NUTRITIONAL AND FUNCTIONAL QUALITY OF SOUTH AFRICAN DRY-BASED SOYA PROTEIN FOODS

BY

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I declare that the dissertation herewith submitted for the degree of MSc (Agric) Food Science and Technology at the University of Pretoria, has not previously been submitted by me for a degree at any other university or institution of higher education.

NUTRITIONAL AND FUNCTIONAL QUALITY OF SOUTH AFRICAN DRY-BASED TEXTURISED SOYA PROTEIN FOODS

ABSTRACT

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As 780 million people in developing countries still do not have access to enough food to meet their basic daily requirements for nutritional well being the provision of affordable and nutritional foods is an on-going challenge. Animal protein resources, e.g., meat, poultry, fish and eggs, are increasingly becoming unaffordable by the disadvantaged masses and their storage is problematic. Alternative protein sources, e.g., seaweed, plankton, single cell protein, fishmeal and legumes are being researched. Soya beans, having a high protein content of 40% is consequently an ideal vehicle for the supply of protein to consumers that cannot afford conventional sources of protein.

In South Africa, soya beans are processed into flavoured dry-based products and marketed extensively as a meat substitute. This is prepared at home as part of a main meal to be consumed with rice or bread. Their affordability and shelf-stability characteristics make them appealing to South Africans constituting the low-income sector and vegetarians.

The quality of mutton and savoury flavoured dry-based soya products manufactured by three Kwa-Zulu Natal companies were investigated and these three manufacturers were designated A, B and C. Proximate analyses and mineral analyses determined the nutritional components. The protein quality in these products was determined by performing Protein Efficiency Ratio (PER) and Net Protein Utilisation (NPU) studies on chickens as biological models. The functionality of these proteins was determined by Nitrogen Solubility Index (NSI) and Protein Dispersibility Index (PDI) studies. The presence of bacteria, yeast, moulds, and mycotoxic fungi were determined by microbiological assay. Consumer acceptability surveys were also undertaken to ascertain appearance, flavour, texture and overall acceptability profiles. The consumer also ranked the products in order of preference. Finally, economic value of these dry-based soya products was determined by comparing the retail price of these products with beef and chicken on a protein basis.

The carbohydrate content of these products was elevated as a consequence of dilution with starch and/ or maize flour. The polysaccharides, raffinose and stachyose which are regarded as causing flatulence in soya products apparently were eliminated during processing as the soya products investigated were devoid of them.

The negative consequence of the manufacturers adding starch and/ or maize flour was that the protein was diluted. The protein content of 25% was significantly lower than the normal protein content of 40% of soya beans.

A diluting effect occurred in the mineral content of iron, zinc and manganese. Calcium, magnesium and phosphorus was also decreased as a consequence of being lost with the soya oil which was removed in the processing of defatted soya flour. In spite of these reductions, soya products are still a good source of calcium, phosphorus and magnesium. The bioavailability of iron may be constrained by phytic acid, which occurs in soya beans.

Amongst the three manufacturers researched, dietary fibre content was least in products from manufacturer C. Flavour also had a significant influence on dietary fibre content as mutton flavoured products had a higher dietary fibre content compared to savoury flavoured products.

These soya products were a good source of polyunsaturated fatty acids with products from manufacturers A and B having the higher fatty acid level content. Savoury flavoured products had a significantly higher fatty acid content compared to mutton flavoured products.

Protein quality differed significantly amongst the three manufacturers. Products from manufacturer A had very low PER and NPU values. Products manufacturers B and C had acceptable PER/ NPU values, similar to peanuts.

Protein functionality values of products indicated that products from manufacturer A were not exposed to severe heat treatment and consequently their anti-nutritional factors were not denatured or inactivated. Consequently their PER/ NPU was depressed. Protein functionality values of products from manufacturers B and C were low indicating extensive heat treatment of TVP. This treatment denatured the anti-nutritional factors thus producing acceptable PER/ NPU values.

Bacterial loads were minimal in these dehydrated products. Some moulds were found indicating fungal contamination, presumably from the air during processing. An absence of mycotoxins confirmed that mycotoxic fungi are not endemic to dry-based soya products. The processing applied to the ingredients used by manufacturers B and C yielded Salmonella/ Shigella free products.

There was no significant difference in the consumer response to mutton flavoured products from all three manufacturers and savoury products from manufacturers B and C were ranked highest by consumers. With respect to products from manufacturer C this could be linked to them having the least moisture, protein and fat content and the highest soluble carbohydrate and ash contents.

Economically, soya protein is far cheaper than beef and chicken protein. Beef protein was calculated to be 140% of the retail price of soya protein and chicken protein was calculated to be 192% of the retail price of soya protein.

While dry-based soya products seems to be nutritionally acceptable and affordable with an extended shelf life, their protein availability and functionality is dependent on processing parameters. These soya products also received an above average acceptance rating by consumers, although textural and appearance qualities could be improved.

This dissertation is dedicated to my late father,

Arunaghary Padayachi,

who passed away on the 19 February 1999.

He laid the foundation for my education and encouraged me further in this direction. For this I will be eternally grateful.

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INTRODUCTION

Despite appreciable world-wide improvements in life expectancy, adult literacy and nutritional status we all view with the deepest concern the unacceptable fact that about 780 million people in developing countries (20% of their combined population) still do not have access to enough food to meet their basic daily needs for nutritional well-being (FAO/WHO, 1992). Thus, it is obvious that the World production of protein must be increased both from conventional and non-conventional sources particularly the latter because of the limitations on land and energy (Anglemier and Montgomery, 1976).

The land available on the earth for cultivation is limited, and it is said that a green arable zone that covers an area twice as big as Belgium turns to desert every year. Therefore, it is clear that the food harvested on the Earth will not be sufficient to feed all of the future population. Furthermore, it may be difficult to produce food in a stable pattern because of the effect of global warming and abnormal weather patterns. At present many people in developing countries are starving, while many in the developed countries, eat excessively and develop health problems related to obesity, e.g. hypertension, diabetes, atherosclerosis and heart disease (Utsumi, 1992).

Many approaches for increasing protein supply and nutritive value have been proposed, and research is in progress on several novel sources. Thus, proteins from oilseeds, grains, legumes, fish, microbes, algae and leaves are being investigated. In recognition of the magnitude of world needs it is expedient to examine all potential sources (Kinsella, 1978).

Excluding energy, the two major factors determining the adequacy of the World's food supply are population and availability of arable land. If the World's population continues to grow at the present rates to a projected 12 billion by 2025, it is anticipated that more cultivated land will be needed. Because of the limited area of new arable land available, expanded food production will depend on increasing energy inputs. However, since fossil energy is a finite resource, the most efficient methods for food production and utilisation must be adopted.

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INTRODUCTION

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The direct use of green leaves would be most efficient, e.g., alfalfa produces 4.2 kJ of protein per kJ of fossil fuel used in its cultivation while soya bean requires about 8.8 kJ per kJ protein produced. Protein production by intensive animal husbandry methods is highly energy consuming, requiring approximately 197.4 kJ per kJ of protein. Therefore, more direct consumption of plant food is inevitable (Kinsella, 1978).

With the rising costs of energy, and limited availability of land greater emphasis on crop agriculture seems inevitable. In the future, plant proteins must provide an even greater proportion of our food protein. Cereals, as they traditionally have, may supply most of this, however, soya bean and, to an increasing extent, sunflower, peanuts, cottonseeds, and other seeds are becoming mayor resources of food proteins for the human population (Kinsella, 1978).

Soya bean is a world-wide source of major nutrients required for normal diets. Annual global production is currently 88 million metric tons (Phillips, 1997). As much as 45 % of the dry matter is protein and the amino acid pattern approaches the optimum by the Food and Agriculture Organisation (FAO) (FAO/WHO, 1992). Among the benefits of soya protein are the good water and fat binding abilities afforded by the soluble proteins which this material contains (Reichert, 1991). Soya bean also contains about 20 % oil, which is very desirable because it contains a large proportion of unsaturated fatty acids (Ologhobo, 1989).

Increased yields of soya, coupled with advances in processing proteins from the soya bean, have improved the opportunity for the further use of soya-protein-based foods in the human diet. Various expert groups and national bodies recommend increasing the relative contribution of plant foods to western-type diet to improve long term health. Therefore, it is important to consider the nutritional qualities of various soya-protein foods for human beings because there may not be a general appreciation for their excellent nutritional characteristics and potential for meeting the physiological needs of human beings at various ages (Young, 1991).

Although soya has long been eaten in the Orient (Young, Wayler, Garza, Steinke, Murray, Rand and Scrimshaw, 1984), a significant contribution by this plant source of protein to the diet of populations in other areas, especially Europe, North America and Africa is a relatively new development.

Genetic improvements of soya bean cultivars have played a key role in developing adapted varieties for these regions and in establishing soya bean as the eighth largest agricultural commodity in the World (Zarkadas, Yu, Voldeng and Minero-Amador, 1993).

The provision of soya-protein-based foods is one strategy for combating protein-energy malnutrition that affects 50% of the World's population (Phillips, 1997). Increasing the amount of meat, milk, eggs or fish is a most difficult task because of lack of refrigeration and adequate regular distribution systems to supply these foods. Therefore, supplying shelf stable foods containing quality protein e.g., soya-protein, in relatively inexpensive, palatable, conventional foods becomes one approach to assisting in solving a portion of this complex problem (Morck, Rusoff, Bednarcyk and Ronai, 1976).

Workers in the mines and agricultural fields require high-energy, balanced diets. At present, South Africa, due to widespread poverty, faces the important issue of under-nutrition. As food scientists, we could perhaps deepen our knowledge and understanding of the cultural and socio-economic diversity of the country's people to meet the demands of a changing society in the country as far as nutrition is concerned. There is also the issue of providing nutritional meals to thousands of migrant and seasonal workers housed in hostels. Also, large proportions of our population are vegetarians, because of their lifestyle or religious beliefs are non-meat consumers. An investigation by Draper, Lewis, Malholtra and Wheeler (1993) suggested that such consumers need appropriate dietary supplements.

To combat these problems of malnutrition and dietary supplementation soya products can provide a solution. In spite of the Western world's scepticism to soya products, there are an increasing number of soya products being introduced commercially onto the South African markets. A local innovative food processing company has recently entered the South African market with the objective of producing soya milk (Penstone, 1996).

Soya milk is available in two forms - liquid or powdered. Both have applications in a variety of industries, including meat and fish processing; simulated milk and nutritional beverages; cereal spreads and soups; and snack foods such as chocolate and sugar confectionery.

The product range can be expanded further through the incorporation of tofu, a concentrated form of soya milk, in reduced-fat salad dressings, cheeses and ice creams (Penstone, 1996).

A novel non-milk drink recently introduced is a range of banana-, strawberry-, and chocolate flavoured instant drinks, mainly for children. As nutritious as flavoured milk drinks, it is different in that it is a non-dairy soya based product that is mixed with water rather than milk (Penstone, 1996). Consequently it works out slightly lower in cost per glass (~R1/ glass). Another product is a nutritious biscuit. It contains bran, milk solids, peanut butter, treacle and micronised soya meal (Penstone, 1996).

A variety of dry-based texturised soya protein products available in numerous flavours, produced by numerous manufacturers are currently available on the South African market. Although there has been extensive studies reported from the West, there is a paucity of information on quality parameters (Prasad, Viswanathan, Swamy and Santhanam, 1995) of texturised vegetable protein (TVP) products. Thus, with the more extensive utilisation of soya proteins in human diets it is necessary to study the latest generation of soya products (Wayler, Queiroz, Scrimshaw, Steinke, Rand and Young, 1983).

Since food processing and preparation may affect the acceptability of, and the physiological response to novel or unconventional sources of protein (Young, Puig, Queiroz, Scrimshaw and Rand, 1984), this study comparatively determines the nutritional, functional and microbial quality, consumer acceptability and economical value of dry-based texturised soya protein foods commercially available in South Africa.

These products are commonly available in 200 g packets, which serves six people after hydration. This research project consequently set out to determine whether these dry-based texturised soya protein food products are not only wholesome, economical, and of good sensory quality but, most importantly, that they carry their fair share of important nutrients.

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CHAPTER 2

LITERATURE REVIEW

2.1 Economic importance

Soya beans were one of the first pulses cultivated by man (Cronjé, 1997). The soya bean (Glycine max (L.) (Merrill, family Leguminosae) undoubtedly originated in the Orient, probably China. As early as the sixteenth century, it was exported from Eastern Asia to Europe. Soya beans were first introduced into the United States in the early 1800s but remained a minor curiosity until the twentieth century, when some farmers started to grow them as a hay crop. It was first cultivated in South Africa in 1903 at Cedara in Natal (Cronjé, 1997). It was not until after 1945 that their value as a supplier of feed and food oil was recognised and exploited (Snyder and Kwon, 1987). In South Africa a genetically improved soya bean with increased pest resistance has just been introduced (Watson, 1997). Soya products are a component of more than 20 000 foodstuffs and so genetically modified soya could have an overwhelming effect on the food market (McGill, 1997).

The spectacular increases in production of soya beans are due to two valuable components - the food oil and the feed (defatted) meal. One metric ton of soya beans yields about 183 kg of oil, and 800 kg of meal (Snyder and Kwon, 1987). When soya beans were first processed, the oil was the valuable component and the meal was considered a by-product. From 1950 to 1960, the value of oil and meal from a single unit of soya beans was roughly the same. However, since 1960, the demand throughout the world for protein foods through the use of good protein feeds has been high, and the meal has become the more valuable component. Currently defatted soya flour is worth R3 000 per metric ton (Mahabeer, Personal communication Buying Department, Robertsons, 1997).

In 1995 South Africa exported 58 tons of soya beans worth R105 000 compared to the 1996 figures (769 tons worth R2 million). Four hundred and sixty six tons of dry-based soya soup worth R 5 million was exported in 1996 (Sawyer. Personal communication: Central Statistical Services, 1997). In 1995 South Africa imported 172 474 tons of soya beans worth R143 million compared to the 1996 figures of 47 210 tons worth R51 million. During this period, South Africa imported 343 tons of dry-based soya soup worth R2.4 million.

2.2 History of soya products

Despite an often negative perception by older generations of soya in Europe, the functional properties of today's soya-protein products (flours, concentrates, isolates and textured products), their appealing nutritional profile (cholesterol-free, low fat) and their competitive price have started to win over both manufacturer and consumer (Tuley, 1996). Both the meat and bakery industries have been major markets for soya proteins, but applications range from calf milk replacers to biodegradable plastics and construction materials. Strengthening consumer interests in both healthy eating, particularly low fat and vegetarianism are bound to be beneficial to further development (Tuley. 1996).

However, the wider appreciation of both the functional and nutritional properties of soya proteins has taken time to establish after negative consumer reaction to many of the products introduced since the Second World War and more recently during the 1970s. The first generation of post-war consumers had a bias against soya and it's products. At that time, soya products positioned as imitation meat and of questionable quality, were rejected by war-weary consumers (Tuley, 1996).

A second "false start" took place in Europe in the 1970s when several important and reputable research companies published reports "promising heaven on up with soya proteins". Encouraged by forecasts of high levels of utilisation, soya manufacturers moved quickly to invest in production, only to find that consumers still were not ready to except soya products, particularly of the quality available (Tuley, 1996).

The bad image of soya required a lot of time to repair. Once soya manufacturers recognised that the promised usage levels of soya were not going to be realised, they changed approach, introducing soya proteins for use as ingredients, in small quantities and for specific technological reasons. These technological reasons include the binding of water and fat; gel formation; emulsification; and improvement of both shelf life and texture of many products. The image of soya as a substitute food was broken. Food manufacturers have realised the functional benefits of using soya at a sensible level in food products (Tuley, 1996).

Since the 1970s soya as a food crop has been poorly understood and that situation still exists. Soya is a foreign crop in South Africa. Consequently people are unfamiliar with it. Therefore, there are sceptics, which inevitably affects demand and attitudes toward soya products. Attitudes towards soya are changing because "not only is soya a good source of protein it has valuable functional properties" (Penstone, 1996).

The concept of using the soya bean as a food ingredient to complement the traditional ranges of beans, pulses and cereals (Reid, 1993) and soya ingredients fitting in well with the theme of health (Reid, 1997) are examples of local literature indicating the recent impact that soya is making in the South African food industry.

2.3 Processing of soya bean

Most soya beans processed into meal and oil are first dehulled, because hulls have a low oil content and their presence would decrease the efficiency of the extractors (Snyder and Kwon, 1987). In addition, for some feeds it is desirable to have a high (49%) protein content, which can be achieved only by using dehulled soya beans. For efficient removal of the hulls, it is desirable to have the soya beans at 10% moisture and so a drying step is required.

During the drying step, heated outside air is forced through a bed of soya beans, causing some loss of moisture. Then cool outside air is used to remove the warm moist air. The beans are then tempered (held to allow for moisture equilibration within the bean) for one to five days (Snyder and Kwon, 1987). This type of drier is energy inefficient, because both air-streams are discharged, and all heat is lost. More efficient driers have been designed and built, in which parts of the cooling and drying air-streams are re-circulated, saving up to 25% of heating fuel (Moore, 1983).

Conventional cracking mills, used to split the soya bean into fragments consist of counter-rotating, corrugated, or fluted rolls. There may be a stack of two or three rolls and soya beans are fed uniformly across the length of the rolls by vibrating feeders. The rolls rotate at different speeds to provide some shearing or nipping action and the corrugations are fewer and deeper in the first set of rolls compared to the second set (Moore, 1983). The size of the cracking rolls is typically 25 cm in diameter with lengths 107cm or greater. Such cracking rolls would give four to six pieces or "meats"' from each soya bean.

Of course, this is impossible to achieve practically, since some fines will be produced and some larger pieces. The hulls are loosened when the beans are cracked and can be separated by aspiration in a multi-aspiration process. The aspirators are set to remove 100% of the hulls, even those hulls that still have meats adhering. These pieces and oversized pieces can be returned to the cracking mills. The meats are sized on vibrating screens, and the fines are separated from the air-stream by cyclone separators (Snyder and Kwon, 1987).

Conditioning of the meats before flaking is a heating step to give proper plasticity to the soya bean particles for optimum flaking. Moisture may be added during conditioning to achieve 11% moisture in the meats. The heating is done by steam with some direct injection, depending on the amount of added moisture that is needed. Rotary horizontal heat exchangers and vertical stacked types are both used for conditioning (Snyder and Kwon, 1987). The heated soya flake is the source of heat for maintaining the solvent extraction system at about 60°Cand so the temperature achieved during conditioning depends on how much heat loss takes place during flaking and conveyance to the extractor. Generally meats are heated to about 65 to 70°C.

The next step in the separation of soya protein from soya oil is to flake the conditioned meats, but before considering that step, the processing needed to produce full-fat soya flour and some newly proposed alternatives to conventional soya bean preparation, need to be reviewed.

The meats after separation of the hulls are the raw material for production of full-fat flours. Two types are produced and both are used mainly in the baking industry. One type is enzyme-active full-fat flour that is important for its bleaching action on wheat flour. This increases the whiteness due to carotene oxidation associated with lipoxygenase activity. At the same time, one gets some flour oxidation that leads to dough with better machinability (Snyder and Kwon, 1987). The limit in utilising enzyme active full-fat soya flour in the baking industry in the United States is 0.5% of enzyme-active full-fat soya flour in wheat flour. The practice of using soya bean flour for its bleaching effect on wheat flour is widespread in Europe and South Africa, The other type of full-fat soya bean flour is made from meats that have been steam treated and dried to inactivate all enzymatic activity.

The grinding of meats to produce flour is done in hammer mills and fineness is achieved such that 97% passes a 150 µm screen (Circle and Smith, 1978). Full-fat flours are difficult to screen, and so sizing is done by air classification primarily. A low-cost process for producing full-fat flour for use as a food ingredient in developing countries has been developed by Mustakas, Albrecht, Bookwalter and Griffen (1967). A more sophisticated version (Mustakas, Albrecht, Bookwalter, McGhee, Kwolek and Griffen, 1970) for producing full-fat soya bean flour makes use of an extruder for the heating step.

The abrupt increase in energy costs during the 1970s was an incentive to improve the efficiency of heating steps in soya bean processing. One improvement is the use of fluidised beds for the heat exchange steps of drying whole beans and conditioning meats (Snyder and Kwon, 1987). Fluidised beds are suspensions of solid particles induced by a strong air-stream entering from below the particles. The air-stream is re-circulated to give rapid heat transfer and energy savings.

For preparation of soya beans by a fluid-bed system (Florin and Bartesch, 1983), the initial drying step to 10% moisture is done in a fluidised bed. The beans are immediately cracked into halves, and hulls are detached by a combination of cracking mills and hammer mills. Then the warm half-beans are further heated in a fluidised conditioner bed, cracked and sent to the flaker. The elimination of cooling and tempering steps of conventional steps saves time and energy, and the fluidised beds allow finer control and more even heating than conventional heat exchange equipment. This process is named the Escher Wyss hot dehulling system (Snyder and Kwon, 1987).

A second innovative process that has been designed for soya bean preparation is to heat the intact beans with microwave energy under vacuum, crack immediately, and dehull. During dehulling, heat from the magnitron microwave generators is used to heat the air-streams and the product (Snyder and Kwon, 1987). Thus no conditioning step is required (if proper moisture is maintained), and the meats can be flaked immediately. Again, there is a saving of time and energy (Snyder and Kwon, 1987).

The conditioned meats are fed directly to flaking mills, which for soya beans are smooth rolls, placed horizontally, with pressure maintained by heavy springs between the two rolls.

The size of these rolls is approximately 70 cm in diameter and 120 cm in length. This single flaking step produces soya bean flakes about 0.25 to 0.37 mm in thickness (Snyder and Kwon, 1987).

Making thin flakes of the soya bean meats in preparation for solvent extraction serves several purposes. These flakes make suitable beds, even when several cm thick, through which solvent can readily flow. The same flow-through capability would not be possible with fine particles. The crushing and shearing action of the flaking rolls tends to disrupt intact cotyledon cells and this disruption may (but this is not certain) facilitate solvent penetration to the lipid bodies (Snyder and Kwon, 1987).

After suitable preparation, the soya beans are ready for separation into oil and meal fractions. This is done throughout the world by solvent extraction. This does not mean that only one type of process is involved. Different solvents, different extraction equipment, and different extraction conditions are used (Snyder and Kwon, 1987). To dissolve and remove oil from soya bean flakes economically, a solvent must have certain properties. Good solubility for triglycerides is desirable, but also one wants some selectivity so that many unwanted compounds are not dissolved. The solvent or at least the residues of the solvent likely to be found in edible products must be non-toxic. Low specific heat and low heat of vapourisation are desirable for low cost of operation. The solvent should not react with the oil seed components or with extraction equipment to form undesirable compounds, nor should it extract undesirable compounds such as pesticides or aflatoxins. Ideally, the solvent would have no inherent safety problems such as explosiveness or flammability and would be cheap and readily available in quantity.

Obviously, no one solvent has all these desirable attributes, but the solvent that comes closest now is commercial hexane. Some undesirable characteristics of hexane are flammability, explosiveness, and high price.

Friedrich and Pride (1984) have shown supercritical carbon dioxide to be an effective extraction solvent for soya bean oil. Carbon dioxide has the advantages of being cheap, readily available, non-toxic (in the amounts used), non-flammable, and readily removed from the oil by simply reducing the pressure. The oil extracted by supercritical carbon dioxide is equivalent to hexane-extracted oil except that less phospholipid is extracted.

The disadvantage of this process is the expensive equipment needed to extract large quantities under pressure.

The principal protein product coming from defatted soya bean flakes is soya bean meal for feed and food purposes. The meal may contain a minimum of 44% protein if hulls have been added back or 47.5 to 49% proteins if free from hulls (Snyder and Kwon, 1987). Trading rules set by the National Soybean Processors' Association require that the type of process used for removing the oil (solvent extraction or expeller/ screw presses) be included as part of the name of the defatted meal. The soya bean meals are not fed directly but are feed components valued mainly for their high-protein quantity and quality (Snyder and Kwon, 1987).

Grinding of defatted flakes to produce meal is done with hammer mills. The specification used by the industry is that all meal should pass a 1 700 µm screen with a maximum of 50% passing a 576 µm screen and a maximum of 1% passing a 200 µm screen (Thomas, 1981). This means that the grinding should be done without excessive production of fines. To minimise fines, the flakes should move through the hammer mill rapidly, and this means there should be ample screen area in the mill. Another factor in moving meal through the mill is good airflow created by the fanlike action of the hammers rotating at 1 800 rpm.

Products intended for human use are called soya bean flour or soya bean grits, depending on the state of subdivision. Soya bean flour is fine enough that 97% will pass a 150 µm screen (Snyder and Kwon, 1987). Soya bean grits are produced in a range of sizes with coarse passing 1 700 to 850 µm screens, medium passing 850 to 425 µm screens and fine passing 425 to 200 µm screens.

There are also full-fat products made for human consumption, as mentioned earlier in this chapter, and some products are made with intermediate amounts of fat. Low-fat flour has 5 to 6% soya oil added and high-fat flour has about 15% soya bean oil added (still less than a full-fat flour at 20%). Both low- and high-fat flours may have lecithin added to a specified level up to 15% (Snyder and Kwon, 1987).

To enhance the protein level in soya protein products above 50%, it is necessary to remove some of the soya constituents other than oil. This is done in the processing of soya protein concentrates and of soya protein isolates.

Soya protein concentrate is manufactured from defatted flakes or flour by removing the oligosaccharides, part of the ash and some of the minor components in one of three ways (Wolf, 1970). The first two methods employing a moist heat/ water leach or an aqueous alcohol leach render the protein insoluble by denaturation, and this obviously reduces its future potential (Seal, 1977). The third procedure uses an acid leach at a pH of 4.2 to 4.5 to remove the soluble oligosaccharides (such as raffinose and stachyose). At this point the major globulins are at their isoelectric point; both the proteins and polysaccharides (such as arabino galactan, acidic pectin type polysaccharides, xylan hemicellulose, and some fibrous celluloses derived from the hull of the bean) are insoluble under these conditions. The wet protein concentrate is then neutralised with sodium hydroxide and spray dried. The final soya protein concentrate product is a cream coloured powder containing a minimum of 70% protein, 20% carbohydrate, 5% ash and 5% moisture.

The soya isolate is also produced from defatted soya flour. The first stage of the process removes the insoluble polysaccharides by dissolving the protein and soluble sugars in an aqueous alkali solution of pH 7 to 8.5. The solute is clarified by centrifugation and then subjected to the isoelectric precipitation process as described in the concentrate process (Seal, 1977). The material is then neutralised and spray dried to yield a product consisting of 90 to 95 % proteins but containing 2 to 4% ash and 3 to 4% minor constituents (Wolf, 1970).

The final group of products in soya processing are the texturised soya proteins which are produced by a relatively simple extrusion process (Seal, 1977). The starting material is defatted soya flour, having a protein dispersibility index (PDI) of 60 to 70%. It is fed into a high-speed mixer along with steam or water and minor additives such as colour and possibly flavours. Passing through the extruder barrel it is subjected to increasing pressure, which melts the particles to a plastic mass. As this mass is forced through the dye (at a pressure of 10.5 kg/cm^2) into the atmosphere, the drop in pressure causes the superheated steam to flash off, causing, a rapid expansion of the material and a puffed texture results. After extrusion the product is dried, cooled, sieved and packaged. A subsequent processor can add further flavouring.

Alternatively, aqueous processing and isolation of protein from soya flour by ultrafiltration membranes can achieve the production of food ingredients from undefatted soya beans (Lawhon, Rhee and Lusas, 1981). These techniques require no petroleum-based solvent and consequently provide increased safety and flexibility of operation.

From the literature reviewed there was an absence of information in the public domain on soya processing in South Africa.

2.4 Nutritional properties of cereals and legumes

According to Utsumi (1992), the protein content of cereal grains ranges from approximately 7 to 15%; the protein content of legume seeds range from approximately 20 to 40% (Table 1). The amino acid compositions of various cereal and legume seed proteins and the suggested pattern of amino acid requirements are shown in Table 2. The data show that the amino acid compositions of cereal grain proteins are adequate for adult requirements but do not satisfy infant and child requirements. Most cereals are deficient in lysine, threonine and tryptophan, whereas most legumes are deficient in the sulphur-containing amino acids and tryptophan (Table 2). Specifically rice, wheat and barley is deficient in histidine and leucine for infants. Rice, maize, wheat and barley are deficient in isoleucine, lysine, threonine, tryptophan and valine for infants. Maize, wheat and barley are deficient in methionine and cysteine for infants.

Soya bean is deficient in leucine, methionine, cysteine, threonine, tryptophan and valine for infants. Pea is deficient in isoleucine, leucine, methionine and cysteine, threonine, tryptophan and valine for infants. Field bean is deficient in isoleucine, leucine, lysine, methionine and cysteine, phenylalanine, tyrosine, threonine, tryptophan and valine for infants. Peanuts are deficient in isoleucine, leucine, lysine, methionine, cysteine, threonine, tryptophan and valine for infants (Table 2).

The protein digestibility of cereal seeds is generally 75 to 90%, whereas that of raw and cooked legume seeds is 15 to 80% and 50 to 90%, respectively (Utsumi, 1992). It is desirable to fortify seed- derived proteins especially cereals and legumes with lysine and sulphur-containing amino acids, respectively, or to consume a blend of these proteins (Utsumi, 1992).

Table 1: Composition of some cereals and legumes [1,2]

Food source	Protein (%)	Fat (%)	Carbohydrates[3] (%)
Cereals			
Rice	7.4	3.0	72.8
Maize	8.6	5.0	70.6
Wheat	10.5	3.0	71.4
Barley	6	2.8	70.8
Oat	13.0	6.2	65.3
Rye	12.7	2.7	70.4
Legumes			
Soya bean	35.3	19.0	28.2
Pea	21.7	2.3	60.4
Field bean	26.0	2.0	55.9
Peanut	25.4	47.4	18.8
Kidney bean	9.9	2.2	57.8

[1]Data from Utsumi (1992).
[2]Dry seed basis
[3]Fibre included

Table 2: Suggested patterns of amino acid requirements and amino acid composition of some seed storage proteins

	Suggested pattern of requirement[a]				Cereal[c]				Legume[d]				
Amino acid	Infant mean (range)[b]	Preschool child (2-5 years)	School-age child (10-12 years)	Adult	Rice	Maize	Wheat	Barley	Soybean	Field Pea	bean	French Peanut	bean
His	26 (18-36)	19	19	16	21	27	21	20	30	26	26	27	30
Ile	46 (45-53)	28	28	13	40	34	34	35	51	41	[illegible]	40	45
Leu	93 (83-107)	66	44	19	77	127	69	67	82	70	71	74	78
Lys	66 (53-76)	58	44	16	34	25	23	32	68	71	63	39	65
Met + Cys	42 (29-60)	25	22	17	49	41	36	37	33	24	21	32	26
Phe + Tyr	72 (68-118)	63	22	19	94	85	77	79	95	76	69	100	83
Thr	43 (40-45)	34	28	9	34	32	28	29	41	36	33	29	40
Trp	17 (16-17)	11	9	5	11	6	10	11	14	9	8	11	11
Val	55 (44-77)	35	25	13	54	45	38	46	52	47	46	48	52

[a,c,d] Data from Utsumi, (1992) Values are in mg/g crude protein.

[b] Amino acid composition of human milk

2.4.1 Soya protein in relation to human protein and amino acid

The nutritional value of processed soya protein (isolated soya proteins and soya protein concentrates) in protein and amino acid nutrition in humans is evaluated on the basis of a review of studies of growth and nitrogen balance in infants, children, adolescents, and adults. Young (1991) showed that well-processed soya protein isolates and soya protein concentrates can serve as the major or even sole source of protein intake and that their protein value is essentially equivalent to that of food proteins of animal origin. However, for new-borns, the data suggest that modest supplementation of soya-based formulae with methionine may be beneficial. Soya proteins have also been found to be of good quality to include in hypo-caloric diets for weight reduction in obese subjects (Young, 1991). To assess the protein quality of an isolated soya protein in relation to meat proteins Wayler, Queiroz, Scrimshaw, Steinke, Rand and Young (1983) evaluated the protein nutritional value of lean beef, isolated soya protein or various combinations of the two sources. No differences in N balance, Digestibility or Net Protein Utilisation (NPU) were observed when the soya protein replaced beef. In a second and similar study, an 84-day metabolic balance experiment was conducted in eight subjects (Young, Wayler, Garza, Steinke, Murray, Rand and Scrimshaw (1984). The sole source of protein intake was provided by the isolated soya protein, given at a level of 0.8 g per kg per day. For comparison, four young men received 0.8 g protein and three subjects 0.68 g protein per kg per day from beef proteins for 60-81 days. Comparative results revealed that the protein nutritional status could be maintained adequately when the isolated soya protein is consumed as the entire source of protein, at a level of 0.8 g per kg per day.

2.5 Factors influencing soya nutritional quality

Adverse factors, notably the protease or trypsin inhibitors in soya beans and in unheated protein products interfere with the digestion and absorption of protein and cause pancreatic enlargement (Doell, Ebden and Smith, 1982). Leiner (1981) has reviewed the mechanism involved. Trypsin inhibitors irreversibly bind trypsin, making the enzyme unavailable for its role in the breakdown of proteins. This causes the intestine to release cholecystokinin to stimulate the pancreas to produce more trypsin. The increased secretory activity causes the pancreas to enlarge.

The amino acids present in trypsin cannot be reabsorbed and thus are lost when the trypsin combines with the trypsin inhibitor.

The loss of the amino acids contained in trypsin has been suggested as being responsible for inhibiting growth (Leiner, 1981). That is, growth inhibition in young animals is caused by excessive losses in faecal matter of proteins secreted by the pancreas. Adult animals do not lose weight when fed soya beans because they have a lower amino acid requirement.

Trypsin contains a large amount (15 to 22%) of the sulphur amino acids methionine and cysteine (as reviewed by Weingartner, 1987). Soya beans are a poor source of these sulphur amino acids. Therefore, when raw amino acids are used as feed, the small quantity of sulphur-containing amino acids does not offset the large losses caused by trypsin inhibitor. Thus, trypsin inhibitor decreases the protein quality of soya beans more than it does foods with large quantities of sulphur amino acids.

There are at least five trypsin inhibitors in soya beans. The Kunitz (Vaintraub and Yattara, 1995) and Bowman-Birk inhibitors have been studied the most. Soya beans contain 1.5% Kunitz inhibitor (as reviewed by Weingartner, 1987) and 0.6% Bowman-Birk inhibitor. Kunitz inhibitor makes up about 50% of the total trypsin inhibitor activity. Trypsin inhibitors reportedly account for 6 to 11.3% of the total soya bean protein (as reviewed by Weingartner, 1987).

Trypsin inhibitors are inactivated by heat, especially moist heat (Leiner, 1994). Atmospheric steaming (100°C) of raw defatted soya bean flakes for 15 min inactivates about 95% of the trypsin inhibitor. Steaming of whole beans for 20 min partially inactivates the inhibitors. However, if the whole beans are adjusted to 20% moisture, atmospheric steaming for 20 min will inactivate almost all the trypsin inhibitor activity. Also, boiling whole soya beans for 20 min will inactivate most of the trypsin inhibitor. If the whole soya beans are soaked overnight (to about 50 to 60% moisture), only a 5 min blanching in boiling water is needed. Methods using dry heat such as roasting, microwaving and extrusion cooking are also effective.

Lectins, formerly known as haemagglutinins, as the name suggests, agglutinate red blood cells. Some are extremely toxic (Leiner, 1994).

They make up 1 to 3% of total protein in defatted soya bean flour. Leiner, (1981) has concluded that they do not adversely affect the nutritional quality of soya bean protein.

Some component in soya beans causes enlargement (goitre) of the thyroid gland in animals and humans; at present, the causal agent is unknown but is partially destroyed by heat (Leiner, 1981). Raw soya beans have caused goitre in rats and chicks. In addition, soya milk, if not supplemented with iodine, has caused goitre in infants (Snyder and Kwon, 1987). In the United States, soya based infant formulae are supplemented with 5 to 75 µg iodine/ 418 kJ formula, a level deemed sufficient to avoid the problems (Hendricks, 1983).

Urease is found in large amounts in raw soya beans (Snyder and Kwon, 1987). It can degrade urea to form ammonia, which is toxic to cattle. Although heat inactivates urease, it takes longer than the treatment for trypsin inhibitors and lectins.

Whole soya beans contain 1 to 2% phytic acid. Phytic acid is found in plant but not in animal tissues and may be one of the plant's methods of storing phosphorus and carbon. Extensive research has been conducted on its chemistry. Phytic acid may decrease the availability of divalent cations, such as calcium, zinc and iron, by the formation of an insoluble protein-phytic acid-mineral complex. It has been cited as causing reduced availability of zinc in soya bean based foods (Snyder and Kwon, 1987) and calcium in whole wheat bread, although fibre probably also plays a role in the latter.

There is conflicting evidence as to whether phytic acid in isolated soya protein is responsible for both mineral deficiency symptoms and calcification problems in humans and animals. However, both effects are overcome by autoclaving (Smith and Circle, 1978). In addition, phytic acid does not interfere with the bioavailability of minerals added to such products (Hendricks, 1983). These findings suggest that mineral supplementation of soya bean based foods, particularly for children, is an effective means of improving diet (Anderson, Chinn and Fisher, 1982).

New methods have been developed to reduce the phytic acid in foods. Leiner (1994) reviewed the effective use of ultrafiltration and ion exchange chromatography as a technique to remove phytic acid from soya beans. Ranhotra, Loewe and Puyat, (1974) reported that phytic acid is hydrolysed during breadmaking by the action of the wheat phytases or the yeast. Duodo (1997) reviewed the reduction of phytate by irradiation of soya beans. He concluded that low dose irradiation could improve the nutritional value of soya beans by lowering the concentration of phytate.

2.6 Soya proteins

The amount of protein in soya beans, 38 to 44%, is higher than the protein content of other legumes, 20 to 30%, and much higher than that of cereals, 8 to 15%. This large quantity of protein in soya beans along with excellent quality increase their value as a feed-stuff and is one of the reasons for the economic advantage that soya beans have over other oil seeds (Snyder and Kwon, 1987).

Proteins of soya beans have been studied after extraction from defatted flakes and compared with proteins extracted from full-fat soya beans. No major differences were found (Hill and Breidenbach, 1974). In this instance lipid extraction was done by Soxhlet extraction, but even when soya bean flakes are extracted commercially with hot hexane (60°C) for 30 to 40 min, there seems to be no major loss of protein solubility, enzymatic or trypsin inhibitor activity.

The proteins of soya beans, as with those of cereals and other legumes, are for the most part devoid of any specific biological activity. Consequently, plant proteins have been separated and named based on the classical solubility pattern: albumins, soluble in water, globulins, soluble in salt solutions; prolamin, soluble in aqueous alcohol; and glutelins, soluble in dilute acid or base. Using this oversimplified pattern the major portion of soya proteins are globulins (Snyder and Kwon, 1987). In contrast, most cereal proteins are prolamins and glutelins.

Although the major fraction of soya bean protein is termed globulin, this fraction can be extracted with water (Wolf, 1970). The solubility of soya proteins in water does vary with pH. If no acid or base is added to the extracting water, the pH will usually be about 6.4 to 6.6, and at this pH range approx. 85% of the soya bean protein is extracted. As the pH is raised with the addition of base, more protein is extracted, but the advantage of increased yields of extracted protein is counterbalanced by the disadvantage of protein damage at pH values above 9 (Snyder and Kwon, 1987). As pH is lowered by addition of acid, the solubility of soya proteins decreases and reaches a minimum in the region of pH 4.5. This solubility pattern forms the basis for some of the processing steps for production of soya concentrates, soya isolates and soya curd.

Based on ultracentrifugation studies, Wolf (1970) categorised the following individual proteins making up 70% of the soya proteins.

Glycinin is the predominant protein in soya beans making up about 35% of the total protein. Its large molecular weight of about 350 000 Daltons indicates that it is a storage protein. The other globulins are α, β and γ-conglycinin (Snyder and Kwon, 1987).

2.7 Functional properties of soya storage proteins

Soya bean seeds are used for making a variety of oriental traditional foods, including Tofu, Kooridofu, Yuba and many others (Kinsella, Damodran and Genman, 1985). Some seed proteins, including wheat and soya bean proteins, can be utilised as food ingredients and for the manufacture of fabricated e.g., texturisation, and processed foods (Kinsella, Damodran and Genman, 1985). Whether seed proteins can be utilised for such foods is determined by their functional properties. In other words, the functional properties of seed proteins determine their food applications in specific food systems, and their acceptability (Kinsella, Damodran and Genman, 1985). Functional properties of importance in food applications are listed in Tables 3 and 4. These properties vary with protein source, protein concentration, protein fraction, prior treatment and environmental conditions (pH temperature ionic strength, etc.) (Kinsella, Damodran and Genman, 1985).

The functional properties of soya integral for the products under review would be solubility, water absorption and binding, viscosity, and the ability to bind onto flavour additives.

Table 3: Functional properties of seed proteins of importance in food applications[1]

General property	Specific functional attribute
Organoleptic	Colour, flavour, odour
Kinesthetic	Texture, mouthfeel, smoothness, Grittiness, turbidity, chewiness
Hydration	Wettability, water absorption, water-holding capacity, swelling, solubility, thickening, gelling, syneresis
Surface	Emulsification, foaming (aeration, whipping), protein-lipid film formation, lipid binding,flavour binding
Structural and Rheological	Viscosity, elasticity, adhesiveness, cohesiveness, stickiness, dough formation, aggregation gelation, network formation, extrudability, texturizability, fibre formation
Other	Compatibility with other food components, enzymatic activity, antioxidant properties

[1]From Kinsella, (1979).

Table 4: Typical properties conferred by seed proteins to food systems[1]

Functional property	Mode of action	Food system
Solubility	Protein solvation	Beverages
Water absorption and binding	Hydrogen bonding of water, entrapment of water (no drip)	Meats, sausages cakes, breads,
Viscosity	Thickening, water binding	Soups, gravies
Gelation	Protein matrix formation and setting	Meats, curds, cheese
Cohesion-adhesion	Protein action adhesive material	Meats, sausages, baked goods, cheeses, pasta
Elasticity	Hydrophobic bonding and disulphide links in gluten, disulphide links in gels	Meats, bakery products
Emulsification	Formation and stabilization of fat emulsions	Sausages, bologna soup, cakes
Fat absorption	Binding of free fat	Meats, sausages, Doughnuts
Flavour binding	Adsorption, entrapment, release	Simulated meats, bakery goods
Foaming	Formation of stable films to entrap gas	Whipped toppings, chiffon dessert

[1]From Kinsella (1979)

The use of soya proteins in the food industry is becoming more widespread. Soya proteins are now employed in many dry-based, canned and frozen "convenience foods" both as an inexpensive extruder for meat and as a functional ingredient. Techniques such as fibre spinning developed over the last fifteen years are used to give the soya bean protein and fibrous structure a "meaty" texture. The many industrial patents indicate the wide interests in this field (Flint and Lewin, 1976).

Soya materials are available in three main forms as set out in Table 5.

Table 5: Composition of soya products[1]

Material	Protein (%)(N x 6.25)	Carbohydrate(%)
Soya flours and grits	40-55	35 (approx)
Soya concentrates	65-70	15
Soya isolates	90	

[1] From Wolf and Cowan (1971)

Soya grits and soya flour especially are used extensively in the baking industry and also forms key ingredients in cereal, dietary and infant foods. Concentrates are also used in baked goods but more widely in the meat industry to reduce shrinkage on cooking as well as to increase the protein content (Flint and Lewin 1976).

2.8 Microbiological quality and shelf life of soya based products

From the studies by Parks, Rhee, Kim and Rhee (1993) on dry-based texturised mix of beef, defatted soya flour and maize starch on shelf-life, it was found to be microbiologically safe during prolonged storage at 37°C. Kinsella (1978) also found that textured soya products had low bacterial counts and under normal storage conditions they can keep for at least a year. Refrigerated (3 to 4°C) soya products have an acceptable shelf life up to 45 days (Wang and Zayas, 1992; Gnanasamandam and Zayas, 1994).

2.9 Methodologies in determining protein quality

In evaluating the nutritional value of food protein products, several methodologies can be followed. The following reviews the methodologies used in this aspect.

The Kjeldahl method for the estimation of the quantity of protein in foods, having high precision and good reproducibility, has made it a universally accepted method (James, 1995). Its disadvantage lies in the fact that it does not give a measure of true protein, since all nitrogen is not in the form of protein, and the use of concentrated sulphuric acid at high temperatures poses a considerable hazard as does some of the use of possible catalysts such as mercury. Titration errors may also occur, as the actual point of colour change, known as the end point, may not truly represent the stoichiometric point.

A basic analytical test in evaluating the protein quality is in compiling amino acid composition of the products. Earlier compilations of the amino acid composition of soya beans were based largely on data obtained by microbiological assay procedures (Smith and Circle, 1978). With the introduction of ion-exchange chromatography and automated techniques for the determination of amino acids much more precise and reliable amino acid data on soya beans and soya bean products have since appeared in the literature. Although knowledge of the amino acid composition of a protein can provide a valuable index as to its potential nutritive value, it is the actual performance of that protein in an intact animal, which must be ultimately, assessed (Smith anal Circle, 1978).

Two of the popular procedures used for the biological evaluation of the nutritive value of proteins are the Protein Efficiency Ratio (PER) and the Net Protein Utilisation (NPU) assays. The PER is defined as the mass gain of a growing animal divided by its protein intake, and, since the value is easily obtainable, the method is commonly used (Anglemier and Montgomery, 1976). Inaccuracies arise on closely analysing the amino acid requirements for rats and for both infant and adult humans. The rat's requirement for sulphur amino acids and lysine is far higher compared to humans. Since sulphur amino acids are limiting in soya protein, the rat assay does not give valid information on how humans would respond to soya protein foods (Snyder and Kwon, 1987). NPU is the product obtained by the digestability of a protein multiplied by its biological value (Smith and Circle, 1978). These determinations done on animal, especially, rat models are difficult to extrapolate to human as rats grow faster than children (Anglemier and Montgomery, 1976). Bioassays are also expensive and time consuming.

Table 3: Functional properties of seed proteins of importance in food applications[1]

General property	Specific functional attribute
Organoleptic	Colour, flavour, odour
Kinesthetic	Texture, mouthfeel, smoothness, Grittiness, turbidity, chewiness
Hydration	Wettability, water absorption, water-holding capacity, swelling, solubility, thickening, gelling, syneresis
Surface	Emulsification, foaming (aeration, whipping), protein-lipid film formation, lipid binding,flavour binding
Structural and Rheological	Viscosity, elasticity, adhesiveness, cohesiveness, stickiness, dough formation, aggregation gelation, network formation, extrudability, texturizability, fibre formation
Other	Compatibility with other food components, enzymatic activity, antioxidant properties

[1]From Kinsella, (1979).

CHAPTER 3

OBJECTIVES

The objectives of this investigation were:

To determine the content of the chemical components in the dry-based soya protein foods on the basis of their proximate chemical analyses.

To determine the presence of any microbial contamination in the dry-based soya protein foods.

To analyse the dry-based soya protein foods for the possible presence of mycotoxins.

To ascertain the quality of the protein in the dry-based soya protein foods by determining the Net Protein Utilisation (NPU) and Protein Efficiency Ratio (PER).

To establish the functional properties of the protein constituents in the dry-based soya protein foods by performing the nitrogen solubility index (NSI) and protein dispersability index (PDI) tests.

To evaluate the acceptance of various dry-based soya protein foods by consumers.

To evaluate the economical value of the various dry-based soya protein foods.

CHAPTER 4

MATERIALS AND METHODS

4.1 Materials

Commercially available soya products, normally purchased from supermarkets by the consumer and cooked at home to be utilised as part of a meal to be consumed with rice/ bread, were acquired from three food processing plants in the Kwa-Zulu Natal Province of South Africa: The first supplier, Imana Foods based in the Pinetown region, was designated "A", Knorrox located in Durban, was designated "B" and Royco located in Pietermaritzburg, was designated "C". Two flavours from each manufacturer were obtained: "mutton" and "savoury". Samples were obtained from the respective factories in 200 g boxes.

4.2 Methods

Each analysis was performed on 3 replicate samples with the sample for each test of the triplicate taken from a different box at random.

4.2.1 Chemical composition

4.2.1.1 Moisture content

The AOAC method 925.10 (air oven method) (Association of Official Analytical Chemists, 1990a) was used. Approximately 2 g sample was accurately massed into previously dried (in a convection oven at 105°C for 1 h), cooled and massed porcelain crucibles. The samples were then dried for a minimum of 3 h in a convection oven at 105°C. Dried samples were cooled to room temperature in a desiccator and massed.

The % moisture content was calculated as follows:

$$\frac{(\text{Mass sample + crucible}) - (\text{Mass dried sample + crucible}) \times 100}{\text{Mass of sample}}$$

4.2.1.2 Protein content

Samples were analysed for protein (N x 6.25) using a Kjeldahl method (Chang, 1994). Approximately 0.5g of sample was massed into Kjeldahl digestion tubes.
Five gram catalyst, consisting of 100 parts K_2SO_4, 6 parts $CuSO_4.5H_2O$ and 2 parts selenium, was added to each tube followed by addition of 20 mL concentrated H_2SO_4. Samples were digested for approximately 2 h in a Buchi Digestion Block (Buchi, Flavil, Switzerland).

The digested samples were then cooled and ammonia distilled into boric acid using a Buchi 322 Distillation Unit. The bluish colour distillate was then titrated against 0.1 M HCl using screened methyl red as an indicator. The end-point was achieved with a colour change to grey.

Protein was calculated as follows:

$$\text{Grams nitrogen in sample} = \frac{\text{Titre} \times 14 \times \text{Molarity of acid}}{1000}$$

$$\%\ \text{Protein} = \frac{\text{grams of nitrogen} \times 6.25}{\text{Mass of sample (g)}} \times 100$$

4.2.1.3. Fat content

Fat determination was done using a Soxhlet method (Min, 1994). Flat-bottomed flasks (500 mL) were dried in an oven for 1 h at 105°. These were then massed and stored in a desiccator.

Approximately 20 g sample was massed accurately and placed in an extraction thimble, which was closed with a wad of cotton wool and then placed in the Soxhlet tube. Approximately 250 mL of petroleum ether (boiling range: 30° to 60°C) was poured into a 500 mL flat bottomed flask. Two anti-bumping beads (pre-dried and massed) were added.

The flask, Soxhlet extractor and condensor were assembled on a heating mantle. The water for condensation and the heating mantle was then turned on. Fat extraction was allowed to proceed for 4 h.

The heating mantle was then switched off and the water supply to the condenser closed. The contents of the flask were evaporated to dryness on a boiling water bath in a fume cupboard.

Any remaining petroleum ether was finally eliminated by placing the flask in a convection oven for 1 h. The flask containing the fat and the anti-bumping beads was then massed.

Fat content was calculated as follows:

$$\% \text{ Fat} = \frac{(\text{Mass of flask + beads + fat}) - (\text{Mass of flask + beads})}{\text{Mass of sample}} \times 100$$

4.2.1.4 Fatty acid profile (FAME) analysis

An oil standard was made using the five fatty acids found in soya products. The FAME derivatives were prepared as detailed below, and the mixture injected into the Supelcowax 10 GC column (polyethylene glycol bonded phase).

For each component, the mean area of three runs was obtained, and then divided by the mass of that individual component to yield a response factor. This factor was then used to calculate the amount of the component in each of the commercial samples.

Approximately 1 g + 0.01 g of the oil sample (ex Soxhlet extraction) was massed into a 200 mL volumetric flask. Five mL of methanolic KOH (0.5 M) solution was pipetted into the flask. A reflux condenser was connected to the flask and boiled until the solution was a clear solution (approx. 3 to 5 min). This was shaken vigorously at intervals. Five mL BF_3-MeOH complex was pipetted to the flask and boiled for 3 min. Solution was allowed to cool (approx. 2 min).

Ten mL iso-octane (2,2,4 tri-methylpentane) was added to the flask. One hundred mL warm saturated salt solution was added and sealed and shaken vigorously for one min. Enough cold saturated salt solution was added to bring the level of the mixture into the neck of the flask.

The two phases were allowed to separate completely. A pasteur pipette was used to withdraw 1 to 2 mL of the upper iso-octane layer, and filtered through anhydrous sodium sulphate into a vial. The solution was ready for injection onto the GC column.

From Standard: Response Factor = Area of peak of interest/concentration.

The standard solution was made up by massing appropriate amounts of the typical components (i.e., C16 P: C18 S: C18:1 O : C18:2 L : C18:3 Ln). This is done individually for each of the fatty acid methyl esters (FAMES). The resultant figure was then adjusted by dividing its sample mass by the mass of the standard (i.e., 1 g) in total.

Key to abbreviations:
P: Palmitic acid, hexadecanoic acid C16: 0
S: Stearic acid, octadecanoic acid C18: 0
O: Oleic acid, cis-9, octadecanoic acid C18: 1
L: Linoleic acid, cis, cis-9,12 octadecadienoic acid C18: 2
Ln: Linolenic acid, cis, cis, cis-9,12,15 octadecatrieonic acid C18: 3

4.2.1.5 Dietary fibre

The official method 985.29 of analysis of the Association of Official Analytical Chemists, (1990b) was followed.

In this method, the sample was first homogenised, dried, ground, and defatted. Then protein and starch were removed via enzymic digestion. The dried residue was massed and corrected for ash and protein content by the following calculation:

Total dietary fibre = Mass residue - Mass (protein + ash)

4.2.1.6 Soluble carbohydrates

The carbohydrate analysis was carried out using a Varian 5000 HPLC with a 5 µm Spherisorb Amine column and a R1-3 refractive index detector with the following reagents and materials used:

Mobile phase: 78% acetonitrile / 22% water
-Flow-rate: 2 mL/min
-Sensitivity: 50
-Attenuation: 16
Carrez I solution made up by dissolving 21.9 g of zinc acetate dihydrate in water containing 3 g acetic acid, and made up to 100 mL.
Carrez II solution made up by dissolving 10.6 g potassium ferricyanide trihydrate in water, and made up to 100 mL.

Internal standards:
The following standards were made up to 50 mL with HPLC water:
537.9 mg fructose
558.9 mg dextrose
1212.7 mg sucrose
532.4 mg maltose
514.8 mg raffinose
548.5 mg stachyose

One gram finely milled sample was massed out accurately into a 50 mL volumetric flask. Twenty-mL HPLC water was added to dissolve the sample. Sample was allowed to stand in a 50°C bath for 10 min. Two mL each of Carrez I reagent and Carrez II reagent was successively added.

The contents of the flask was sealed and shaken thoroughly. The flask was placed in an ultrasonic bath for 10 min. The flask was placed at ambient temperature for 5 min. The flask was cooled to 20°C. The solution was then made up to the 50 mL mark with HPLC water. The contents of the flask were filtered through with a Whatman # 4 filter paper. The filtrate was passed through a 0.45 µm filter. The solution was ready for injection onto the HPLC column.

The area under the curves was determined by the HPLC software to calculate the individual soluble carbohydrate contents for the respective samples.

4.2.1.7 Carbohydrate content

The % carbohydrate content was obtained by subtraction as in the following formula:

100 - (% Moisture + % Protein + % Fat +% Dietary fibre + % Ash)

4.2.1.8. Ash content

AOAC method 942.05 for ash was used (Association of Official Analytical Chemists, 1990c). Approximately 5 g of sample was accurately massed into previously heated, cooled and massed porcelain crucibles. This was then ignited in a muffle furnace set at 600°C for 6 h. The resultant whitish-grey ash was cooled to room temperature in a desiccator and massed.

Ash content was calculated as follows:

$$\% \text{ Ash} = \frac{(\text{Mass of sample + ash}) - (\text{Mass of empty crucible})}{\text{Mass of sample}} \times 100$$

4.2.1. 9 Mineral content

The levels of the inorganic nutrients in soya-based products were determined by Atomic Absorption Spectrophotometry.

Total inorganic matter was determined by dry ashing of the soya samples. The residual ash was then digested in hydrochloric acid and the metal chlorides were taken up into an appropriate volume of diluted acid.

The soya-based samples were digested and treated as follows:

A known mass was moistened (approx. 1 g dried product) with a few drops of water. Five mL of nitric acid was added and evaporated to moist salts. The digestion was repeated until no visible charred material was present. Five mL of cooled nitric acid and 5 mL of perchloric acid was added. This was heated slowly until the solution had cleared and then evaporated to moist salts. The residue was dissolved in 2 mL nitric acid and diluted to 50 mL with the addition of 200 mg/L potassium chloride.

An Atomic Absorption Spectrometer equipped with a Deuterium background facility, the appropriate lamps and data storage was employed.

In the determination of calcium, phosphorus in the matrix depressed the calcium absorbance by approx. 40%. This was overcome by the use of a nitrous oxide-acetylene flame, which also overcame other inter-element interferences. Calcium was however, partially ionized in this flame. To suppress ionization, potassium nitrate or chloride was added to give a final concentration of 2000 mg/L potassium in all solutions including the blank. Background correction was advisable.

In the determination of sodium the Na standards all contained 2000 mg/L potassium chloride.

During the determination of magnesium in the air-acetylene flame chemical interferences were overcome by the addition of a releasing agent such as strontium or lanthanum.

Analysis in the nitrous oxide-acetylene flame was free from inter-element interference. The magnesium standards were made up with 1N ammonium acetate, and each contained 5 000 mg/L potassium chloride.

For potassium determination, the calibration standards were made up with 1 M ammonium acetate.

The standards for zinc determination were made up with 0.5M EDTA solutions.

Phosphorus was determined as the phosphate using the UV spectrophotometric molybdenum blue method based on Deniges reaction. This involved the addition of ammonium molybdate to convert the orthophosphate and the phosphomolybdate via stannous chloride reduction to the molybdenum blue compound.
Principle:
The sample was digested by the use of aqueous nitric acid (1:1). The digest was mixed with molybdic acid, which was then reduced by Fe^{2+} to produce molybdenum blue complex. Absorbance was then measured at 660 nm using a spectrophotometer.

The following reagents were prepared for use:

-Ammonium molybdate: 0.0355 M
To a 500 mL volumetric flask, 200 mL distilled water was added. Cooled 45 mL concentrated sulphuric acid and then 22 g of ammonium molybdate was added. This volumetric flask was sealed and shaken thoroughly. This solution was made up to the 500 mL mark with distilled water. This reagent was stable.

-Phosphorus Standards
Phosphorus 1.62 mmol/L (5mg/100mL) standard was made by dissolving 0.220 g KH_2PO_4 (dried at 110°C for 1 h) in water in an IL volumetric flask. This solution was diluted to the mark with distilled water. A few drops of chloroform were added as a preservative and stored in a polyethylene bottle. This phosphorus standard was discarded if there were signs of microbial growth.

-Iron-trichloroacetic acid, stabilised
In a 500 mL volumetric flask, 50 g Tri-chloroacetic acid was dissolved in 300 mL water; 5 g Thiourea and 15 g ferrous ammonium sulphate hexahydrate (Mohr's salt) was added. This solution was made up to mark with distilled water and stored in an amber bottle.

Ten mL of iron-trichloroacetic acid to 23 mL sample or standard or blank was added. This was mixed well and allowed to stand for 10 min. One mL of molybdate reagent was added, mixed well and read on spectrophotometer after 20 min. The colour was stable for approx. 2h.

4.2.1.10 Caloric value

The caloric value was measured in a bomb calorimeter. In a bomb calorimeter, the food sample is burnt under pressurised oxygen and the energy that is given off is measured in the form of kJ. The model utilised was a DDS CP500 automatic calorific processor with a DDS CP501 solid state cooler, a DDS CP502 filling station and a DDS CP503 universal interface as accessory units (Digital Data System, Northcliff, Gauteng, South Africa). The method as laid down in the manual accompanying this equipment was followed:

An accurately massed sample of approx. 0.5 g was measured into the metal dish. Ten cm of microchrome wire was placed into the groove and the ferules were slid over it. This was placed into the DDS CP500 filling station and oxygen to 600 kPa was filled in. Sample ID number and mass of sample was entered. Samples were burnt and caloric values recorded. The metal dish was then placed into the DDS CP50I solid state cooler for cooling.

4.2.2 Microbiological assessment

4.2.2.1 Petrifilm E. coli count plates

The Petrifilm *E. coli* count plate (3-M, Boksburg, South Africa) is a reliable, sample-ready medium system for enumerating *Escherichia coli* and coliforms. Petrifilm *E. coli* count plates contain violet red bile nutrients, a cold water soluble gelling agent, a glucuronidase indicator to identify *E. coli* and a tetrazolium indicator to enhance the visualisation of other gram negative (non-*E. coli*) bacteria.

The sample was cultured according to the instruction pamphlet that accompanied this test system as follows:

The *E. coli* count plates were placed on a flat surface. The top film was lifted and I mL of the 10^{-1}, 10^{-2} and 10^{-3} diluted samples were dispersed onto the centre of the bottom film. This was done in duplicate.

The top film was slowly rolled down onto the sample to prevent the entrapment of air bubbles. The sample was distilled evenly within the circular well using a gentle downward pressure on the centre of the plastic spreaders. The plates were incubated in a horizontal position with the clear side up in stacks not exceeding 20 plates. The plates were incubated for 24 h. and examined for coliform and *E.coli* growth.

The petrifilm *E.coli* count plates were accurately counted on a standard colony counter. *E.coli* colonies appeared blue and blue colonies with gas are confirmed *E.coli*.

Other coliform colonies were red and associated with gas.

4.2.2.2. MPN Technique for the identification of bacteria belonging to Enterobacteriaceae

The presumptive method using Lauryl Sulphate Broth was followed and the differential method using Brilliant Green Bile Broth was followed. Confirmation tests on Chromocult Coliform agar and Eosin Methylene-blue lactose sucrose agar was carried out.

Other culture media utilised for non-coliforms:

Malt extract agar, Shigella-Salmonella agar and Baird-Parker media were also utilised.

4.2.2.3 Analysis of mycotoxins

The extraction, dialysis, thin layer chromatography and detection of aflatoxins, moniliformin and fumonisin (toxins produced by fungi belonging to the mycotoxin group) was achieved by adhering to the procedure abridged by Dutton (1996):
Dialysis method:
A. Neutral Fraction:

Twenty five grams of the milled sample was milled and placed in a homogeniser (or wrist action shaker) flask. One hundred mL of acetonitrile, 4% w/v aqueous potassium chloride (9:1) was added. This was shaken for 1 h. The residue was filtered through a Whatman No. 1 filter paper on the Buchner apparatus and the residue was washed with a little more of the solvent mixture (about 10 mL). The residue was saved if presence of moniliformin or fumonisin was suspected.

The total filtrate was transferred to a 250 mL separating funnel and extracted twice with two equal volumes of iso-octane.

Fifty mL of chloroform was added to the defatted aqueous extract and then the chloroform layer (bottom) was run through a small bed of anhydrous sodium sulphate (5 to 10 g) in a folded 11 cm filter paper in a small filter funnel placed in the neck of a 250 mL rotary evaporator flask. The aqueous layer and sodium sulphate bed was retained for Step B.

The combined chloroform extracts was evaporated to dryness on the rotary evaporator with the water bath not exceeding 60°C. The residue, dissolved in 2 mL acetonitrile, was carefully transferred into a pre-wet knotted dialysis sac and immersed in 30% v/v acetone overnight and gently shaken overnight. The dialysate was then extracted with three 25 mL portions of chloroform. Each extract was passed through a bed of anhydrous sodium into a clean rotary evaporation flask as previously.

The extract was evaporated on a rotary vacuum evaporator to dryness and 2 mL of chloroform was added using a pipette and safety filler. After dehydration, this was termed the neutral fraction.

B: Acid fraction:

Fifty mL of 1 M sulphuric acid was slowly added to the retained aqueous fraction (from which the neutral fraction has been extracted) and the resultant effervescence was allowed to subside. Three 25 mL portions of chloroform was carefully extracted, and any buildup of gas was released. Gentle inversion with opening of the tap at each inversion was allowed. This 75 mL of chloroform solution was then passed through the saved bed of anhydrous sulphate and treated in exactly the same way as for the neutral extract but with omission of the dialysis step. This was stored in the refrigerator as the acid fraction.

C: M Fraction:

For the detection of moniliforrnin or fumonisin the solid residue was taken after filtering off the extract (in A: Neutral Fraction) and blended with methanol (100 mL) for 1 h. This extract was filtered and evaporated to dryness in a rotary evaporator. The extract was reconstituted in 0.5 mL 75% methanol/water to give M fraction.

TLC plates were prepared and run in a CEI (9 mL chloroform 0.5 mL ethyl acetate and 0.5 mL isopropanol) tank. The solvent was allowed to reach the top of the plate and then immediately removed from the tank.

The plate was dried well using warm air (over-heating the plate was avoided). The plate was cooled and placed at right angles to the first run into the second solvent in a tank (6 mL toluene, 3 mL ethylacetate and 1 mL formic acid). The origin was in the bottom right hand corner.

The solvent was allowed to run to the top off the plate, and the plate removed from the tank and dried.

Evaluation of results:

The plates were carefully inspected under good daylight and any visible spots were marked with a pencilled circle and identified with a suitable code. The plate was viewed under both long and short wave ultra-violet light.

4.2.3. Protein quality

The PER test is based on growth methods, whereas the NPU method measures nitrogen balance. The methodology followed was according to Wessels (1970):

Broiler chickens were reared to seven days of age on a commercial starter feed. There-after the birds were placed on the experimental treatments which consisted of soya-product plus a supplement of vitamins and minerals and a filler to ensure that the protein content of the mixture was fixed at ten percent of the feed. A nitrogen-free feed was offered to one group of chickens.

Each treatment was replicated four times, with eight chickens per replication. Feed consumption was measured during the 14 day trial period, as was the gain in mass of the birds.

At the end of the test period four broilers from each replication (16 broilers per treatment) were sacrificed, massed individually and dried in a force-draft oven at 90°C.

The moisture content of the chickens were determined from which the nitrogen content was predicted using the following equation:

Body nitrogen (in mg) = 121.6 + 33.1 (x)
(Where x represents body moisture content in g)

$$PER = \frac{\text{Mass gain}}{\text{Protein intake (g)}}$$

$$NPU = \frac{\text{Body N of test group - Body N of group fed non-protein diet}}{\text{N consumed by test group}}$$

4.2.4 Protein functionality

4.2.4.1 Protein dispersibility index (PDI)

This fast stir official method Ba 10-65 of analysis of the American Oil Chemists Society (1973) was followed:

Ten g sample in 150 mL distilled water was blended in an automatic blender at 8 500 rpm for 10 min. The slurry was decanted, allowed to settle and the liquid portion decanted into 50 mL centrifuge tubes. The suspension was centrifuged for 10 min. at 600 x g. The clear supernatant liquid was analysed for nitrogen by the Kjeldahl procedure.

The PDI was calculated by first determining the percentage of water-dispersible protein:

$$\text{Water-dispersible protein} = \frac{\text{Mass of nitrogen in supernatant x 6.25}}{\text{Mass of sample (g)}}$$

$$PDI = \frac{\text{Water-dispersible protein (\%) x 100}}{\text{Total protein (\%)}}$$

4.2.4.2 Nitrogen solubility index (NSI)

The NSI or the slow-stir method, based on the solubility of protein, is detailed as the American Oil Chemists Society Official Method Ba 11-65 (1973):

Five g of sample was stirred in 200 mL distilled water for 120 min at 30°C mechanically at 120 rpm. The solution was diluted to 250 mL. Forty mL was decanted into a centrifuge tube and centrifuged for 10 min at 200 x g.

The clear supernatant liquid was decanted through glass wool in a funnel and the nitrogen content was determined by Kjeldahl analysis.

The NSI was calculated by first finding the percentage of water-soluble nitrogen:

o

$$\% \text{ Water-soluble nitrogen} = \frac{\text{Mass of nitrogen in supernatant} \times 100}{\text{Mass of sample (g)}}$$

$$\text{NSI} = \frac{\text{Water-soluble nitrogen (\%)} \times 100}{\text{Total nitrogen (\%)}}$$

4.2.5 Consumer evaluation

Hundred packages containing one flavour from three different processors were distributed amongst M L Sultan Technikon and University of Natal staff and students. The group thus consisted of middle to upper income literate and adult socio-economic members from a mixture of racial categories. In a similar manner a further 100 packages containing the second flavour was distributed.

Accompanying each package was the following procedure, which detailed the instruction to each household sampling these soya products:

Method of preparation of soya products:

Empty the contents of sample packet into a saucepan. Add 4 cups (800 mL) of water. Bring to the boil with continuous stirring and let simmer for 10 min. Serve preferably with rice.

The "Consumer evaluation of soya products" questionnaire that was provided to all the consumers evaluating the samples is shown in the following two pages:

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

CONSUMER EVALUATION OF SOYA PRODUCTS

Field worker : . . . . . .

Household ID # : . . . . . .

We would like you to take part in a consumer taste test of three soya products. Please evaluate the products using the forms supplied. A field worker will collect your completed evaluation sheets on . . . . . .

We are interested in how much you and at least one other member of your family like each of the three soya products. A 100 g serving of this product translate to 1471 kJ. Participants for this test should be 25 years or older and should consume soya products regularly. You will be asked to evaluate how much you like the appearance, flavour, texture and overall impression of the products. Since this a is a "blind" taste test, the products will be identified by 3-digit codes. Your responses will be coded; no reference will be made to your individual identity.

NB : There is no beef or pork extracts added to these products.

In order to keep the variables to a minimum, we ask that you evaluate the three products, using bread as a carrier, in the order as indicated on the evaluation from and use sips of water to cleanse your palate between different products.

1. You have received three containers of soya products.

2. For identification purpose each product has a separate 3-digit code number written on the packaging.

3. At least two members of the household should independently complete an evaluation sheet. Please note that there are no correct or incorrect answers - just give your honest opinion.

4. Use bread as a carrier and evaluate how much you like the appearance, flavour, texture and overall impression of the products.

5. You are welcome to write down any comments and/or suggestions regarding the samples or testing procedure.

We appreciate your willingness to help evaluate these products.

Department of Biological Sciences
Faculty of Science
M L Sultan Technikon
P.O. Box 1334
DURBAN
4000
Tel. No. : (031) 308-5286
Fax. No. : (031) 308-5286'

Participant # : .....Household ID # .....

2. 1 How much did you like soya product # .....? Mark with a [X] in the appropriate block.

	APPEARANCE	FLAVOUR	TEXTURE	OVERALL ACCEPTABILITY
Like extremely				
Like very much				
Like moderately				
Like slightly				
Dislike slightly				
Dislike moderately				
Dislike very much				
Dislike extremely				

Comments .....
.....

2.2 How much did you like soya product # .....? Mark with a [X] in the appropriate block.

	APPEARANCE	FLAVOUR	TEXTURE	OVERALL ACCEPTABILITY
Like extremely				
Like very much				
Like moderately				
Like slightly				
Dislike slightly				
Dislike moderately				
Dislike very much				
Dislike extremely				

Comments .....
.....

2.3 How much did you like soya product # .....? Mark with a [X] in the appropriate block.

	APPEARANCE	FLAVOUR	TEXTURE	OVERALL ACCEPTABILITY
Like extremely				
Like very much				
Like moderately				
Like slightly				
Dislike slightly				
Dislike moderately				
Dislike very much				
Dislike extremely				

Comments .....
.....

2.4 Now that you have evaluated all three samples, please indicate your order of preference.

Most preferred		Least preferred
Sample code:	Sample code:	Sample code:

Each soya sample was given a 3 digit code obtained from a Random Numbers Table and the order of sampling was randomised. Responses were converted to a hedonic scale of 1 to 8 where 1 was most unfavourable and 8 intensely liked.

4.2.5.1 Statistical analysis of order of preference for savoury flavoured dry-based soya products.

i) Calculation of rank sum total:

The last response in each consumer evaluation of the dry-based savoury flavoured soya products questionnaire was converted to a numerical score by allocating one point to each best preferred manufacturer, two points to the second-best preferred manufacturer and three points for the least preferred manufacturer.

i) Calculation of χ^2 using the Friedman Test:

$$\chi^2_R = \frac{12 \quad x \quad (\Sigma R^2) \quad - \quad 3n\,(k+1)}{nk(k+1)}$$

$$\text{Therefore } \chi^2_R = \frac{12 \quad x \quad 120866 \quad - \quad 3(100)(3+1)}{100\,(2)\,(3)}$$

$$= 8.66$$

From the $\chi 2$ table: $\chi 2$ for $p < 0.05$ (df2) = 5.991
Therefore, the ranks for this data set differ significantly at $p < 0.05$.

iii) "Least significant rank difference" for the Friedman Test

We now have to determine which products were ranked significantly differently from each other; to do that we have to calculate the "least significant rank difference" ("lsrd") for the Friedman test:

$$\text{lsrd} = t_{\alpha,\infty} \quad x \quad \frac{[nK(K+1)]^{0.5}}{[\quad 6 \quad]}$$

Where t is from the t-table: t for $p < 0.05$ (df2) = 1.96

$$\text{Therefore, lsrd} = t \quad x \quad \frac{[100(3)4]^{0.5}}{[\quad 6 \quad]}$$

$$= 27.7$$

4.2.5.2 Statistical analysis of order of preference for mutton flavoured dry-based soya products

I) Calculation of rank sum total:

The last response in each consumer evaluation of the mutton flavoured dry-based soya products questionnaire was similarly converted to a numerical scale by allocating one point to the best preferred manufacturer, two points to the second best preferred manufacturer and three points to the least preferred manufacturer.

I) Calculation of χ^2_R:

$$\chi^2_R = \frac{12}{nk(k+1)} \times (\Sigma R)^2 - 3n(k+1)$$

$$= 35.12$$

From the χ^2 table χ^2 for $p < 0.05$ (df2) = 5.991
Therefore, the ranks for this data set differ significantly at $p < 0.05$.

iii) " Least significant rank difference" for the Friedman Test:

$$lsrd = t_{\alpha,\infty} \times \frac{[nk(k+1)]^{0.5}}{[6]}$$

$$= 1.96 \times \frac{[1200]^{0.5}}{[6]}$$

$$= 27.7$$

4.2.6 Statistics

All statistics were analysed according to the One-Way Analysis of Variance using the Statgraphics Version 5.0 Program. Any differences were subjected to the Least Square Difference (LSD) treatment to determine if the difference was significant ($p<0.05$).

CHAPTER 5

RESULTS

5.1 Chemical composition

5.1.1 Moisture content

The moisture content of mutton and savoury flavoured soya products from three different manufacturers is given in Table 6.

Table 6. Influence of manufacturer and flavour on the moisture content of dry-based soya products (g/100g) on an "as is" basis

Manufacturer	Flavour		Manufacturing effect[1]
	Mutton	Savoury	
A	6.54[2]	7.06	6.80c[3]
B	7.06	4.84	5.95b
C	4.64	3.76	4.20a
Flavour effect	6.07b	5.23a	

[1]Values obtained after raw ingredients have been processed in the individual soya processing plants to manufacture dry-based soya products according to their own set procedures.

[2]Mean moisture content of three replicates.

[3]Mean values with different letters differ significantly from each other ($p<0.05$).

The moisture content of the dry-based soya products from manufacturer A was the highest and from manufacturer C the lowest. In general, the moisture contents of the mutton flavoured dry-based soya products were significantly higher than those of the savoury soya products.

5.1.2 Protein content

The protein content of mutton and savoury flavoured soya products from three different manufacturers is given in Table 7.

Table 7. Influence of manufacturer and flavour on the protein content of dry-based soya products (g/100 g) on an "as is" basis

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	28.4[1]	22.6	25.5a[2]
B	26.5	24.8	25.6a
C	26.0	24.3	25.2a
Flavour effect	26.9b	23.9a	

[1]Mean protein content of three replicates

[2]Mean values with different letters differ significantly from each other ($p<0.05$).

There was no significant difference in the protein content of the dry-based soya products amongst all three manufacturers. However, there was a significant flavour affect on the protein content as the mutton flavoured dry-based soya products had a higher protein content than the savoury soya products.

5.1.3 Fat content

The fat content of mutton and savoury flavoured soya products from three different manufacturers is given in Table 8.

Table 8. Influence of manufacturer and flavour on the fat content of dry-based soya products (g/100 g) on an "as is" basis

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	7.43[1]	7.94	7.68b[2]
B	7.31	7.71	7.51b
C	5.33	7.31	6.32a
Flavour effect	6.30a	7.55b	

[1]Mean fat content of three replicates.

[2]Mean values with the different letters differ significantly from each other ($p < 0.05$).

The fat contents of the dry-based soya products from manufacturers A and B were significantly higher than in products from manufacturer C. There was a significant flavour affect as the fat content of the mutton flavoured dry-based soya products was lower than the savoury soya products.

5.1.4 Fatty acid profile

5.1.4.1 Palmitic acid (C 16: 0) content

The palmitic acid content of mutton and savoury flavoured soya products from three different manufacturers is given in Table 9.

Table 9. Influence of manufacturer and flavour on the palmitic acid (C 16: 0) content of dry-based soya products (g/100 g) on an "as is basis

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	0.741[1]	0.921	0.831c[2]
B	0.635	0.738	0.687b
C	0.531	0.637	0.578a
Flavour effect	0.635a	0.762b	

[1]Mean palmitic acid (C 16: 0) content of three replicates.

[2]Mean value with different letters differ significantly from each other ($p < 0.05$).

There was a significant difference in the palmitic acid content of the dry-based soya products amongst all three manufacturers with products from manufacturer A having the highest and products from manufacturer C generally having the lowest palmitic acid content. There was also a significant flavour affect on the palmitic acid content. The mutton flavoured dry-based soya products had a lower palmitic acid content compared to the savoury soya products.

5.1.4.2 Stearic acid (C 18: 0) content

The stearic acid content of mutton and savoury flavoured soya products from three different manufacturers is given in Table 10.

Table 10. Influence of manufacturer and flavour on the stearic acid content of dry-based soya products (g/100 g) on an "as is" basis

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	0.333[1]	0.314	0.323b[2]
B	0.318	0.325	0.321b
C	0.218	0.316	0.267a
Flavour effect	0.289a	0.318a	

[1]Mean stearic acid content of three replicates.

[2]Mean values with different letters differ significantly from each other ($p < 0.05$).

The stearic acid content of the dry-based soya products from manufacturer C was generally lower than products from manufacturer A and B. However, there was no significant flavour affect on stearic acid contents of the mutton and savoury flavoured soya products.

5.1.4.3 Oleic acid (C 18: 1) content

The oleic acid content of mutton and savoury flavoured soya products from the different manufacturers is given in Table 11.

Table 11. Influence of manufacturer and flavour on the oleic acid (C 18: 1) content of dry-based soya products (g/100 g) on an "as is" basis

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	1.63[1]	1.68	1.66b[2]
B	1.72	1.63	1.68b
C	1.13	1.51	1.32a
Flavour effect	1.49a	1.61b	

[1]Mean oleic acid content of three replicates.

[2]Mean values with different letters differ significantly from each other ($p < 0.05$).

Products from manufacturer C had a significantly lower oleic acid content than products from manufacturers A and B. In general mutton flavoured products had a lower oleic acid content than savoury flavoured soya products.

5.1.4.4 Linoleic acid (C 18: 2) content

The linoleic acid content of mutton and savoury flavoured soya products from three different manufacturers is given in Table 12.

Table 12. Influence of manufacturer and flavour on the linoleic acid (C 18: 2) content of dry-based soya products (g/100 g) on an "as is" basis

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	4.15[1]	4.35	4.25b[2]
B	4.23	4.13	4.18b
C	3.09	4.11	3.60a
Flavour effect	3.82a	4.19b	

[1]Mean linoleic acid content of three replicates.

[2]Mean values with different letters differ significantly from each other ($p < 0.05$).

Products from manufacturer C generally had a lower linoleic acid content than those from manufacturers A and B. In general the mutton flavoured products had a lower linoleic acid content than savoury flavoured products.

5.1.4.5. Linolenic acid (C 18: 3) content

The linolenic acid content of mutton and savoury flavoured soya products from three different manufacturers is given in Table 13.

Table 13. Influence of manufacturer and flavour on the linolenic acid (C 18: 3) content of dry-based soya products (g/100 g) on an "as is" basis

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	0.584[1]	0.667	0.626b[2]
B	0.612	0.699	0.656c
C	0.501	0.624	0.562a
Flavour effect	0.565a	0.663b	

[1]Mean linolenic acid content of three replicates.

[2]Mean values with different letters differ significantly from each other ($p < 0.05$).

Products from manufacturer C had the lowest linolenic acid content whereas products from manufacturer B had the highest. The mutton flavoured products had a significantly lower linolenic acid content than the savoury flavoured soya products.

5.1.5 Dietary fibre

The dietary fibre content of mutton and savoury flavoured soya products from three different manufacturers is given in Table 14.

Table 14. Influence of manufacturer and flavour on the dietary fibre content of dry-based soya products (g/100 g) on an "as is" basis

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	5.24[1]	4.86	5.05b[2]
B	6.76	3.84	5.30b
C	3.24	3.16	3.20a
Flavour effect	5.07b	3.97a	

[1]Mean fibre dietary content of three replicates.

[2]Mean values with different letters differ significantly from each other ($p < 0.05$).

Soya products from manufacturers A and B were significantly higher in dietary fibre than those from manufacturer C. The mutton flavoured products generally had a higher dietary fibre content compared to savoury flavoured soya products. This was particularly evident between the mutton and savoury flavoured soya products that emanated from manufacturer B.

5.1.6 Soluble carbohydrates

The individual soluble carbohydrates of mutton and soya flavoured products from different manufacturers are shown in Table 15.

Table 15. Soluble carbohydrates[1] in mutton and savoury flavoured soya products from different manufacturers (g/100 g) on an "as is" basis

Manufacturer and Flavour	Fructose	Glucose	Sucrose
A: Mutton	0	0.123	0.662
A: Savoury	0.012	0.033	0.738b
B: Mutton	0	0	0.793b
B: Savoury	0	0	0.778b
C: Mutton	0	0	0.842c
C: Savoury	0	0	0.829c

[1]Mean soluble carbohydrate content of three replicates.

[2]Mean values with different letters differ significantly from each other ($p<0.05$).

Fructose and glucose only occurred in savoury flavoured dry-based soya products from manufacturer A. Sucrose occurred in all the products with the highest concentration in the products from manufacturer C.

5.1.7 Carbohydrate content

The carbohydrate content of mutton and savoury flavoured soya products from three different manufacturers is given in Table 16.

Table 16. Influence of manufacturer and flavour on the carbohydrate content of dry-based soya products (g/100 g) on an "as is" basis

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	42.65[1]	49.18	45.92a[2]
B	45.13	49.04	47.08a
C	50.61	51.09	50.85b
Flavour effect	46.13a	49.77b	

[1]Mean carbohydrate content of three replicates.

[2]Mean values with different letters differ significantly from each other ($p < 0.05$).

The carbohydrate content of the dry-based soya products from manufacturer C was significantly higher than the products from manufacturers A and B. The savoury flavoured products generally had a higher carbohydrate content compared to mutton flavoured products.

5 1.8. Ash content

The ash content of mutton and savoury flavoured soya products from three different manufacturers is given in Table 17.

Table 17. Influence of manufacturer and flavour on the ash content of dry-based soya products (g/100 g) on an "as is" basis

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	9.74[1]	8.36	9.05b[2]
B	7.24	9.77	8.51a
C	9.98	10.38	10.18b
Flavour effect	8.99a	9.50a	

[1]Mean ash content of three replicates.

[2]Mean values with different letters differ significantly from each other ($p < 0.05$).

In general the ash contents in the dry-based soya products from manufacturer B were lower than in soya products from manufacturers A and C. There was no significant flavour affect in the ash content between the mutton and savoury flavoured products.

5.1.9 Mineral content

5.1.9.1 Calcium

The calcium content of mutton and savoury flavoured soya products from three different manufacturers is given in Table 18.

Table 18. Influence of manufacturer and flavour on the calcium content of dry-based soya products (mg/100 g) on an "as is" basis

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	150[1]	110	130a[2]
B	130	140	135a
C	130	130	130a
Flavour effect	137a	127a	

[1]Mean calcium content of three replicates.

[2]Mean values with different letters differ significantly from each other ($p < 0.05$).

There was no significant difference in the calcium content of the dry-based soya products between the three manufacturers. There was also no flavour affect on the calcium content between the mutton and savoury flavoured soya products.

5.1.9.2 Magnesium

The magnesium content of mutton and savoury flavoured soya products from three different manufacturers is given in Table 19.

Table 19. Influence of manufacturer and flavour on the magnesium content of dry-based soya products (mg/100 g) on "an is" basis

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	160[1]	130	145b[2]
B	150	140	145b
C	140	130	135a
Flavour effect	150b	133a	

[1]Mean magnesium content of three replicates.

[2]Mean values with different letters differ significantly from each other ($p < 0.05$).

There was a significant difference in the magnesium content of the dry-based soya products. Those from manufacturer C were lower than those from manufacturers A and B. The mutton flavoured soya products had a higher magnesium content than the savoury flavoured products.

5.1.9.3 Sodium

The sodium content of mutton and savoury flavoured soya products from three different manufacturers is given in Table 20.

Table 20. Influence of manufacturer and flavour on the sodium chloride content of dry-based soya products (mg/100 g) on an "as is" basis

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	2 738[1]	3 836	3 287a[2]
B	4 380	3 983	4 182b
C	3 483	3 934	3 708a
Flavour effect	3 533a	3 918b	

[1]Mean sodium chloride content of three replicates.

[2]Mean values with different letters letter differ significantly from each other ($p < 0.05$).

There was a significant difference in the sodium content of the dry-based soya products. Those from manufacturer B were higher in sodium than those from manufacturers A and C. Sodium levels were generally higher in the savoury flavoured products than in the mutton flavoured products.

5.1.9.4 Zinc

The zinc content of mutton and savoury flavoured soya products from three different manufacturers is given in Table 21.

Table 21. Influence of manufacturer and flavour on the zinc content of dry-based soya products (mg/ 100 g) on an "as is" basis

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	3.0[1]	3.0	3.0a[2]
B	4.0	3.0	3.5b
C	4.0	3.0	3.5b
Flavour effect	3.7a	3.0a	

[1] Mean zinc content of three replicates.

[2] Mean values with different letters differ significantly from each other ($p < 0.05$).

There was a significant difference in the zinc content of dry-based soya products. Those from manufacturer A generally had less zinc than those of products from manufacturers A and B. However, there was no significant flavour affect on the zinc contents between the mutton and savoury soya products.

5.1.9.5 Manganese

The manganese content of mutton and savoury flavoured soya products from three different manufacturers is given in Table 22.

Table 22. Influence of manufacturer and flavour on the manganese content of dry-based soya products (mg/100 g) on an "as is" basis

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	2.0 [1]	2.0	2.0a[2]
B	2.0	2.0	2.0a
C	2.0	2.0	2.0a
Flavour effect	2.0a	2.0a	

[1]Mean manganese content of three replicates.

[2]Mean values with different letters differ significantly from each other ($p < 0.05$).

There was no significant difference in the manganese content of soya products amongst all three manufacturers. There was also no significant flavour affect on manganese content.

5.1.9.6 Potassium

The potassium content of mutton and savoury flavoured soya products from three different manufacturers is given in Table 23.

Table 23. Influence of manufacturer and flavour on the potassium content of dry-based soya products (mg/100 g) on an " as is" basis

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	1 310[1]	940	1 120a[2]
B	1 130	1 120	1 120a
C	1 160	1 060	1 110a
Flavour effect	1 200b	1 040a	

[1]Mean potassium content of three replicates.

[2]Mean values with different letters differ significantly from each other ($p < 0.05$).

There was no significant difference in the potassium content of the soya products between the three manufacturers. However, there was a significant flavour affect on the potassium contents as the mutton flavoured soya products had higher potassium content.

5.1.9.7 Phosphorus

The phosphorus content of mutton and savoury flavoured soya products from three different manufacturers is given in Table 24.

Table 24. Influence of manufacturer and flavour on the phosphorous content of dry-based soya products (mg/100 g) on an "as is" basis

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	280[1]	230	255a[2]
B	240	250	245a
C	260	230	245a
Flavour effect	260b	237a	

[1]Mean phosphorus content of three replicates.

[2]Mean values with different letters differ significantly from each other ($p < 0.05$).

There was no significant difference in the phosphorus content of the soya products between the three manufacturers. However, there was a significant flavour affect on the phosphorus content as the mutton flavoured soya products generally had a higher phosphorus content.

5.1.9.8 Iron

The iron content of mutton and savoury flavoured soya products from three different manufacturers is given in Table 25.

Table 25. Influence of manufacturer and flavour on the iron content of dry-based soya products (mg/100 g) on an "as is" basis

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	9.0[1]	6.0	8.0a[2]
B	9.0	7.0	8.0a
C	8.0	8.0	8.0a
Flavour effect	8.7b	7.0a	

[1]Mean iron content of three replicates.

[2]Mean values with different letters differ significantly from each other ($p < 0.05$).

There was no significant difference in the iron content of the products between the three manufacturers. However, the mutton flavoured products generally had a higher iron content than the savoury flavoured products.

5.1.10 Caloric value

The caloric value of mutton and savoury flavoured soya products from the three different manufacturers is given in Table 26.

Table 26. Influence of manufacturer and flavour on the caloric content of dry-based soya products (kJ/100 g) on an "as is" basis

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	620[1]	583	602a[2]
B	590	610	600a
C	613	603	608a
Flavour effect	608a	599a	

[1]Mean caloric content of the three replicates.

[2]Mean values with different letters differ significantly from each other ($p < 0.05$).

There was no significant difference in the caloric content of the dry-based soya products from the three manufacturers. Also, flavour did not significantly influence the caloric content.

5.1.11. Summary proximate composition

The mean proximate composition of the six dry-based soya samples compared to beef is shown in Table 27.

Table 27. Summary proximate composition of the six dry-based soya products compared to beef (g/ 100 g) on an "as is" basis

Nutrient component	Dry-based soya products	Beef[1]
Moisture	5.65	70-73
Protein	25.43	20-22
Fat	7.21	4-8
Dietary fibre	4.52	0
Carbohydrate	47.95	5
Ash	9.24	1

[1] Composition of lean muscle tissue from Anglemier and Montgomery, (1976).

5.2 Microbiological assessment

5.2.1 Petrifilm *E.coli* plate count

The petrifilm *E.coli* plate count of mutton and savoury flavoured soya products from three different manufacturers is given in Table 28.

Table 28. Influence of manufacturer and flavour on the *E.coli* plate count of dry-based soya products (cfu/ g)

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	0[1]	25	12.5b[2]
B	41	12	26.5c
C	0	0	0.0a
Flavour effect	13.7b	12.3a	

[1]Mean petrifilm *E.coli* plate count of four replicates.

[2]Mean values with different letters differ significantly from each other ($p < 0.05$).

There was a significant difference in the *E coli* plate count of the dry-based soya products between the three manufacturers with products from manufacturer C having zero *E. coli* contamination and products from manufacturer B generally having the highest *E. coli* contamination levels. Flavour, also had a significant effect on the *E. coli* plate count. In general mutton flavoured products had a higher *E. coli* count.

5.2.2 *Enterobacteriaceae* organisms observed on eosin methylene blue (EMB) agar and chromocult plates

The *Enterobacteriaceae* organisms found on EMB agar and chromocult plates were identified as shown in Table 29.

Table 29. *Enterobacteriaceae* organisms observed on eosin methylene blue (EMB) agar and chromocult plates

Medium	Organisms
EMB Chromocult	*Enterobacter aerogenes* *Klebsiella sp.*

Enterobacteriaceae organisms were present on all the products from the three manufacturers.

5.2.3 Other culture media to test for moulds, yeasts, *Shigella*, *Salmonella* and *Staphylococcus*

The results from malt extract agar (ME), *Shigella-Salmonella* (SS) agar and Baird-Parker medium (BP) are shown in Table 30.

Table 30. Growth on malt extract agar (ME), *Shigella-Salmonella* agar (SS) and Baird - Parker medium (BP) plated with 10^{-1}, 10^{-2} and 10^{-3} dilutions of dry-based soya products

Manufacturer and flavour	Media								
	ME			S-S			B-P		
	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
A : Mutton	+[1]	+	+	+	-[2]	-	+	+	+
A : Savoury	+	+	+	+	-	-	+	+	+
B : Mutton	+	+	+	+	-	-	+	+	+
B : Savoury	+	+	+	+	-	-	+	+	+
C : Mutton	+	+	+	-	-	-	+	+	+
C : Savoury	+	+	+	-	-	-	+	+	+

[1] : Growth or [2] : No growth

Mutton and savoury flavoured products from manufacturer C were devoid of *Shigella* and *Salmonella* contamination. Products from manufacturers A and B had minimal *Shigella* and *Salmonella* contamination. All the products had mould and *E.coli* present.

5.2.4 Analysis for aflatoxin, moniliformin and fumonisin

Table 31 shows the results for mycotoxin analysis of extracts from mutton and savoury flavoured soya products run on thin layer chromatography (TLC).

Table 31. Influence of manufacturer and flavour on the presence of aflatoxin, moniliformin and fumonisin in dry-based soya products

Manufacturer	Flavour	
	Mutton	Savoury
A	-[1]	-
B	-	-
C	-	-

[1]: Absence of any aflatoxin, moniliformin or fumonisin

All the products were free from aflatoxin, moniliformin and fumonisin contamination.

5.3 Protein quality

5.3.1 Protein efficiency ratio (PER)

The PER of mutton and savoury flavoured soya products from three different manufacturers is given in Table 32.

Table 32. Influence of manufacturer and flavour on the PER of dry-based soya products

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	0.2[1]	1.2	0.7a[2]
B	2.3	2.9	2.6b
C	2.3	2.9	2.6b
Flavour effect	1.6a	2.3a	

[1]Mean PER acid content of four replicates with eight chickens per replicate.

[2]Mean values with different letters differ significantly from each other ($p < 0.05$).

There was a significant difference in the PER of the soya products between manufacturers. Products from manufacturers B and C had significantly better PER than those from manufacturer A. However, there was no significant flavour affect on the PER of the mutton and savoury flavoured soya products.

5.3.2 Net protein utilisation (NPU)

The NPU of mutton and savoury flavoured soya products from three different manufacturers is given in Table 33.

Table 33. Influence of manufacturer and flavour on the NPU of dry-based soya products

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	35.2[1]	14.2	24.7a[2]
B	66.3	35.0	50.8b
C	69.9	28.5	50.8b
Flavour effect	58.2b	25.9a	

[1]Mean NPU values of four replicates with eight chickens per replicate.

[2]Mean values with different letters differ significantly from each other ($p < 0.05$).

There was a significant difference in the NPU of the soya products between manufacturers. Products from manufacturers B and C had better NPU values than those from manufacturer A. Mutton flavoured products had a higher NPU compared to savoury flavoured products.

5.4 Protein functionality

5.4.1 Protein dispersibility index (PDI)

The PDI of mutton and savoury flavoured soya products from three different manufacturers is given in Table 34.

Table 34. Influence of manufacturer and flavour on the PDI of dry-based soya products on an "as is" basis

Manufacturer	Flavour		Manufacturing Effect
	Mutton	Savoury	
A	75.48[1]	64.65	70.06c[2]
B	28.72	30.56	29.64b
C	16.82	18.00	17.42a
Flavour effect	40.34b	34.74a	

[1]Mean PDI of three replicates.

[2]Mean values with different letters differ significantly from each other ($p < 0.05$).

There was a significant difference in the PDI values of the soya products between the three manufacturers with those from C having the lowest PDI and products from manufacturer A having the highest PDI. Mutton flavoured products generally had higher PDI compared to the savoury flavoured products.

5.4.2 Nitrogen solubility index (NSI)

The NSI or protein solubility index (PSI) of mutton and savoury flavoured soya products from three different manufacturers is given in Table 35.

Table 35.Influence of manufacturer and flavour on the NSI of dry-based soya products on an "as is" basis

Manufacturer	Flavour		Manufacturing effect
	Mutton	Savoury	
A	7.59[1]	7.74	7.66b[2]
B	3.30	3.53	3.42a
C	3.36	3.60	3.48a
Flavour effect	4.75a	4.96a	

[1] Mean NSI of three replicates.

[2] Mean values with different letters differ significantly from each other ($p<0.05$).

Products from manufacturer A had a higher NSI than those from manufacturers B and C. However, there was no a significant flavour affect on the NSI.

5.5 Consumer evaluation

5.5.1 Consumer evaluation of savoury flavoured soya products

The evaluations of appearance, flavour, texture and overall acceptance of savoury flavoured dry-based soya product from three different manufacturers is given in Table 36.

Table 36. Influence of manufacturer on the appearance, flavour, texture and overall acceptance of savoury flavoured dry-based soya products[1]

Manufacturer	Appearance	Flavour	Texture	Overall acceptance
A	5.48a[2]	5.73a	5.53a	5.59a
	(0.18)[3]	(0.21)	(0.18)	(0.20)
B	6.13b	6.35b	6.22b	6.27b
	(0.17)	(0.20)	(0.17)	(0.19)
C	6.42b	6.50b	6.25b	6.40b
	(0.18)	(0.19)	(0.20)	(0.20)

[1] Mean response from 100 consumers indicating their evaluation on a hedonic scale of 1 to 8 where 1 = Dislike intensely and 8 = Like intensely.

[2]Means with different letters in columns differed significantly from each other ($p < 0.05$).

[3]Values in parenthesis indicate standard error.

Savoury flavoured products from manufacturer B and C were equally liked in their appearance, flavour, texture and overall acceptance categories. Manufacturer A produced savoury flavoured products that were least liked in the four categories.

5.5.1.1 The influence of manufacturer on order of preference of savoury flavoured soya products

The influence of manufacturer on order of preference of savoury flavoured soya products is given in Table 37.

Table 37. Influence of manufacturer on order of preference of savoury flavoured soya products

Manufacturer	Rank Totals[1]
A	224b
B	187a
C	189a
lsrd	27.7

[1]Rank totals with different letters differing significantly from each other ($p < 0.05$).

Products from manufacturers B and C were ranked better than those from manufacturer A. There was no significant difference in the ranking of products from manufacturers B and C.

5.5.2 Consumer evaluation of mutton flavoured soya products

The evaluation of appearance, flavour, texture and overall acceptance of mutton flavoured dry-based soya product from three different manufacturers is given in Table 38.

Table 38. Influence of manufacturer on the appearance, flavour, texture and overall acceptance of mutton flavoured dry-based soya products[1]

Manufacturer	Appearance	Flavour	Texture	Overall acceptance
A	6.10a[2]	6.10a	5.94a	5.92a
	(0.20)[3]	(0.22)	(0.21)	(0.21)
B	6.10a	6.17a	6.20a	6.07a
	(0.20)	(0.23)	(0.20)	(0.23)
C	6.37a	6.57a	6.53a	6.58a
	(0.17)	(0.20)	(0.18)	(0.19)

[1]Mean response obtained from 100 consumers indicating their evaluation on a hedonic scale of 1 to 8 where 1 = Dislike extremely and 8 = Like extremely.

[2]Means with different letters in column differed significantly from each other ($p < 0.05$).

[3]Values in parenthesis indicate standard error.

There was no significant difference in consumer response amongst the three manufacturers in the four evaluative categories.

5.5.2.1 The influence of manufacturer on order of preference of mutton flavoured soya products

The influence of manufacturer on order of preference of mutton flavoured soya products is given in Table 39.

Table 39. Influence of manufacturer on order of preference of mutton flavoured soya products

Manufacturer	Rank Totals[1]
A	236b
B	210b
C	154a
lsrd	27.7

[1]Rank totals with different letters differ significantly from each other ($p < 0.05$).

Products from manufacturer C were ranked the best. However, there was no significant difference in the ranking order of products from manufacturers A and B.

5.6 Overview of the nutrient content per serving of the dry-based soya products and contribution to the recommended daily dietary allowance (RDA)

Table 40 shows an overview of the nutrient content per serving of the dry-based soya products and contribution to the RDA.

Table 40. Nutrient content per 33.3 g serving of the dry-based soya products and % contribution to the recommended daily dietary allowance (RDA)

Nutrient	Mean content	%RDA
Energy (kJ)	603	4.8[1]
Protein (g)	8.48	15.2
Calcium (mg)	43.9	5.5
Phosphorus (mg)	82.8	10.4
Iron (mg)	1.3	7.2
Magnesium (mg)	47.2	11.8
Zinc (mg)	0.56	3.7

[1] Figures represent % contribution to the RDA (Government Gazette, 1966).

A single serving of the dry-based soya products makes a significant contribution in protein, phosphorus, iron and magnesium to the RDA.

5.7 Economic comparison of soya protein with beef/ chicken protein

Table 41 shows the economic comparison of soya protein with beef/ chicken protein.

Table 41. Economic comparison of soya protein with beef/ chicken protein on an "as is" basis

Food	Protein content (g%)	Retail price/kg	Price/kg protein
Beef	20	R23.95	R119.75
Chicken	20	R32.99	R164.95
Dehydrated soya	25	R21.45	R85.80

Dehydrated soya products retailing at R4.29 per 200 g (Personal observation: Pick and Pay Hypermarket by the Sea, Durban, 16/6/99) packet was more economical on a price per kg protein basis than filleted chicken retailing at R32.99 per kg (Personal observation: Filleted chicken breasts at Pick and Pay Hypermarket by the Sea, Durban, 28/5/99). Beef protein was also more expensive than soya protein. Chicken protein was the most expensive amongst these three protein sources and soya protein the most cheapest.

CHAPTER 6

DISCUSSION

6.1 Chemical composition

The low moisture contents (approx. 5.7%) of these dry-based soya products enable them to have a long storage life. The moisture content of raw, dried soya beans is 8.5% (Langenhoven, Kruger, Gouws and Faber, 1991) and that of stored mature soya beans is usually about 12 to 14% to ensure storage stability (Snyder & Kwon, 1987). The mean moisture content reported here was similar to the moisture content of textured soya protein concentrate of 5.8% reported by Doell, Ebden, and Smith (1982) and that of in defatted soya flour (7%) reported by Kinsella (1978). The thermal processing applied to defatted soya flour maybe responsible for the further reduction in moisture content to the levels found here.

In Britain, Wenlock, Sivell and Agater (1985) in compiling moisture data for dehydrated cereal and legume-containing products also reported a flavour influence on moisture content. The flavourants utilised to create the mutton and savoury flavours may be responsible for the differing moisture contents between the two flavours. The different levels of moisture levels amongst the three manufacturers could be attributed to the differing intensity or duration of thermal treatment utilised by the manufacturers.

The mean protein content of these soya products (25.4%) was very much lower than the protein content of raw, dried soya beans which varies from 36.5% (Langenhoven, Kruger, Gouws, and Faber, 1991) to 38% (Ologhobo, 1989) and 40% (Smith and Circle, 1978). The protein content of defatted soya flour, concentrate and isolate is reported as 56, 72 and 96%, respectively (Kinsella, 1978). This is evidence that the natural protein concentration in the locally manufactured, dry-based soya products may have been diluted by the addition of other ingredients e.g., starch.

The higher protein content in mutton flavoured products is indicative of the hydrolysed vegetable protein (HVP) present in the mutton flavoured extracts used to convey a mutton flavour. Spices contributing to a savoury flavour, being of low protein content, presumably depressed protein content values.

Of interest was the lack of rancidity complaints received during the consumer evaluation of these soya protein foods. Many of the off flavour compounds in novel proteins originate via the oxidation of the lipid components. Deterioration caused by lipids is a general problem with protein concentrates. Though present at few parts per million, these off flavours adhere to proteins and may persist in the product through processing (Kinsella, 1978). It is probable that the local manufacturers are incorporating an antioxidant in their formulation.

The fatty acid profile (obtained by determining the mean content of each fatty acid and expressing it as a % of the mean fat content) of 9.7% palmitic acid, 4.2% stearic acid, 21.5% oleic acid, 55.9% linoleic acid and 8.6% linolenic acid closely resembles the fatty acid profile of soya oil reported by Weingartner (1987) (9% palmitic acid, 4% stearic acid, 24% oleic acid, 54% linoleic acid and 8% linolenic acid). The conventional cooking oils e.g., coconut, palm kernel, olive and sunflower are devoid of linolenic acid (Langenhoven, Kruger, Gouws and Faber, 1991). The only other edible oil with linolenic acid is low erucic rape oil but the rape oil fatty acid profile (5% palmitic acid, 2% stearic acid and 9% linolenic acid) (Snyder and Kwon, 1987) does not match the fatty acid profile reported here. These data indicate that soya oil was added back to these soya protein foods.

The fatty acid profiles amongst the manufacturers were consistent which possibly suggests that different quantities of vegetable oil were added. Manufacturer C had the lowest content for all three fatty acids and also the lowest fat content.

The general finding that mutton flavoured product had the lower content for all five fatty acids in comparison with savoury flavoured products could be attributed to savoury flavourants being added in the form of oleoresins. Different formulations from the three manufacturers and different formulations to produce the mutton and savoury flavour account for these flavour differences in fatty acids.

The percentage fatty acid composition reported here was similar to the fatty acid profile of dehydrated banana soya flakes found by Ruales, Pólit and Nair (1990): palmitic acid-11%, stearic acid-3%, oleic acid-22%, linoleic acid-56% and linolenic acid-8%.

Doell, Ebden and Smith (1982) also reported a similar fatty acid composition in tofu, which is a fermented soya product: palmitic acid-12%, stearic acid-4%, oleic acid-17%, linoleic acid-60% and linolenic acid-6%.

Volatile compounds resulting from lightly roasting soya bean cotyledons are also known to be derived from lipids in the beans (Macleod and Ames, 1988). Consequently, consumers reported "soya" odours from the aromatic compounds that were liberated during the cooking process.

The mean dietary fibre content of the soya bean products (4.5%) was slightly higher than the insoluble carbohydrate content (3.5%) (Smith and Circle, 1978) and fibre content (4%) (Weingartner, 1987) reported for defatted soya flour. Whole, raw soya bean is reported to have a fibre content of 6%, half of which resides in the hull (Smith and Circle, 1978). Fibre levels vary according to the variety of soya bean and to the extent of processing. Ologhobo (1989) reported that fibre increased in germinated soya beans and values obtained ranged from 2.61% in decorticated soya to 7.16% in germinated soya. The results here fall within this range.

The constituents of raw soya bean fibre are 20% crude cellulose, 50% crude hemicellulose and 30% pectin (Snyder and Kwon, 1987). Dehulled soya bean flour contains 6.2% neutral detergent fibre, 5.7% detergent fibre, 4.6% crude cellulose, 0.5% crude hemicellulose and 1.3% lignin (Snyder and Kwon, 1987).

In recent years, the possible beneficial effects of dietary fibre have received much attention (Snyder and Kwon, 1987). Fibre, besides providing bulk to the diet stimulates propulsive movements (peristalsis) and thus counteracts constipation (Meyer, Meij and Meyer, 1997). It has been claimed that people who live on a diet containing large amounts of vegetable and fruit fibre have a low incidence of diverticulitis (inflammation of the colon diverticula), cancer of the colon, diabetes mellitus and coronary heart disease – *inter alia* because of the high fibre content of these foods. Inglett (1997) has reported on soluble fibre contributing to better blood composition by lowering blood cholesterol.

Very little soluble carbohydrate occurred in the soya protein foods analysed (0.774%). Normally in mature soya beans, the quantities of soluble carbohydrate are about 10%, with approx. 5% sucrose, 1% raffinose, and 4% stachyose (Smith and Circle, 1978).

Kinsella (1978) reported 4.5% sucrose, 3.7% stachyose and 1.1% raffinose in defatted soya flour. Since the samples here were devoid of raffinose and stachyose, the oligosaccharides seem to have been eliminated during the processing stages but leaving behind residual sucrose. Alternatively, all the soluble carbohydrates could have been washed out and small additions of fructose/glucose or sucrose could have been made.

The finding that the major soluble carbohydrates found in all three manufacturer's products was sucrose, and that glucose only occurred in mutton and savoury flavoured soya products from manufacturer A and further that fructose only occurred in savoury flavoured dry-based soya product from manufacturer A could be accounted for by different individual manufacturer's formulation.

Raffinose and stachyose are not digestible by enzymes produced by humans, although they can be used by the gut microflora, producing gaseous waste products (Brody, 1994). This gave rise to a negative attribute of soya products that contained these oligosaccharides as the resulting gas production causes flatulence (Steggerda, Richards and Rackis, 1966). The absence of the trisaccharide raffinose and tetrasaccharide stachyose is thus beneficial to the consumer, as the problem of flatulence to soya protein food consumers is eliminated.

The soluble carbohydrates can be extracted from defatted soya bean flakes in three principal ways and with many minor variations (Snyder and Kwon, 1987). All processes require that the protein be insolubilised in some way, so that it is not extracted. The three methods for insolubilising the proteins are by heating, by acid and by ethanol. Details of these processes are commercial secrets and not readily available, but the general principles are as described.

Carbohydrates in soya beans according to Snyder and Kwon (1987) are normally around 30% (inclusive of approx. 3% dietary fibre). The higher value obtained here (45.2%) is possibly indicative of the starch that was added during the manufacturing process.

Different levels of carbohydrates amongst the manufacturers and flavours is indicative of the different formulations adhered to in processing these products i.e., different levels of starch addition.

The mean ash content, which is the total mineral content, of 10.2%, was double the content in soya bean (5%) (Snyder and Kwon, 1987) and also far greater than the content in defatted soya flour (5.8%) (Kinsella, 1978).

The higher ash values in these products compared to the ash content of soya bean and defatted soya flour is probably indicative of the added salt and monosodium glutamate that were used in the manufacturing process to enhance the flavourants added. The higher ash values in the savoury flavoured products are possibly indicative of the addition of extra salt and monosodium glutamate than in the mutton flavour. Different manufacturer formulations may account for the different manufacturing effect on ash content.

The soya bean is a good source of minerals (Nelson, Wei and Steinberg, 1980). The major minerals in soya beans are potassium, sodium, calcium, magnesium, sulphur and phosphorus (Snyder and Kwon, 1987). Snyder and Kwon (1987) stated that both the type of soil and growing conditions can influence the mineral content of soya beans.

Compared with the mineral content of soya flour, these products revealed similar drastically reduced quantities with all minerals except sodium. Elevated levels (7.6 g%) of sodium content in products out of all the manufacturers is indicative of the large quantities of salt and monosodium glutamate that was added to enhance taste qualities. O' Dell (1979) and Smith and Circle (1978) reported that calcium and magnesium in soya bean each constituted 300 mg/100 g. Erdman and Fordyce (1989) reported a calcium content of 241 mg/100 g soya flour and a magnesium content of 290 mg/100 g soya flour. In comparison the levels here were slightly more than half the normal calcium (130 mg/100 g) and slightly less than half the normal magnesium content (140 mg/100 g) found in defatted soya flour. This can be accounted for by the phenomenon that calcium, magnesium, and phosphorus can be extracted with the phospholipids and become part of the oil during the oil extraction process (Snyder and Kwon, 1987). In addition, the dilution effect during the processing of these products could have depressed mineral values.

The zinc content of the soya products was lower than the zinc content in flakes of banana pulp and soya flour (Ruales, P∧lit and Nair, 1990). Values found here was slightly less than the 5.2 mg/100 g in defatted soya beans (Snyder and Kwon, 1987).

In soya bean products likely to be phytate rich (1.0 to 2.2 g%) (Anderson and Wolf, 1995), the zinc is poorly available to animals and this may have implications for human nutrition. The phytate-protein complexes formed during processing are probably responsible for the variability of zinc utilisation in diets containing isolates manufactured by different processes (Erdman and Fordyce, 1989). Consequently, the bioavailability of zinc may be compromised by the presence of phytates but this is overcome by the adequate quantity of zinc that is available from soya products.

Manganese was significantly lower than in soya beans (2.8 mg/100 g) (Circle and Smith, 1978) and 3.8 mg/100 g in defatted soya beans (Snyder and Kwon, 1987). Dilution during manufacture of the soya products was probably the cause. The mean potassium content of 1.1 g% was also lower than the 1.7 g% reported in soya beans (Snyder and Kwon, 1987). This could be attributed to the dilution effect of added starch.

The mutton flavoured dry-based soya products had a significantly higher potassium content (1.2 g%) than savoury flavoured dry-based soya products (1.0 g%) which may be attributable to the different flavourants used. Generally this seemed to be the pattern as the mutton flavoured dry-based soya products had higher mineral content in all cases, except sodium and manganese, compared to the savoury flavoured dry-based soya bean products.

A level of 13.7 mg/100 g of iron has been reported for defatted soya flour (Snyder and Kwon, 1987). The lower levels of iron in these products (7.9 mg/100 g) could be attributed to the dilution effect of starch being used as a bulking agent during processing. Iron has a significant pro-oxidant effect in soya bean oil and excessive levels in soya oil containing products have to be avoided (Snyder and Kwon, 1987).

In a survey conducted by Draper, Lewis, Malhotra and Wheeler (1993) amongst vegetarians, it was reported that iron intakes were high due to their high consumption of soya bean products. They quoted other workers who found lower plasma ferritin in vegetarians than omnivores. They also reported an absence of anaemia in long-term vegetarian women.

Textured vegetable protein	Ground maize
Hydrolysed vegetable protein	Spices
Meat extract	Salt flavour enhancer
Vegetable	Rice
Herbs	Colourants
Sugar	Maize flour
Flavourants	Acidifiers
Acidifiers	Vegetable fat

This mixture is then passed through a screen to ensure a product of a fixed particle size is mixed further and subsequently conveyed through a final screen before being stored in large drums. It is subsequently packaged into required product sizes (200 g or 500 g) (Personal communication: Colin Pillay, Production Supervisor: Robertsons, 1998).

6.2 Protein quality

PER values of soya bean (1.7) reported by Tolstoguzov, Braudo and Gurov (1981) and for defatted soya flour (1.8) (Smith and Circle, 1978) are rather lower than the values obtained for these soya products (mean of 2.1).

Inclusion of PER data from manufacturer A drastically decreased mean PER values. This phenomenon was repeated with products from manufacturer A having low NPU values also. This probably indicates an inferior protein quality in these products. Products from manufacturers B and C exhibited excellent PER (mean value of 2.6). This is better than the PER of cows milk (2.5), peanut (1.7), rice (1.9), maize (1.2) and wheat (1.0) (Snyder and Kwon, 1987). Only beef muscle with a PER of 3.2 is higher.

Young (1991) reported that raw soya beans are of low nutritional value. This is due to the prevalence of trypsin inhibitors. Heat processing denatures these protein inhibitors and consequently the reported mean of 2.1 in these soya foods analysed is higher than the PER of soya beans. Seal (1977) also stated that the greater the heat treatment applied to the soya protein the greater its availability to the body. This could be due to heating making the protein more digestible (Johnson, 1976).

The PER values are slightly higher compared with PER of dry-based texturised soya chunks of 1.7 to 2.0 (Prasad, Viswanathan, Swamy, and Santhanam, 1995) and the PER of soya fortified unleavened flat bread (1.7) (Rawat, Singh, Mital and Mittal, 1994).

The calcium, iron, manganese, phosphorus, potassium, sodium, and manganese contents were far higher than in pan-fried, minced beef (7, 2.4, 21, 161, 312, 70, 0.02 mg/ 100 g respectively). (Langenhoven, Kruger, Gouws, and Faber, 1991). Only the zinc content (5.44 mg/100 g) was higher in minced, pan-fried beef.

There has been a major concern from a nutritional perspective of the role that phytic acid play on the mineral bioavailability in soya products (as reviewed by Snyder and Kwon, 1987). Phytic acid forms complexes with seed proteins, some of which sequester ions, making them unavailable for the animal to absorb (Jaffe, 1981). Most plant foods have poor iron availability for humans. The low solubility of ferric complexes with phytic acid adds weight to the assumption that this acid may be responsible for the significant differences in iron availability in animal and vegetable sources (Erdman and Fordyce, 1989). In contrast, Jaffe (1981) has reported that there is now ample evidence that phytate is not a major factor in the absorption of iron into the blood stream.

According to Snyder and Kwon (1987), numerous authors (Cook, Morck and Lynch, 1981; Morck, Lynch, Skikne and Cook, 1981; Schriker, Miller and Van Campe, 1983; Bodwell, 1983) have shown that women, men and children fed amounts of soya protein in feeding programmes are not adversely affected in their iron bioavailability. Consequently phytate does not seem to be a problem in soya products.

Phytic acid is stable to cooking and probably is not degraded during texturisation by extrusion (Anderson and Wolf, 1995). As it is reported as not having a detrimental effect on the bioabsorption of minerals, except zinc, its presence does not appear to be a significant issue (Anderson and Wolf, 1995). Research suggests that phytic acid may have anticarcinogenic properties as well (Messina and Barnes, 1991).

The dry-based soya products from the different manufacturers are made from textured vegetable protein purchased from National Protein Products, Potgietersrus, South Africa. A calculated amount is then added into a cone mixer and formulated amounts of fat, flavourant, salt and a variety of confidential additives are added, which probably account for the manufacturing and flavouring effects on certain fatty acids, fat, dietary fibre, carbohydrate, and certain minerals. The following is the complete list of ingredients constituting a soya product from one of the manufacturers:

The mean NPU value of 42 falls below the range of 48 to 61 for soya bean (Smith and Circle, 1978) and higher than the NPU value of 31 for textured soya flour (Kinsella, 1978).

Inclusion of NPU data from manufacturer A again drastically reduced mean NPU values. Products from manufacturers B and C had a mean NPU of 51 that is within the literature range of 48 to 61 for soya bean (Snyder and Kwon, 1987; Jain, 1988).

The savoury products gave consistently lower NPU values apparently because the chickens ate less, presumably because they did not like the savoury taste (Personal communication, Prof. R.M. Gous, Department of Animal and Poultry Science, University of Natal, 1996). Both the PER and NPU reveal that the manufacturer A's protein quality was drastically inferior compared to manufacturers B and C. This may be attributed to inadequate thermal processing by manufacturer A which may not have denatured the protein anti-nutritional constituents. This possibility is supported by the higher nitrogen solubility data for products from manufacturer A in comparison to products from manufacturers B and C (see section 6.3).

The NPU of these products are better than the NPU of peanut (49) (Snyder and Kwon, 1987). The cereals rice (70), maize (52) and wheat (52) are slightly better in NPU compared to these products. Conventional protein sources like egg (91.5), cows milk (86), beef (73.5) and salmon (71) have better NPU values than these products. Work on the nutritional quality of flakes made of banana pulp and full fat soya gave a NPU of 56 (Ruales, Pólit and Nair, 1990) which was slightly higher than the NPU values reported here.

Studies of Kies and Fox (1973) and Kies (1974) have shown that textured soya protein is a valuable source of protein for both adolescents and adults. Soya bean products are also important as a nutrient in the diet of vegetarians as they can contribute 10% of their protein intake (Draper, Lewis, Malhotra and Wheeler, 1993). Since vegetarians rely largely on soya for their protein intake, protein quality becomes important, as their protein sources are limited. If the soya protein source were inactivated through inadequate heat processing then these soya consumers would be existing on an inferior diet and could suffer from kwashiorkor.

A problem with the protein quality of soya bean products is that methionine is the first limiting amino acid (Bookwalter, Warner, Anderson, Mustakas and Griffin, 1975). PER values on methionine fortified soya based foods indicated that the addition of DL-methionine can improve the protein nutritional quality of dry soya bean-based foods. The inclusion of methionine also significantly improved biological values of the protein in dehydrated soya products (Horon and Wolff, 1976).

6.3 Protein functionality

The functional property of soya bean protein of stabilisation of a gel which acts as a matrix for holding moisture and gives desirable chewiness (Wolf, 1970) is essential for a well developed texture in soya based mixtures (Kazemzadeh, Diehl, Rhee and Dahm, 1986). This desired functional property could only be achieved if the protein has the appropriate solubility. Texture of soya foods is thus dependent on soya proteins probably because proteins contribute to the skeletal structure of the texturised product in which the carbohydrates are dispersed. A decrease in solubility on heating is a reflection of protein denaturation, which occurs as a result of the protein molecule becoming unfolded and more asymmetrical (Anglemier and Montgomery, 1976). This would expose more hydrophobic residues and would decrease solubility of the protein. Thus, PDI and NSI determinations reflect the intensity and extent of heat processing that the test material has undergone. Snyder and Kwon (1987) stated that the PDI was always higher than NSI because the rapid shearing action of the blender blades used during the PDI determination disperses more protein than slow stirring.

Soya bean meal is normally toasted (steamed at atmospheric pressure) before processing and this step affects the NSI (Walker and Kochlar, 1982). The step is vital in inactivating numerous anti-nutritional factors e.g., trypsin inhibitors, lectins, goitrogens and phytates, and consequently adds to the full nutrition potential of soya protein (Leiner, 1981). Consequently, a compromise is made between the reduction of the anti-nutritional factors and the loss of functionality by controlling the extent of heat treatment (Wolf, 1970).

PDI and NSI values of soya flours vary widely depending on the duration of heat treatment (Wolf, 1970).

Traina and Breene (1994) on analysing eight commercial full-fat soya flours reported PDIs varying from 31 to 95.

Soya flours having minimal heat treatment had the highest PDI, whereas those exposed to harsh heat processes had the lowest PDI. Smith and Circle (1978) reported NSI decreasing from 80 to 20 when raw soya flakes were treated with steam in an autoclave at atmospheric temperature.

The NSI values of 3-7 in the soya products fall below the 10-20 NSI range for soya incorporation into crackers and infant foods (Wolf, 1970), probably because of heat treatment during removal of the solvent from the soya flakes following solvent extraction to remove the soya oil. The low NSIs may indicate inferior protein functionality.

Mutton flavoured products, which had the higher protein content, had higher PDI and NSI than the savoury flavoured soya products. Severe heat treatment tends to create a protein mesh on the outside of soya products retarding fat absorption (Snyder and Kwon, 1987). This may be the reason that the mutton flavour products having, a lower fat content (6.3%) than the savoury products (fat content of 7.6), had a statistically significant lower NSI (4.75) compared to savoury flavoured soya products (4.96).

Lapvetelainen, Kerrola and Linko (1991) reported that sodium chloride increases the solubility of soya proteins at the isoelectric pH range. Savoury flavoured products having higher mean sodium content (3 918 mg/100 g) than mutton flavoured products (3 533mg /100g) also had a higher NSI value (4.96) compared to 4.75. This finding is in line with the fact that salt enhances the solubility of soya proteins.

The mean PDI of mutton and savoury flavoured product from manufacturer A of 70.1 fell within the range, indicating a lightly toasted soya flour (60 to 80) (Snyder and Kwon, 1987). The lower PDI values emanating from the products of manufacturers B and C (29.6 and 7.4 respectively) indicate that the soya flour had been moderately heated to heavily toasted. The probable minimal heat treatment of products from manufacturer A may not have inactivated the protease inhibitors which could possibly explain their very low PER and NPU values.

6.4 Microbiological assessment

The bacterial count for these products was low (Table 27), and under normal storage conditions they could keep for at least a year, on the basis of research on dry-based soya balls by Prasad, Viswanathan, Swamy and Santhanam (1995).

Salmonella and *Shigella* were both present on both flavours out of manufacturer A and manufacturer B. An absence of *Salmonella* and *Shigella* may indicate hygienic manufacturing procedures are being adhered to by manufacturer C. The low PDI values in manufacturer C's products, indicative of high heat processing, may have also contributed to a sterilising effect on microorganisms. All three products indicated minimal *E. coli* contamination.

These dry-based texturised soya protein foods contained normal populations of microorganisms associated with dry-based foods, with products from manufacturer C probably being processed most hygienically as they were *Samonella/ Shigella* negative.

In malt extract agar which is a mould indicative medium, the products from all three manufacturers showed the presence of some mould on both flavoured products. As these are not sterilised products contamination could originate from the exposure to ambient air during production. These fungi could be *Diaporthe phaseolorum*, *Colletotrichum dematium* var. *truncata* and *Cercospora kikuchii* as these fungi are known to damage soya beans (Weingartner, 1987).

Parks, Rhee, Kim and Rhee (1993) working with high-protein extrudates of defatted soya flour, maize starch and beef as a dry-based snack food also found low counts of moulds, yeasts, coliform bacteria, and *E.coli*, within the normal microbiological profile range of dry-based foods.

All six products were free from aflatoxin, moniliformin and fumonisin contamination. This result is in keeping with general mycotoxin studies in soya foods (Click, 1998). Aflatoxin is not considered a problem in soya bean storage (Weingartner, 1987). He reported Shortwell *et al.*, (1969) finding two of 866 samples that were positive for aflatoxin, and they were grades that would have been rejected for human use. He also reported Hesseltine *et al.*, (1966) as being unable to detect aflatoxin on soya beans inoculated with *Aspergillus flavus*.

Probably foods with a high level of poly-unsaturated fatty acids such as soya beans are not susceptible to invasion by mycotoxic fungi (Click, 1998).

6.5 Consumer acceptability

Concerning the savoury flavoured products the products from manufacturers B and C were the best liked in appearance, flavour, texture and overall appearance. Analytically both manufacturers had a lower moisture and a higher soluble carbohydrate content compared to products from manufacturer A.

Four respondents commented that the texture of the products from manufacturer A was too fine. This may be the reason that savoury flavoured products from manufacturer A obtained significantly the lowest response (5.53) in texture ratings (Table 36). The significantly high sodium content analytically determined in savoury flavoured products from manufacturer B was substantiated by consumers stating that these products were "too salty".

Contributory factors to flavour are the spice and fatty acid levels as the flavour stability is dependent on low levels of saturated and poly-unsaturated fatty acids. Savoury flavoured product from manufacturer C had the highest ash content (Table 17) probably indicating adequate spice availability. This is based on the assumption that spices contain inorganic salts and ash content reflects this constituent (Ihnat, 1984). Tables 9-13 reflected that products from manufacturer C had the lowest fatty acid levels. Consequently adverse and rancid off-odours may be prevented from materialising and volatile flavourants are stabilised in products emanating from manufacturer C.

Some of the texture attributes can be correlated to levels of fat (Parks, Rhee, Kim and Rhee, 1993) and fibre. Table 8 revealed that product from manufacturer C had the least dietary fibre content. As products from B and C obtained the best texture rating, this indicates that low levels of fat and dietary fibre probably contributed to an acceptable texture.

For mutton flavour, the products from manufacturers A, B and C all had equal consumer acceptability.

As in the texture of savoury flavoured product from manufacturer A, mutton flavoured product from manufacturer A also received comments that it was "too fine". Three consumers substantiated the highest sodium content analytically determined (4 380 mg/100 g) in mutton flavoured product from manufacturer B by requesting that the salt content in this product be reduced.

The data from the least significance rank difference test showed that most of the consumers preferred product C of the mutton flavoured products as their number one choice. Consumers consequently preferred purchasing product C than product A/B. This was reinforced by one consumer stating that he would definitely buy the mutton flavoured products from manufacturer C and another consumer stating that these products "were really good."

For savoury flavoured products, consumers preferred to purchase products from manufacturers B and C instead of A.

This anomaly in consumer preference between mutton and savoury may lie in the finding that in savoury products from manufacturers B and C the fat and dietary fibre contents were lower than in product A. This compositional relationship was not prevalent in mutton flavoured products.

In a related study on dry-based textured soya protein (TSP) chunks, Prasad, Viswanathan, Swamy and Santhanam (1995) reported that the product remained acceptable over a 12-month period from the point of view of sensory qualities. Assessment of several attributes (colour, aroma, taste and texture) on TSP chunks from five different commercial sources gave an overall acceptability score of 6.7 to 7.4 on a nine-point scale. This is similar to the hedonic evaluation of 5.6 to 6.4 for savoury and 5.9 to 6.6 (on a eight-point scale) for mutton flavoured products evaluated on these South African products. These overall acceptability ratings on the South African products also indicated that mutton flavoured product had very slightly better acceptability ratings than savoury flavoured product. This could also be linked to mutton flavoured products having a significantly greater PDI than savoury flavoured products.

6.6 Economic comparison with alternative protein foods

Raw soya beans retail for R5.50 per kg (Personal observation: Haribhai and Sons (Pty) Ltd Spices, Durban on 28/6/99).

Based on 40% protein content (Snyder and Kwon, 1987) this equates to R13.75 per kg protein.

Defatted soya flour is industrially available at R3.00 per kg (Personal communication: Mahabeer, Planning Dept., Robertsons, Durban, July 1998).

Based at a protein content of 51.5% protein content (Kinsella, 1978) this equates to R5.82 per kg protein but defatted soya flour can only be used as a food ingredient industrially and not as a food material to be prepared and consumed domestically.

Soya protein was cheaper than beef or chicken protein on a price per kg protein basis. This occurs because soya beans are a primary agricultural product, whereas meat is a secondary product since the animal must convert plant protein to its own protein. Kinsella (1978) reported that soya bean cultivation requires about 9 kJ per kJ protein produced, whereas protein production by intensive animal husbandry is highly energy consuming, requiring 197 kJ per kJ of protein. Approximately 10 kg of plant protein is required to produce one kg of animal meat protein. Thus, meat protein is far more expensive than plant protein.

CHAPTER 7

CONCLUSIONS AND RECOMENDATIONS

The low moisture content of these dry-based soya products enables them to have a long storage life. The different levels of moisture levels amongst the three manufacturers could be attributed to the differing intensity or duration of thermal treatment utilised by the processors of the TVP.

The mean protein content of these soya products (25.4%) was very much lower than the protein content of 40% of raw dried soya beans or that of 56% in defatted soya flour. This is evidence that the protein concentration in these dry-based products may have been diluted by the addition of other ingredients e.g., starch. Mutton flavoured products have a higher protein content compared to savoury flavoured products possibly because of the added hydrolysed vegetable protein used in creating a mutton flavour.

The higher carbohydrate content (45% as opposed to the normal carbohydrate content in soya beans of 30%) is indicative of the starch and/ or maize flour added during the manufacturing process. This consequently diluted the protein content (40% to 25%).

These products are a good source of potassium, sodium, calcium, magnesium, sulphur and phosphorus. Iron, zinc and manganese content in these products are lower than their content in soya beans. This may be a further consequence of the dilutory effect of the added carbohydrates. The presence of phytic acid may have an influence on the bioavailability of zinc. Animal model studies may clarify this issue.

Mean PER values of the products from manufacturers B and C of 2.6 are better than the PER of cow's milk, peanut, maize and wheat. However, lean beef has a higher PER compared to the PER of these products. The PER of product from manufacturer A was far lower than the PER of cow's milk, peanut, maize and wheat.

The NPU values of the products from manufacturers B and C is higher than the NPU of peanut but lower than the NPU of rice, maize, wheat, eggs, milk, beef and salmon. Methionine is probably the limiting amino acid in these products as methionine is the limiting amino acid in soya beans. Consequently, these products are consumed with rice, which complements the methionine deficiency.

The low NSI and PDI values from manufacturers B and C is indicative of the exposure of soya defatted flour to excessive heat treatment during the manufacture of TVP. The low NSIs may indicate inferior protein functionality from these two manufacturers. The high PDI of products from manufacturer A indicates lightly toasted soya flour. This inadequate heat treatment may not have inactivated the protease inhibitors, which will explain the low PER and NPU values obtained for these products.

As all three manufacturers purchase their TVP from National Protein, it may be of value to conduct research on the consistency of the protein quality of TVP being produced or on the maintenance of processing temperatures being adhered to at National Protein.

The bacterial counts in these dehydrated products are in the acceptable range ensuring extended keeping quality. Manufacturer C probably has the most hygienic manufacturing process amongst all three manufacturers as their products are devoid of *Salmonella* and *Shigella* contamination. The presence of mould on all six products requires more research to ascertain the source of contamination and to identify the fungal contaminant. The absence of mycotoxins indicates that these fungi are not mycotoxic.

From a consumer acceptability perspective there was no significant difference in the consumer response to mutton flavoured products, whereas savoury products from manufacturer B and C has the best ratings in terms of appearance, flavour, texture and overall acceptance. Savoury flavoured products from manufacturer B and C having the least fat and fibre content in comparison with the products from the manufacturer A may contribute to them having the best overall acceptance. Particle size differentiation, which was not undertaken, could be researched as a factor determining texture acceptability as consumers commented that some products are "too fine." These savoury products from manufacturer A have the least texture ratings. Levels of sodium chloride also play a role in flavour acceptability, as products from manufacturer B are excessively salty.

Consumers possibly had a slight preference for mutton flavoured product compared to savoury product. This could be linked to mutton flavoured products having a significantly greater PDI than savoury flavoured products.

These soya products are a cheaper source of protein compared to the price of chicken (192% of the price of soya protein) or beef (140% of the price of soya protein). In South Africa, where the affordability of nutritional foods is of concern this is an important finding. To enhance the marketing of soya products this fact could be exploited commercially.

While dry-based soya products seems to be nutritionally acceptable and affordable with an extended shelf life, their protein availability and functionality is dependent on processing parameters. These soya products also received a 77% acceptability rating (a mean response of 6.13 out of 8 expressed as a %age) indicating above average acceptance by consumers but with room for improvement in appearance and textural characteristics.

CHAPTER 8

REFERENCES

American Oil Chemists Society, 1973. Official and Tentative methods. 3rd ed. Tentative methods Ba 10-65 and Ba 11-65. American Oil Chemists Society, Campaign, I11inois. pp Ac4-41.

Anderson, R.L. and Wolf, J. W., 1995. Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soya bean processing. *Journal of Nutrition* **125**, 581S-588S.

Anderson, S.A., Chinn, H.1. and Fisher, K.D., 1982. History and current status of infant formulas. *American Journal of Clinical Nutrition* **35**, 381 -397.

Anglemier, A. F. and Montgomery M. W., 1976. Amino acids, peptides and proteins. In Principles of Food Science Part 1 Food Chemistry (Ed. O.R. Fennema) Marcel Dekker, New York. pp 205-284.

Association of Official Analytical Chemists, 1990a. Method 925.10 Air Oven Method - final action. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th ed. Arlington, Virginia. pp 777.

Association of Official Analytical Chemists, 1990b. Method 985.29 Total Dietary Fibre Method - final action. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th ed. Arlington, Virginia. pp 1105.

Association of Official Analytical Chemists, 1990c. Method 942.05 Ash Direct Method-final action. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th ed. Arlington, Virginia. pp 70.

Bodwell, C.E., 1983. Effects of soy protein on iron and zinc utilisation in humans. *Cereal Foods World* **28**, 342-344.

Bookwalter, G.N., Warner, K., Anderson K., Mustakas G. C. and Griffin, E.L., 1975. Fortification of dry soya bean-based foods with DL-methionine. *Journal of Food Science* **40**, 266-270.

Brody, T., 1994. Nutritional Biochemistry, Academic Press, London. pp 85-88.

Chang, S.K.C., 1994. Protein Analysis. In Introduction to the Chemical Analysis of Foods (Ed. S. S. Nielsen) Jones and Bartlet, Boston, Massachusetts. pp 207-219.

Che Man, Y.B., Abdul Karim, M.N.B. and Kun, T.T., 1992. Evaluation of four high-protein rice-soya snack formulations. *International Journal of Food Science and Technology* **27**, 715-719.

Circle, S.J. and Smith, A.K., 1978. Processing soya flours, protein concentrates and protein isolates. In Soyabeans: Chemistry and Technology, Vol. 1, Proteins (Eds. A.K. Smith and S.J. Smith). AVI Publishing, Westport, Connecticut. pp 294-338.

Click, M., 1998. General identification of mycotoxins in foods. Paper read at the Mycotoxin Seminar, ML Sultan Technikon. Durban, July, 1998.

Cook. J.D., Morck, T.A. and Lynch, S.R., 1981. The inhibitory effect of soy products on nonheme iron absorption in man. *American Journal of Clinical Nutrition* **34**, 2622-2699.

Cronjé, J., 1997. Basic Food is the Best. Consumer Council, Pretoria. pp 16- 17.

Doell, B.H., Ebden, C.J. and Smith, C.A., 1982. Trypsin inhibitor activity in conventional soya foods. *Quality Plant foods in Human Nutrition* **31**, 139 150.

Draper, A., Lewis, J., Malhotra, N. and Wheeler, E., 1993. The energy and nutrient intakes of different types of vegetarian: a case of supplements? *British Journal of Nutrition* **69**, 3-19.

Duodu, K.G., 1997. Nutritional quality of irradiated sorghum porridge and spinach relish. M Inst Agrar (Food Processing) dissertation. University of Pretoria, Pretoria. pp 70-74.

Dutton, M.F., 1996. Methods for the analysis of mycotoxins and related techniques. University of Natal, Durban. pp 4-10.

Erdman, J. W. and Fordyce, E. J., 1989. Soy products and the human diet. *American Journal of Clinical Nutrition* **49**, 725-737.

FAO/WHO, 1992. World Declaration on Nutrition. Final Report of the International Conference on Nutrition. FAO/WHO, Rome. pp 9-14.

Flint, F.D. and Lewin, Y.A., 1976. The histochemical demonstration of soyabean products in foodstuffs. *Journal of Food Technology* **11**, 137-142.

Florin, G. and Bartesch, H.R., 1983. Processing of oilseeds using fluid bed technology *Journal of the American Oil Chemists Society* **60**, 193-197.

Friedrich, J.P. and Pride, E.H., 1984. Supercritical CO_2 extraction of lipid bearing materials and characterization of the products. *Journal of the American Oil Chemists Society* **61**, 223.

Gnanasambandam, R. and Zayas, J.F., 1994. Chemical and bacteriological stability of frankfurters extended with wheat germ, corn germ and soyabean proteins. *Journal of Food Processing and Preservation* **15**, 159-171.

Government Gazette, 1966. Act on Foodstuffs, Cosmetics, and Disinfectants (Act 54 of 1972). Regulations concerning Recommended Daily Dietary Allowances. Government Gazette No. 3666, 17 March. Lex Patria Publishers, Johannesburg. Annex page.

Hendricks, K.M., 1983. Soy-protein formulas: Recommendations for use in infant feeding. *Pedriatics* **72**, 359-361.

Hill, J.E. and Breidenbach, R.W., 1974. Proteins of soya bean seeds 1. Isolation and characteristics of the major components. *Plant Physiology* **53**, 742.

Horon, F.E. and Wolff, H. 1976. Meat analogs-a supplement. In New Protein Foods. (Ed. A.M. Altschul). **2B,** 260-279.

Hu, L., Huff, H.E., Heymann, H. and Hsiegh, F., 1996. Effects of emulsifier and soyabean fibre addition on sensory properties of corn meal extrudate. *Journal of Food Quality* **19**, 57-77.

Ihnat, M., 1984. Atomic absorption and plasma atomic emission spectrometry. In Modern Methods of Food Analysis (Eds. K.K. Stewart and J.R. Whitaker) AVI Publishing, Westport, Connecticut. pp 129-166.

Inglett, G.E., 1997. Development of a dietary fibre gel for calorie reduced foods. *Cereal Foods World* **42**, 382-385.

Jaffe, G., 1981. Phytic acid in soya beans. In Proceedings of the Conference on Soya Processing and Utilisation. *Journal of the American Oil Chemists Society* **58**, 493-495.

Jain, M., 1988. Educating health workers and villagers on the dietary uses of soya foods in Madhya Pradesh, India. *Food and Nutrition Bulletin* **10**, 41-43.

James, C.S., 1995. Analytical Chemistry of Foods. Blackie Academic and Professional, London. pp 207-219.

Johnson, D.W., 1976. Oilseed vegetable protein concentrates. *Journal of the American Oil Chemists Society* **53**, 321-324.

Kazemzadeh, M., Diehl, K.C., Rhee, K.C. and Dahm, P.F., 1986. Mechanical and structural evaluation of texturised soya proteins of varying protein content. *Cereal Chemistry* **63**, 304-310.

Kies, C., 1974. Nutritional implications of textured protein products. *Cereal Foods World* **19**, 450-455.

Kies, C. and Fox, H.M., 1973. Effect of varying the ratio of beef and textured vegetable protein nitrogen on protein nutritive value for humans. *Journal of Food Science* **38**, 1211-1213.

Kinsella, J.E., 1979. Functional properties of soya bean proteins. *Journal of the American Oil Chemists Society* **56**, 242-258.

Kinsella, J.E., 1978. Texturised proteins: Fabrication, flavouring and nutrition. *CRC Critical Reviews in Food Science and Nutrition* **10**, 147-207.

Kinsella, J.E., Damodran, S. and Genman B., 1985. Physiological and functional properties of oilseed proteins with emphasis on soya bean proteins. In New Protein Foods. Vol. 5: Seed Storage Proteins (Eds. A.M. Altschul and H.L. Wilke) Academic Press, Orlando, Florida. pp 107-179.

Langenhoven, M., Kruger, M., Gouws, E. and Faber, M., 1991. Medical Research Council Food Composition Tables. 3rd ed., Medical Research Council, Parow. pp 22 & 51.

Lapvetelainen, A., Kerrola, K. and Linko, R., 1991. Effect of phytic acid hydrolysis on the functionality of soya protein isolate. *Lebensmittel-Wissenschaft-und-Technologie*, **24**, 71-75.

Lawhon, J.T., Rhee, K.C. and Lusas, E.W., 1981. Soy protein ingredients prepared by new processes-Aqueous processing and industrial membrane isolation. *Journal of the American Oil Chemists Society* **3**, 377-379.

Leiner, I.E., 1981. Factors affecting the nutritional quality of soya products. In Proceedings of the Conference on Soya Processing and Utilisation. *Journal of the American Oil Chemists Society* **58**, 406-415.

Leiner, I.E., 1994. Implications of antinutritional components in soya bean foods. *Critical Reviews in Food Science and Nutrition* **34**, 31-67.

Macleod, G. and Ames, J.M., 1988. Soya flavour and its improvement. *CRC Critical Reviews in Food Science and Nutrition* **27**, 219-400.

McGill, A.E.J., 1997. It's all in a day's food. *Food Industries of South Africa* **50**, (3), 14-15.

Messina, M. and Barnes, S., 1991, The role of soya products in reducing the risk of cancer. *Journal of Natural Cancer Institute* **83**, 541-546.

Meyer, B.J., Meij, H.S. and Meyer, A.C., 1997. Human Physiology. Juta and Co., Cape Town. pp 9.23-9.33.

Min, D.B., 1994. Crude fat analysis. In Introduction to the Chemical Analysis of Foods (Ed. S.S. Nielsen) Jones and Bartlett Publishers, Boston, Massachusetts. pp 184-186.

Moore, N.H., 1983. Oilseed handling and preparation prior to solvent extraction. *Journal of the American Oil Chemists Society* **60**, 189-192.

Morck, T.A., Rusoff, I.I., Bednarcyk, N.E. and Ronai, K.S., 1976. Protein-fortified shelf-stable products. *Cereal Foods World* **21**, 492-496.

Morck, T.A., Lynch, S.R., Skikne, B.S. and Cook, J.D., 1981. Iron availability from infant food supplements. *American Journal of Clinical Nutrition* **34**, 2630-2634.

Mustakas, G.C., Albrecht, W.J., Bookwalter, G.N. and Griffin, E.L. Jnr., 1967. Full-fat Soyabean Flours by a simple process for Villagers. Agricultural Research Service, USDA, Washington DC. pp ARS 71-34.

Mustakas, G.C., Albrecht, W.J., Bookwalter, G.N., McGhee, J.E., Kwolek, W.F. and Griffin, E.L. Jnr., 1970. Extruder processing to improve nutritional quality, flavour and keeping quality of full-fat soya bean flour. *Food Technology* **24**, 1290- 1294.

Nelson, A.I., Wei, L.S. and Steinberg, M.P., 1980. Foods from whole soya bean. In World Soya bean Research Conference II: Proceedings. (Ed. F.T. Corbin) Westview Press, Boulder, Colorado. pp 745-761.

O'Dell, B.L., 1979. Effect of soy protein on trace mineral availability. In Soy Protein and Human Nutrition (Ed. H.L. Wilke, D.R. Hopkins and D.H. Waggle). Academic Press, New York. pp 125-139.

Ologhobo, A.D., 1989. Improving nutritive value of soya bean (*Glycine max* (L. Merr.) through processing: Biochemical and protein quality studies. *Tropical Agriculture (Trinidad)* **66**, 290-296.

Parks, J., Rhee, K.S., Kim, B.K. and Rhee, K.C., 1993. High-protein texturised products of defatted soyabean flour, corn starch and beef: shelf-life, physical and sensory properties. *Journal of Food Science* **58**, 21-27.

Penstone, K., 1996. Nutritional quality of cereal and legume storage proteins. *Food Review* **5**, 5.

Phillips, R.D., 1997. Nutritional quality of cereal and legume storage proteins. *Food Technology* **51**, 62-66.

Prasad, N.N., Viswanathan, K.R., Swamy, M.S. and Santhanam, K., 1995. Studies on the protein quality and storage stability of textured soya bean proteins. *Journal of Food Science and Technology* **32**, 202-205.

Ranhotra, G.S., Louwe, R.J. and Puyat, L.V., 1974. Phytic acid in soy and its hydrolysis during bread making. *Journal of Food Science* **39**, 1023-1025.

Rawat, A., Singh, G., Mital, B.K. and Mittal, S.K., 1994. Effect of soya fortification on quality characteristics of chapatis. *Journal of Food Science and Technology* **31**, 114-116.

Reichert, J. E., 1991, The use of soy protein in meat products. *Die Fleischere* **8**, 3-5.

Reid, R. 1993. Sunrise for soya. *Food Review*, **20,** (4) 12-13.

Reid, R. 1997. Soya shows its versatility. *Food Review*, **24** (6), 12-14.

Ruales, J., Pólit, P. and Nair, B.M., 1990. Evaluation of the nutritional quality of flakes made of banana pulp and full fat soya flour. *Food Chemistry* **36,** 31-43.

Schricker, B.R., Miller, D.D. and Van Campe, D., 1983. Effects of iron status and soy protein on iron absorption by rats. *Journal of Nutrition* **113**, 996-1001.

Seal, R. 1977. Soya products: A food processors guide. *Chemistry and Industry*, **4,** 441-446.

Smith, A.K. and Circle, S.J., 1978. Chemical Composition of the Seed. In Soybeans: Chemistry and Technology, Vol 1, 2nd ed. (Eds. A.K. Smith and C.J. Circle). AVI Publishing, Westport, Connecticut. pp 61-92.

Snyder, H.E. and Kwon T.W., 1987. Soya Bean Utilisation, Van Nostrand Reinhold Company, New York. pp 31-70; 74-92; 145-147; 188-214.

Steggarda, F.R., Richards, E.A. and Rackis, J.J., 1966. Effects of various soybean products on flatulence in the adult man. *Proceedings of the Society of Experimental Biological Medicine* **121**, 1235-1239.

Sugimoto, H., Nishio, M., Horiuchi, T. and Fukushima, D., 1981. Improvement of organoleptic quality of fermented soyabean beverage by additions of propylene glycol alginate and calcium lactate. *Journal of Food Processing and Preservation* **5**, 83-93.

Tolstoguzov, V.B., Braudo, E.E., and Gurov, A.N., 1981. On the protein functional properties and the methods of their control. Part 2. On the methods of control of functional properties of proteins and gel-forming systems. *USSR Academy of Sciences* **25**, 817-823.

Thomas, G.R., 1981. The art of soyabean meal and hull grinding. *Journal of the American Oil Chemists Society* **58**, 194-196.

Traina, M.S. and Breene, W.M., 1994. Composition,functionality and some chemical and physical properties of eight commercial full-fat soya flours. *Journal of Food Processing and Preservation* **18**, 229-252.

Tuley, L., 1996. Healthy outlook for soya proteins. *International Food Ingredients* **5**, 23-28.

Utsumi, S., 1992. Plant food protein engineering. *Advances in Food and Nutrition Research* **36,** 89-208.

Vaintraub, I.A. and Yattara, H.B., 1995. Proteolysis of Kunitz soya bean trypsin inhibitor: Influence on its activity. *Journal of Agricultural and Food Chemistry* **43**, 862-866.

Walker, A.F. and Kochlar, N., 1982. Effect of processing including domestic cooking on nutritional quality of legumes. *Proceedings of the Nutritional Society* **41,** 41-51.

Wang, C.R. and Zayas, J.F., 1992. Comparative study of shelf-life of frankfurters containing plant protein additives. *Journal of Food Quality* **15,** 211-224.

Watson, T.G., 1997. Genetically modified foods - A South African perspective on biosafety issues. Paper presented at: Harnessing Food Science and Technology for Sustainable Development Conference, CSIR, Pretoria.

Wayler A., Queiroz E., Scrimshaw, S.N., Steinke, F.H., Rand, W.M. and Young, V.R., 1983. Nitrogen Balance studies in young men to assess the protein quality of an isolated soya bean protein in relation to meat protein. *Journal of Nutrition* **113**, 2485-2491.

Weingartner, K.E., 1987. Processing, nutrition and utilisation of soya beans. In Soybeans for the Tropics (Eds. S.R. Singh, K.O. Richie and K.E. Dashiel) John Wiley and Sons, Chichester. pp 149-177.

Wenlock, R.W., Sivell, L.M. and Agater, I.B., 1985. Dietary fibre fractions in cereal and cereal containing products in Britain. *Journal of the Science of Food and Agriculture* **36,** 113-121.

Wessels, J.P.H., 1970. The amino acid supplementation of different animal protein sources on their own or together with plant proteins in chicken rations. *Agroanimalia* **2**, 21-27

Wolf,W.J. 1970. Soya bean proteins: Their functional, chemical and physical properties. *Journal of Agricultural and Food Chemistry* **18,** 969-976.

Wolf, W.J. and Cowan, J.C., 1971. Soya beans as a Food Source. Butterworth, London. 1-10.

Young, V.R., 1991. Soya bean protein in relation to human protein and amino acid nutrition. *Journal of the American Dietetic Association* **91,** 828-834.

Young, V.R., Puig, M. Queiroz, E., Scrimshaw, N.S. and Rand W.M., 1984. Evaluation of the protein quality of an isolated soya bean protein in young men: relative nitrogen requirement and effects of methionine supplementation. *American Journal of Clinical Nutrition* **39**, 16-24.

Young, V.R., Wayler, A., Garza, C., Steinke, F.H., Murray. E, R., Rand, W.M. and Scrimshaw, N.S., 1984. A long- term metabolic balance study in young man to assess the nutritional quality of an isolated soyabean protein and beef proteins. *The American Journal of Clinical Nutrition* **39**, 8-15.

Zarkadas, G.C., Yu, Z., Voldeng, H.D. and Minero-Amador, A., 1993. Assessment of the protein quality of a new high protein soya bean cultivar by amino acid analysis. *Journal of Agricultural and Food Chemistry* **41**, 616-623.