibre blends can also act as fillers. Fibre blends consisting of prebiotic soluble fibres (inulin and pectin) can be incorporated with texture supporting fruit fibres to produce breads of superior functionality yet similar quality as the wheat bread product.

- *Fibre Enriched White Bread*

DF content of flour used in the baking of bread is approximately 3 g/100 g. It can be doubled by addition of 5% fibre isolate. Inglett and Carriere (2001) mentioned that breads produced with increased DF often have: improved dough stability, improved bread yield, reduction in baking losses, improved bread crumb and prolonged fresh keeping due to water binding of fibres (>10 g/g fibre).

- *Bread for Special Diets*

Gluten free bread requires a component to compensate the dough stabilising function of cereal glutens. Gluten can be replaced with fruit fibres, particularly those fibres with high pectin content as these often stabilise the dough through the formation of a heat stable three dimensional network (Inglett and Carriere, 2001).

- *Low Calorie Baked Goods*

Fibre isolate can be used in baked goods to produce low calorie baked goods. These fibres can lead to less butter being used in recipes with minimal change to the sensory properties but with increased yield and at lower cost (Inglett and Carriere, 2001).

1.7.2. DF in Beverages

Soluble fibres are used in beverages as they improve the viscosity but do not change the character of the beverage. Low viscosity pectins have been developed and used for the specific purpose of fortification of beverages.

1.7.3. DF in Meat Products

The inclusion of DF in meat products can improve the texture, juiciness and stability of the final product. Examples of meat products are listed below (Inglett and Carriere, 2001):

- *Mince Meat Products*

Most mince meat products use 2-3% inclusion of fruit fibres with a high WBC. The addition of fibre can reduce the costs by 9% as it improves the yield and quality factors, and reduces calories.

- *Boiled Sausages*

Fruit fibres can also be used in these products, as a natural alternative to phosphate and soy or milk proteins. The soluble DF is known to support the gell formed by the solubilised proteins and fat. Fruit DF addition also aids the texture stability and reduces the loss of moisture during cooking.

- *Salami Like Sausage*

In most cases not more than a 1% inclusion of fruit fibres is used in this mixture. This improves the ripening period of the curd sausage as the WBC of the fruit fibres plays a significant role.

- *Fillings*

Fibres, usually from fruit, improve the speed of drying and enhance the flavour, viscosity and texture of the main filling components. In filled pasta, DF increases the soaking speed due to the capillary action of the fibre.

1.7.4. DF in Ice-cream or Sorbet

In ice-creams up to 30 g of DF per product could be used without negative affects on the sensory properties of the products. Technological benefits (Inglett and Carriere, 2001) include:

- *Stabilisation of the ice milk*
- *Allows freezing at lower temperatures without negatively affecting the ice crystals*
- *More homogenous air bubble formation*
- *More consistency after freezing*
- *Enhanced melting stability*
- *Prolonged shelf life*
- *Enhanced stability during transportation.*

The inclusion of DF as an ingredient improves the melting properties of the products and improves the mouth feel, freeze/thaw stability and offers nutritional value.

1.7.5. DF in Dairy Products

Fruit DF can be used in place of gelatine or modified starch to prevent protein coagulation and subsequent phase separation.

1.7.6. DF in Pasta

It is important that fibre enrichment of pasta does not result in negative effects on the taste, colour or texture. Citrus fibres are the preferred fibre source in pastas because of the associated advantages (Inglett and Carriere, 2001) which include:

- *Improved yields*
- *Reduced cooking time*
- *Improved drying properties*
- *Enhanced cooking stability*
- *Enhanced shelf life due to the improved WBC of the fibre ingredient.*

Pasta's that contain high levels of low viscosity pectin, or β-glucans and that would have an effect on the cholesterol levels are being developed for special diets (Inglett and Carriere, 2001).

1.8. Objective

This project focused on developing a DF product from the by-products of the pineapple canning process which could target the food and health-care markets. The by-product, which comprises the peels, cores and trimmings, is a potential source of fibre, essential oils, flavours, proteases and other commercial organic compounds.

This involved the development of a process for the preparation of functional fibre from the cores and peel, which would retain valuable bioactives. This could be achieved by controlling the drying and milling steps of the process. The preferred processing parameters would be established by assessment of the composition and functionality of the fibre products after the various treatments. This includes the effect of temperature, particle size and chemical treatment on the performance characteristics of the DF. Modification to enhance the market value of the fibre by improving key functional properties such as water binding capacity (WBC) would be considered.

As performance characteristics such as WBC, significantly affect the product price, modification of the fibre to achieve this was attempted.

CHAPTER 2

2. CHARACTERISATION OF PINEAPPLE WASTE MATERIAL

2.1. Introduction

The main DF sources are from plant cell wall components such as cellulose, hemicelluloses, lignin and pectic substances. In addition, there are also non-structural components such as gums and mucilage's that include industrial additives such as modified cellulose, modified pectin, commercial gums and algae polysaccharides (Grigelomo-Miguel *et al.* 1999).

DF can be categorised according to its dispersion in water, as either soluble or insoluble. As mentioned earlier each fraction has specific health benefits. The insoluble fraction is associated with increased WBC and intestinal regulation, while the soluble fraction plays an important role in the reduction of cholesterol and glucose in the blood. A well balanced proportion of soluble to insoluble DF ratios is suggested as being 50-70% insoluble DF components to 30-50% soluble DF components (Grigelomo-Miguel *et al.* 1999).

There is an increasing interest in finding DF fruit sources because the ratio between soluble and insoluble fractions is more balanced compared to cereal brans which are the most commonly used DF source in food products. In addition fruit fibres have better oil binding capacity (OBC), better colonic fermentability and lower phytic acid and caloric values (Larrauri *et al.* 1999).

Larrauri *et al.* (1999) predicted that there would be a demand for DF derived from fruits in the future particularly those that retain most of the bioactive components such as flavonoids, polyphenols and carotenoids. These compounds may prove to have higher health promoting effects than the DF itself. Vitamins and enzymes are bioactive components prominent in most plant material and it is of particular importance to establish the effects of processing on their activity. These components are sensitive to heat and light and therefore processing effects in the canneries could lead to the deterioration of these activities (Greenwood and Munro, 1979).

As the colour and functional properties of the final fruit DF is influenced by many factors such as variety and maturity of the fruit, and the drying process, it was important to obtain a detailed understanding of the nature of the raw material to be able to benchmark any changes that occurred during processing. The nutritional carbohydrate and bioactive composition of the fresh peel and core materials were profiled to provide this baseline. In particular the soluble and insoluble DF ratio as well as the bioactive components such as the vitamins C and E and the enzyme bromelin which are sensitive to various processing techniques, were determined.

2.2. Materials and Methods

2.2.1. Sample Preparation and Collection

Three batches, each ten kg in mass, and collected at monthly intervals were used (Figure 2.1) for the analysis of the pineapple canning waste from the fruit *Ananas comosus.* These batches comprised aliquots taken at intervals directly from Collondale pineapple canning lines and were representatives of the day's production. They were couriered in a cooler box (packed with ice) to the CSIR in Rosebank Cape Town. It was essential that the material was kept cold during transportation as fluctuations in temperature could affect the antioxidant activity.

Batches one and two were divided into three sub samples and batch three was divided in four sub samples.

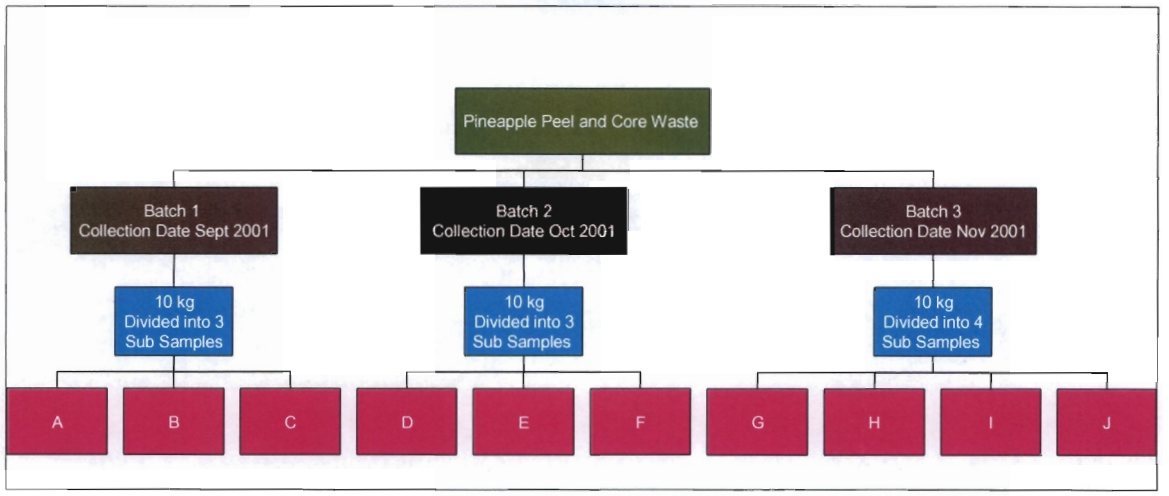


Figure 2.1: Process used to Determine Nutritional Profile of the Fresh Pineapple Waste Material

2.2.2. Nutritional Profile

Nutritional analysis was conducted on each of the samples (A-J Figure 2.1) on the day of arrival resulting in a total of ten sets of results being collected. The three different sub samples from each batch were used to take care of natural variation due to the homogeneous nature of the raw material. A duplicate analysis on each sub sample was performed to ensure accuracy of measurement. The required nutritional, carbohydrate and bioactive analyse were carried out on the fresh material. The nutritional and mineral analysis of the waste material included the determination of the ash, moisture, protein, fat, and carbohydrate and total DF contents. The caloric value was determined from these results. A further investigation of the carbohydrate content involved the determination of Klason lignin, pectin contents and the individual neutral sugars present. Sub samples A-J were assayed in duplicate and the arithmetic mean calculated. This provided the baseline for assessing processing effects.

2.2.3. Methods Used for Nutritional Analysis (Proximate analysis)

The following standard methods were used (see appendix for detail):

- *Ash: AOCS Ba 5a-49. Revised 1973. Third edition.*
- *Fat: Bligh and Dyer method J. Sci. Food Agric. 1985,6, 177-185.*
- *Moisture: Oven Moisture method. AOCS Ba 2-38. Revised 1982. Third edition.*
- *Protein: Bradstreet R.B. 1996. The Kjeldahl Method for Organic Nitrogen. Academic Press.*

2.2.4. Mineral Profile

The metals were determined using an atomic absorption spectrophometric method suitable for the determination of all the metals except for the phosphorous, which was determined by a colorimetric method. The following methods were used for mineral analysis:

- *Metals: SA National Scientific Programmes Report No. 44, July 1981.*
- *Phosphorous: AOAC 1984, p. 167.*

The arithmetic mean of ten sets (Sub samples A-J) of duplicates was calculated.

2.2.5. Bioactive Profile

The antioxidant activity is important to establish as fruit based DFs can be classified as an Antioxidant DF (AODF) if it is above a certain level. The following vitamin and enzymatic analysis were conducted on a composite sample of the three batches (1-3) of fresh pineapple peel and core waste material:

- *Total antioxidant activity*
- *Vitamin C*
- *Vitamin E*
- *Total Phenols*
- *Bromelin*
-

Total Antioxidant Activity

The modified ferric thiocynate (FTC) method reported by Larrauri *et al.* (1997) was followed. The antioxidant activity is measured by recording the change in absorption at 500 nm of ferric thiocynate due to oxidation of the compound. The total antioxidant activity is expressed as antioxidant units per mg dry mass in 100 ml assay mixture. The FTC method can be used to measure the amount of peroxide in initial stages of lipid oxidation, where peroxide gradually decomposes to form lower molecular compounds. Kikuzaki and Nakatani (1993) used this method successfully for the determination of the total antioxidant capacity of pineapple processing waste.

Vitamin C

Vitamin C was determined according to a fluorimetric method (AOAC 984.26) that determines the total vitamin C (ascorbic and dehydroascorbic acid) in food. It is applicable to both fresh and stored food and in particular those of complex composition, such as fruit and fruit juices. Vitamin C is determined by the reaction of dehydroascorbic acid with O-phenylenediamine to give 3-(1,2 dihydroxyethyl) furo 3,4-b-quinozaline 1-one, which is fluorescent. Vitamin C is calculated on a zero percent moisture basis and is recorded as mg/kg.

Vitamin E

The Munné-Bosch *et al.* (1999) method was used for the determination of Vitamin E. The fresh pineapple samples were ground in liquid nitrogen and a 0.1 g sample was extracted with 5 mℓ methanol (GR grade) and 4 mℓ hexane (GR grade) for ten minutes. The mixture was centrifuged at 1500g for ten minutes and the hexane layer removed and evaporated to dryness with nitrogen. The dried residue was dissolved in 2 mℓ methanol and then analysed by HPLC using a Phenomexex prodigy 5 ODS 2 (5 micron) column (250 x 4.5 mm).

Total Phenols

The total phenols were determined by the Folin Ciocalteau method as adapted by Singleton *et al.* (1999).

Bromelin

Bromelin commonly found in the pineapple stems and core of the fruit is an enzyme that hydrolyses proteins, peptides, amides and esters of amino acids. Bromelin activity was determined by a colorimetric method based on the method in Preparations for Biochemistry from Merck Catalogue number: 1651. The bromelin activity was calculated as Anson units, which are defined as that quantity of enzyme that under test conditions liberates 1 mmole of Folin positive amino acids per minute calculated as tyrosine units (calculated as tyrosine-Sigma Aldridge T 3379). The activity was reported as National Fine Units (NFU) using the following conversion ratio: 54 000 NFU units are equivalent to 830 tyrosine units (Anson).

2.2.6. Food Carbohydrates

The total carbohydrates were determined using the described methods. Sub samples A-J were tested in triplicate except for the water soluble neutral sugars that were tested in duplicate.

Total DF Content

The current method used for determination of DF content (AOAC 985.29) measures the indigestible carbohydrate portion including lignin but excludes the oligosaccharides. An extension of this method involves the acid hydrolysis of the residue after enzymatic treatment and subsequent analysis of sugars and uronic acids. In this method the

resistant starch is also measured and although this is not a NSP it should be included under the umbrella of DF as it behaves in a manner similar to other DF components in that it resists digestion in the small intestine.

The following TDF tests were selected for this study to as they include quantification of the insoluble and soluble portions.

- *Soluble and insoluble DF: AOAC 991.43.*
- *TDF: Bioquant enzymatic determination. Merck Method No. 14 also referred to as the AOAC method 991.85.*

Uronic acids and Pectin Determination

The SDF component of pineapple samples consists mostly of pectin and neutral sugars. The pectin content (galacturonic acid) was determined by the colorimetric method as uronic acids are not volatile (because of their carboxyl groups) and therefore cannot be quantified using the GC method used for the sugar determination. Two different methods were used as neither was directly applicable to the raw material. The Rouse and Atkins (1955) method is suitable for fruit juices. The other method was a colorimetric assay used by Gianotti (1989) for the determination ofglycosaminoglycans (GAGS) in human tissue.

Neutral Sugars

According to Larrauri *et al.* (1997), soluble carbohydrates found in pineapple peel consisted mostly of uronic acids and neutral sugars such as arabinose, xylose, mannose, galactose and glucose. A gas chromatographic method with flame ionization detection was used for the quantification and identification of sugars as the alditol acetates.

Sugars present in the water soluble fraction of the fresh and dried (104°C for eight hours) pineapple peel and core were determined using the following procedure. This was done to investigate the possibility of valuable sugars in the wet material that could have lost during processing. Each of the ten sub samples of pineapple peel and core were extracted with hot water (65°C), the extract evaporated with a rotovac and the residue freeze-dried. The sample collected after freeze-drying was tested for neutral sugar content. The samples were tested in duplicate and the results were calculated on a 0 % moisture basis.

The polysaccharides were first hydrolysed to their constituent monosaccharides, then reduced and acetylated in order to make them volatile (Holligan and Drew, 1971).

Insoluble Components

Cellulose and Klason lignin are the two main components that comprise the insoluble portion of DF.

- *Cellulose*

Although the cellulose was not measured, it can be calculated by difference if the total insoluble, lignin and pectin contents are known. The cellulose and hemicelluloses forms an integral part of the insoluble dietary component of the pineapple DF.

- *Klason lignin*

Klason lignin was determined according to the modified method developed by Larrauri, *et al.* (1997). The insoluble DF residue remaining after enzymatic treatment was hydrolysed with 12 M sulphuric acid at room temperature for one hour, followed by a further hydrolysis with 1 M sulphuric acid at 100°C for 90 minutes. After hydrolysis the insoluble residue was collected by centrifugation and filtration. The final residue was oven dried at 105°C (to constant mass) and quantified as Klason lignin.

2.3. Results and Discussion

These results are tabulated and described in the following sections.

2.3.1. Nutritional Profile

The results tabulated below represent arithmetic averages obtained from duplicate testing of the prepared sub samples. The nutritional profile of the fresh material was determined and the total carbohydrates were calculated by difference (100 - total of the ash, fat, protein, TDF and moisture).

Table 2.1: Nutritional Profile of Fresh Pineapple Peel

Analysis (g/100 g)	**Batch 1** (A-C) Mean of 6 results (g/100 g)	**Batch 2** (D-F) Mean of 6 results (g/100 g)	**Batch 3** (G-J) Mean of 8 results (g/100 g)	**Overall Mean** [a] (g/100 g)
Ash	0.5 ± 0.07	0.6 ± 0.04	0.7 ± 0.07	0.6 ± 0.03
Protein	0.6 ± 0.05	0.6 ± 0.06	0.6 ± 0.02	0.6 ± 0.05
Fat	0.3 ± 0.08	0.3 ± 0.08	0.3 ± 0.06	0.3 ± 0.09
Moisture	86.4 ± 3.2	85.4 ± 4.5	83.4 ± 2.0	85.1 ± 3.2
TDF[b]	7.9 ± 2.0	9.0 ± 4.3	6.0 ± 4.3	7.7 ± 0,4
CHO[c]	4.3 ± 0.3	4.1 ± 3.3	9.0 ± 4.3	5.4 ± 0.03
Calories/	36 ± 0.8	35 ± 4.3	66 ± 6.4	45 ± 0.08
Energy	90 ± 4	87 ± 3.4	165 ± 5	108 ± 4

[a]The data presented are the arithmetic mean of 10 sets of duplicate results of samples A-J [b]TDF= total dietary fibre, [c]CHO = carbohydrates by difference (100 – sum of protein moisture fat ash and TDF)

Table 2.2: Nutritional Profile of Fresh Pineapple Core

Analysis (g/100 g)	Batch 1 (A-C) Mean of 6 results (g/100 g)	Batch 2 (D-F) Mean of 6 results (g/100 g)	Batch 3 (G-J) Mean of 8 results (g/100 g)	Overall Mean [a] (g/100 g)
Ash	0.3 ± 0.08	0.3 ± 0.08	0.3 ± 0.08	0.3 ± 0.06
Protein	0.4 ± 0.08	0.5 ± 0.07	0.3 ± 0.08	0.4 ± 0.03
Fat	0.2 ± 0.08	0.2 ± 0.08	0.2 ± 0.03	0.2 ± 0.04
Moisture	84.3 ± 3.0	85.3 ± 4.0	83.4 ± 0.5	84.3 ± 2
TDF[b]	8.0 ± 3.3	10.0 ± 2.0	7.2 ± 0.7	8.4 ± 0.7
CHO[c]	6.8 ± 0.06	3.7 ± 0.08	8.6 ± 0.4	6.4 ± 0.4
Calories	49 ± 0.9	30 ± 5.0	60 ± 1.8	45 ± 0.6
Energy	123 ± 0.9	75 ± 2.0	150 ± 0.8	117 ± 3.0

[a]The data presented are the arithmetic mean of ten sets of duplicate results of samples A-J, [b]TDF= total dietary fibre [c]CHO = carbohydrates by difference (100 – sum of protein moisture fat ash and TDF)

The assay results of the raw material (Tables 2.1 and 2.2) indicate that when calculated on a dry basis the peel contains a higher fat, ash and protein content than the core. The high fat content could be due to the presence of wax in the peel. According to Duncan (2001), natural waxes are released from the peel during the pressing operation. Similar results for the moisture, fat and ash were obtained by Guerra *et al.* (2002), except for the total carbohydrate value that was almost double. Although the same species of

pineapple was used the variation of the results could be attributed to seasonal variation and geographical location.

2.3.2. Mineral Composition

The results of the mineral composition profile are shown in Table 2.2 and compared with published values of the fresh edible portion. Values have been calculated on a "0% moisture basis" to allow for comparisons.

Table 2.2: Mineral Content in Fresh Pineapple Peel (at 0 % moisture basis)

Analysis (mg/kg)	Batch 1 (A-C) Mean of 6 results (mg/kg)	Batch 2 (D-F) Mean of 6 results (mg/kg)	Batch 3 (G-J) Mean of 8 results (mg/kg)	Overall Mean (mg/kg)	[a] Fresh Fruit (mg/kg)
Sodium	322 ± 5	389 ± 0.4	400 ± 0.4	370 ± 30	74 ± 0.5
Calcium	349 ± 4	378 ± 0.5	390 ± 0.4	372 ± 20	52 ± 0.4
Potassium	12475 ± 7	13000 ± 5	12478 ± 20	12651 ± 5	8370 ± 40
Iron	79 ± 3.6	90 ± 9	60 ± 0.2	76 ± 2	30 ± 4
Zinc	8 ± 2.1	3 ± 0.3	7 ± 0.3	6 ± 0.3	6 ± 0.08
Magnesium	130 ± 3	129 ± 2.4	119 ± 0.4	126 ± 0.4	103 ± 0.4
Phosphorous	3963 ± 3,3	3498 ± 0.9	4050 ± 30	3837 ± 30	518 ± 0.5

[a]Gouws and Langenhoven, 1986 Food Composition Tables NRIND - MRC

Table 2.3: Mineral Content in Fresh Pineapple Core (at 0% moisture basis)

Analysis (mg/kg)	Batch 1 (A-C) Mean of 6 results (mg/kg)	Batch 2 (D-F) Mean of 6 results (mg/kg)	Batch 3 (G-J) Mean of 8 results (mg/kg)	Overall Mean (mg/kg)	[a] Fresh Fruit (mg/kg)
Sodium	321 ± 5	389 ± 0.4	400 ± 0.4	370 ± 30	74 ± 0.5
Calcium	349 ± 4	378 ± 0.5	390 ± 0.4	372 ± 20	52 ± 0.4
Potassium	11475 ± 7	12000 ± 5	11478 ± 20	11651 ± 5	8370 ± 40
Iron	79 ± 3.6	90 ± 9	60 ± 0.2	76 ± 2	30 ± 4
Zinc	8 ± 2.1	3 ± 0.3	7 ± 0.3	6 ± 0.3	6 ± 0.08
Magnesium	130 ± 3	129 ± 2.4	119 ± 0.4	126 ± 0.4	103 ± 0.4
Phosphorous	3963 ± 3,3	3498 ± 0.9	4050 ± 30	3837 ± 30	518 ± 0.5

[aa]Gouws and Langenhoven, 1986 Food Composition Tables NRIND- MRC

The results show that the peel is generally higher in minerals, as expected from the higher ash content. Both the peel and the core contain comparatively high potassium contents relative to the rest of the minerals. The peel contains approximately 3312 mg/kg more potassium than the core and the potassium value obtained for the core is approximately 969 mg/kg more than the value recorded by Gouws and Langenhoven (1986) for the flesh of the fruit. The high potassium content in the pineapple peel and core is important for a DF product as potassium plays a key role in many of the body's functions. The core and peel contains higher amounts of Mg, Ca, P and Na compared to what is found in the flesh of the fruit reported by Gouws and Langenhoven (1986). The

zinc and the iron were found to occur at comparatively low levels compared to the remaining metals assayed.

2.3.3. Carbohydrate Profile of Pineapple Waste

The insoluble and soluble dietary component levels as well as the Klason lignin, uronic acids are shown in Table 2.4.

Table 2.4: Carbohydrate Profile of Pineapple Peel

Analysis	Batch 1 (A-C) Mean of 6 results (g/100 g)	Batch 2 (D-F) Mean of 6 results (g/100 g)	Batch 3 (G-J) Mean of 8 results (g/100 g)	Overall Mean [a] (g/100 g)
TDF [b]	8.8 ± 4.0	9.5 ± 0.2	7.0 ± 0.5	7.7 ± 0.4
SDF	0.4 ± 0.03	0.1± 0.03	0.5 ± 0.04	0.3 ± 0.02
IDF	8.4 ± 4.0	9.4 ± 0,2	6.5 ± 0.6	7.4 ± 0.4
Pectin [c]	0.8 ± 0.03	0.7 ± 0.4	0.9 ± 0.3	0.8 ± 0.3
Klason Lignin	5.9 ± 0.3	4.6 ± 0.5	3.4 ± 0.4	4.6 ± 0.5
Moisture Content	85.8 ± 0.10	85.4 ± 0.8	85.0 ± 0.5	85.4 ± 0.4

[a] Mean of ten sets of duplicate results,[b]Addition of SDF and IDF, [c]Using Rouse Adkins method

Table 2.5: Carbohydrate Profile of Pineapple Core

Analysis	Batch 1 (A-C) Mean of 6 results (g/100 g)	Batch 2 (D-F) Mean of 6 results (g/100 g)	Batch 3 (G-J) Mean of 8 results (g/100 g)	Overall Mean [a] (g/100 g)
TDF [b]	8.9 ± 0.9	10.0 ± 0.9	6.4 ± 0.3	8.4 ± 0.3
SDF	1.0 ± 0.3	0.7 ± 0.09	0.5 ± 0.04	0.7 ± 0.03
IDF	7.0 ± 0.8	9.3 ± 0.3	5.9 ± 0.9	7.7 ± 0.7
Pectin [c]	1.3 ± 0.7	1.1 ± 0.4	1.5 ± 0.4	1.3 ± 0.3
Klason Lignin	6.6 ± 0.5	7.8 ± 0.9	5.7 ± 0.7	6.7 ± 0.8
Moisture Content	84.1 ± 2.0	85.0 ± 5.0	84.0 ± 09.0	84.4 ± 6.0

[a] Mean of ten sets of duplicate results, [b]Addition of SDF and IDF, [c]Using Rouse Adkins method

Based on the results in Table 2.4 and 2.5, the core contains 0.3% more SDF than the peel. This is expected as the core contains 0.5% more pectin (a SDF) than the peel. On a dry mass basis, the TDF is the most prominent component other than water comprising 53% and 54% of the peel and the core respectively. Although pectin is a SDF, it is present with a content greater than the SDF content. This discrepancy is probably due to the fact that the pectin method (Rouse Adkins) is more specific and accurate than the SDF method that is known to under-recover some of the low molecular weight soluble carbohydrate components due to loss during the filtration step (Asp 2003). It is also very difficult to measure the pectin that is trapped within the cell wall structure and this could also lead to discrepancies. The core contains more Klason lignin than the peel; this is expected as the Klason lignin plays a supporting role in maintaining the structure and strength of the cell walls, which is one of the key functional roles of the core of the pineapple fruit.

The amounts of Klason lignin found in the samples tested (6.7 g/100 g) are lower than those reported by Larrauri et al. (1997), however they stated that variation is acceptable as it can be attributed to the different structures in the mass fraction of the different fruit fibre fractions.

Larrauri et al. (1997) have also reported a high IDF content comprising 99% of the TDF which is comparable to the results obtained for the peel and the core where the IDF comprise more than 90% of the TDF composition.

2.3.4. Neutral Sugar Profile

The neutral sugar content of the aqueous extracts of fresh and dried extracts pineapple peel and core material was determined.

2.3.5. Water Soluble Component

Table 2.6: Neutral Sugar Content of Soluble Component of Fresh and Dried Material

Monosaccharide	% Monosaccharides in Total Carbohydrate [a]			
	Fresh		Dried	
	Peel	Core	Peel	Core
Rhamnose	1.6 ± 0.4	n/d	2.8 ± 0.8	1.1 ± 0.2
Arabinose	1.8 ± 0.3	1.1 ± 0.3	4.7 ± 0.3	1.0 ± 0.3
Xylose	0.7 ± 0.3	1.5 ± 0.4	2.5 ± 0.2	1.1 ± 0.3
Mannose	21.6 ± 0.8	17.9 ± 0.9	14.9 ± 0.9	18.5 ± 0.9
Galactose	3.4 ± 0.7	2.1 ± 0.4	4.8 ± 0.6	2.2 ± 0.3
Glucose	71.0 ± 7.0	77.3 ± 0.39	72.1 ± 7.0	76.7 ± 8.0
Total NS in sample (g/100 g) [b]	**61.0**	**64.0**	**51.3**	**63.4**

The arithmetic mean of ten sets of results obtained from the ten sub samples are reported,[a] Percentage monosaccharides of the total carbohydrate determined by difference, [b]Percentage carbohydrate in the actual sample determined by subtracting from the proximate analysis, n/d = not detected

From the results in Table 2.6 the neutral sugar content and the monosaccharides profile of the soluble portion of the fresh and the dried core are similar. The peel has 10% less neutral sugars than the core. This may be due to browning of the neutral sugars by caramelisation, pyrolysis or the Maillard reaction during drying. In particular the hexoses are easily charred during drying even though the other sugars are more fragile

(Holdsworth 1979). It appears that the core was not as affected as the peel and the reasons for this are unknown at this stage. The neutral sugars detected comprise mostly of hemicellulosic components of the IDF. These results conform to the results obtained by Larrauri *et al.* (1997), who found that the neutral sugar composition of the dried and washed pineapple peel consisted primarily of glucose and mannose followed by trace amounts of arabinose and galactose. Rhamnose was not reported, as it was not detected.

2.3.6. Bioactives

A baseline for the bioactives which included the following: Total antioxidant activity, vitamins C and E, total phenols and bromelin activity was important as these components would add value to the final product if they were to be consumed.

Total Antioxidants

Bioactive assays were conducted on composite samples of the ten sub samples and the arithmetic mean is presented in Table 2.7 except for the vitamin E where only three composites from batches one, two and three (refer to Fig 2.1) were tested. Arthimetic means of the results obtained are presented in Table 2.7.

Table 2.7: Bioactives of the Fresh Peel and Core Pineapple Samples

(Calculated on 0 % moisture basis)

Composition	Number of Samples Tested	Peel	Core
Total Antioxidant Activity [a](AO units)	10	19	15
Vitamin C (mg/kg)	10	188	97
Vitamin E (mg/kg)	3	0.5	0.2
Total phenols (mg/kg)	10	0.3	0.3
Bromelin Activity [b](NFU units/kg)	10	3.3	1.3

[a]AO = antioxidant units, [b]Conversion factor of 830 Tyrosine units = 54 000 NFU (J. van Aswegen 2002)

The ferric thiocynate method used in the determination of total antioxidant capacity is commonly used in *in vitro* studies to evaluate the efficacy of prevention of oxidation. The results showed that the pineapple fresh material has a relatively high antioxidant capacity. These results are similar to the results reported by Larrauri *et al.* (1996). In a later publication, Larrauri *et al.* (1997) reported that the antioxidant capacities in the pineapple material could be attributed to polyphenols such as catechin and myricetin. The current work showed the concentrations of phenols were similar in the peel and the core.

The results of the vitamin C analyses were in agreement with those of the total antioxidant activity test in that the peel had higher antioxidant content than the core. Surprisingly the fresh peel had almost double the amount vitamin C than the core had

even though the core with more flesh is expected to have a higher content. It appears that the pressing of the core to remove the juice may have destroyed most of the vitamin C.

The levels of bromelin activity recorded were very low. The peel contains nearly 40% more activity than the core, which was unexpected as most of the bromelin is purportedly found in the core and the stem of the pineapple fruit. (Watson 1989). A previous study on the bromelin activity of the *Ananas comosus* from a cannery in the same region recorded levels of up to 2800 NFU/ 100 g , and indicated that the bromelin enzyme activity was stable at temperatures up to 40 °C (Watson 1994).

Based on the low levels of the associated bioactives vitamins E and C and bromelin, it is unlikely that they contribute much to the antioxidant activity of the peels and core waste material. These vitamins were possibly destroyed during the peeling and pressing processes in the cannery, as a result of their sensitivity to heat and light. Although vitamin E is heat resistant, it is readily destroyed during processing through oxidation reactions.

Larrauri *et al.* (1996), who have attributed the high antioxidant activity to the polyphenols, have also suggested that the uronic acids may enhance the antioxidant capacity of polyphenols. Larrauri *et al.* (1997) have reported that pineapple DFs exhibit a 52.1% higher antioxidant capacity than the orange, lemon and apple commercial DFs. They have attributed this to specific polyphenols such as myricetin, which comprises 59% of the polyphenols present. Identified but less prominent phenols such as salicylic,

tannic, *trans*-cinnamic and *p*-coumaric acids exhibited a lower antioxidant activity. According to Wen and Wrolstad (2002), phenol composition is important to establish the authenticity of the juice and to evaluate the effects of processing on juice quality.

2.4. Conclusion

The pineapple waste consists mostly of DF, and neutral sugars. The fresh peel and core consist predominately of IDF, which has a large Klason lignin component. The high IDF content makes this raw material a suitable source for the manufacture of a DF product. Ideally any DF product has to satisfy the certain specifications (Laraurri 1999 and Figuerola *et al.* 2005)

Since the neutral sugar content is responsible for the sweet taste and odour of the final product, it is important to remove these sugars to enable production of a competitive product of neutral colour and taste (Laraurri 1999). As a result a washing step may be necessary as this will help in the removal of the neutral sugars and thereby reduce the risk of product discoloration as well as reduce microbial contaminants that are associated with pineapple, due to its close contact with the soil.

The neutral sugar composition of the soluble component of the fresh material consists mostly of the hexoses glucose and mannose. It is known that these hexoses are easily caramelised or destroyed when exposed to high temperatures during the drying process. Therefore, the removal of these sugars may be an important process to consider should a high temperature process be selected for the manufacture of a suitable DF product.

High temperature drying may need to be introduced to successfully prevent a higher than acceptable microbial load. However, this will be at the expense of the heat sensitive bioactives, such as vitamin C. This may not be a major problem as most of the vitamin C and E has already been destroyed during the processing in the cannery. The retention of the bioactives is important in the development of an antioxidant rich DF as this can give the pineapple DF a commercial advantage over the current commercial cereal DFs which do not have many bioactives.

Chapters three and four investigate the processes that effectively reduce the microbial load and the neutral sugar content in the pineapple waste material thereby producing a suitable DF product that is safe to use as a food ingredient and is of neutral colour and taste.

CHAPTER 3

3. DEVELOPMENT OF A PROCESS FOR THE REMOVAL OF THE MICROBIAL CONTAMINATION

3.1. Introduction

The canning process at Collondale cannery is Hazard Analysis Critical Control Points (HACCP) approved but the by-product exits the cannery on a belt under non HACCP conditions. As a result the pineapple raw material by-product has a high microbial load, this could be attributed to factors such as soil contaminants, the use of recycled water during the washing process and the fact that the waste product is not treated under (HACCP) conditions.

Microbial contamination is an important issue and it is therefore imperative that all the processing parameters ensure that the microbial load is reduced to within the specified limits as provided by Larrauri (1999) for without this it may not be used as a food ingredient.

In addition to the manufactured DF product meeting the microbial specification limits of a food ingredient, it must not contain pathogenic micro-organisms (Laraurri 1999). The specifications listed in Table 3.1 were provided by a commercial company (Van Schalkwyk 2004) and apply to most commercial fibres. These values compared well to the values provided by the Department of Health for dried food products. This may be

found in the guidelines for environmental health officers on the interpretation of microbial analysis data of food.

Table 3.1: Microbial Specifications of Commercial DF

Microbial	Specified Limit (CFU/gram)
Yeast and mould	Less than 1000
TMA	Less than 1000
Salmonella	0
Clostridium	0

(http;//www.doh.gov.za/docs/regulations/foodstuffs/microbiological.pdf)

Since high levels of yeast and mould are expected in the pineapple peel, several processing options need to be explored to determine the best method of reducing the microbial contamination to acceptable levels.

3.2. Objective

The objective was to develop a process that was most effective in the removal of the high microbial load associated with pineapple waste material.

3.3. Materials and Methods

3.3.1. Sample Preparation and Collection

Representative 30 kg batches of peel and core were collected from Collendale Cannery in April 2002 and couriered to Cape Town under low temperature conditions. On arrival the batches were divided into three sub samples to allow for comparison of the most effective method for the removal of the microbial load.

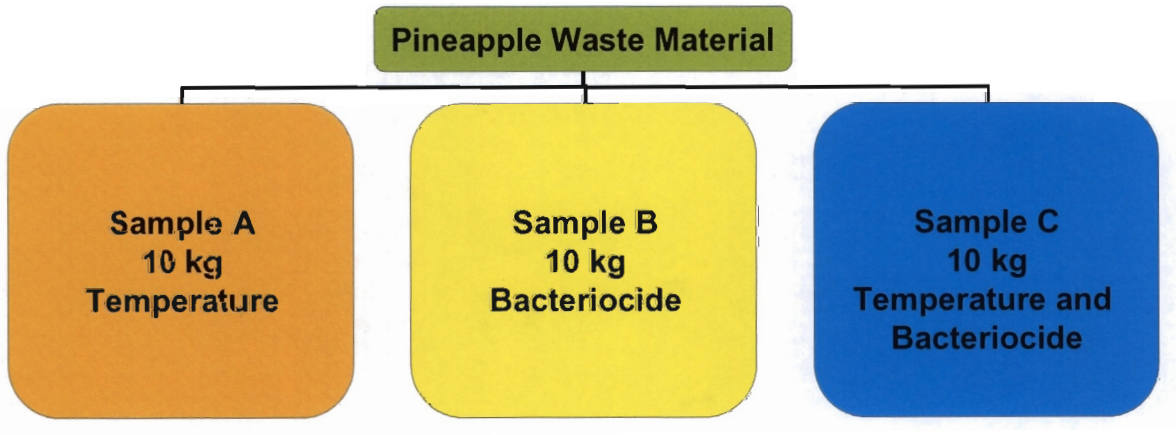


Figure 3.1: Process Used for the Removal of the Microbial Load

3.3.2. Microbial Methods

Microbial testing was subcontracted to Swift Micro Laboratories (Pty) Ltd: the following methods were used:

- *Total Microbial Assay (TMA) count (Method No. 35 SWJM)*
- *Yeast and Mould Count (Method No. 50 SWJM)*

- *Clostridium Method (Method No SWJM 16)*
- *Salmonella (Method No SWJM 42)*

(Confirmation tests were done using the same method numbers for both *Clostridia* and *Salmonella*)

3.3.3. Heat Treatment

Three ten kilogram samples were dried in an oven at 36, 50 and 105°C. Three representative fractions were used for microbial analysis.

3.3.4. Bacteriocide Treatments

Inspexx and Oxonia bacteriocides were obtained from Ecolab (Pty) Ltd. These solutions were made up to 0.02% (w/v) in distilled water and a ratio of 1:10 (w/v) of pineapple material to bacteriocide solution was used to wash the pineapple peel and core for a period of one hour. The washed material was placed in a previously sterilized Trespade sausage screw press to remove the excess liquid. Three representative aliquots of the solid material were submitted for microbial analysis. For the washed sample a 500 g sample was placed in a pan sieve with an aperture size of 125 μm. The sample was washed with running cold or hot tap water for a period of five minutes to yield the cold and hot wash samples respectively.

3.3.5. Heat and Bacteriocide Treatment

A ten kilogram sample of pineapple material was washed according to the process mentioned in 3.3.4 and then the pineapple material was dried according to process 3.3.3. Three representative aliquots were submitted for microbial analysis.

3.4. Results and Discussion

The microbial contents were evaluated (Table 3.2, 3.3 and 3.4) after drying at different temperatures and after various pre-treatment steps with two types of bacteriocides as described and the results compared with those obtained on untreated material. *Clostridia* and *Salmonella* were not detected in the starting raw material and it was therefore not necessary to test for these under the specific processing treatments.

Table 3.2: Microbial Loads of the Peel and the Core Material Processed at Different Temperatures

Temperature (°C)	Total Microbial Assay (CFU/gram)		Yeast (CFU/gram)		Mould (CFU/gram)	
	Peel	Core	Peel	Core	Peel	Core
Fresh	3×10^6	1×10^6	14×10^3	0.7×10^6	N/G	340
36	100×10^6	1.6×10^6	75×10^6	720×10^3	0	340
50	21×10^3	2.3×10^3	26×10^3	30	8×10^3	120
104	10	20	N/G	N/G	N/G	N/G

The data presented are the means of three replicates, N/G = No Growth

The results in Table 3.2 indicate that the Total Microbial Assay (TMA), yeast and the mould growths are destroyed at high temperatures. Both the peel and the core have a reduction of more than 10^6 TMA counts while all the yeast and mould growth is prevented with increased drying temperatures from 36° to 104°C. Drying at 104°C is effective in reducing the TMA, yeast and mould counts so that it meets the required specifications.

Table 3.3: Effect of Pre-treatment Washing on the Microbial Growth

Pre-Treatment	TMA (CFU/gram)		Yeast (CFU/gram)		Mould (CFU/gram)	
	Peel	Core	Peel	Core	Peel	Core
Fresh	3×10^6	1×10^6	14×10^3	14×10^3	N/G	340
Inspexx then Cold wash	2×10^6	6×10^3	18×10^6	720×10^3	N/G	N/G
Oxonia then Cold Wash	212×10^3	37×10^3	N/G	240	N/G	260

The data presented are the means of three replicate, N/G = No Growth

From the results in Table 3.3, although Oxonia is more effective than Inspexx in reducing the TMA and yeast counts, however the product does not meet the required specifications

Table 3.4: Effect of Bacteriocide, Washing and High Temperature (104°C) Drying on the Microbial Growth

Pre-Treatment	TMA (CFU/gram)		Yeast (CFU/gram)		Mould (CFU/gram)	
	Peel	Core	Peel	Core	Peel	Core
Fresh	3.7×10^6	1.6×10^6	14×10^3	720×10^3	N/G	340
Cold wash[a]	10	20	N/G	N/G	N/G	N/G
Hot wash[a]	N/G	30	N/G	N/G	10	N/G
Inspexx and Cold Wash	N/G	N/G	N/G	N/G	N/G	N/G
Oxonia and Cold Wash	10	N/G	N/G	N/G	N/G	N/G

The data presented above are the means of three replicates [a] without bacteriocide treatment, N/G = No Growth

The results (Table 3.4) confirmed that drying at 104°C destroys all the TMA, yeast and mould irrespective of the pre-treatment and therefore indicates that high temperature drying is an effective method of reducing the microbial load in the DF product.

3.5. Conclusion

The microbial stability is very important for a DF product and one of the concerns regarding the processing of pineapple waste is trying to reduce the high yeast and mould count. Drying at a high temperature (104°C) for a minimum time of 5 hours is the only practical solution to reduce the TMA and the yeast and mould counts to below allowed limits. Although Inspexx and Oxonia bacteriocides were used the results have shown that they are ineffective, they were only effective if the samples were subsequently dried at high temperatures (104°C). Although the high temperature can provide a microbiologically stable product, it is important to be aware that it may destroy the bioactives and cause browning reactions that discolour the DF thereby reducing its commercial value. Should the raw material be used for commercial production better handling conditions on the processing line should limit the initial load, and minimize problems during the fibre production.

CHAPTER 4

4. DEVELOPMENT OF A PROCESS FOR THE EFFECTIVE REMOVAL OF NEUTRAL SUGARS

4.1. Introduction

An extensive review of the literature indicates that the main use of fruit waste is as a fermentation substrate for the production of organic acids or as a matrix for extraction of proteases (Watson 1994). These processes have been pursued worldwide with varying degrees of success, but investigation shows that they do not appear to be commercially feasible in the South African context (citric acid and ethanol can be produced more effectively by fermentation of sugar cane and the demand for bromelin – proteolytic enzyme, is too small). The opportunity lies in exploitation of the carbohydrate fraction for its intrinsic value rather than as a substrate. The carbohydrates, comprising more than 90% of the solids, are largely fibrous in nature and contain both soluble and insoluble fibre. DF is gaining increasing importance in the health field and soluble fibre is currently being cited as an important factor in the control of GIT disease as it promotes the growth of colonic *Bifidobacteria.* These bacteria are instrumental in the production of short chain fatty acids that lower cholesterol, lower the pH of the colon and help establish a suitable colon environment.

Although various methods have been employed for the development of DF from fruit waste material, according to Larrauri (1999), the DF must meet the following characteristics:

- *Total DF content not less than 9%*
- *Low lipid (fat) content*
- *Low caloric value (< 8.4 kJ/g)*
- *No nutritionally objectionable components*
- *Be as concentrated as possible so that minimal amounts may have maximum physiological effect.*
- *The fibre must be bland in taste, colour, texture and odour.*
- *Have a balanced composition (IDF and SDF fractions) and adequate amounts of associated bioactive compounds.*
- *It should also have low microbial content, have a good shelf life and not have any adverse affects on the food it is added to. In addition, it must be compatible with the food processing methods.*
- *The fibre should also have a reasonable price and positive consumer opinion.*

According to Larrauri (1999) the following are some developments in the manufacturing of DFs.

- *Increased the level of soluble fibre and improved texture from vegetable material by enzymatic treatment. Gould (1989) treated DF from plant straw with alkaline hydrogen peroxide that resulted in the removal of lignin and left the cellulose and hemicelluloses thereby producing a fibre with improved WBC and swelling capacities.*
-

- *Sugar beet peel and roots can be washed with antioxidant and bacteriocide solutions, thereby producing effective DF for weight loss programmes.*

- *The DF content may be improved through fermentation processes with Aspergillus oryzae. This can also lower the caloric value and improve the water and oil binding capacity of the prepared fibre.*

Ground oat hulls can be treated with approximately 5% sodium hydroxide solution in an autoclave at 100-200 °C and at 0.1 MPa to 1.0 MPa to remove the silica and lignin from the oat hulls. The digested material is filtered, neutralized and bleached to form the desired end product.

Internationally, the demand for DF is increasing due to its health benefits. In South Africa DFs are typically imported at between R10.00 and R80.00 per kilogram. Locally produced fibre will replace imports and it is expected that a unique product will be developed by virtue of the associated bromelin, vitamins, and antioxidants (which include polyphenols) and show much greater activity than those from oranges, lemons and apples (Larrauri *et al.* 1997). This will add a competitive advantage in the market place.

Based on the high levels of microbial contamination and sugar content of the pineapple waste material, processing options were explored in an attempt to reduce both the neutral sugars and the microbial load. These processes were based on the basic DF processes assessed by Larrauri (1999) who evaluated wet milling and washing processes used in DF production from fruit by-products, as these were effective in the

removal of the neutral sugars and the microbial load in the raw material. Products of these processes were evaluated by comparing the biochemical and functional properties of the fibre at the various stages.

4.2. Objective

The main aim was to develop a process that was effective in the removal of the neutral sugars in the pineapple waste raw material and to evaluate the effects that basic processing techniques have on the functional properties of the product.

4.3. Materials and Methods

4.3.1. Sample Collection and Processing Treatments

In January 2002, a 40 kg representative batch of each of pineapple peel and core were collected. This batch was sub-divided into four sub samples (Figure 4.1 A-D). Each sub samples were treated according to processes 1-4 that are described in Figure 4.2 and the analysis was done in triplicate on each of the processed sub samples.

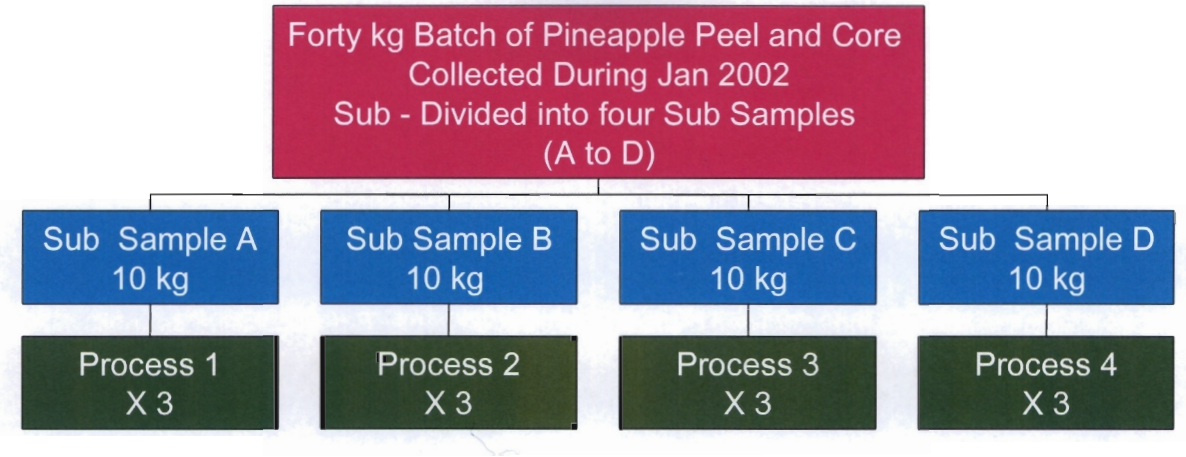


Figure 4.1: Process used to Evaluate the Effect of Basic Processing Practices on DF Characteristics

The processing treatments that were used are described in Figure 4.2

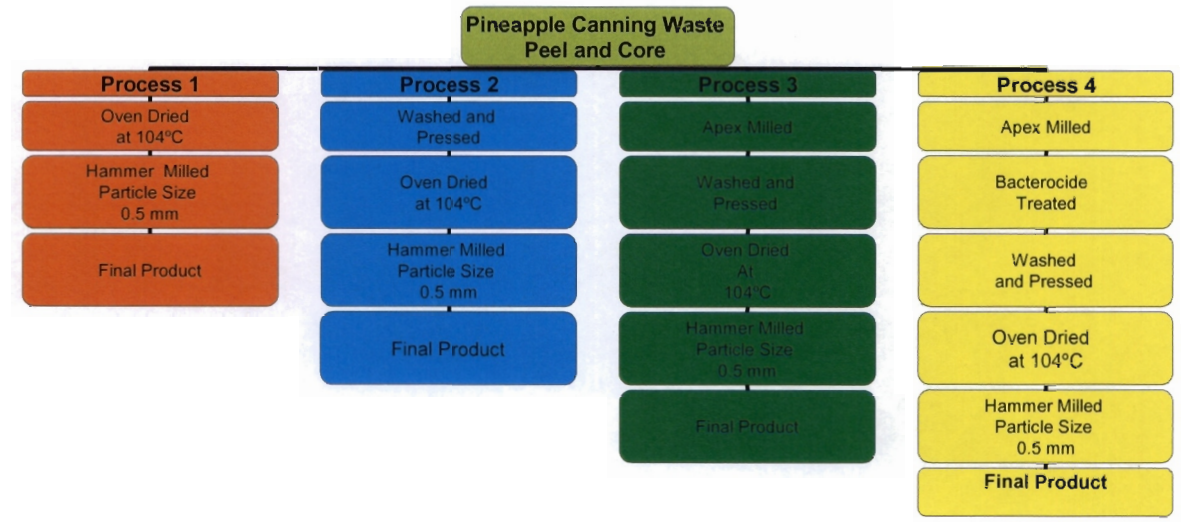


Figure 4.2: Processes Used in the Preparation of Pineapple Peel and Core

- *Wet Milling*

The Apex mill was used to reduce the particle size to 75 mm to facilitate the drying and subsequent dry milling processes.

- *Washing Treatment*

A 500 g sample was placed in a pan sieve with an aperture size of 125 μm. The sample was washed with running cold tap water for a period of five minutes.

- *Bacteriocide Treatment*

The pineapple peel and core samples were soaked in a 5% (v/v) solution of Inspexx bacteriocides for a period of five minutes prior to washing under running cold tap water for a period of two minutes.

- *Pressing Treatment*

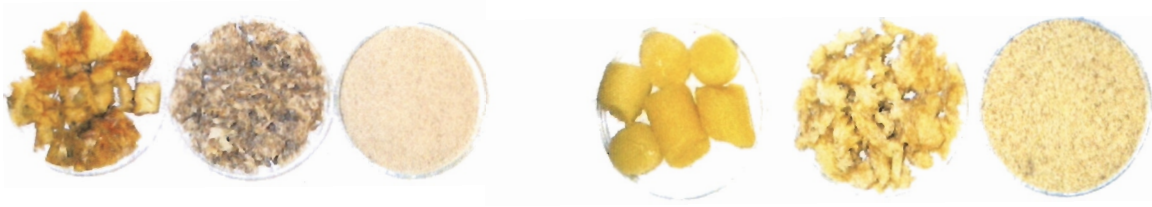
After the washing process was completed the sample was pressed in a Trespade sausage screw press to remove 80% of the excess water.

- *Drying Process*

A convection oven or a tunnel dryer set at 104 °C were used for drying, and the samples were held at designated temperature until constant mass was obtained. Trays were presterilized with a bacteriocide (Oxonia), and the temperature was monitored during the process with data trace probes (supplied by MESA laboratories Model Number FRB.)

- *Dry Milling*

After drying, the samples were comminuted in a hammer mill (Scientec RSA No 423) with a sieve size of 0.5 mm. The dry DF product was collected and placed in a sealed sterilized glass container. Figure 4.3 shows the fresh, dried and milled pineapple peel and core samples.



(A) (B)

Figure 4.3: Fresh, Dried and Milled Samples of the Peels (A) and the Core (B) during Processing

The final fibre products produced from the four different processing methods were characterised at each stage of the processing in the same way as the fresh raw starting material. Functional behaviour was also evaluated.

4.3.2. Analytical Methods

The mineral and ash content, carbohydrate and bioactive methods used in the evaluation of the produced DF have been described in detail in Chapter two. The neutral sugar (NS) content was determined in conjunction with the colour L/*b* ratio as this provided an indication of the amount of NS present. The higher the sugar content the darker the dried product will be due to the browning reaction that occurs during the

drying process. The colour results were compared to the sugar profile to establish which process was most effective in the removal of neutral sugars.

Vitamin C, vitamin E, polyphenols as well as the enzyme bromelin were evaluated before and after the drying steps, since the vitamins are sensitive and can be destroyed at high temperatures. As most of the polyphenols are soluble in water and some of them are also sensitive to heat, it was important to evaluate the level of polyphenols that remained after the washing and drying steps.

4.3.3. Functional Properties

The functional properties measured included the following: water binding capacity (WBC), oil binding capacity (OBC), density and pH. These tests were applied during the different processing steps and on the final dried product. Each test was conducted in triplicate.

Water Binding Capacity

The WBC was determined using the technique described by Chau *et al.* (2003). One gram of sample was weighed into 10 mℓ of distilled water and the solution was stirred for one minute. The fibrous suspension was centrifuged at 2200g for 30 minutes and the supernatant volume measured. The WBC was expressed as grams of water held per gram of sample.

Oil Binding Capacity

The OBC was determined by the method described by Chau *et al.* (2003). In this case one g of sample was mixed with ten mℓ of oil (sunflower oil, density 0.92). The suspension was vortexed followed by centrifugation at 2200g for ten minutes at 25 °C. The OBC was expressed as grams oil held by gram of sample.

Density and pH

The pycometer was used for the determination of the density and kerosene was used instead of water, as DFs are insoluble in kerosene.

A pH meter Copenhagen Radiometer PHM 82 with a combined electrode was used for the pH measurements that were conducted on one g of the sample that was mixed with ten mℓ of distilled water.

Colour

Colour was determined by using the Gardner Color guard system / 05 (BYK Gardner).

The following co-ordinates were determined:

- *Redness (a: negative to positive corresponds from green to red)*
- *Yellowness (b: negative to positive corresponds from blue to yellow)*
- *Lightness (L: 0 to 100 corresponds from black to white)*

The colour results were expressed as L/*b* ratio. A high L/*b* ratio will indicate a colour closer to white while a low L/*b* ratio indicates a colour closer to yellow.

4.4. Results and Discussion

Processes one to four represents samples A to D respectively.

4.4.1. Effect of Processing on Carbohydrate Composition

The carbohydrate composition of the peel and core samples processed by each of the four methods was assessed. This included the ratio of insoluble and soluble DF as well as the determination of pectin and the Klason Lignin content; these comprise a high proportion of the insoluble and soluble DF components. The neutral sugar content and the caloric values were also determined. The results at a 0% moisture basis are presented in Tables 4.1 and 4.2 for the peel and core, respectively.

Table 4.1: DF Components of Processed Peel

Process	TDF[a] (g/100 g)	IDF (g/100 g)	SDF (g/100 g)	Pectin (g/100 g)	Lignin (g/100 g)	Total Carbohydrates (g/100 g)	Energy (KJ/100 g)
Dry and Mill (1)	60.4	60.4	n/d	10.8	27.3	30.4	664
Wash, Dry and Mill (2)	70.7	70.7	n/d	8.9	23.9	14.9	520
Wet Mill, Wash, Dry and Mill (3)	81.1	81.1	n/d	10.0	28.4	8.3	369
Wet Mill, Bacteriocide, Wash, Dry and Mill (4)	75.8	75.8	n/d	9.0	25.3	13.5	376

The data presented is the arithmetic mean of three sets of results on each on the sub samples A-D Fig 4.1,[a]TDF = Sum of IDF and SDF, n/d = not detected

Table 4.2: DF Components of Processed Core

Process	TDF[a] (g/100 g)	IDF (g/100 g)	SDF (g/100 g)	Pectin (g/100 g)	Lignin (g/100 g)	Total Carbohydrates (g/100 g)	Energy (KJ/100 g))
Dry and Mill (1)	59.2	59.2	n/d	9.5	34.3	31.4	609
Wash, Dry and Mill (2)	88.8	88.8	n/d	4.8	30.6	8.6	361
Wet Mill, Wash, Dry and Mill (3)	79.3	79.3	n/d	6.8	32.8	17.8	350
Wet Mill, Bacteriocide, Wash, Dry and Mill (4)	83.0	83.0	n/d	4.8	28.4	14.2	308

The data presented is the arithmetic mean of three sets of results on each on the sub samples A-D Fig 4.1, [a]TDF = Sum of IDF and SDF, n/d = not detected

The results show that the washing and wet milling processes have reduced both the carbohydrate and caloric values. This could be attributed to the removal of the fats, proteins and neutral sugars during the washing process. This change has led to subsequent increases in the TDF content. This can be seen in Table 4.1 where process 1 has the highest total carbohydrates (30.4 g/100 g) and total calories of 664 g/100 g while process three has the highest TDF (81.1 g/100 g) and highest IDF and subsequently the lowest carbohydrate content (8.3 g/100 g by difference), and the lowest calories (369 calories/100 g). No SDF was found in the peel samples and the levels of lignin and pectin contents in the peel are relatively constant throughout the various processes.

Higher pectin levels were obtained in the peel samples compared to the cores even though the core is expected to contain higher levels, since pectin is often associated with the fruit rather than the skin of the pineapple fruit. This result could be due to the juice extraction processes in the cannery which may have removed most of the pectin.

The limitations of the TDF methodology made interpretation of the results difficult. It has been found that NSP with a degree of polymerisation less than ten are solubilized by the proteolytic enzymes and these components are often lost during the filtration step and therefore not included as TDF (McCleary 2003). In addition, some non-digestible oligosaccharides are soluble in ethanol and therefore lost during the filtration process and are also not included in the DF estimates. This underestimation of TDF could explain the high pectin and low SDF contents obtained.

4.4.2. Effect of Processing on Mineral Composition

From the results in Table 4.3 and 4.4 the inclusion of the washing steps (process two, threeand four) resulted in the reduction of the mineral content, reflected in the ash contents of both the peel (1.9 g/100 g) and the core (1.2 g/100 g). The highest loss was the potassium content that has decreased by 0.9 g/100 g in the peel and by 0.8 g/100 g in the core this may suggest that it occurs in a more soluble form and is therefore easily removed. The washing step has also reduced the iron and phosphorous contents in the peel. Due to instrumental problems it was not able to measure the calcium and magnesium as done previously.

Table 4.3: Mineral Composition of the Processed Pineapple Peel

Process used	Na (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	P (mg/kg)	K (mg/kg)	Pb (mg/kg)	Ash (g/100 g)
Dry and Mill (1)	322	8	79	3963	12557	10	3.8
Wash, Dry and Mill (2)	384	15	39	3023	3688	12	2.6
Wet Mill, Wash, Dry and Mill (3)	426	8	88	3013	3500	7	1.9
Wet Mill, Bacteriocide, Wash, Dry and Mill (4)	315	16	64	3001	3200	8	2.6
[a]Flesh of the Fruit (Dry Basis)	74	6	30	518	8370		

The data presented is the arithmetic mean of three sets of results on each on the sub samples A-D Fig 4.1,[a]Gouws and Langenhoven, 1986 Food Composition Tables NRIND- MRC

Table 4.4: Mineral Composition of the Processed Pineapple Core

Process used	Na (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	P (mg/kg)	K (mg/kg)	Pb (mg/kg)	Ash (g/100 g)
Dry and Mill (1)	352	6	54	1124	9339	9.8	4.0
Wash, Dry and Mill (2)	373	4	46	1130	1682	10.5	1.0
Wet mill, Wash, Dry and Mill (3)	429	6	20	1150	1690	9.5	1.2
Wet Mill, Bacteriocide, Wash and Dry Mill (4)	470	15	27	1100	1723	8.1	0.9

The data presented is the arithmetic mean of three sets of results on each on the sub samples A-D Fig 4.1.

4.4.3. Effect of Processing on the Sugar Content

Xylose is the most prominent neutral sugar in both the peel and the core, followed by arabinose and galactose then glucose and mannose (Table 4.5).

Table 4.5: Percentage Monosaccharide Content in the Processed Fibre Products

Sample	Process	%Monosaccharides of Total CHO (g/100 g)					%Total CHO [a] in sample
		Ara	Xyl	Man	Glc	Gal	
Peel	Dry and Mill (1)	26.1	59.1	2.4	8.4	4.1	44.1
	Wash, Dry and Mill (2)	16.2	31.2	1.8	5.5	2.8	32.3
	Wet mill, Wash, Dry and Mill (3)	27.7	64.8	5.8	4.6	n/d	28.5
	Wet Mill, Bacterocide, Wash and Dry Mill (4)	11.3	12.7	8.9	8.8	8.6	17.7
Core	Dry and Mill (1)	21.9	29.6	15.0	16.2	17.4	45.9
	Wash, Dry and Mill (2)	36.6	47.3	2.0	10.6	3.5	45.8
	Wet mill, Wash, Dry and Mill (3)	34.2	43.7	4.0	11.1	7.0	37.8
	Wet Mill, Bacteriocide, Wash and Dry Mill (4)	31.8	44.1	2.9	21.2	9.7	34.4

The data presented is the arithmetic mean of three sets of result on each on the sub samples A-D Fig 4.1, [a]CHO = Carbohydrates calculated by dividing the sum of all monosaccharides by the total mass used. Ara = Arabinose, Xyl = Xylose, Man = Mannose, Gal = Galactose, Glc = Glucose, n/d = not detected

The results in Table 4.5, shows the dry milling process produced a DF with the highest neutral sugar content, due to the omission of the washing and wet milling steps. The washing process appeared to remove some of the water-soluble neutral sugars whilst the wet milling process apparently released more neutral sugars through the breakage of the cell wall material.

For the peel, process 4 reduced the xylose and arabinose monosaccharides content compared to process 1. This could mean that insoluble material composed of associated cellulosic material such as arabinoxylans was removed.

4.4.4. Effect of Processing on Colour of Final Product

It is clear from the results that NSs were washed out resulting in a changed L/*b* ratio (Table 4.6).

Table 4.6: Colour Results of the Processed Peel and Core

Process	Peel				Core			
	Colour			NS	Colour			NS
	L	*b*	L/*b*		L	*b*	L/*b*	
Dry and Mill (1)	58.7	19.9	2.9	44.1	63.1	19.7	3.2	45.9
Wash, Dry and Mill (2)	60.9	19.9	3.1	32.3	77.6	20.7	3.7	45.8
Wet mill, Wash, Dry and Mill (3)	61.8	18.1	3.4	28.5	87.5	17.0	5.1	37.8
Wet Mill, Bacteriocide, Wash and Dry Mill (4)	49.3	17.5	2.8	17.7	74.7	21.3	3.5	34.4

The data presented is the arithmetic mean of three sets of results on each on the sub samples A-D Fig 4.1.

From the results presented in Table 4.6, the processes two and three resulted in a product with a more favourable colour; this was more noticeable with the core material. The inverse relationship between colour and neutral sugars content confirms the theory that neutral sugar behaviour is a relevant factor. Lario *et al.* (2004) have shown that high temperature drying can cause browning of the NS which results in the reduction of the L values and the increases in the *b* values. The L/*b* ratio therefore reflects the ratio of lightness or darkness of the fibre and can be used to determine the effectiveness of a process in the removal of the NS – the more NS removed during processing the higher

the L/*b* ratio and the more neutral the colour of the final end product. Process one had a lower L/*b* ratio as compared to processes two and three because the neutral sugars were not removed and they could have possibly caramelised during drying resulting in browning and the discoloration of the final end product.

In process 4 the treatment with the bacteriocide appears to have lowered the L/*b* ratio for both the peel and the core samples. It is also possible that the bacteriocide treatment could also have contributed to the darkening of the product possibly by oxidation of other components such as the phenols.

4.4.5. Effect of Processing on the Associated Bioactives

As all four processes dry the product at 104°C, and no precautions were taken to prevent oxidation, it can be assumed that components such as enzymes and antioxidants that are generally sensitive to heat, oxygen and light are destroyed during the drying process. The vitamin E content of the fresh raw material was too low to be deemed important (Chapter two) and was therefore not monitored.

Assessment and comparison of total antioxidant activity, vitamin C, total phenols and bromelin showed that, as expected (Table 4.7) the high temperature drying destroyed the bromelin activity and reduced the vitamin C substantially, while the reduction of the total antioxidant activity was comparably less. It appears that any potential antioxidant activity after drying would be present as phenolics as they were not affected.

These results indicate the need to investigate a low temperature drying process using alternative technology such as the incorporation of more effective bacteriocide processes to control the microbial contamination if a bioactive DF product is desired.

Table 4.7: Effects of Drying at 104°C on the Bioactive Activity

Bioactives	Units	Fresh		Dried	
		Peel	Core	Peel	Core
Total Antioxidant Capacity	AO units	19	15	13	8
Vitamin C	mg/kg	188	97	18	12
Total Phenols	mg/100g	33	38	3	3
Bromelin	NFU /kg	3.3	1.3	n/d	n/d

The data presented is the arithmetic mean of three sets of results on each on the sub samples A-D Fig 4.1, n/d = not detected.

4.4.6. Effect of Processing on Functional Properties

The results of the functional properties are shown in Table 4.8.

Table 4.8: Effects of Processing on the Functional Properties

Process	Peel				Core			
	WBC (g/g)	OBC (g/g)	Density	pH	WBC (g/g)	OBC (g/g)	Density	pH
Dry and Mill (1)	4.5	3.0	1.8	4.3	3.6	1.7	2.2	4.2
Wash, Dry and Mill (2)	5.7	3.0	1.9	4.2	4.9	2.9	2.3	4.3
Wet mill, Wash, Dry and Mill (3)	5.8	3.1	1.9	4.6	4.6	3.1	2.1	4.2
Wet Mill, Bacteriocide, Wash and Dry Mill (4)	2.8	2.0	2.0	4.2	4.8	3.1	2.5	4.1

The data presented is the arithmetic mean of five sets of results on each on the sub samples A-D Fig 4.1

The results show an increase in the WBC of the peel from processes one to three. The lower results obtained when a bacteriocide is used is unexplained as the process does not affect the core. The densities and the pH were unaffected by the different processing.

4.5. Conclusion

Process one produced a DF product with suitable functional properties but inferior colour and low TDF content due to the high amount of neutral sugars present. Processes two and three resulted in improved quality of the fibre product by removing and improving the colour, WBC and OBC. Although peel DF did not compare well with commercial DF's such as Apple Q plus fibre which has a WBC of 15 g/g (Van Schalkwyk, 2004) it compared well to cereal bran that had a WBC of 2 g/g (Van Schalkwyk, 2004). While process four, which makes use of a bacteriocide-washing step, appears to have adversely affected the colour, WBC and OBC reasons for this are not known at this stage.

Based on the requirements for commercial DF, certain modifications need to be made to the pineapple processing parameters to improve the DF composition to reflect a soluble component and better WBC. Although the neutral sugars have been removed during the washing step, additional steps are necessary to produce a competitive DF with a neutral colour and flavour.

Several modified processes such as temperature, chemical and mechanical processing parameters will be explored and the effect of these processes on the functional and chemical composition of the DF will be investigated in the following section (Chapter five).

CHAPTER 5

5. THE EFFECT OF CHEMICAL, TEMPERATURE AND MECHANICAL MODIFICATION ON DF CHARACTERISTICS

5.1. Introduction

The effect of chemical, thermal and mechanical processes that may modify the carbohydrate, bioactives contents and the functional properties of the DF were evaluated. It has been reported that such modifications may lead to increases in the DF content with concomitant increases in the WBC and therefore an improvement in the commercial value of the DF (Caprez *et al.* 1986; Cadden 1987; Meuser 2001).

The debate regarding the quantity of the DF versus the quality of DF remains unresolved because of the link between the molecular structure and functionality. In some cases the quantity of TDF may not correlate with good WBC because of the induced changes to the structure and the composition of the DF during processing. These changes are due to the hydrolytic enzyme reactions, chemical degradation or physical or mechanical stress. Changes in the DF particle size, surface area, and porosity can affect the rheological properties of the fibre in the food matrix as well as the sensory quality and the stability of the food.

5.1.1. Chemical Modification

Meuser (2001) conducted an extensive amount of work on the chemical modification of DFs using a variety of acids, alkalis, alcohols and enzymes such as protease and amylase. In some cases it was found that the modified product was not always superior to the unmodified product when tested in baking trials where the chemically modified

bran product had inferior dough forming abilities. Hydrogen peroxide treatment of DF's often produced a product with an off flavour whilst enzymatic modifications of the DF products enhanced the baked products by improving the colour and the crumb texture of the final product (Meuser 2001). In this section (5.1.1.1) the effect of using an alkali peroxide treatment during processing on the carbohydrate, mineral, bioactive and functional properties of the DF was evaluated.

Delignification with Alkali Peroxide

Agricultural by-products of the fruit and vegetable industries have inherent value and provide an inexpensive and readily available renewable source of lignocellulosic material. According to Gould (1986), the biggest problem is the fact that micro-organisms and enzymes are unable to convert the polysaccharide portion to monomeric sugars. This can be attributed to either the unavailability of cellulose and hemicelluloses due to its physical and chemical association between lignin and polysaccharides in plant cell walls or the high degree of crystallinity within the cellulose polymer.

Alkaline hydrogen peroxide treatment has been used to remove lignin that can comprise a substantial proportion of the fibres of non-woody lignocellulosic portions of plant fruits. The delignification process releases the celluloses and hemicelluloses and makes them readily available as carbohydrates sources. This results in enhanced WBC in the final product as well as increased solubility (Gould 1986).

Lignin can prevent cellulose degradation by acting as a barrier between the cellulolytic enzyme and its substrate. It has been found that the enzymatic cellulose degradation of

lignocellulosic material is inversely related to the lignin content (Gould 1986). Even when the lignin levels are low, the hydrolysis of the cellulose is limited by its own physical properties of the polysaccharides as the amorphous regions of the cellulose molecule are hydrolysed at higher rates than the microcrystalline regions.

Hydrogen peroxide is known to react with lignin under certain conditions and has found applications in the timber industry in the bleaching of wood pulps. A recent process developed by Gould (1986) involves delignification with alkaline hydrogen peroxide at a temperature between 80 and 120°C. However, it was found that this process caused partial degradation of the cellulose and did not preserve the hemicellulose B portion (soluble component) which is normally required for use as a high energy product. Sangnark and Noornhorn (2004) have shown that the cellulose content increases while the hemicellulose A and lignin decreases when the DF material is treated with alkali peroxide. During the delignification process hemicellulose A (insoluble component) is converted to hemicellulose B and the lignin is solubilised by the alkaline hydrogen peroxide treatment.

A patented process (US Patent 4,806 475) by Gould (1989) has been developed to preserves the hemicellulose carbohydrate portion after hydrolysis; it involves treating the substrate with hydrogen peroxide in a highly alkaline aqueous solution in a pH controlled environment. This process delignifies the substrate to a level where all the polysaccharides are made available by disrupting the crystal structure of the cellulose thereby making the product able to easily swell in water. Approximately 40-60% of the total original lignin content can be removed during this process. The success of this process is pH dependent and therefore requires control of the pH during the reaction. A pH of 11.5 is most effective; a pH below 11.2 will inhibit the delignification process;

and a pH above 11.8 will cause the hemicelluloses to solubilise resulting in an inferior DF product. This process is most effective at room temperature (25°C) and is facilitated by mechanical forces such as stirring which opens the fibre structure exposing its hydroxyl groups allowing it to bind to water more readily (Sangnark and Noornhorn 2004).

5.1.2. Thermal Modification

Thermal processing such as drying can lead to measured increases in the DF content through the formation of protein-fibre complexes. These protein-fibre complexes form aggregates that are resistant to enzymes used in the DF analysis and they therefore behave as DF (Caimre and Flint 1991). Caprez *et al.* (1986) also found that boiling and extrusion processes can increase water-binding capacity of the DF product and improve the DF composition by increasing the soluble DF fraction. However, their results have shown that drying the product at temperatures above 60°C could decrease the WBC.

Thermal processing can induce changes to the properties of DF in a variety of ways. Svenberg *et al.* (1997) showed that heat processing of vegetables hydrolyses the intermolecular bonds of the polysaccharide chains, resulting in the reduction of the molecular weight and consequent viscosity of these polysaccharides.

Thermal stress applied to cereals can lead to an increase in the soluble fibre content; it may also cause an increase in the solubility; and cause partial depolymerisation of soluble DF (Meuser 2001).

Extrusion involves combined heat and mechanical processes that cause extensive damage to the polysaccharide structure (Meuser 2001). Theander *et al.* (1990) have also

shown that drying catalyses the Maillard reaction as a result of the high temperatures used during the processing.

Since thermal processing can play a significant role in the modification of DF it is important to evaluate the effect that temperature has on the structure and functional properties of the DF. In the preparation of fibres the double drum dryer is often selected as this is based on favourable capital and operation costs. However, it causes excessive darkening of the fibre product. The severe heat breaks the cell wall membrane releasing all the cell contents and therefore affects the stability of the polysaccharides particularly the pectin. The current project tested temperatures of 36, 50 and 104°C.

5.1.3. Mechanical Modification

Mechanical methods like particle size reduction combined with a separation process can bring about changes to physicochemical, nutritional and functional properties of the DF product (Cadden 1987). It has been reported that grinding effects of milling will negatively affect the hydration characteristics of the fibre as well as its texture (Cadden 1987). Most commercial DF powders have a particle size distribution of between 0.43 and 0.15 mm (for this project the smallest possible particle size that could be achieved with the hammermill). Milling to a smaller particle size had improved shelf life stability of the fibre and enhanced the sensory attributes of the final product. The reasons for this are not known, but it is believed that the microbial contaminants are retained in the coarser fraction (Larrauri *et al.*1997). Mechanical stress such as milling can lead to changes in the fibre length and polysaccharide structure.

The pineapple peel and the core fibres have been shown to contain mainly IDF, which is predominantly cellulose, and could therefore have similar characteristics to cereals with respect to the relationship of particle size and fibre length with WBC.

5.2. Objective

The aim was to evaluate the effect that chemical, mechanical and thermal modification processes have on the nutritional, bioactive, carbohydrate and mineral composition and functional behaviour of the DF.

5.3. Methods and Sample Preparation

The nutritional, mineral, bioactive, carbohydrate and functional tests described in Chapters three and four were used in the evaluation of the modified DF. Methods specific to each chapter will be further elaborated in the relevant chapters.

The protocol for collection and preparation of the pineapple peel and core samples is depicted in Figure 5.1.

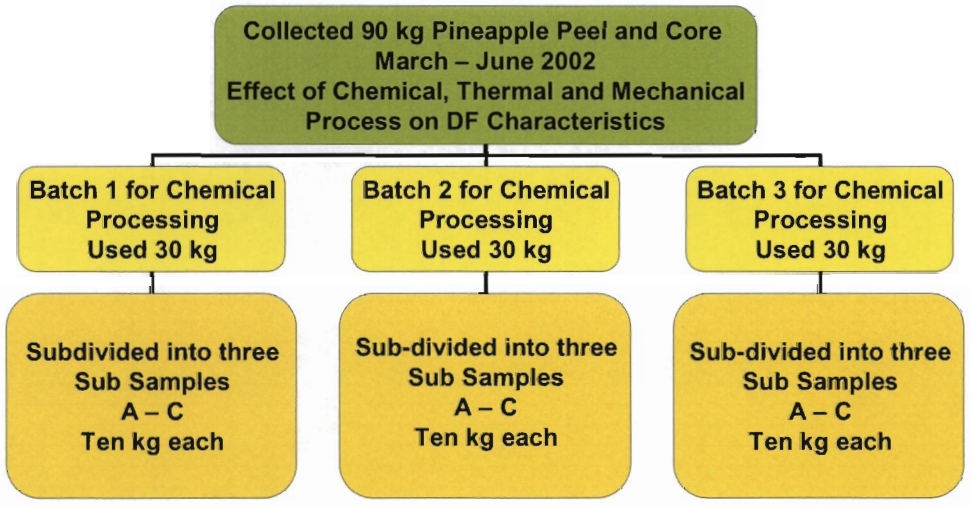


Figure 5.1: Process used to Evaluate the Effect of Modified Processing on the DF Characteristics

5.3.1. Procedure for Alkali Peroxide Treatment

The pineapple peel and core matrices were washed prior to alkaline peroxide treatment to remove the undesirable matter (Gould 1989). This step is not necessary if the material provided is free of microbial contamination; exceptions are made for substrates with high sugar levels as this often inhibits the drying process. In each case two kg aliquots of sub samples of pineapple peel or core were placed in 20 ℓ of one percent alkaline peroxide solution at pH 11.2 and paddled stirred for a period of eight hours. The mixture was filtered using a 1.77 mm pan sieve and the retained insoluble fraction washed with tap water until pH was neutral, prior to pressing and drying (Gould 1989). This process, depicted in Figure 5.2, was repeated three times.

Figure 5.2: Process for Chemical Modification

5.3.2. Procedure for Thermal Treatment

A 30 kg batch of starting material was milled, washed and pressed as described in Figure 5.2 before being aliquoted into three sets of ten kilogram samples. These were dried at three different temperatures (Figure 5.3). The final processed samples were tested to determine the effect that thermal processing has on the final product.

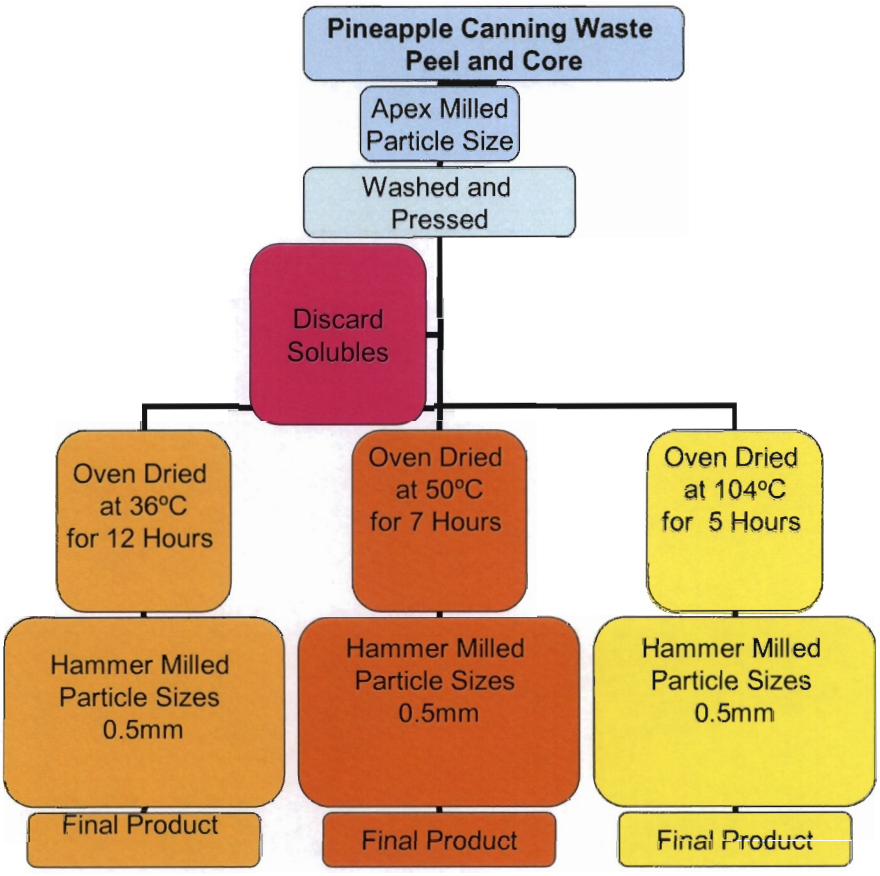


Figure 5.3: Process Used to Prepare Fibres for Comparison of Effect of Different Drying Temperatures on the Final Product

5.3.3. Procedure for Mechanical Treatment

A 90 kg batch of starting material was wet milled, washed, pressed and dried at 104°C before being divided into three sets of ten-kilogram aliquot sub samples. These were hammer milled to different particle sizes (using sieve sizes of 0.5, 0.8 and 2.0 mm), 0.5 mm was the smallest sieve size that could be used for the type of hammer mill used in this project as shown in Figure 5.4. The resultant product was analysed for nutritional and carbohydrate profiles, bioactive content and functional behaviour.

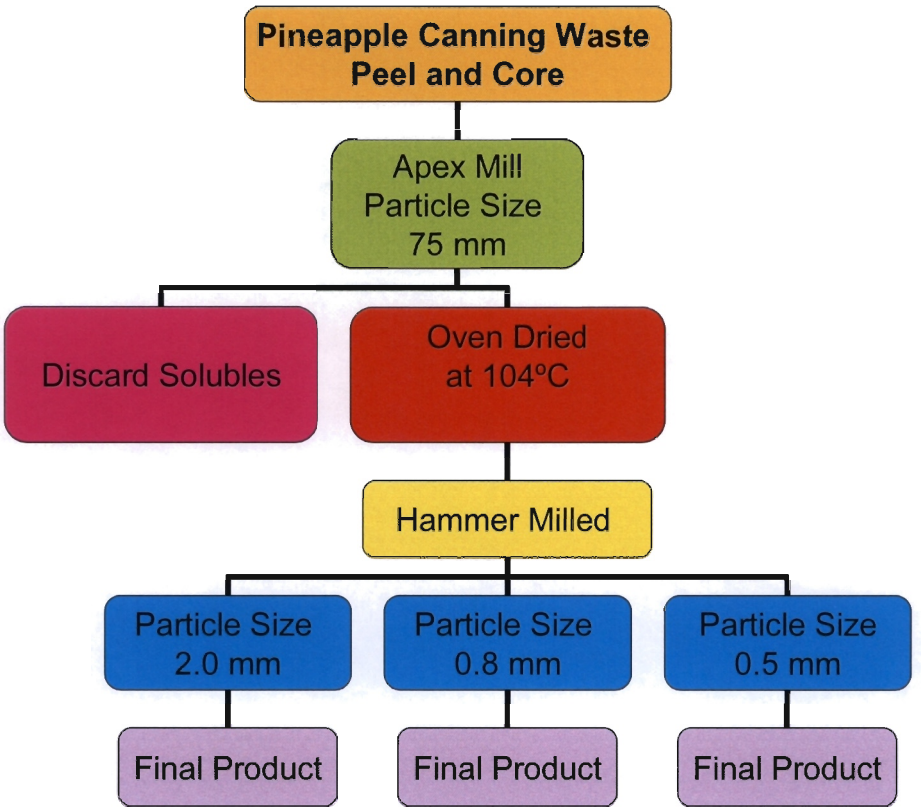


Figure 5.4: Process Used to Evaluate the Effects of Particle Size on the DF Characteristics

5.4. Results and Discussion

The effect of processing can only be quantitatively assessed by comparison to the unprocessed sample (baseline process) form a different batch which was harvested at a different time and therefore would have a different profile Consequently only trends can be discussed in this section.

5.4.1. Effect of Alkali Treatment on DF Characteristics

5.4.1.1 Effect of Alkali Peroxide Treatment on Carbohydrate and Caloric Profile

The carbohydrate profile and caloric content of the alkali peroxide treated pineapple peel and core samples are displayed in Table 5.1.

Table 5.1: Effect of Alkali Peroxide Treatment on the Carbohydrate and Caloric Composition (at 0% moisture basis)

Composition (g/100 g)	Baseline Process		Alkali Peroxide Treatment	
	Peel	Core	Peel	Core
TDF	60.4	59.2	87.0	88.5
SDF	n/d	n/d	n/d	n/d
IDF	60.4	59.2	87.0	88.5
Pectin/TDF	17.9	16.0	2.5	7.2
Klason Lignin	27.3	34.3	27.0	36.3
Klason Lignin/TDF	45.2	57.9	31.0	41.0
Calories (C/100 g)	664	609	43	25
Non Fibre CHO [a]	24.4	32.0	0.6	0.7

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1

[a] CHO = Carbohydrates, n/d = not detected

From the results presented in Table 5.1, it appears that there was an increase in the TDF contents of the alkali treated peel and core compared to the product from the basic process one Table 4.1, i.e. peel from 60.4 g/100 g to 87.0 g/100 g and the core 59.2 g/100 g to 88.5 g/100 g). Because different starting material was used for the two processes there is a considerable difference between the DF contents. This could be associated with the removal of the fats, proteins and neutral sugars in the treated material which would result in the reduced caloric values of the peel and core material. Decreases from 664 to 43 calories and 609 to 25 calories have been recorded for the peel and the core respectively. The alkali treatment has removed the soluble material i.e. pectin and SDF and the intended lignin. The comparison between the klason lignin and TDF ratios of the baseline and the alkali treatments, indicates that the alkali treatment has decreased the percentage klason lignin and TDF ratio for the peel and core by 14.2 and 16.9% respectively. In the peel, the pectin content has decreased from 10.8 to 2.2 g/100 g while the core has decreased less from 9.5 to 6.4 g/100 g this is expected as the washing process is capable of removing the hemicelluloses (Larrauri 1999).

5.4.1.2 Effect of Alkali Peroxide Treatment on Mineral Composition

The results of the mineral composition of the alkali processed peel and core are presented in Table 5.2.

Table 5.2: Comparison of the Mineral Composition of Alkali Treated and Process 1

Minerals	Baseline Process		Alkali Peroxide Treated	
	Peel	Core	Peel	Core
Sodium (mg/kg)	322	352	372	300
Iron (mg/kg)	179	54	8	4
Zinc (mg/kg)	8	6	6	0
Phosphorous (mg/kg)	3963	1124	3966	1000
Potassium (mg/kg)	12557	9339	3561	4001
Lead (mg/kg)	10	10	12	0
Total Ash (g/100 g)	3.8	4.0	3.2	3.0

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1

The results in Table 5.2 show that compared to the baseline process the alkali peroxide treatment removed some of the minerals particularly the potassium. The potassium content will always be greater due to the starting level. As a result the ash content in the peel and the core were also reduced by 0.6 g/100 g and 1.0 g/100 g respectively. The peel has also lost 171 mg/kg of iron through this treatment and the core 50 mg/kg. This is expected, since minerals are often removed during the washing process or as a result of the effect of the alkali peroxide on cell wall structure.

Effect of Alkali Peroxide Treatment on Neutral Sugars

The neutral sugar content of the alkali treated material and the product from the baseline process is shown in Table 5.3:

Table 5.3: Effect of Alkali Peroxide Treatment on the Monosaccharide Content

Monosaccharides	Monosaccharides of Total NS (g/100 g)			
	Baseline Process		Alkali Treated	
	Peel	Core	Peel	Core
Rhamnose	n/d	n/d	n/d	n/d
Arabinose	26.1	21.9	27.1	26.7
Xylose	59.1	29.6	65.3	59.2
Mannose	2.4	15.0	1.3	0.0
Galactose	8.4	16.2	4.0	10.8
Glucose	4.1	17.4	2.3	3.3
%Total NS in sample (g/100 g)	44.1	45.1	31.0	33.7

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1
n/d = not detected

The alkali peroxide treatment has reduced the total NS content of the core by 11.4% compared to the untreated process. This is attributed to the removal of the hexoses, as decreases of 15.0%, 7.6% and 14.1% are recorded for mannose, galactose and glucose respectively.

Table 5.4: The Effect of Alkali Processing on the Colour and NS Content

Process	Peel				Core			
	Colour			NS	Colour			NS
	L	*b*	L/*b*	(g/100 g)	L	*b*	L/*b*	(g/100 g)
Baseline Process	58.7	19.9	2.9	44.1	63.1	19.7	3.2	45.1
Alkali Peroxide	61.1	17.5	3.5	31.0	76.3	17.9	4.2	33.7

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1
NS = Neutral Sugars

The L/*b* colour ratio is higher in treated samples than the baseline study (i.e. peel by 20.7% and the core by 31.3%) showing that the alkali peroxide treatment improved the colour in that it yielded a product with a more neutral colour. These increases can be related to the reduction of the neutral sugars during processing, particularly the hexoses that caramelise during heating, resulting in a darker fibre product, and a low L/*b* ratio.

Effect of Alkali Peroxide Treatment on Bioactives

The results of the effects of the alkali peroxide treatment on the bioactive components are shown in Table 5.5.

Table 5.5: Effect of Alkali Peroxide Treatment on the Bioactive Components

Bioactive	Peel		Core	
	Baseline Process	Alkali Peroxide Treatment	Baseline Process	Alkali Peroxide Treatment
Vitamin C (mg/100 g)	188.0	n/d	97.0	n/d
Vitamin E (mg/100 g)	0.5	0.2	0.2	0
Total Phenol (mg/100 g)	33	0.9	38	0.6
Bromelin Activity (NFU Units/Kg)	3.3	n/d	1.3	n/d

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1
n/d = not detected

From the results displayed in Table 5.5, the vitamin E and C and the bromelin activity were destroyed during the alkali peroxide treatment. Only trace amounts of the phenolics were retained after the processing in the peel and the core respectively. Given that most the bioactives are destroyed during alkali treatment, this process is not suitable for retaining the bioactives in the fibre sample.

5.4.1.5 Effect of Alkali Peroxide Treatment on Functional Properties

The results of the effect of the alkaline peroxide activity on the functional activities are presented in Table 5.6. A comparison has been made with commercial products to benchmark the pineapple fibres.

Table 5.6: Effect of Alkali Peroxide Treatment on the on Functional Properties

Functional Property	Baseline Process		Alkali Peroxide Treatment		Commercial fibre	
	Peel	Core	Peel	Core	AQ plus Apple	Cereal Bran
WBC (g/g)	4.5	3.6	6.9	4.4	15	2
OBC (g/g)	3.0	1.7	3.2	3.0	2	2

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1 note each set is the mean of five individual readings, AQ plus apple fibre: reference Dolcre Herbacel product, Cereal Bran: commercial bran used in bread.

It is possible that the removal of hemicellulose A and Klason lignin components through the alkali treatment could have resulted in a 53% and 22 % improved WBC in both the peel and the core; this is in agreement with the conclusions deduced by Sangnark and Noornhorn (2004). OBC was also enhanced, with the core showing the bigger effect. These effects can be explained by the theory of Sangnark and Noornhorn (2004) that the alkali peroxide treatment removes celluloses and hemicellulose as it breaks the crystalline structure of the cellulose components by disrupting the H-bonding pattern between the polysaccharide chains. As a result a highly opened cellulose structure is formed which has more free hydroxyl groups available for binding to water molecules and thus a higher WBC was observed. An additional advantage of

this process is that it is irreversible and that this structure persists after drying. Since the pineapple DF consists predominantly of cellulose, lignin and hemicelluloses based on the findings by Sangnark and Noornhorn (2004), one may assume the WBC is effectively increased through the solubilisation and the removal of hemicellulose A components (insoluble components).

In summary the alkali treatment is a suitable treatment for the product of DF because it increased the IDF content through the removal of components such as the fats, proteins and neutral sugars and as a result decreased the caloric content of the DF product. The alkali treatment is also effective in the removal of some minerals such as potassium, and some of the soluble sugars, particularly the hexoses such as glucose and galactose. These sugars are responsible for the browning reactions and their removal resulted in a product having an improved colour after drying. The other benefit of the alkali process was to change structural carbohydrate components to allow for more functionality. The disadvantage was the destruction of most of the bioactives.

5.4.2. Effect of Temperature on DF Characteristics

Effect of Temperature on Carbohydrate and Caloric Profiles

The carbohydrate profile and mineral values for materials processed at different temperatures are presented in Tables 5.7 and 5.8. These results were all calculated at a 0% moisture basis.

Table 5.7: Effect of Temperature on the Carbohydrate Profile of the Peel

Temperature of Processing (°C)	TDF (g/100 g)	IDF (g/100 g)	SDF (g/100 g)	Pectin (g/100 g)	Klason Lignin (g/100 g)	Total CHO (g/100 g)
36	81.4	81.4	n/d	6.8	34.9	6.5
50	83.5	83.5	n/d	6.8	34.3	4.8
104	81.5	81.5	n/d	5.5	31.5	8.3

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1

n/d = not detected

Table 5.8: Effect of Temperature on the Carbohydrate Profile of the Core

Temperature of Processing (°C)	TDF (g/ 100g)	IDF (g/ 100g)	SDF (g/ 100g)	Pectin (g/ 100g)	Klason Lignin (g/ 100g)	Total CHO (g/ 100g)
36	81.3	81.3	n/d	5.8	44.4	14.1
50	79.5	79.5	n/d	7.1	35.6	15.9
104	79.0	79.0	n/d	9.3	41.5	17.8

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1

n/d = not detected

From the results in Tables 5.7 and 5.8 it is evident that processing at different drying temperatures did not alter the carbohydrate composition as expected of the peel or core. as expected during processing. Although the increase in temperature resulted in slight reductions in the Klason Lignin contents of the peel and the core, this variation may be an artefact associated with the complicated methodology involved in these determinations.

Effect of Temperature on Mineral Composition

The mineral composition of the processed peel and core are displayed in Tables 5.9 and 5.10:

Table 5.9: Mineral Composition of the Processed Pineapple Peel

Temperature of Processing (°C)	Na (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	P (mg/kg)	K (mg/kg)	Pb (mg/kg)	Ash (g/100 g)
36	372	7	148	3963	3397	11	1.9
50	319	7	213	3696	3192	11	2.2
104	384	15	139	3540	3688	13	1.9

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1

Table 5.10: Mineral Composition of the Processed Pineapple Core

Temperature of Processing (°C)	Na (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	P (mg/kg)	K (mg/kg)	Pb (mg/kg)	Ash (g/100 g)
36	303	4.3	38	1124	1558	11.1	1.3
50	301	4.5	45	1135	1192	11.3	1.1
104	373	2.3	46	1163	1682	10.5	1.3

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1

The results indicate that temperature did not have any influence on the mineral content of the peel and core material. Variance measured could be attributed to analysis errors and difficulty of representative sampling of the rough heterogeneous raw material.

Effect of Temperature on Neutral Sugar Composition

The neutral sugar profile of the whole peel and the core are given in Table 5.11.

Table 5.11: Neutral Sugar Content of Processed DFs

Monosaccharides	% Monosaccharides of Total NS (g/100 g) [a]					
	Peel			Core		
	Temperature (°C)			Temperature (°C)		
	36	50	104	36	50	104
Rhamnose	n/d	n/d	n/d	n/d	n/d	n/d
Arabinose	28	27	26	25	24	28
Xylose	53	63	59	50	53	50
Mannose	2	3	2	3	3	n/d
Galactose	12	1	8	12	11	10
Glucose	4	5	4	8	7	n/d
Total NS in Sample (g/100 g) [b]	43	34	44	32	33	31

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1,
[a] Percentage monosaccharides of the total carbohydrate determined by difference
[b] Percentage carbohydrate in the actual sample determined by subtracting from the proximate analysis, n/d = not detected; NS = Neutral Sugars

The hexoses (mannose, galactose and glucose) of the core material showed a slight decrease with increased temperature unlike the peel material where the results were variable.

The remaining results of the monosaccharides in the peel and core were inconsistent with an increase in temperature. Some results increased such as the xylose and arabinoses of the core while the others fluctuated with increased drying temperature. The reasons for these fluctuations may be due to analysis variation. During the heating,

the hydrogen-bonded networks are altered which results in the increased solubility of certain sugars. This could be an additional factor that causes variation in the results for the neutral sugar profile. Greenwood and Munro (1979) have mentioned that hexoses char more readily at higher temperatures than pentoses; this could explain the reduction in concentration of the glucose and mannoses in the core as drying temperatures increase (36° to 104°C). According to Greenwood and Munro (1979), monosaccharides and oligosaccharides are susceptible to browning when exposed to heat. This browning effect can be due to caramelisation, pyrolysis or the Maillard reaction. Caramelisation is due to the removal of water from the sugar molecule, while pyrolysis is a result of the cleavage of the carbon bonds. The Maillard reaction is a non-enzymatic browning process in which the reducing end of a sugar molecule reacts with an amino group of an amino acid or protein. The Maillard reaction is dependent upon temperature, pH and water activity. This reaction can be inhibited by sulphur dioxide. This reaction rate can increase should the pH and temperature increase. Greenwood and Munro (1979) have shown that a 10°C increase in temperature can double the Maillard reaction rate. In addition increases in the pH and water activity can also be responsible for increases in the Maillard reaction rate – [the main component reported by Holdsworth (1979), which characterises the intense odour of "burnt pineapple", is 2,5-dimethyl-4-hydroxy-2, 3-dihydro-3-furanose] acetic acid, furfural, formaldehyde, acetaldehyde and acetone are other products that are associated to thermal breakdown.

This information is confirmed by evaluation of colour of heat treated fibres. In the case of the pineapple peel and core, the increase in drying temperature has lead to charring of the hexoses probably through caramelisation and pyrolysis processes rather than the Maillard reaction. These browning effects can affect the nutritional value of the food negatively as in the case of the Maillard reaction, which causes a drop in the protein

levels. The monosaccharide profile is compared to the L/*b* ratio of the colour results in Table 5.12. L/*b* ratios are higher (except for the peel at 36°C) than those found measured for the baseline study.

Table 5.12: Comparison of the Neutral Sugar Content (NS) with the Colour L/*b* Ratio

Process	Peel				Core			
	Colour				Colour			
	L	*b*	L/*b*	NS	L	*b*	L/*b*	NS
Baseline Study	58.7	19.9	2.9	44.1	63.1	19.7	3.2	45.1
36°C	33.8	18.5	1.8	43.6	78.6	15.5	5.0	32.6
50°C	65.2	18.2	3.6	34.7	78.7	15.1	5.2	33.9
104°C	63.1	17.7	3.7	44.2	74.1	17.2	4.3	51.1

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1, NS = Neutral Sugars

The sugar content in pineapple material undergoes a caramelisation or pyrolysis reaction during the drying, which results in the darker colour at higher temperatures. In Table 5.12 the core shows a decrease in the L/*b* ratio as the temperature increases from 50 to 104°C, which signifies a darker colour. A lower temperature drying system is therefore required to obtain a neutral colour and this has to be looked at in conjunction with the removal of the soluble sugars with washing and control of the microbial load.

5.4.2.4 Effect of Temperature on Bioactive Activity

- **Vitamin C Activity**

The effects of drying at different temperatures on the Vitamin C content are presented in Figure 5.5

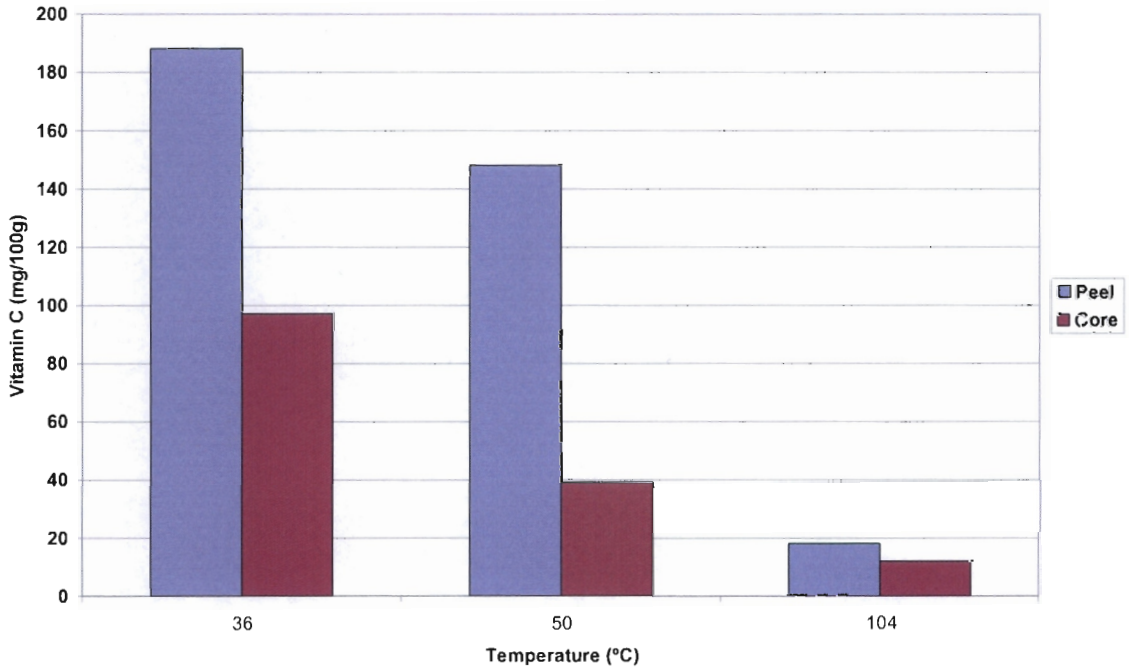


Figure 5.5: The Effect of Drying on the Vitamin C Contents of the Peel and Core

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1

An increase in drying temperature to 104°C lead to the decrease in the vitamin C activity of the peel and the core, of 90% and 88% respectively. Vitamin C is destroyed upon heating (Holdsworth 1979). This can be attributed to the oxidation processes that occurs upon exposure to high temperatures and light.

This activity can be lost if it is oxidised to 2,3-diketogulonic acid (DKGA) as this reaction is irreversible (Holdsworth 1979). The rate of oxidation is influenced by several factors including pH, trace metals, the presence of oxygen and an increase in temperature.

All these factors apply to the DF process. Priestley (1979) has mentioned that the aerobic oxidation of vitamin C produces dehydro-ascorbic acid and hydrogen peroxide.

- **Total Polyphenols**

Some phenols are sensitive to temperature and can oxidise at certain temperatures, therefore it is important to evaluate the effects that temperature has on the total phenol content. Two approaches were used; the one determined the effect of temperature (36°C, 50°C and 104°C) on the phenol activity; the other monitored the phenol activity during low temperature drying at 36°C as a function of time. A convection oven or a tunnel dryer set at 104 °C were used for drying the samples were dried until constant mass was obtained. Trays were sterilized with a bacteriocide (Oxonia), and the temperature was monitored during the process with data trace probes (supplied by MESA laboratories Model Number FRB).

(1) Effects of Drying Temperature on the Total Polyphenol Content

The phenolic content of the dried peel and the core at each of the drying temperatures are presented in Table 5.13.

Table 5.13: Effects of Processing on Total Phenol Composition

Temperature of Drying (°C)	Total Phenolic Content (mg/kg)	
	Peel	Core
36	5.2	5.7
50	3.7	4.8
104	3.3	3.8

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1

The results indicate that approximately 30% of the total phenols were destroyed in both the peel and the core samples when the drying temperature is increased from 36 to 104°C.

(2) Effects of Low Temperature Drying on the Total Phenols

The effect of low temperature drying (36°C) on the phenolic composition over eight hours is presented in Figure 5.6. Samples were taken every hour from the drying apparatus (tunnel dryer) and tested for total polyphenol activity.

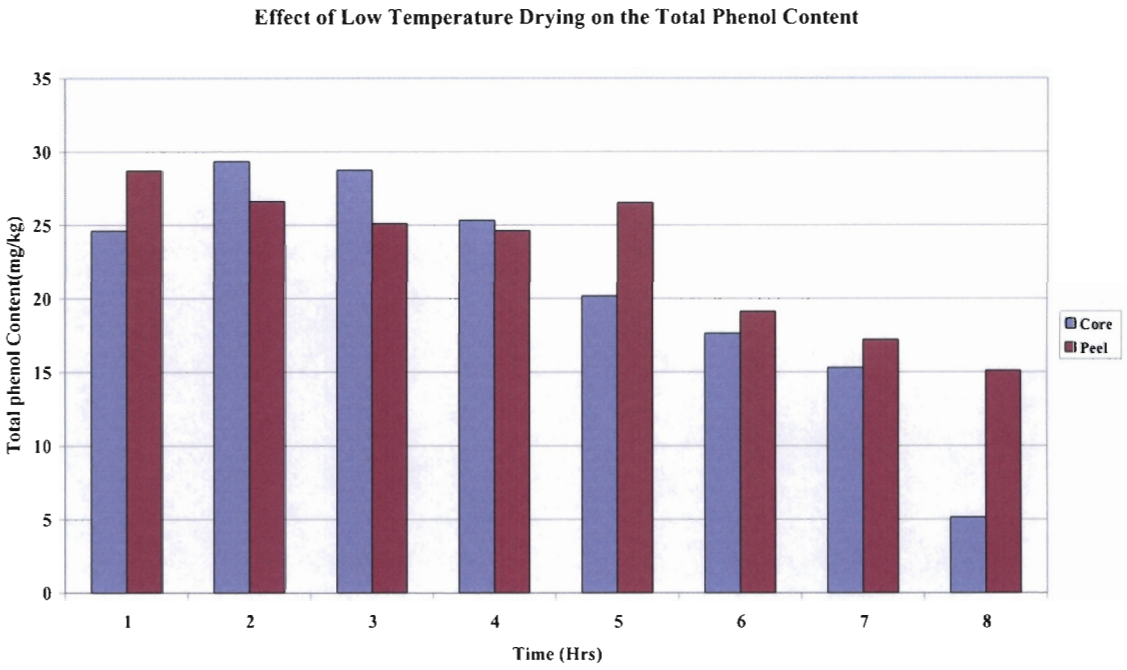


Figure 5.6: Effect of 36°C Temperature with Time on the Total Polyphenols in Peel and Core

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1

The results indicate that the total polyphenols are gradually destroyed with time even at a relatively low temperature. The reduction of the total phenols in the pineapple peel samples with an increase in duration of exposure to a constant temperature is supported by studies by Larrauri (1999) who also reported a 18% reduction but at 100°C and for

polyphenols in grape skins. Similar trends were also observed with cassava and wort, in which temperatures of 65-75°C resulted in significant reductions of polyphenols.

Larrauri (1999) suggests that the loss of polyphenols in fruit during heating can be due to three types of mechanisms:

- Release of bound phenolic compounds.
- Partial degradation of lignin which can also result in the release of phenolics.
- Thermal degradation of phenolic compounds.

- **Bromelin Activity**

The activity of the proteolytic bromelin enzyme is known to be stable at temperatures of up to 36°C, (Watson 1989), which presents the opportunity of retaining bromelin activity in processes that use low temperature drying operations. Figure 5.7 shows that after just one hour at 36°C, activity is greatly reduced. Loss of moisture to the atmosphere as opposed to incubation conditions at the same temperature may be instrumental in the destruction of enzyme efficacy.

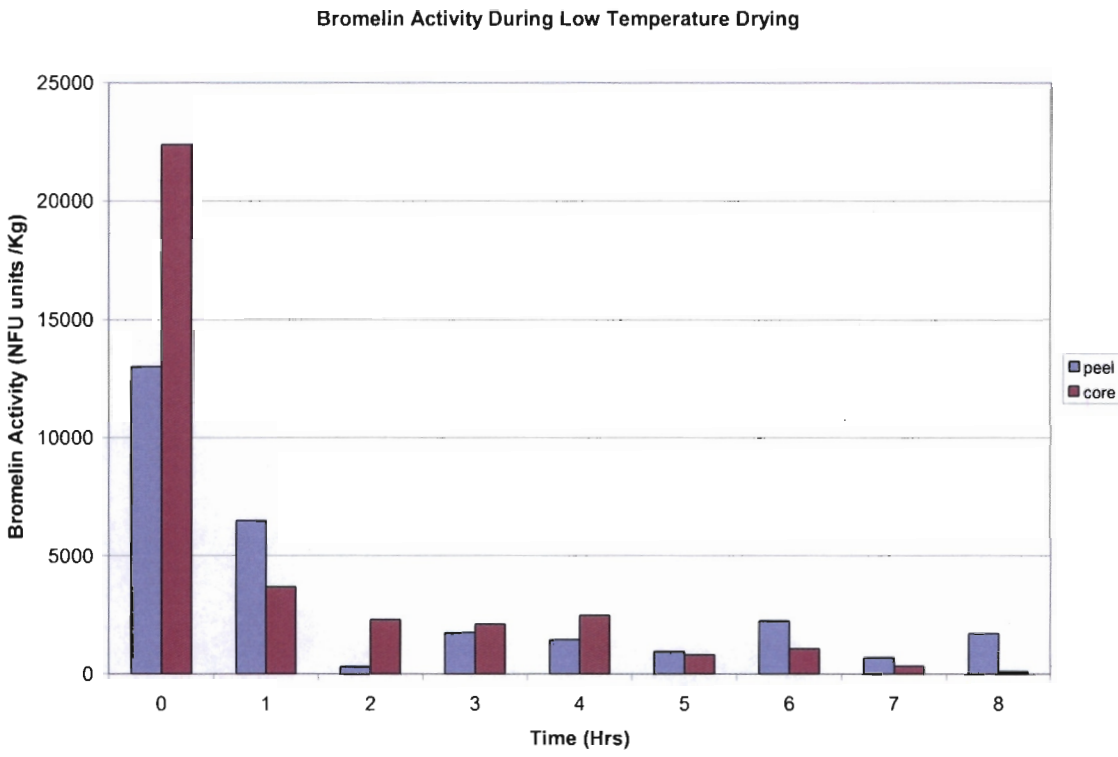


Figure 5.7: The Effects Drying at 36°C on the Bromelin Content

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1

Effect of Temperature on Functional Properties

The effects of drying at different temperatures on the functional properties are shown in Table 5.14.

Table 5.14: Effect of Thermal Processing on the Functional Properties

Temperature	Peel			Core		
	WBC (g/g)	OBC (g/g)	Density (g/g)	WBC (g/g)	OBC (g/g)	Density (g/g)
36°C	6.6	3.0	1.8	5.6	2.6	2.2
50°C	5.9	3.3	1.9	5.2	3.3	2.3
104°C	5.6	3.2	1.9	5.0	3.2	2.2

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1

Table 5.14 shows a decrease in the WBC in the peel (18%) and the core (12%) with increased drying temperatures from 36 to 104°C. The OBC and density results are not as affected by the different temperature used during the processing.

The increased processing temperature did not affect the carbohydrate, mineral or sugar contents of the final DF product, however it did destroy most of the bioactive activity. As a result of the high microbial load of the pineapple waste, a high temperature drying process is required to comply with specified limits and unless an alternative low temperature anti-microbial process is used, the chances of producing an antioxidant-rich based fibre product (AODF) are slim. Although drying is expensive, it is an important and critical step as it improves the shelf life of the pineapple DFs without

requiring the use of preservatives. In addition, drying reduces both the packaging and transport cost.

5.4.3. Effect of Particle Size on DF Characteristics

Effect of Particle Size on Carbohydrate and Caloric Composition

The carbohydrate composition of the peel and core samples assayed at each of the three particle sizes (0.5, 0.8 and 2.0 mm). Although these are larger than the particle sizes used in some commercial applications, they are adequate to demonstrate the effect of size reduction. The ratio of insoluble and soluble DF, pectin and Klason lignin contents are also reported as they comprise a high proportion of DF. Results presented in Tables 5.15 and 5.16 are calculated on a 0% moisture basis.

Table 5.15: The Effect of the Mechanical Modification on the Carbohydrate and Caloric Profile of the Peel

Particle Size (mm)	IDF (g/100 g)	SDF (g/100 g)	Klason Lignin (g/100 g)	Pectin (g/100 g)	CHO[a] (g/100 g)	Calories (g/100 g)
0.5	81.6	n/d	24.0	10.2	9.5	88
0.8	83.9	n/d	25.7	5.3	5.5	72
2.0	85.8	n/d	31.6	4.1	7.9	85

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1, [a]CHO= Carbohydrates

Table 5.16: The Effect of the Mechanical Modification on the Carbohydrate and Caloric Profile of the Core

Particle Size (mm)	IDF (g/100 g)	SDF (g/100 g)	Klason Lignin (g/100 g)	Pectin (g/100 g)	CHO [a] (g/100 g)	Calories (g/100 g)
0.5	79.0	n/d	35.4	6.8	17.8	69
0.8	84.9	n/d	37.6	6.9	11.2	58
2.0	87.3	n/d	31.6	9.1	8.5	42

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1

[a]CHO = Carbohydrates

From the results presented in Tables 5.15 and 5.16, more carbohydrate and hence a higher caloric value appear to be related with a decrease in particle size. This could be related to complexities in the methodology and experimental error associated with each method.

The results in Table 5.15 indicate that the particle size seems to affect the pectin content of the peel with a higher value being obtained when the particle size decreased (59% decreases in pectin from 0.5 to 2.0 mm change in particle size). This is the opposite to observations for the core where a higher value for pectin content with larger particle size. The opposites for the peel and the core could be due to the molecular and structural changes that the mechanical shear forces display on the structural polysaccharides within the cell wall structure that hold the pectin components together.

Since pectin is a hemicellulose, some of the pectin that is bound to the cell wall could be released proportionately with increased mechanical shear forces. Comminution to a small particle size could therefore result in a better IDF: SDF ratio due to release of more pectin as more rupturing of the cell wall occurs.

Effect of Particle Size on Mineral Composition

The results of the effect of mechanical processing on the mineral composition of the peel and the core are in Tables 5.17 and 5.18:

Table 5.17: Effect of Particle Size on the Mineral Composition on the Processed Pineapple Peel

Particle size (mm)	Na (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	P (mg/kg)	K (mg/kg)	Pb (mg/kg)	Ash (g/100 g)
0.5	384	15	139	3964	3688	13	1.9
0.8	330	14	127	3654	3685	14	2.0
2.0	432	8	142	3210	2785	15	1.9

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1

Table 5.18: Effect of Particle Size on the Mineral Composition of the Processed Pineapple Core

Particle size (mm)	Na (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	P (mg/kg)	K (mg/kg)	Pb (mg/kg)	Ash (g/100g)
0.5	370	4	46	1130	1682	10	1.3
0.8	261	4	30	1235	1594	11	1.1
2.0	317	4	24	1321	1316	10	1.1

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1

The results in Table 5.17 and 5.18 for the peel and core show that as expected there is no change in the ash content with an increase in particle size (0.5 to 2.0 mm).

Although some variation was found no distinct trends linking mineral content with particle size was noted, except for the potassium that has decreased with an increase in particle size in both the peel and the core As mineral content is measured by hydrolysing the material and removing organic compounds, particle size is unlikely to play a role.

Effect of Particle Size on Neutral Sugar Composition and Colour

Results of the effect of mechanical processing on the neutral sugar content and the colour L/b ratio of the peel and the core DF are presented in Table 5.19.

Table 5.19: Effect of Particle Size on the Neutral Sugar Content and Colour of Processed Fibre Products

Monosaccharides	% Monosaccharides of Total NS					
	Peel			Core		
	Particle size (mm)			Particle size (mm)		
	0.5	0.8	2.0	0.5	0.8	2.0
Rhamnose	n/d	n/d	n/d	n/d	n/d	n/d
Arabinose	35.5	26.0	32.0	38.7	33.7	25.0
Xylose	49.5	59.0	57.1	50.9	43.7	55.9
Mannose	2.4	2.4	n/d	n/d	4.1	3.7
Galactose	9.4	8.4	10.9	10.4	11.3	9.8
Glucose	3.2	4.1	n/d	n/d	7.1	5.7
Total NS (g/100 g)	50.0	44.1	37.2	51.1	36.1	32.2
Colour ratio L/*b*	4.8	3.5	3.3	4.3	4.3	4.1

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1, n/d= not detected

From the results both the peel and the core show a decrease in the overall NS content and the L/*b* ratio with an increase in particle size (0.5 to 2.0 mm). More NS have been removed from the core with the change in particle size from 0.5 mm to 2.0 mm (19%) compared to the 13% lost in the peel. This could be attributed to analytical variation as well as the different structure and composition between the peel and the core as it is not expected that a change in particle size will change the neutral sugar profile. The trend to a lower L/*b* ratio indicates that smaller particles have a preferable colour (closer to white).

Effect of Particle Size on Bioactive Components

The effect on bioactive compounds was not evaluated as the samples were dried at 104°C prior to milling and this high temperature has been shown to destroy most of the bioactives.

Effect of Particle Size on Functional Properties

Mechanical processing effects on the functional properties of the DF are presented in Table 5.20.

Table 5.20: Effects of Particle Size on the Functional Properties

Particle Size (mm)	Peel			Core		
	WBC (g/g)	OBC (g/g)	Density (g/g)	WBC (g/g)	OBC (g/g)	Density (g/g)

0.5	5.6	3.1	2.0	5.2	2.7	2.2
0.8	5.4	3.1	2.0	4.1	2.9	2.3
2.0	5.8	3.5	1.9	4.7	3.3	2.4

The data presented is the arithmetic mean of three sub samples indicated in Fig 5.1

Surprisingly different trends were noted for the two raw materials. The WBC of core material improved slightly with a decrease in the particle size whereas the peel did not Increased surface area allowing for increase water absorbency may play a role, however as the pineapple DF is largely a cellulosic type DF a decrease in WBC is expected with a decrease in particle size. This relationship between particle size and WBC is supported by Cadden (1987), who noted that cellulosic type fibres rely on their cellulosic matrix structure to bind and to hold onto water. The reduction in particle size can cause changes to this structure thereby reducing the ability of the fibre to bind to water (Sangnark and Noornhorn 2004). The OBC and the density were not affected by the change in the particle size.

The increase in particle size has lead to increases in the IDF and a decrease in the neutral sugar contents this is an artefact be due to analytical variation and methodology. Although the ash content remains similar, some of the minerals have been affected such as the potassium that has decreased with an increase in particle size in both the peel and the core. The WBC has not been largely affected by the change of particle size.

5.5. Conclusion

Modification of the DF through chemical, thermal or mechanical changes resulted in changes to the DF profile, with respect to the colour and the monosaccharide profile. The pineapple DF product could be a suitable DF source for use in a variety of food applications, even though the bioactive content was much lower than anticipated.

In some cases, a single process or a combination of modifications will be necessary to obtain the DF product that could be used in higher concentrations without sacrificing the quality of the final product. These modifications will be needed to improve the composition, structure, sensory and physicochemical properties of the DF product. The behaviour in the food products should also be evaluated, if possible by a relevant food science organisation or appropriate industrial partner.

CHAPTER 6

6. GENERAL CONCLUSION

The pineapple waste consists mostly of DF and neutral sugars. The fresh peel and core consist predominately of IDF, which has a large Klason lignin component. Since the neutral sugar content is responsible for the sweet taste and odour of the final product, it is important to remove these sugars to enable production of a competitive product of neutral colour and taste. A washing step is necessary as this will help in the removal of the neutral sugars and thereby reduce the risk of product discoloration as well as reduce microbial contaminants that are associated with pineapple, due to its close contact with the soil.

The neutral sugar composition of the soluble component of the fresh material consists mostly of the hexoses glucose and mannose. It is known that these hexoses are easily caramelised or destroyed when exposed to high temperatures during the drying process. Therefore, the removal of these sugars may be an important process to consider should a high temperature process be selected for the manufacture of a suitable DF product.

High temperature drying is necessary to successfully prevent a higher than acceptable microbial load. However, this will be at the expense of the heat sensitive bioactives, such as vitamin C. The retention of the bioactives is important in the development of an antioxidant rich DF as this can give the pineapple DF a commercial advantage over the current commercial cereal DFs which do not have many bioactives. The microbial stability is very important for a DF product and one of the concerns regarding the processing of pineapple waste is trying to reduce the high yeast and mould count. Drying at a high temperature (104°C) for a minimum time of 5 hours is the only practical solution to reduce the TMA and the yeast and mould counts to below allowed

limits. Although Inspexx and Oxonia bacteriocides were used the results have shown that they are ineffective, they were only effective if the sample were dried at high temperatures (104°C). Although the high temperature can provide a microbiologically stable product it is important to be aware that it may destroy the bioactives and cause browning reactions that discolour the DF thereby reducing its commercial value. Should the raw material be used for commercial production better handling conditions on the processing line should limit the initial load, and minimize problems during the fibre production.

The baseline process produced a DF product with suitable functional properties but inferior colour and low TDF content due to the high amount of neutral sugars present. A baseline process which includes a washing step resulted in improved quality of the fibre product by removing the sugars and improving the colour, WBC and OBC. Although peel DF did not compare well with commercial DF's such as Apple Q plus fibre which has a WBC of 15 g/g it compared well to cereal bran that had a WBC of 2 g/g.

Based on the requirements for commercial DF, certain modifications need to be made to the baseline processing parameters to improve the DF composition to reflect a soluble component and better WBC. Although the neutral sugars have been removed during the washing step, additional steps are necessary to produce a competitive DF with a neutral colour and flavour. Modified processes such as temperature, chemical and mechanical processing parameters have been explored and the effect of these processes on the functional and chemical composition of the DF have shown that the

alkali treatment is a suitable treatment for the product of DF because it increased the IDF content through the removal of components such as the fats, proteins and neutral sugars and as a result decreased the caloric content of the DF product. The alkali treatment was also effective in the removal of some minerals such as potassium, and some of the soluble sugars, particularly the hexoses such as glucose and galactose. These sugars are responsible for the browning reactions and their removal resulted in a product having an improved colour after drying. The other benefit of the alkali process was to change structural carbohydrate components to allow for more functionality. The disadvantage was the destruction of most of the bioactives. Modification of the DF through chemical, thermal or mechanical changes resulted in changes to the DF profile, with respect to the colour and the monosaccharide profile.

The behaviour in the food products should also be evaluated, if possible by a relevant food science organisation or appropriate industrial partner. Although this was not tested in this study, based on the DF measured nutritional and functional properties, there are several areas in the food industry where pineapple DF may be used. Examples include health bars, cereals, fruit juices, baked products and processed meats. In order to improve the pineapple DF so that a broader application base may be achieved, it may require further modification to the current process. In some cases, a single process or a combination of modifications will be necessary to obtain the DF product that could be used in higher concentrations without sacrificing the quality of the final product. These modifications will be needed to improve the composition, structure, sensory and physicochemical properties of the DF product. In addition it may be important to consider the seasonal variation and its impact on processing. Consensuses on the DF assay methods make comparisons of various DF products more viable.

CHAPTER 7

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APPENDIX

General Methods

Gas Chromatography Methods: Method for the Determination of Sugars

Materials and Equipment

The following materials and equipment were required for the analysis:

Chemicals (all the chemicals were GR grade)

Trifluoroacetic acid, $NaBH_4$, Acetic acid (CH_3COOH), Methanol (CH_3OH), Pyridine, Acetic anhydride, Chloroform ($CHCl_3$) Internal Standard Inositol.

Equipment

The GC model: Varian 3300

Column DB 225 capillary column (supplied by Anatech)

Detector: Hydrogen Flame Ionisation Detector (FID)

Isothermal temperature of 230°C

Nitrogen Pressure of 20psig

Hydrolysis

The 5 mg sample is hydrolysed with 1 mℓ of 2 M Trifluoroacetic acid (TFA) at 125°C for 1 hour

(3) After cooling the extract transfer into a round bottom flask

(4) Add methanol and rotovac at 40°C to remove the excess TFA as an ester.

(5)

Reduction

(6) Add 1 mℓ distilled water and add a "spatula tip" of sodium borohydrate ($NaBH_4$.) and leave at room temperature for an hour.

(7) Add acetic acid to the mixture drop by drop until the effervescence stops.

(8) Add 2 mℓ 10% acetic acid/methanol solution to this and rotovac` at 40 °C.

(9) Repeat this step four times.

(10) Then add methanol only and 'rotovac` to remove the borate and the methyl esters.

(11) The borate formed in the complex must be removed before the acetylating step because borate complexes involving polyhydroxy compounds are liable to interfere with acetylation.

(12)

(13)

(14)

(15)

(16)

Acetylation

(17) Add 0.4 mℓ pyridine then 1 mℓ acetic anhydride and reflux for 1 hour at 100°C.

(18) Evaporate with the rotovac.

(19) Wash the contents with methanol and chloroform and transfer to glass centrifuge tube.

(20) Add water then vortex and centrifuge gently.

(21) After centrifugation remove the top aqueous layer.

(22) Repeat the vortex and centrifuge steps with acidified water, water, water with bicarb and then water.

(23) Remove the water layer then dry the chloroform layer with anhydrous Na2SO4. (Keep in the oven). Let it cool before adding.

(24) Transfer chloroform layer through cotton wool into clean vial. Place on top of the oven to evaporate the $CHCl_3$

(25) Check for traces of acetic anhydride (this must be removed otherwise it will damage the column) if there are traces then it must be washed again. (Step 6)

(26) When all the acetic anhydride is removed add a small amount of chloroform

(27) Add anhydrous sodium sulphate to $CHCl_3$ layer. Filter the $CHCl_3$ layer through Whatman No 41 with Na_2SO_4

(28) Add a piece of cotton wool and pipette the chloroform layer. This extract is prepared for injection into the GC.

Determination of Ash

The AOCS Ba 5a-49 (Revised 1973. Third edition) method was used for the determination of ash. The dried sample was weighed in a crucible and then placed on an open flame to burn off the combustible organic material then placed in a muffle set at a temperature of 550°C for 8 hours. The material that remains after incineration in the muffle is quantified as ash.

Determination of Fat

The Bligh and Dyer method (*J. Sci. Food Agric.* 1985,6, 177-185) was used for the determination of fat. In this method the fat is extracted with a mixture of a known amount of 40 mℓ chloroform and 40 mℓ methanol. 20 mℓ of chloroform is removed and placed in a preweighed beaker. The beaker is placed on a hot plate (70°C) for the chloroform to evaporate. The remaining residue is quantified as percentage fat.

Determination of Moisture

The oven moisture method was used for the determination of moisture (AOCS Ba 2-38. Revised 1982. Third edition.) The sample was weighed into a preweighed glass petri dish and then placed in a convection oven at the temperature of 104°C for 8 hours. The moisture content was quantified by the difference in weight of the sample and petri dish after drying.

Determination of Protein

The following Kjeldahl method was used for the determination of nitrogen (Bradstreet R.B. 1996 The Kjeldahl Method for Organic Nitrogen. Academic Press). The weighed sample is digested with potassium copper sulphate and concentrated sulphuric acid on a digester block at 420°C for 1 hour. After digestion step the sample is placed in the Kjedahl instrument where the free nitrogen is liberated and quantified as a protein by automatic titration.

Determination of Minerals

Two grams of the dried material was weighed into an acid washed crucible and ashed according to the Ash method AOCS Ba 5a-49 (Revised 1973 Third edition) to destroy all the organic material. The ashed residue was washed into 100mℓ volumetric flask and was made up to volume with ten % nitric acid (v/v). The solution was filtered with Whatman 41 filter paper before being read by atomic absorption using the required standards.

Total Antioxidant Activity

Sample preparation was according to Larrauri *et al.* (1997). The dried powder 50 g was extracted sequentially with 40 mℓ of 50% (v/v) methanol solution and then extracted

with 40 mℓ of 70% (v/v) acetone solution at room temperature over a 60-minute time interval.

The combined extracts were centrifuged at 2500g for 15 minutes. The supernatant was collected, evaporated and freeze-dried. The pellet was dissolved in ethanol. This extract is stable for a month when frozen and 0.5 mℓ of this solution was used in the analysis.

Vitamin C

Vitamin C was determined according to a fluorimetric method (AOAC 984.26) that determines the total vitamin C (ascorbic and dehydroascorbic acid) in food. Samples were processed immediately as vitamin C is rapidly oxidised by air. Wet samples (3-4 g) were weighed into a container and 10 mℓ metaphosphoric acid added. This mixture was homogenised for 30 seconds. The solution was then transferred quantitatively into a 100mℓ flask and made up to the 100 mℓ mark with meta phosphoric acid. The ascorbic acid was first oxidised to dehydroscorbic acid by treatment with activated carbon. The development of the fluorescent derivative of the vitamin is prevented by formation of a boric acid dehydroascorbic acid complex before the diamine solution was added. This allowed for the differentiation between the fluorescence from the vitamin and that from possible "interfering substances." The fluorescence was measured after samples had stood for 60 minutes in the dark, at an excitation wavelength of 356 nm and an emission wavelength of 440 nm. The levels of vitamin C were quantified by comparison with the ascorbic acid standard curve ranging from 5 to 50 mg/kg.

Vitamin E

The Munné-Bosch *et al.* (1999) method was used for the determination of Vitamin E. The fresh pineapple samples were ground in liquid nitrogen and a 0.1 g sample was extracted with 5 mℓ methanol (GR grade) and 4 mℓ hexane (GR grade) for 10 minutes. The mixture was centrifuged at 1500g for 10 minutes and the hexane layer removed and evaporated to dryness with nitrogen. The dried residue was dissolved in 2 mℓ methanol and then analysed by HPLC using a Phenomexex prodigy 5 ODS 2 (5 micron) column (250x4.5 mm).

Total Phenols

The total phenols were determined by the Folin Ciocalteau method as adapted by Singleton *et al.* (1999).

The pineapple peel and core samples of 2-4 g of the raw material were pulverized using a pestle and mortar and then extracted with chloroform and methanol mixture until all the chlorophyll was removed. This was achieved once a clear solution of chloroform was obtained, then the sample was extracted with methanol, by pulverising, centrifuging and then removing the methanol extract. This step was repeated until the methanol was clear. The solid material was re-dissolved in deionised water, and freeze-dried.

The sample was stored for two hours at 23°C before measuring the absorbance at 760 nm. Gallic acid (range 5 to 100 mg/ℓ) was used for the preparation of the standards.

Determination of Pectin

Four grams of sample were made up to 40 mℓ with hot ethanol in a centrifuge tube. This mixture was heated in a water bath at 85°C for 10 minutes and then made up to 50 mℓ with ethanol, centrifuged, and the supernatant discarded. The pellet was extracted with ethanol once more in the same manner described above. The residue was then washed into a 100 mℓ flask to which 5 mℓ (1 M) NaOH was added made up to 100 mℓ with distilled water and then filtered. The filtrate was collected and used for the colorimetric reaction using galacturonic acid as the standard.

Bromelin

Bromelin activity was determined by a colorimetric method based on Preparations for Biochemistry from Merck Catalogue number: 1651.The wet pineapple samples (0.5 g) were mixed in a solution of 6 mℓ ammonium citrate (0.1 M) and Anson haemoglobin (20 mg/ℓ). The pH was adjusted to pH 6. This solution was incubated at 35.5°C and the reaction was terminated after 10 minutes by the addition of 5 mℓ trichloroacetic acid solution (0.3 M). This mixture was centrifuged and an aliquot 2 mℓ of the supernatant combined with 10 mℓ of sodium hydroxide (0.5 M) and 3 mℓ of 50% Folin reagent and

the absorbance of this solution determined at 750 nm this was quantified against a standard curve.