BIOCHEMICAL, PHYSICO-CHEMICAL AND NUTRITIONAL PROPERTIES OF SWEET POTATO (*Ipomoea batatas*) AND ITS PROCESSING INTO AN INFANT WEANING FOOD

A thesis submitted to the University of Surrey in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Science

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December 2004

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Sweet potato varieties, Tanzania (Tz), Nasport (Nsp) and Spk004 (Spk), identified as potential raw material for baby food formulation, were characterised for proximate composition, mineral content and physicochemical characteristics. The interaction of sweet potato flour and proteins (soya and fish) were investigated. Starch was the most abundant nutrient in sweet potato (58 to 79 g/100g flour dry weight). Resistant starch ranged between 17 and 29%; these values were lower in sun-dried samples compared with freeze-dried ones and were reduced significantly on boiling. The *in vitro* starch digestibility was high, particularly in the sun-dried samples (80-85%), indicating sweet potato carbohydrates may be utilised efficiently. Lipids (1.1-2.3 g/100g) and proteins (2.7-5.0 g/100g dry weight) were low. Aspartic acid and glycine were the most abundant amino acids, cysteine and methionine were limiting. The orange-fleshed varieties Nsp and Spk contained high levels of β-carotene (provitamin A), which were reduced in sun-dried and oven-dried samples. The main elements were K, Na, Mg and Ca with an average of 1190, 408, 698 and 576 mg/kg, dry weight respectively and moderate iron (37 mg/kg) and Zn (40 mg/kg). The *in vitro* digestion method showed that Ca, Mn, Mg and Zn in sweet potato are moderately bioavailable (55, 51, 45 and 65 % respectively). Polyphenols identified in sweet potato were caffeoylquinic acids, *p*-coumaroylquinic acids, feruloylquinic acids, dicaffeoylquinic acids and caffeoylferuloylquinic acids. Caffeoylferuloylquinic acids have not been reported before in sweet potato.
Small deformation rheology indicated that sweet potato flour had lower elastic modulus, $G'$ values compared with extracted starch. A mixture of sweet potato and soya (5% sp: 15 soya) and that of soya and fish (10%: 10%) w/w in water had lower $G'$ and $G''$ values than a mixture of sweet potato and fish (10% sp: 10% fish), which could be useful in making soft products. A sweet potato-based infant food using the Microdiet program was developed with nutritional and rheological characteristics similar to commercial baby foods. Lipid oxidation increased on storage of the dried product for 16 weeks at 22 °C, particularly in product containing fish but reduced in the presence of antioxidants (350, 500 mg vitamin C, 350,500 mg vitamin E and 100 mg citric acid/ Kg). Products containing sweet potato and soya (recipe B) were more stable in the absence of antioxidants.
OBJECTIVES

- To assess the biochemical, physicochemical and nutritional properties of sweet potato varieties from Uganda.

- To assess the Physicochemical and rheological properties of sweet potato flour and starch

- To assess the physicochemical and rheological properties of sweet potato starch and protein (soya and fish) mixtures.

- To formulate an infant food by combining sweet potato with other local raw materials such as soya flour and fish to produce an optimum product of nutrient content and composition using Micro-diet a nutrition database to comply with FAO/EU guidelines.

- To assess the rheological properties of the product.

- To assess the biochemical and nutritional changes in the product on storage and to identify methods of minimising lipid oxidation using antioxidants
## ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>BHA</td>
<td>Butylated hydroxy anisole</td>
</tr>
<tr>
<td>BHT</td>
<td>Butylated hydroxy toluene</td>
</tr>
<tr>
<td>Con A</td>
<td>Concanavalin A</td>
</tr>
<tr>
<td>CFQA</td>
<td>Caffeoylferuloylquinic acid</td>
</tr>
<tr>
<td>CQA</td>
<td>Caffeoylquinic acid</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DDW</td>
<td>Deionised distilled water</td>
</tr>
<tr>
<td>DiCQA</td>
<td>DiCaffeoylquinic acid</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulphoxide</td>
</tr>
<tr>
<td>DNSA</td>
<td>Dinitrosalicylic acid</td>
</tr>
<tr>
<td>FAAS</td>
<td>Flame atomic absorption spectroscopy</td>
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<tr>
<td>FQA</td>
<td>Feruloylquinic acid</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>GOPOD</td>
<td>Glucose oxidase peroxidase</td>
</tr>
<tr>
<td>HPLC</td>
<td>High pressure liquid chromatography</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>Inductively coupled mass spectrometry</td>
</tr>
<tr>
<td>IVDS</td>
<td><em>In vitro</em> digestibility of starch</td>
</tr>
<tr>
<td>IMS</td>
<td>Industrial methylated spirit</td>
</tr>
<tr>
<td>LCMS</td>
<td>Liquid chromatography followed by mass spectrometry</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>MOPS</td>
<td>Morpholinopropanesulphonic acid</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometer</td>
</tr>
<tr>
<td>NAARI</td>
<td>Namulonge Agriculture and Animal Research Institute</td>
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<tr>
<td>NSP</td>
<td>Nasport</td>
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*iv*
<table>
<thead>
<tr>
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<tr>
<td>PV</td>
<td>Peroxide value</td>
</tr>
<tr>
<td>PDA</td>
<td>Photodiode array</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acid</td>
</tr>
<tr>
<td>PVDF</td>
<td>Polyvinylidene difluoride filter</td>
</tr>
<tr>
<td>PITC</td>
<td>Phenyl isothiocyanate</td>
</tr>
<tr>
<td>SP</td>
<td>Swelling power</td>
</tr>
<tr>
<td>sp</td>
<td>Sweet potato</td>
</tr>
<tr>
<td>SPK</td>
<td>Spk 004</td>
</tr>
<tr>
<td>TBARS</td>
<td>Thiobarbituric acid reactive substance</td>
</tr>
<tr>
<td>TEA</td>
<td>Trethylamine</td>
</tr>
<tr>
<td>TFH</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TZ</td>
<td>Tanzania</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra violet</td>
</tr>
<tr>
<td>VIS</td>
<td>Visible</td>
</tr>
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<td>WSI</td>
<td>Water soluble index</td>
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ACKNOWLEDGEMENTS

I would like to thank my supervisor, Professor Nazlin Howell for her guidance, support, and encouragement through out the course of the study and for the preparation of this thesis. She has always been available to provide help when needed.

I am grateful to Dr. Ward (Chemistry) for his assistance in elemental analysis and his comments on the same aspect.

I would like to express my thanks to Professor Clifford for his supervision of Phenol analysis and helpful comments

A huge thank you to my beloved husband who has supported me all the way and looked after our two sons Joshua and Emmanuel while I was busy in the laboratory.

Many thanks to Hajo and all my friends in Food Safety laboratory (Sue Saeed, Sue Knight, Sekai, Farah, Sami, Sarah, Badri, Wengu, Saad, Derick for the assistance offered in the laboratory. Special gratitude to Naren Amlani for all the computing and IT help and guidance.

Many thanks to Makerere University, for permitting me to undertake this study.

Scholarship from the Association of Commonwealth Universities is greatly appreciated.

Finally above all I thank the Almighty God for without his support and care I would not have been able to complete this study.
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CHAPTER ONE
1.0 INTRODUCTION

1.1 Historical background of sweet potato

The sweet potato (Ipomoea batatas. L) is a dicotyledonous plant which belongs to the family convolvulaceae and produces edible starch storage roots. It is said to have originated around 6,000 – 8,000 B.C (Austin, 1983). The crop later spread to most of the Neotropics with the exception of the temperate zones of the new world. Europeans introduced the crop into Africa in early 1500 (Yen, 1982). By 1900 sweet potato was already an important crop in central and parts of western Uganda. Greenway (1944) pointed out that the crop entered Uganda from both east and western parts of the African continent. Sweet potato cultivation spread to northern Uganda during the early part of the 19th century (Drisberg, 1923). In the 1950s sweet potato occupied about 9% of the food crops in Uganda and is currently ranked as the most important crop after millet, bananas and cassava.

1.2 Utilisation of sweet potato in Uganda

It has been estimated that sweet potato production in developing countries exceeds 133,000 metric tonnes per annum, representing over 34% of the roots and tubers cultivated in these regions (FAO, 2002). The roots and foliage of the plant are important commodities to small-scale farmers in Africa, Latin America and Asia. Over 90% of the production in developing countries is in Asia, especially China where the crop has been estimated to provide up to 10% and 5% respectively of the intakes in calories and protein (Tian et al., 1991). In certain regions of Africa, South America, the Carribean and the Pacific the sweet potato is important as a staple calorie source.
In Uganda, sweet potato is one of the three most important staple food crops. In fact, Uganda is the second largest sweet potato producing country in Africa and the third largest (Table 1.1) in the world (FAO, 2002). Sweet potato production is estimated at 2.5 million tonnes per year from 400,000 hectares per cultivate.

Table 1.1: Principal producers of sweet potato worldwide, 2002

<table>
<thead>
<tr>
<th>Country</th>
<th>Production (1000) tonne</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>120,000</td>
</tr>
<tr>
<td>Nigeria</td>
<td>2,522</td>
</tr>
<tr>
<td>Uganda</td>
<td>2,515</td>
</tr>
<tr>
<td>Vietnam</td>
<td>1,655</td>
</tr>
<tr>
<td>Indonesia</td>
<td>1,600</td>
</tr>
<tr>
<td>India</td>
<td>1,200</td>
</tr>
<tr>
<td>Philippines</td>
<td>545</td>
</tr>
<tr>
<td>Kenya</td>
<td>535</td>
</tr>
<tr>
<td>Papua New Guinea</td>
<td>485</td>
</tr>
</tbody>
</table>

Source: FAO Production Yearbook, 2002

Sweet potato is an important food security crop that fits well into Uganda’s farming and food systems. It stores well underground as a famine reserve crop, withstands extreme dry weather conditions and performs well in marginal soils. Sweet potato is more tolerant than other crops to late planting and can be grown throughout the year (Bashaasha et al., 1995).
A typical household owns a sweet potato plot of less than one acre and cultivates more than five varieties, each identified by a name in the local language. This nomenclature is based on varietal characteristics such as yield, maturity period, root size and shape and other factors such as place of their origin and the person who introduced the variety (Bashaasha & Vanegas, 1991).

Although sweet potato is a major staple crop in the subsistence economy system of the country, its processing is remarkably limited. Usually sweet potato roots are steamed or roasted in the fresh form. Home or village level processing of the crop is relatively uncommon in Uganda. However in some parts of the country with a long hot dry season, serious attacks by weevils limit the length of time that the roots can be stored in the ground and farmers harvest, chip, sun-dry the roots as a way to preserve and store the crop. Industrial processing of the sweet potato into animal feed, flour, starch or noodles and other biotechnological uses in Uganda are non-existent. The limited range of ways in which sweet potato is utilised limits the potential benefits of the crop to farmers and other users. Therefore more possibilities of using sweet potato as a raw material for infant food formulation should be explored.

1.3 Sweet potato proximate composition

Sweet potato is a nutritious crop and previous reports indicate that the roots provide substantial amounts of energy, appreciable amounts of proteins, vitamins, lipids and minerals (Ravindran et al., 1995).
1.3.1 Dry matter

In common with other roots and tubers the sweet potato has a high moisture content resulting in relatively low dry matter content. The average dry matter content is approximately 30%, but this varies widely depending on such factors as cultivars, location, climate, soil type and cultivation practices (Bradbury & Holloway, 1988). Dry matter content varies from 13.6% to 37.1% in a number of sweet potato cultivars (Anon, 1981). The average composition of root dry matter is as shown below.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>70.0</td>
<td>36.0</td>
<td>72.1</td>
</tr>
<tr>
<td>Total sugars</td>
<td>10.0</td>
<td>21.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Total protein</td>
<td>5.0</td>
<td>4.7</td>
<td>4.4</td>
</tr>
<tr>
<td>Ash</td>
<td>3.0</td>
<td>3.3</td>
<td>2.9</td>
</tr>
<tr>
<td>Total fibre</td>
<td>10.0</td>
<td>5.3</td>
<td>2.4</td>
</tr>
<tr>
<td>Lipid</td>
<td>1.0</td>
<td>4.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Vitamin</td>
<td>&lt;1.0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Key

1, 2 and 3 are sources of data.

1 Woolfe, 1992  3 Ravindran et al., 1995  2 Nnam, 2001
1.3.2 Starch

Starch is an important biopolymer produced during photosynthesis and is utilised by both plants and animals to provide energy in many biochemical processes. Starch is a mixture of two polymers, amylose, which is linear and amylopectin, which is highly branched. Most starches are 20-25% amylose, but there are exceptions: pea starch contains about 60% amylose and the waxy varieties of maize have little or none (Coultate, 1996). Amylose consists of long chains of α-D-glucopyranosyl residues linked α 1-4 glycosidic bond. Amylopectin is a highly branched molecule, which consists of short chains of (1-4) linked alpha glucose with 1-6 alpha linked branches. It is reported that the molecular size of amylopectin is almost too large to be determined accurately but light scattering studies indicate a value of 10^6 D-glucosyl residues per molecule; this makes it one of the largest naturally occurring macromolecules (Kennedy et al., 1993 & Coultate, 1996). The ratio of amylose to amylopectin varies with starch source but it is typically 1:4 (Orfold et al., 1987).

1.3.2.1 Granular characteristics

Starch occurs in plant tissues in the form of discrete granules whose characteristics of size; shape and form are unique to each botanical species (Woolfe, 1992). Fasihuddin (1999) also reported that starches from different sources differ in overall structure including size distribution of the granules, shape, amylose and lipid content, distribution of chain length in amylopectin, and crystalline structure.

Sweet potato granules have been reported to be oval, round or polygonal with a central hilum and within an individual cultivar, they vary greatly in size. Between cultivars the mean granule size ranged from 12.3 to 21.5 μm (Madamba et al., 1975).
The average granule size of sweet potato starch is reported to be similar to that of cassava starch (Lii & Chang, 1978). Similarly Dreher & Berry (1983) reported that sweet potato granules are of a similar size to that of cassava and maize, but are smaller than those of potato starch, which also have a large range of granule size.

According to Rasper (1971), particle size including size distribution is one of the characteristics that markedly affect the functional properties of starch granules. Smaller granules are reported to have both higher solubility and water absorption capacity (Goering & Dehaas, 1972), as cited by Tian et al., 1991. As with most types of starches, sweet potato granules are made up of amylopectin and amylose molecules. The sweet potato starch amylopectin: amylose ratio is variable but generally about 3:1 or 4:1 (Woolfe, 1992). The amylose content of sweet potato starch granules has been determined as ranging from 17.5 to 38% in cultivars from the United States, Philippines and Korea (Shin & Ahn, 1983)

1.3.2.2 Gelatinisation

Gelatinisation refers to the swelling of starch granules caused by polymer hydration when the granules are treated with boiling water or with a solution of hot ethanol. Gelatinisation increases the accessibility of the starch polymer to either a solubilising or hydrolysing agent. Starch gelatinisation may be described as a loss of macromolecular organization over a temperature range characteristic of the starch source. Reports indicate that gelatinisation properties vary with different plant species (Noda et al., 1998). Large differences in gelatinisation properties have been reported among the waxy rices (Tester & Morrison, 1990). Gelatinisation temperature for sago
starch is high compared to corn, pea and potato (Tian et al., 1991; Valetudie et al., 1995) and ranges from 69.4°C to 70°C.

Gelatinisation of sweet potato starch is of the single state type (Madamba et al., 1975) and is reported to take place at 58°C and 69°C (Lii & Chang, 1978). McPherson & Jane (1999) similarly reported that the gelatinisation onset temperature for sweet potato is 57.8°C. In addition Noda et al. (1997) demonstrated that the gelatinisation onset of ten different sweet potato starches ranged between 55.8°C and 61.3°C. A number of factors affect the gelatinisation temperature including granule size, molecular weight, crystallinity, internal granular organisation and the distribution of the amylopectin chain length (Cowburn, 1989 & Noda et al., 1998). The gelatinisation temperature appears to be greater in sweet potato species than in the cassava, potato or wheat but is similar to that of rice (Zobel, 1988; Tian et al., 1991).

1.3.2.3 Viscosity

In the presence of water and heat, starch granules swell by absorbing water and begin to break down resulting in a paste, which then forms a gel on cooling. The consistency of the paste and the properties of the gel are important for many industrial applications as they influence important quality characteristics (Leelavathi et al., 1987). Hoover (2001) reported that the legume and yam starches exhibit a higher pasting temperature and thermal stability than other starches, suggesting the presence of strong binding forces within the granule interior. The tuber and root starches, with the exception of yam starches, exhibit a peak viscosity similar to cereals. The absence of a peak viscosity is characteristic of legume pasting curves (Hoover & Sosulki, 1991). The pasting temperatures of sweet potato starches vary between 66.5°C and
86.3°C (Lii & Chang, 1978). Sweet potato starch has also been described as having a high peak viscosity and thins rapidly with prolonged cooking before thickening on cooling (Anonymous, 1968).

1.3.2.4 Retrogradation

Retrogradation involves reorientation of the starch molecules and subsequent hydrogen bonding to form a crystalline structure. These molecular interactions are time and temperature dependent (Hoover, 2001). During retrogradation, amylose forms double helical associations of 40 to 70 glucose units whereas amylopectin crystallization occurs by association of the outermost short branches (Ring et al., 1987). Del Rosario & Pontiveros (1983) reported that retrogradation is affected by the amylose and amylopectin concentrations and the presence of other molecules such as sugars, salts, emulsifiers, molecular size, temperature and pH. Retrogradation varies with starch source and is known to be important in the staling of bread and changes in the texture of canned soups and of other foods with high starch concentrations. Sweet potato starch was found to retrograde more slowly and to a lesser extent than some other starch sources, which apparently explained a slower rate of staling in the sweet potato-substituted breads as compared with whole wheat bread (Del Rosario & Pontiveros, 1983)

1.3.2.5 Digestibility

Digestibility is the proportion of a foodstuff absorbed from the digestive tract into the blood stream, it is measured as a difference between intake and faecal output. The efficient digestion of starch is especially important to specific groups of people such as infants under 6 months of age. Ruminants must also be provided with highly
digestible starch to ensure maximum reproduction (Dreher et al., 1984). Digestibility of native starches among species are affected by the following factors, starch source (Ring et al., 1998; Dreher et al., 1984), granular size (Snow & O’Dea, 1981), amyllose and amylopectin ratio (Hoover & Sosulsku, 1991), extent of molecular association between starch components and degree of crystallinity (Dreher et al., 1984 & Ring et al., 1998). Processing treatments, storage conditions, chemical modification and genetic breeding all affect the digestibility of starch (Dreher et al., 1984). Cereal starches are generally known to be more digestible than root/tuber and legume starches. The digestibility of these starches can be improved by cooking and malting (Alonso et al., 2000).

Sweet potato starch is reported to be more digestible than potato but less digestible than cassava (Hizukuru et al., 1988; Kainuma, 1988). Varietal differences among sweet potato digestibility have been reported to be highly significant (Madamba et al., 1975). Zhang (1995) suggested that the starch digestibility of sweet potato could be improved to the same level as that of corn through conventional breeding.

1.3.3 Protein

1.3.3.1 Background

Proteins play an important role in biological and food systems and include biocatalysts (enzymes), structural components of cells and organs (for example collagen, keratin, elastin), contractile proteins (actin and myosin), hormones (insulin, growth factor), transport proteins (serum albumin, transferrin, hemoglobin), antibodies (immunoglobulins), protective proteins (toxins and allergens) and storage proteins (seed proteins, casein micelles, egg albumen) as nitrogen and energy source
for embryos (Bender, 1993). Proteins are large polymers of 20 different amino acids joined together by peptide bonds. Every amino acid contains an amino group (NH$_2$) and a carboxyl group (-COOH). Amino acids have the amino and carboxyl groups attached to the same carbon atom, for example glycine (NH$_2$-CH$_2$-COOH). The general formula of these amino acids is CH$_2$R-NH$_2$COOH, where the nature of R can vary in chemical complexity from the simple methyl group of alanine to the aromatic ring structures of phenylalanine, tyrosine and tryptophan (Coultate, 1996). Because of the variety of side chains that occur when these amino acids are linked together, the different proteins may have different chemical properties and different structures (DeMan, 1990). The four structures that exist in proteins include; primary, secondary, tertiary and quaternary.

1.3.3.2 Primary structure

The primary structure of a protein denotes the linear sequence in which the constituent amino acids are linked via peptide bonds. It is known to be different for each protein. The chain length and the amino acid sequence of the polypeptide determines its three dimensional structure in solution (Holme & Hazel, 1998; Coultate, 1998). The primary structure is determined by the gene containing the information for that protein and this determines the shape of the protein and its biological function.

1.3.3.3 Secondary structure

Secondary structure is the three dimensional shape of a polypeptide chain or a portion of a chain. This structure assumes a helical structure similar to that shown in Fig.1.1, which is stabilised by the intra-chain hydrogen bonds formed between the amide
hydrogen of one peptide bond and the oxygen of a carbonyl group (Holme & Hazel, 1998). In the secondary structure the polypeptide chains fold up in a variety of ways due to two main types of chemical interactions:

i) Hydrogen bonds between oxygen of one peptide bond and the nitrogen of another

ii) Interaction between the side chains of amino acids.

Fig. 1.1 Secondary structure of proteins adapted from Holme & Hazel, 1998

(a) A regular backbone structure due to the bond angles involved

(b) Helical form is stabilised by hydrogen bonds between —NH group of one peptide bond and the —CO group of another Peptide bond
Depending on the nature of the side chains different regions of the chain may fold into alpha helix or beta-pleated sheet. In the alpha-helix the peptide backbone of the protein adopts a spiral form, for example myosin. The helix has 3.6 residues per turn and a pitch of 5.4Å. It is generated by a repeating sequence of dihedral angles ($\phi = -60^\circ$ and $\psi = -50^\circ$) that orient NH groups of the peptide toward the N-terminus and C=O groups toward the C-terminus of the helix. It is stabilized by intra-chain hydrogen bonding between the N-H of the $i^{th}$ residue and the C=O group of the $i + 4^{th}$ residue (Nakai & Moddler, 1996). The $\alpha$-helical structures in proteins are predominantly amphiphilic, that is half of the helical surface is hydrophilic and the other half is hydrophobic. Generally in native proteins, the hydrophobic surface of the $\alpha$-helix faces the interior of the protein and is engaged in hydrophobic interactions with other non-polar groups in the interior. Such interactions are generally said to contribute to the stability of the folded form of the protein (Damodaran, 1996).

The $\beta$-sheet structure is an extended structure in which the C=O and N-H groups are oriented perpendicular to the direction of the backbone. In this configuration, hydrogen bonding can occur only between sheets. Generally $\beta$-type proteins are more hydrophobic than the $\alpha$-type proteins. They exhibit high denaturation temperatures. Polypeptide segments in which the consecutive amino acid residues assume random combinations of $\phi$ and $\psi$ angles have disordered or aperiodic structures. Proteins with high levels of proline residues usually assume a random structure. This is because of their pyrrodine ring structure in which the $\phi$ is fixed at $70^\circ$C and their inability to form hydrogen bonds; $\alpha$ and $\beta$-caseins consist of about 8.5 and 17% proline residues respectively. The uniform distribution of these residues in
its sequence hinders formation of α-helical and β-sheet structures and these proteins exist predominantly in disordered state. Other food proteins that contain a large amount of proline residues include cereal proteins such as gliadins and glutenins. Since the main biological function of caseins and cereal proteins is to provide nitrogen and energy sources for infants and germinating seeds, a disordered structure is necessary to ensure high susceptibility of these proteins to proteolytic digestion (Damodaran, 1996).

1.3.3.4 Tertiary structure

This refers to the spatial arrangement of the entire polypeptide chain with secondary structure segments into a compact three-dimensional folded form. Since the way in which a protein chain folds is determined by interactions between amino acid side chains and its environment, it obviously depends on both the sequence of amino acids and the way in which different regions are brought near each other by the secondary structure (Holme & Hazel, 1998). The tertiary structure reflects the sum of all non-covalent interactions possible within a protein and between the protein and the solvent. During the formation of tertiary structure, there is relocation of the non-polar residues to the interior. However, not all the hydrophobic groups are buried, for example in globular proteins. Lee & Richards (1971) reported that about 40-50% of the water accessible protein surface is occupied by non-polar residues. Likewise some polar residues are inevitably buried in the interior of proteins where they are hydrogen bonded to each other or engaged in electrostatic interaction with oppositely charged residues. Being very strong in low dielectric environment, these buried ion-pair interactions contribute significantly to the stability of proteins.
The folding of a protein into a compact tertiary structure is accompanied by a reduction in the interfacial area between non-polar groups of the protein and the surrounding solvent water. The distribution of hydrophilic and hydrophobic residues and their relative fraction in the amino acid sequence influences several physico-chemical properties such as shape, surface topography and solubility of the protein (Damodaran, 1996). The shape adopted is characteristic of the particular protein and is essential for structural and catalytic functions.

1.3.3.5 Quaternary structure

The quaternary structure refers to the spatial arrangement of subunits (Nakai & Modler, 1996). Each polypeptide chain is known as a subunit, and the quaternary complex is referred to as the oligomeric structure. These subunits may be identical polypeptide chains or chemically distant species (Zubay et al., 1995; Berg & Tymoczko, 2002). An example of protein aggregation is human haemoglobin, which contains 4 polypeptide chains (α2 β2) held non-covalently, in a specific conformation required for its biological function. Many food proteins such as legumes and oilseeds are oligomeric proteins with several subunits. Soy globulins and cereal proteins typically contain more than 35% hydrophobic amino acid residues and exist in complex oligomeric states (Bushuk & MacRitchie, 1989).

1.3.3.6 Sources and quality of proteins

Important sources of proteins include animal flesh and their products; they provide high quality and quantity of protein to man. Plants particularly pulses also provide a good source of protein. The roots, plantains and tubers provide protein in low quantities. In developed countries people obtain much of their protein from animal
products whereas in other parts of the world the major protein is derived from plant products. Many plant proteins are deficient in one or other of the essential amino acids. The quality of proteins can be determined by its protein content, the type and amounts of essential amino acids it contains, and the degree to which the protein is digested and absorbed by the body (Fox & Cameron, 1999). In general animal proteins are of higher quality than plant proteins.

1.3.3.7 Denaturation

Protein denaturation is a process by which a protein loses its native structure (only the secondary and tertiary structure are affected). The process is peculiar to proteins and affects different proteins to different degrees. Denaturation can be caused by a variety of agents of which the most important are heat, salts, pH, chemicals and surface effects. These factors disrupt the weak non-covalent forces, which hold the protein in its organised structure and cause chains to unfold (Fox & Cameron, 1999). When proteins are denatured their properties are completely altered, biological activity is destroyed, solubility decreased and viscosity increased. Many proteins are denatured by heat for example if egg white is heated coagulation begins at about 60°C. Higher temperature causes the protein (albumin) to separate out as a solid. The proteins of egg-white are denatured by surface forces when the egg white is whipped to a foam (Howell, 1992).

Denaturation of proteins is important in nutrition as the compact structure of proteins is resistant to the action of digestive enzymes. Proteins, which have been denatured lose their compact structure and many more peptide bonds are accessible to digestive
enzymes, which is why cooked proteins are more readily digested (Alonso et al., 2000; Yvonne et al., 2001; Rosario & Jayashree, 2000).

1.3.3.8 Functional properties of proteins

In addition to their nutritional function, protein plays an important role in the expression of sensory attributes of foods. Food preferences by consumers are predominantly based upon organoleptic properties such as flavour; odour, colour, taste, texture and mouth feel of foods (Damodaran, 1996). Proteins represent a most important class of functional ingredients because they possess a range of dynamic functional properties as indicated in Table 1.3. According to Nakai & Modler (1996) the functional properties routinely exploited are gelling, foaming and emulsifying properties. Since these functional properties are influenced by protein-water interactions this section will focus on properties such as solubility, viscosity, gelation and emulsification.

Solubility

Solubility is often considered to be a prerequisite for performance of a protein in several food applications (Kinsella, 1976). Several functional properties such as thickening, emulsification and gelation of proteins are affected by protein solubility. Solubility of a protein is related to its hydrophilicity/ hydrophobocity balance. Thus the amino acid composition of a protein inherently affects its solubility characteristics. Solubility of a protein is a manifestation of the energetics of protein-protein interactions and protein-solvent interactions. Bigelow (1967) reported that the solubility of a protein is related to the average hydrophobicity of the amino acid residues and charge frequency of the protein. A low average hydrophobicity and high
charge frequency results in high solubility. However, it has been pointed out that although the solubility characteristics of several proteins follow this empirical relationship, there are several exceptions (Damodaran, 1996). On a fundamental level, the solubility characteristics of proteins depend not simply on their average hydrophobicity and charge frequency but on the physical and chemical characteristics of the protein surface and thermodynamics of its interaction with the surrounding solvent as well.

The solubility of proteins is markedly affected by pH, ionic strength, ion types, temperature, solvent, polarity and processing conditions. These agents affect the solubility of proteins by mainly causing alteration in the ionic, hydrophilic and hydrophobic interactions at the protein surface. The insolubility of most proteins at their isoelectric points is due to neutralization of charge repulsion among protein molecules; this promotes aggregation via hydrophobic interaction (Nakai & Modler, 1996).
Table 1.3: Functional roles of food proteins in food systems

<table>
<thead>
<tr>
<th>Function</th>
<th>Mechanism</th>
<th>Food</th>
<th>Protein type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility</td>
<td>Hydrophilicity</td>
<td>Beverages</td>
<td>Whey Protein</td>
</tr>
<tr>
<td>Viscosity</td>
<td>Waterbinding, Hydrodynamic, size</td>
<td>Soups, gravies, salads,</td>
<td>Gelatin</td>
</tr>
<tr>
<td></td>
<td>shape</td>
<td>dressings, desserts</td>
<td></td>
</tr>
<tr>
<td>Cohesion</td>
<td>Hydrophobic, ionic, hydrogen bonding</td>
<td>Meats, pasta, sausages</td>
<td>Muscle, egg and whey proteins</td>
</tr>
<tr>
<td>Adhesion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelation</td>
<td>Water entrapment, Immobilization</td>
<td>Meat, gels, cakes, bakeries, cheese</td>
<td>Muscle, egg and milk proteins</td>
</tr>
<tr>
<td>Elasticity</td>
<td>Hydrophobic interactions, disulphide cross links</td>
<td>Meat and Bakery products</td>
<td>Muscle and cereal proteins</td>
</tr>
<tr>
<td>Emulsification</td>
<td>Adsorption and formation at interfaces</td>
<td>Sausages, soups, cakes, dressings</td>
<td>Muscle, egg, milk proteins</td>
</tr>
</tbody>
</table>

Source: Nakai and Modler, 1996 pp 169
Solubility and viscosity are two experimentally measurable properties that can provide information about the functional behaviour as well as the physicochemical nature of proteins. Many proteins, upon hydration, absorb water and swell thereby causing changes in their hydrodynamic properties that are reflected in thickening and concurrent increases in viscosity (Kinsella, 1976). A knowledge of flow properties and viscosity behaviour of protein dispersions are of practical significance in relation to the processing and design of pumping, feed rate, spray drying and heat exchange. Viscosity can be used as an index of structural changes in proteins and subsequently of the hydrodynamic/rheological properties of modified food proteins.

Gelation

Gelation is a rheological property and its definition is based on the observer and the techniques used to measure it, for example gels may be described by their capacity to immobilize a liquid, their macromolecular structure and by their textural or rheological properties (Kinsella, 1976; Howell, 1992). Gelation is an important functional property of food properties because of their great potential use in fabricating novel and structured products (Kinsella, 1976). Proteins are thought to contribute to the rheological properties of food by the formation of an orderly, three-dimensional network of associated or aggregated protein molecules that are capable of entrapping large amounts of water within the matrix (Hermansson, 1978).

The formation of a gel occurs under conditions that disrupt the native protein structure (Nakai & Moddler, 1996). It follows that denaturation of a protein is a pre-
requisite for protein gelation. Comfort & Howell, 2002 pointed out that the ability of
denatured proteins to associate and form gels depend on their properties and the
environmental conditions (such as protein concentration, other proteins, other
components, pH) to which they are exposed. The type of gels formed will also depend
on the above conditions. Protein may interact with each other in a number of ways to
affect the properties of gels formed and the overall texture of the food. Howell (2000)
reviewed various gels formed as a result of protein interacting with other proteins and
these included; synergistic or compatible gels, aggregates and phase separated gels.

Synergistic type of interaction results in greater gel strength. This occurs due to
similarities in molecular weight, for example plasma-egg albumen protein mixtures
when heated at temperature of 85 °C produced gels of greater strength than predicted
from the contribution of the individual proteins in the mixture. During heating the
buried hydrophobic, sulphydryls and other reactive groups are exposed during
denaturation and can participate in protein-protein interactions.

Aggregation occurs mainly through electrostatic interaction and Howell (1995)
reported electrostatic interaction of lysozyme with bovine serum albumin, which
enhanced foaming properties.

Phase separation occurs because the mixed protein or other macromolecules do not
associate due to physical and chemical differences. An example of a phase-separated
system is a mixture of soya and whey proteins. The proteins phase separate due to
differences in molecular weight, i.e. soya isolate proteins are large (MW 140,000-
190,000 for 7S globulins and 300,000-400,000 for 11S globulins) compared with
whey proteins (MW 14,000 for α-lactalbumin and 18,000 for β-lactoglobulin). Phase separation also occurs with mixtures of protein and polysaccharides (Ledward, 1993).

In food systems in addition to imparting unique rheological properties to the food, the three dimensional gel network provides a structural matrix for holding water, flavour and other food ingredients (Nakai & Moddler, 1996).

Emulsification

An emulsion is a mixture of two or more immiscible liquids such as vegetable oil and water. During mechanical agitation, the liquid that is dispersed as droplets is discontinuous, or the internal phase and the surrounding liquid is the continuous phase. If oil is the dispersed phase and the water is the continuous phase, the emulsion is an oil in water type or oil-water emulsion, whereas if water is the dispersed phase and oil the continuous phase, the emulsion is a water-in-oil type or water-oil (Kinsella et al., 1994). Food emulsions are quite complex because of additional compounds contained in the different phases: dispersed solids in cakes and sausages, partially solid in milk, butter and dessert or partially crystalline liquid and gas phases (ice cream and whipped toppings) (Dickinson and Stainsby, 1982).

The interfacial tension between water and oil is quite high, therefore emulsions are thermodynamically unstable and phase separation occurs over time. The stability of emulsions can be improved by the addition of amphiphilic surface-active molecules that adsorb at the oil-water interface and reduce the interfacial tension. Proteins being amphiphilic are well suited to act as macromolecular surfactants (Nakai & Moddler, 1996). Factors affecting emulsifying properties include the amount of protein
adsorbed, rate of adsorption at the oil-water interface, extent of conformational rearrangement at the interface and ability to form a continuous, cohesive and viscous film via covalent and non-covalent interaction. The stability of emulsions is affected by the following factors; electrostatic repulsion of charged groups, protein conformation denaturation, solubility of the emulsifier, pH, salts, temperature and emulsifier concentration (Damodaran, 1996).

1.3.3.9 Sweet potato protein

The overall picture of the sweet potato crop as an important world protein source is impressive. It has been estimated that sweet potato yields an average of 184 kg protein/ha, comparing favourably with the estimated average yields for wheat protein (200 kg/ha) and rice (168 kg/ha). Sweet potato is one of the major global crops that has the potential to provide about 2 million tonnes of protein world wide (Walter et al., 1984).

However to consumers the concentration of protein in the food as eaten is of first concern. The total protein content of sweet potato is on average about 5 g/100g dry weight or 1.5% fresh weight (Woolfe, 1992). Wide variations occur in total protein level in the different parts. Total protein content of sweet potato from the United States and Taiwan is evenly distributed throughout the root. A higher concentration has been reported in the outer layer of flesh close to the skin, which is removed by peeling (Makki et al., 1986) than in the rest of the flesh. The total protein content of the sweet potato varies according to variety. Other factors affecting protein concentration include environment. This may vary according to location, season or year of growth and entails changes due to climate, soil and incidence of pests (Purcell
et al., 1978). The protein quality of some sweet potato cultivars has been studied. Ameny et al. (1994) studied African white-fleshed sweet potato varieties and investigated their protein quality by the protein efficiency ratio method. Their results indicated that one of the varieties (T1702) had a higher protein content and high protein quality. Ravindran et al. (1995) reported that the in vitro digestibility of sweet potato protein is 75.8% indicating that sweet potato protein is well utilised.

1.3.4 Lipids

Lipids are organic compounds, which contain carbon, hydrogen and small amounts of oxygen. They are soluble in organic solvents and most of them are derivatives of fatty acids for example oils, fats, waxes and phospholipids. Oils and fats are of nutritional importance and are used in cooking, salad making, and in many manufactured foods. They are important in nutrition as the most compact energy source available and play an important role in body metabolism. Lipids provide a more concentrated form of fuel than proteins or carbohydrates. As a storage fuel, lipids have an advantage in that they can be stored in anhydrous form and represent more energy for less bulk than the glycogen stored in the liver or muscle which is heavily hydrated (Gurr & Harwood, 1991).

The sources of oils and fats are mainly animal products and plants such as sunflower, legumes and cereals particularly corn. Roots and tubers are not good sources of lipids. Much of the oils and fats can be synthesized by the body tissues of humans. However some fatty acids have to be supplied by the diet because they cannot be synthesized endogenously; these are referred to as essential fatty acids. Fatty acids are the
building units of lipids and can be grouped into two classes namely, saturated and unsaturated fatty acids.

1.3.4.1 Saturated fatty acids

Saturated are fatty acids that have no double bonds in their structure. Most of them are straight chain structures with an even number of carbon atoms. A few of the saturated acids are branched. Acids from C2 to C30 have been reported but the most common lie in the range of C12 to C22. The more saturated acids are shown in table 1.4 with some physical properties and their occurrence.

**Table 1.4: Properties of saturated fatty acids**

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0</td>
<td>Dodecanoic</td>
<td>Lauric</td>
<td>44.8</td>
<td>130</td>
<td>Coconut oil</td>
</tr>
<tr>
<td>14:0</td>
<td>Tetradecanoic</td>
<td>Myristic</td>
<td>54.4</td>
<td>149</td>
<td>Coconut oil</td>
</tr>
<tr>
<td>16:0</td>
<td>Hexadecanoic</td>
<td>Palmitic</td>
<td>62.9</td>
<td>167</td>
<td>Cotton seed, palm oil</td>
</tr>
<tr>
<td>18:0</td>
<td>Octadecanoic</td>
<td>Stearic</td>
<td>70.1</td>
<td>184</td>
<td>Ruminant depot, cocoa</td>
</tr>
<tr>
<td>20:0</td>
<td>Icosanoic</td>
<td>Arachidic</td>
<td>76.1</td>
<td>204</td>
<td>Minor component of seed fat</td>
</tr>
<tr>
<td>22:0</td>
<td>Docosanoic</td>
<td>Behenic</td>
<td>82.0</td>
<td>-</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

**Source:** Gunstone & Norris, 1983.
Saturated acids are solid with a melting point, which increases with molecular weight. In general the melting point of a natural fat depends on the proportion of saturated and unsaturated fatty acids it contains, and to a small extent on the chain length of the fatty acids (Gunstone & Norris, 1983; Gurr & Harwood, 1991).

1.3.4.2 Unsaturated fatty acids

Unsaturated fatty acids have double bonds in their structure and include the monoenoic acids and polyunsaturated fatty acids (PUFA). Monoenoic acids have one double bond in their structure. Over a hundred of them have been identified of which oleic acid (octadec-9-enoic) acid is the most common. Polyunsaturated acids have two to six cis double bonds arranged in a methylene interrupted pattern as in linoleic acid ($\text{CH}_3(\text{CH}_2)_4\text{CH}=$CHCH$_2$CH=CH(CH$_2$)$_7$COOH). All dienoic acids are derived from monoenoic acids by removing hydrogen only from carbon atoms between an existing double bond and carboxyl group. This is done in mammals with the help of enzymes known as desaturases (Gurr & Harwood, 1991). Natural fats contain complex mixtures of all the three types of fatty acids (saturated, monounsaturated and polyunsaturated). Fats with the highest constituent of saturated fatty acids are categorized as saturated fats.

1.3.4.3 Lipid autoxidation

Saturated fats are very stable but unsaturated fatty acids are susceptible to oxidation. The unsaturated bonds present in all fats and oils react with oxygen leading to the formation of primary, secondary and tertiary oxidation products that make the fat containing foods unsuitable for consumption. Oxidation of lipids leads to rancidity (oxidative deterioration of fatty foods) (Osawa, 1999). The lipid oxidation reaction
occurs in three stages namely initiation, propagation and termination. In the initiation process, hydrogen is abstracted from the fatty compound, which is initiated by free radicals such as hydroxyl radicals.

\( \text{LH} + \text{OH}^- \rightarrow \text{L}^+ + \text{H}_2\text{O} \)

This reaction takes place at the carbon next to the double bond. A free radical then combines with oxygen to form a peroxy-free radical, which can in turn abstract hydrogen from another unsaturated molecule to yield a peroxide and a new free radical, thus starting the propagation reaction.

\( \text{L}^+ + \text{O}_2 \rightarrow \text{LOO}^\cdot \)

\( \text{LOO}^\cdot + \text{LH} \rightarrow \text{LOOH} + \text{L}^+ \)

In the terminal reaction, the free radicals react with themselves to form stable products. Termination may also take place in the presence of certain antioxidants such as Vitamin C, E and BHT. They react with radicals generated during propagation and serves as a defence mechanism to protect against oxidative stress by lipid peroxidation (Halliwell, 1993).

The lipid hydroperoxides formed are very unstable and breakdown to aldehydes, hydroxides, alcohols and hydrocarbons, which are responsible for the oxidised flavour of fats (Deman, 1992; Coultate, 1996). The rate of oxidation depends primarily on the composition of the fat, its degree of unsaturation and the type of unsaturated fatty acid present. High temperatures increase lipid oxidation. Light and ionizing radiation are accelerators of oxidation. Therefore foods with fat have to be handled under an atmosphere of inert gas or kept away from oxidants by vacuum packing.
1.3.4.4 Sweet potato lipids

Lipid concentration in sweet potato has been reported to range from 0.1% to 0.8% in the raw root (Collazos et al., 1974; Haytowitz & Mathews, 1984). Analysis of the lipid fractions for their fatty acid composition showed that palmitic and linoleic acid are most abundant in all fractions comprising of 29.3% and 44.7% respectively of the total lipids (Walter, Hansen & Purcell 1971). Equal amounts of linoleic and oleic acids were found in Korean sweet potato lipids (Lee, 1972). Oleic was the most abundant unsaturated acid found in a Nigerian sample (Fabuya, 1981). Sweet potato lipid is highly unsaturated. Rhee & Watts (1966) reported that sweet potatoes are relatively high in lipid oxidizing activity. The uptake of oxygen by stored dehydrated sweet potato flakes and consequent auto-oxidation of carotenoid pigments and unsaturated fatty acids, leading to loss of colour and production of off odours and off-flavours is quite common.

1.3.5 Vitamins

Vitamins are organic compounds that are required in small quantities and their absence leads to deficiency diseases such as pellagra, scurvy, beriberi and night blindness. Vitamins must be provided by the diet because the body is unable to synthesize them from other nutrients (Garrow et al., 2000).

Sweet potatoes are substantial sources of ascorbic acid and contain moderate amounts of thiamin, riboflavin, niacin as well as pyrodixine, folic acid and pantothenic acid. They contain satisfactory quantities of vitamin E (Woolfe, 1992). The ranges of thiamin, riboflavin and niacin contents in some South Pacific Island cultivars range
from 0.04–0.12, 0.02–0.06 and 0.26–0.89 mg/100g fresh-weight (fwb) (Kwiatkowska 
et al., 1989).

Sweet potatoes produce variable and sometimes large quantities of carotenoids which 
act as precursors of vitamin A. It has been reported that the total carotenoid levels are 
high in dark yellow or orange-fleshed cultivars (Woolfe, 1992; Solomons, 1997). 
White fleshed sweet potatoes from the South Pacific were analysed and reports 
indicate that they contained low levels of β-carotene (5.5 to 21.4 μg/100g) (fwb) and 
no α-carotene was found (Singh & Bradbury, 1988). The carotenoid content varies 
with cultivars. The carotenoid content of 17 cultivars grown in Taiwan ranged from 
0.4 to 24.8 mg/100g fwb (Wang & Lin, 1969). The carotenoids of 26 cultivars grown 
in the Philippines with flesh colour from white through cream and yellow to carrot-
like varied from traces to 11.45 mg/100g fwb (Garcia et al., 1970).

Most root and tuber staples lack or are extremely low in β-carotene resulting in 
negligible quantities of retinol equivalents. The only starchy staple apart from sweet 
potato with a significant amount of β-carotene is the plantain. Cereals, with the 
exception of some cultivars of yellow maize, which are commonly grown in Uganda, 
have no provitamin A activity. The only consumed vegetable, which has a high 
carotene content, comparable to that of carotene-rich sweet potato is the carrot. Even 
tomatoes which many consumers consider being high in provitamin A are much 
lower in biologically active carotenoids than many cultivars of sweet potato (Woolfe, 
1992). The analysis of one sweetened dried sweet potato product, made in the 
Philippines and eaten as a snack or dessert, revealed a much higher concentration of 
beta-carotene than dried fruit products such as apricots and peaches (Woolfe, 1992).
In Guatemala, sweet potato is known to be the main source of vitamin A. Solomons et al. (2001) studied sweet potato vitamin A content and found that 49% to 67% of the daily recommended retinol equivalents is provided by 28 g of sweet potato (yellow cultivar). Thus sweet potato is a good source of vitamin A that could combat vitamin A malnutrition in developing countries (Solomons, 1997).

1.3.6 Minerals

The ash (inorganic residue) content of sweet potatoes averages approximately 1% of the fresh root weight and about 3-4% of the dry weight. Ash contains a variety of minerals and trace elements. Some of these minerals are absorbed in varying quantities depending on their concentration in the soil in which the roots are grown or are derived from fertilizers or sprays used during cultivation (Woolfe, 1992). In general, potassium is the element present in greatest concentration, followed by phosphorus, calcium and magnesium (Picha, 1985; Holloway et al., 1989). Other elements present include sodium, iron, copper, zinc, manganese and sulphur (Ohtsuka et al., 1984; Monro et al., 1986). Variation of mineral content has been observed in different varieties of sweet potato. Significant differences in K, P, Ca and Mg were reported in six American cultivars analysed immediately after harvest (Picha, 1985).
Table 1.5: Mineral content of sweet potato roots from various countries

<table>
<thead>
<tr>
<th>Element</th>
<th>Fresh weight basis (ppm)</th>
<th>Dry weight basis (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Ca</td>
<td>240</td>
<td>290</td>
</tr>
<tr>
<td>K</td>
<td>3960</td>
<td>2600</td>
</tr>
<tr>
<td>P</td>
<td>410</td>
<td>510</td>
</tr>
<tr>
<td>Mg</td>
<td>200</td>
<td>260</td>
</tr>
<tr>
<td>Fe</td>
<td>6.9</td>
<td>4.9</td>
</tr>
<tr>
<td>Na</td>
<td>210</td>
<td>520</td>
</tr>
<tr>
<td>Cu</td>
<td>1.6</td>
<td>1.7</td>
</tr>
<tr>
<td>Zn</td>
<td>2.4</td>
<td>5.9</td>
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<td>1.1</td>
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<td>Al</td>
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</tr>
<tr>
<td>Bo</td>
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<td>1.0</td>
</tr>
<tr>
<td>Pb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Mo</td>
<td>2.4</td>
<td></td>
</tr>
</tbody>
</table>

1 Monro et al., 1986
2 Bradbury and Holloway, 1988
3 Collazos et al., 1974
4 Tan et al., 1981
5 Kim et al., 1981
6 Nnam 2001 (ppm dry weight basis)
7 Ravindran et al., 1995 (ppm dry weight basis)
1.3.7 Anti-nutritional factors

The anti-nutritional factors commonly found in sweet potato are trypsin inhibitors and oxalic acid. Oxalic acid is known to bind to calcium thereby preventing the bioavailability of calcium to the body (Holloway et al., 1989). Ravindran et al. (1995) reported that the oxalate levels do not pose a nutritional hazard since greater than 60% of the oxalates in sweet potato are in the water-soluble form. Water-soluble oxalates are known to leach out during cooking in water and can be removed by discarding the water (Libert & Franceshi, 1987). Trypsin inhibitors are a broad class of polypeptides and proteins occurring in plants and other life forms, which inhibit the action of proteolytic enzymes, for example trypsin and chymotrypsin. These inhibitors have been identified in a variety of plants, especially the Graminea, Leguminoseae and Solanaceae families (Ryan, 1981). Trypsin inhibitors are thought to play a role in plant protection by inhibiting the digestive enzymes of invading insect, pests or pathogens.

The first non-leguminous plant reported with a trypsin inhibitor was the sweet potato (Sohonie & Bhandarker, 1954). The strong inhibition of trypsin, which has been demonstrated to occur in vitro with sweet potato trypsin inhibitor could indicate an interference with protein digestion in vivo. This has nutritional implications in humans, especially those whose protein intake is marginal (Woolfe, 1992). The degree of trypsin inhibitor in sweet potato varies with cultivar and environment. Bradbury et al. (1985) reported that there was a small amount of chymotrypsin inhibitor activity detected in cultivars from the Solomon Islands.
For safety reasons it is important to identify the conditions for destroying sweet potato inhibitors and oxalate inhibitors. Trypsin inhibitors are destroyed by heat (Dickey & Collins, 1984). Similarly Ravindran et al. (1995) observed that moist heat treatment completely destroyed trypsin inhibitory activity in sweet potato tubers.

### 1.3.8 Phenolic compounds

Phenolics are functional compounds that occur naturally in plants; fruits, vegetables, coffee, tea, alcoholic beverages and herbal medicines. They occur in animal tissues but to a very limited extent (Knight, 2003). Phenolic compounds in plants are thought to play a defensive or protective role. They act as barriers to insects, which normally feed on leaves containing them (Harborne & Williams, 2000). They also act as repellants to predators (animal/microbial) by precipitating proteins or by immobilizing enzymes impeding the invasion of the host by the parasite (Haslam, 1989). Phenolic compounds have a harsh, astringent taste and produce in the mouth a feeling of dryness and roughness, which render many plant tissues unacceptable as food sources to potential predators.

Plant phenolics are broadly classified as simple phenols and polyphenols.

#### 1.3.8.1 Simple phenols

Simple phenols consist of a single aromatic ring with one or more aromatic hydroxyl groups attached to one of the carbon atom of the ring (Fig. 1.2). Other substituents present in most simple phenols include \(-\text{OCH}_3\) and \(-\text{COOH}\). Simple phenols occur as conjugates with sugars (esters and glycosides) or hydroxy acids. The most important subclass of simple phenols is the phenylpropanoids and these include cinnamates (e.g. hydroxycinnamic acids which include ferulic, caffeic and \(p\)-coumaric acids) and
associated conjugates such as chlorogenic acids (Clifford 2001). Chlorogenic acids (CGA) are a family of esters formed between certain trans cinnamic acids and (−) quinic acid (1-L-1(OH), 3,4/5-tetrahydroxyclohexane carboxylic acid) (Clifford, 2000). CGA are widely distributed in plants (Clifford 2000, 2003) and these include caffeoylquinic acids, feruloylquinic acids, dicaffeoylquinic acids and caffeoylferuloylquinic acids that have been recently characterised by Clifford et al., (2003).

![Chemical Structures]

Fig.1.2: Examples of structures of simple phenols

1.3.8.2 Polyphenols

Polyphenols are defined as compounds containing at least two aromatic rings each having at least one hydroxyl group. These include flavonoids; flavanones, flavonols, flavanols and flavones, which occur naturally as glycosides (Manach et al., 2004) in vegetables and fruits such as berries, tomatoes, potatoes, squash and broccoli; tannins are also polyphenols that have the ability to produce leather from hides and these include condensed, hydrolysable and derived tannins (Clifford, 2001).
1.3.8.4 Phenols in sweet potato

There are a considerable number of phenols that have been reported in sweet potato. Sweet potato phenolic compounds were reported by Rudkin & Nelson (1947) to consist of chlorogenic acid and other similar compounds, which were not clearly and fully identified due to the limited techniques at that time. Increase in concentration of these acids was observed when sweet potato was mechanically injured or attacked by the sweet potato weevil (Uritana et al., 1960; McClure, 1960); this was probably for protection against predators or as healing action.

Uritani et al. (1955) separated three polyphenols from sweet potato, which on decomposition, gave caffeic acid and quinic acid as fragments. These fragments were identified by their melting points, which is not a reliable method because other substances can have the same melting points. Thin layer chromatography was the commonly used technique for the separation of the phenolics in sweet potato but this does not provide sufficient information for the identification and elucidation of the phenolic structures of substance separated. Other phenolics that have been identified are anthocyanins, mainly present in purple and deep orange-fleshed varieties (Goda et al., 1996); these anthocyanins can be used as food colorants. Several authors have reported that the major colouring constituents in storage roots of sweet potato are acylated anthocyanins (Goda et al., 1996). Nuclear magnetic resonance was used to elucidate the structures of anthocyanins.
1.4 Weaning

The term ‘weaning’ is defined by Felicity et al. (1993) as the process of introducing foods other than breast milk to a child and gradually increasing the amount so that eventually the child gets enough energy and nutrients from ordinary family food. Weaning enables infants to meet their changing nutritional needs as they become less dependent on milk. It coincides with a period of rapid growth and development when an adequate diet is crucial to meet nutrient needs (DoH, 1995). The same report on health and social subjects recommends that solid foods should be introduced between the ages of 4 to 6 months because by this age the infants’ physiology and development have matured to cope with the weaning diet and most infants are ready to experience new tastes and textures. The Department of health and Social Security, DHSS (1988) in infant feeding have pointed out that there is a wide range of rate of growth and of maturing in the months after birth and acknowledged that some infants might begin weaning at 3 months. The Working Group on Weaning and Weaning Diet, DoH (1995) recommends that a start to weaning no earlier than age 4 months is the most appropriate advice for the great majority of infants.

1.4.1 Weaning and weaning foods in the developing world

As one of the aims of this research was to develop an infant weaning food for the developing world, it is important to consider weaning in the developing world. Weaning is one of the most important dietary events in an infant’s life. Due to the conditions in many developing countries, weaning frequently gives rise to a life or death situation partly because of a lack of nutritionally suitable weaning foods and also due to diet related diarrhoeal disease.
Sandra et al. (1994) reported that over a quarter of all the deaths occur among children in developing countries, under five years of age, mostly during the first three years of life. About 70% of child mortality is due to acute respiratory infections, diarrhoea and peri-natal and neonatal deaths. The World Health Organisation (WHO) estimates that malnutrition contributes to over 25% of these deaths. Children are particularly vulnerable to malnutrition during the weaning process. WHO (1998) reports that Protein–Energy malnutrition is the most common deficiency disease in the world, especially in the developing countries. The precipitative factors are related to poor food quality, insufficient food intake and severe and repeated infections (Onis, 1997). Sandra et al. (1994) also reported that in developing countries, these foods are woefully inadequate in calories, protein and micro-nutrients resulting in growth faltering and malnutrition.

The traditional weaning foods in the developing countries are mainly porridges from maize, sorghum and millet, which do not satisfy the energy and nutrient needs of infants (WHO, 1998). The gruels are either too watery and thus have a low energy content or are too bulky (thick porridge) and cannot be consumed in sufficient quantity by young children. Bulkiness of gruels is due to the effect of heat on starch structure. On cooking, the starch granules swell and bind large amounts of water. These results in gruels of high viscosity, which need to be further, diluted with water to give a consistency that is appropriate for child feeding. The dilution decreases the energy and nutrient densities of the gruel and makes it practically impossible for the child to meet his/her requirements due to the limited functional gastric capacity of children (200-250 ml) (Svanberg, 1987). Walker & Pavitt, (1989) indicated that factors limiting energy intake of an infant weaned on a low energy gruel are the
volume that the infant can consume at any one time and frequency of feeding. Therefore, in formulating weaning foods for the developing world, factors that increase the energy and nutrient density need to be taken into account.

1.4.1.1 Development of weaning foods

It is important that knowledge and technical know-how for formulating baby foods that meet the daily requirements to developing countries is made available. In developed countries, manufacturing baby foods that are appropriate in nutritional composition, texture and taste to the infant's needs and that have regard to the infant's physiological limitation is usually not an issue (Morgan, 1998). However, in the less developed countries, improving formulation of weaning foods is often hindered by a lack of resources. Therefore consideration needs to be given to use of raw materials that are appropriate and readily available.

The study carried out by WHO, 1981 on the survey of breast-feeding and weaning practices in seven developing countries (Ethiopia, Nigeria, Zaire, Chile, Guatemala, India and Philippines) indicated that growth was poorest in cases where breast-feeding was most widely practised and food supplements least given. WHO (1981) suggested that other inputs are needed to improve infant health in developing countries in addition to breast-feeding (after four months). Among these inputs are the provision of high quality and nutritious weaning foods and instructions on how to use these foods in an appropriate and sanitary manner. Weaning foods should provide the nutritional needs of the infant or young child according to national and international standards. Developing countries need to develop the capacity to produce their own weaning foods locally.
Most infant foods in developing countries tend to be based on cereals. Clark & Laing (1991) suggested that cereals tend to be used by tradition because they mix easily with water and milk. However the Department of Health and Social Security report (1974) and more recently the Department of Health (1994), discouraged wheat cereal due to the infant's susceptibility to coeliac disease. Some of the formulations found in developing countries are discussed below.

1.4.1.2 Cereal based weaning food

The most common weaning foods in developing countries of Africa are porridges from maize, sorghum and millet. The products are liked by most children, are soft and easy for a baby to swallow, easy to prepare and cheap. But plain porridges are not that nutritious because they are not rich in energy and some other nutrients such as proteins and vitamins. To enrich this porridge a paste of either groundnuts or sesame and a little oil is added (Felicity et al., 1993). Sometimes the porridge is fermented to reduce on bulkiness. Nnam (2001) reported that fermentation improves the nutritional quality, flavour, aroma and safety of traditional foods. In addition, cereals blended together with legumes in good proportion can produce nutritionally adequate formulations (mixture) that are suitable for infant weaning (Yvonne et al., 2001).

1.4.1.3 Soya-based infant weaning food

There is an increase in the use of soy flour in baby meals. The overall positive nutritional value, low cost, high availability, excellent functional properties and innovative food product development explains this increased use (Edman & Fordyce, 1989). The Working Groups on Weaning and Weaning Diet, DoH (1995) reported
that soy-protein based infant formulae meet the requirements for essential amino acids for young infants and they continue to provide a useful protein source for children beyond the first year of life. The essential amino acid content and the quality of protein in soy infant formulae are important adjuncts to vegetarian weaning diets (Report of the Scientific Committee for Food, 1989).

The appropriateness of soy products for infants was reviewed by Torun et al. (1981). Both these studies concluded that well processed soy-protein products were comparable with milk/casein in protein quality and anti nutritive factors such as trypsin inhibitors are removed during manufacturing of concentrates and isolates. Apart from protein quality, use of soy in baby foods also relates to functional properties. The use of soy flour in infant foods such as packet meals is aided by its dispersibility and solubility (Comfort, 1995). In this study soy flour will be used for the above qualities.

1.4.2 The legislation on infant food

The Codex Alimentarius Commission (2003) proposed a model composition for supplementary foods designed to be fed to infants from 4-6 months onwards and young children (1-3) years. This food standard is shown in Table 1.6 below. The Codex specifies the protein effeciency ratio (PER) of the protein in the mixture is 70% of that of the reference protein (casein). In cases where the home diet is limiting in lysine, it is desirable that the supplementing food supplies excess lysine thus improving the quality of the home diet as well as supplying additional high quality protein.
In general, the supplemented food should supply all the required vitamins and minerals. In developing countries minerals such as calcium and phosphorous are in short supply and must be provided by the supplement. In view of the widespread occurrence of vitamin A deficiency in many developing countries and its importance in growth and disease resistance, vitamin A is also needed in generous amounts in the supplement.

A good source of iron is also required because of the prevalence of nutritional anemia in the developing world and this should be supplied together with ascorbic acid to assist in making it available (Jansen 1992). The Codex standard allows up to 3 g/100g crude fibre in the supplement. Jansen et al. (1981) suggested that the crude fibre be limited to a maximum of 2 g/100g just to help in reducing the bulk and improving digestibility.

1.4.2.1 Nutrients added to manufactured weaning foods

Codex Alimentarius/FAO/WHO (2003) outlined the conditions for the additions of nutrients to manufactured baby foods. There are a number of reasons for the addition of nutrients to baby foods, the most common being the fortification/enrichment purpose. The rationale for this is to prevent or correct a demonstrated deficiency in a specific population. However Codex Alimentarius/FAO/WHO (2003) points out that fortification is a responsibility of national authorities since the amounts needed are determined by the needs of a particular population. A list of permitted food additives was published by Codex Alimentarius/FAO/WHO (2003) and specifies that there is a maximum limit of contaminants such as toxins, trace elements, heavy metals and solvent residues. Ingredients used for infant foods must be free from pesticide
residues and no exposure to ionising radiation is permitted. There are good physiological reasons for not introducing additives into the diet of a young infant very early. The British Nutrition Foundation report (1997) suggests that certain additives impose a risk to the infant’s health because they compromise kidney function by imposing a high renal solute load, associated with dehydration as the infant cannot concentrate urine, and high volumes of water are excreted to pass out solutes.

1.4.2.2 Salt
The dietary requirement for sodium for a 6 months child is 140 mg/day. Infants are less efficient in excreting an excess sodium and for this reason sodium intakes of infants should be moderated. There is evidence that habitual sodium intakes in adult populations are a determinant of prevailing levels of high blood pressure (Law, 1991). Excessive intakes of salt during infancy can result in high blood sodium levels and therefore the possible role of sodium in the pathogenesis of high blood pressure in later life (Baker et al., 1992). Since most foods contain naturally occurring salt, care should be taken not to add salt to infant foods.

1.4.3.3 Sugar
Additional sugars should be limited to that needed for palatability of sour fruits.
It is advisable to keep levels of sugar as low as possible to prevent dental carries (DOH, 1995).
### Table 1.6: Proposed model composition for supplementary foods

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Per 100g</th>
<th>per 100 Kcal</th>
<th>Per 100 KJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g)</td>
<td>21</td>
<td>5.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>13</td>
<td>3.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Crude fibre (g)</td>
<td>3</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Vitamin A as retinol (µg)</td>
<td>400</td>
<td>100</td>
<td>24</td>
</tr>
<tr>
<td>Vitamin D (µg)</td>
<td>12</td>
<td>3.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>12</td>
<td>3</td>
<td>0.7</td>
</tr>
<tr>
<td>Ascorbic acid (mg)</td>
<td>96</td>
<td>25</td>
<td>5.7</td>
</tr>
<tr>
<td>Thiamin (mg)</td>
<td>2</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>1.6</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>17</td>
<td>4.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Vitamin B₆ (mg)</td>
<td>1.3</td>
<td>0.35</td>
<td>0.08</td>
</tr>
<tr>
<td>Folic acid (µg)</td>
<td>193</td>
<td>50</td>
<td>12</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>800</td>
<td>200</td>
<td>48</td>
</tr>
<tr>
<td>Phosphorous (mg)</td>
<td>800</td>
<td>200</td>
<td>48</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>12</td>
<td>3.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Iodine (µg)</td>
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<td>35</td>
<td>8.1</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>7.7</td>
<td>2.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Mg (mg)</td>
<td>154</td>
<td>40</td>
<td>9.2</td>
</tr>
</tbody>
</table>

Data from Joint FAO/WHO; Food Standards Programme, Codex Alimentarius Commission 25th session, Rome, 2003.
1.4.4.4 Food hygiene

DOH (1995) advises that that foods given during weaning should be prepared, handled and stored in a hygienic way. Commercial foods are required by food law to be safe up to the point of sale.

1.5 Conclusion

In Uganda, infants are weaned on cereal-based foods including maize, millet and sorghum. Currently, there is a high demand for cereals as food, raw material for alcohol preparation and as cash crops. This trend has affected the availability of weaning foods at affordable prices. In attempting to reduce the incidence of malnutrition among infants, substitutes of raw material for weaning food preparation have to be used and these would include tubers and roots such as cassava, yams and sweet potatoes. In this study, sweet potato was chosen as the possible alternative raw material because of its favourable features such as its adaptability to diverse environments and its high yielding ability. Sweet potato is also a good source of carbohydrates and energy (O’Hair, 1984). The digestibility of carbohydrate fraction of sweet potato roots is reported to be greater than 90% (Yoshida & Moromoto, 1958). This study is therefore aimed at examining the potential that sweet potato may have as an alternative raw material in the preparation of infant weaning food.
1.6 OBJECTIVES OF THE STUDY

• To assess the biochemical, physicochemical and nutritional properties of sweet potato varieties from Uganda.

• To assess the rheological properties of sweet potato flour and starch

• To assess the rheological properties of sweet potato starch and protein (soya and fish) mixtures.

• To formulate an infant food by combining sweet potato with other local raw materials such as soya flour and fish to produce an optimum product of nutrient content and composition using Micro-diet a nutrition database to comply with FAO/EU guidelines.

• To assess the rheological properties of the product.

• To assess the biochemical and nutritional changes in the product on storage and to identify methods of minimising lipid oxidation using antioxidants
2.0 MATERIALS AND METHODS

2.1 Materials

Sweet potato roots varieties, Nasport (Nsp) and Spk were obtained from Namulonge Agriculture and Animal Research Institute (NAARI), Kampala, Uganda and Tanzania (Tz) variety was obtained from the Eastern part of Uganda (Soroti). Nasport and Spk (orange fleshed cultivars) and Tanzania were harvested six months after planting. Tanzania is a yellow fleshed cultivar which is a commercially important crop widely grown in the drier northeastern districts of Kumi and Soroti. On the basis of morphological, performance and quality characteristics, the Tanzania variety, which is also widely grown in Tanzania is known as SPN10, in Malawi it is known as ‘Kenya’ and in Kenya the same Tanzania variety is known as ‘Enaironi’. The Tanzania variety is the most widely grown sweet potato cultivar in sub-Saharan Africa (Mwanga et al., 2001). Medium roots (weight 200 g, length 20 cm and diameter 6 cm) of the three varieties were selected, sliced and sun dried for four days, at Makerere University, Uganda. In addition some fresh roots were airfreighted to the University of Surrey, Guildford, UK and non-damaged material was selected and sliced into small pieces and either freeze-dried or oven-dried at 100°C for 12 h 100 °C prior to analysis. The dried products were stored at 22 °C in sealed polythene bags for further tests. Fresh sweet potatoes were analysed for proximate composition immediately after arrival.
2.2 Methods

The proximate composition of sweet potato was determined according to the AOAC (1995) methods.

2.2.1 Moisture and dry matter

2.2.1.1 Principle

The sweet potato is dried in an oven at 100°C for 12 h; the weight loss incurred is quantitatively determined.

2.2.1.2 Procedure

A dried moisture dish (7-10 cm in diameter) together with a small glass rod were placed in the 100°C oven for 30 minutes, cooled in a desiccator and weighed. Chopped sweet potato samples were weighed out accurately (20 ± 0.1g) into the dish. The sample was evenly spread over the base of the dish with the glass rod. The dish with the glass rod was left overnight (12 h) in an oven at 100°C. The samples were then removed and cooled in the desiccators and weighed. The loss in weight taken as the moisture content and was determined as shown follows:

\[
\% \text{ Moisture} = \frac{\text{Weight loss on drying at } 100^\circ C \times 100}{\text{Weight of sample}}
\]

\[
\% \text{ Dry matter} = \frac{\text{Weight of oven dry sample} \times 100}{\text{Weight of fresh sample}}
\]
2.2.2 Determination of ash

2.2.2.1 Principle

Ash is the inorganic residue remaining after the organic matter has been burnt away. The ash obtained is not necessarily the same composition as the mineral content as there may be some loss due to volatilisation.

2.2.2.2 Procedure

Sweet potato dried samples (2 g) were weighed into a pre-weighed porcelain crucible in triplicate. The crucible and sample were heated on a bunsen flame to remove moisture. The crucibles were heated in a muffle furnace at 550°C overnight. The crucibles were transferred into a desiccator, cooled and weighed immediately. Ash content of the samples was calculated as follows:

\[
\text{Weight of ash (g)} = \text{(weight of crucible + Ash)} - \text{(weight of crucible)}
\]

\[
\% \text{ Ash} = \frac{\text{Weight of ash} \times 100}{\text{Weight of dry sample}}
\]

2.2.3 Crude Protein

This was determined by the Kjeldahl's method (AOAC, 1995).

2.2.3.1 Principle

The Kjeldahl method is based on the digestion of the sample with hot concentrated sulphuric acid in the presence of a metallic catalyst. Organic nitrogen in the sample is reduced to ammonia; this is retained in the solution as ammonium sulphate. The digest is made alkaline, and then distilled to release the ammonia. The ammonia is trapped in dilute acid and titrated.
2.2.3.2 Procedure

Either fresh sweet potato (2 g) or finely ground freeze-dried or sun-dried sweet potato samples (1 g) weighed in triplicate in a filter paper and transferred to digestion tubes. A catalyst tablet (5 g K$_2$SO$_4$, 0.15 g CuSO$_4$, 0.15 g TiO$_2$) was added. Digestion acid 95% sulphuric acid (20 ml) was added and mixed by swirling gently. The digestion tubes were transferred to the pre-heated block digester and left for two hours until the digestion was complete. The tubes were then removed and allowed to cool for 5 min. Demineralised water (75 ml) was added to the cooled digest.

Boric acid 4% (25 ml) in a titration receiver flask was placed on the distillation unit. The tube containing the digested sample was attached to the distillation unit and the start button started to effect the metered addition of sodium hydroxide solution and to initiate the steam distillation. After 5 min the distillation was stopped and the flask was removed. Methyl red indicator was added to the contents of the flask and titrated with 0.05 M hydrochloric acid solution to a grey end point. A blank determination was also carried out following the same procedure described above but the sample was omitted.

1 ml of 0.05 M hydrochloric acid is equivalent to 0.0007 g N.

Calculation of total nitrogen

\[
% \text{ Total Nitrogen} = \frac{0.0007 \times (\text{titration (ml)} - \text{blank (ml)}) \times 100}{\text{Weight of sample}}
\]
The crude protein content was obtained by multiplying the total nitrogen content by a factor of 6.25.

2.2.4 Crude lipid determination

2.2.4.1 Principle
Lipid content was determined using Soxhlet extraction method. The free lipid content and free fatty acids can be determined by extracting the dried material with a light petroleum fraction in a continuous extraction apparatus. The solvent is distilled off and the extract is dried and weighed.

2.2.4.2 Procedure
Sweet potato samples (3 g) were weighed in triplicate into the thimbles and a defatted cotton plug was put on the top of the sample. The thimbles were inserted into the condensers; each knob was moved to the boiling position.

To six extraction cups were added boiling chips and 30 ml of the solvent (petroleum ether 60-80°C). The samples were extracted by boiling for 3 hours after which the extraction mode knobs were moved to the rinsing position. After rinsing, the remaining solvent was collected in the condenser and evaporated. The thimbles and cups weighed and the weight of the lipid was determined by difference.
2.2.5 Small deformation oscillatory measurement

2.2.5.1 Principle

The oscillation technique on a constant stress Rheometer is a non-destructive test, which measures simultaneously the viscous and the elastic behaviour of a material.

The sample is placed between two parallel plates. The upper plate is oscillated sinusoidally at some frequency ($\omega$) in rads per second and amplitude $d$ in mm, while the other plate remains stationary. With no slippage at either side a deformation gradient is created across the thickness of the sample. The output signals are analysed and using established methods the rheological parameters are computed.

For an ideal elastic material (Hookean solid) the stress strain waves would be in phase as the sample is able to store the applied energy and then return the energy to the system when relaxed. In contrast, a Newtonian fluid will dissipate the applied stress energy and the phase difference will be exactly 90° out of phase with the strain (Fig. 2.1). For the visco-elastic material the behaviour lies somewhere in between. The ratio of in phase stress to strain is known as storage modulus ($G'$) and the 90° out of phase stress to strain is the loss modulus ($G''$). Their ratio ($G'/G''$) is the tangent of the phase angle difference between the strain and the total stress wave (Ferry, 1980).

Rheology provides a diagnostic tool in understanding consumer perception of texture and elaborates on its relationship to food structure (Borwankar, 1992). In this study, the rheological measurement is particularly useful for quantifying viscoelasticity and for quality control purposes. Viscosity can be related to mouth feel, which is of particular importance to the infant or to appearance, which is of importance to the mother in preparation of the gruel.
Out of phase axis, G" 

In phase axis, G'

Fig. 2.1 Principle of small deformation oscillation technique

a) Sine wave analysis to determine viscoelasticity

b) Determination of G' and G"
2.2.5.2 Procedure

Pastes (10%, 20% or 30% w/w in distilled water) were prepared using sun-dried and freeze-dried flour and extracted starch. The samples were analysed using a Rheometrics controlled stress rheometer 200, fitted with a 40 mm parallel plate geometry with a gap of 0.3 mm. Silicone oil was applied to prevent evaporation during heating. A frequency of 1 rad/s was used and applied stress of 0.5-0.8 Pa. The stress was varied to produce a fairly constant strain throughout the temperature cycle. The temperature of the Peltier plate was programmed to ramp, at a rate of 2 °C per minute, from 20 to 90 °C then back to 20 °C. The $G'$ (storage modulus) and $G''$ (loss modulus) values were noted at 20 °C before heating, at 90 °C and after cooling at 20 °C.
CHAPTER THREE
3.0 NUTRITIONAL AND BIOCHEMICAL PROPERTIES OF DRIED SWEET POTATO (*IPOMOEA BATATAS* L)

3.1 Introduction

The sweet potato (*Ipomoea batatas*. *L*) is a dicotyledonous plant, which belongs to the family convolvulaceae, and produces edible starch storage roots. It is an important food crop in sub-Saharan Africa and ranks seventh among the most important food crops worldwide (Scott, 1992). In Uganda, sweet potato is one of the three most important staple food crops. Uganda is the second largest sweet potato producing country in Africa and fourth largest in the world (FAO, 2002).

Sweet potato is an important food security crop that fits well into Uganda’s farming system and stores well underground as a famine reserve crop; it withstands extreme dry weather conditions and performs well in marginal soils. Sweet potato is more tolerant than other crops to late planting and can be grown throughout the year (Bashaasha *et al.*, 1995). Sweet potato is a nutritious crop and previous reports indicate the roots serve as a good source of energy and nutrients (carbohydrates, β-carotene) and can provide reasonable amounts of protein (Ravindran *et al.*, 1995; Van Hal, 2000). Sweet potato can add natural colour, sweetness and flavour to processed products, and has been used for its specific properties to form new products making a significant contribution to β-carotene intake. Yellow or orange colouration can be obtained by utilising sweet potato flour rich in β-carotene, which is absent in cereals grown in Uganda (maize and rice). It has been reported that addition of orange-fleshed sweet potato in buns, chapattis and mandazis (sweet fried bread) greatly increased the content of total carotenoids (Low *et al.*, 1997; Hagenimana & Low, 2000).
In sweet potato, carbohydrates account for the bulk of the flour (84 and 95%) and total starch varies between 57 and 90%. Sugar levels comprise 7 - 23% (Van Hal, 2000). The carbohydrate content varies depending on the cultivar and also location of growth (Woolfe, 1992). The content of sugars depends on the location and time of harvest. The protein content in sweet potato is generally low ranging from 1.0 to 8.5% on a dry weight basis but interestingly, the biological value of sweet potato protein is reported to be good in both fresh and dry form (Ravindran et al., 1995). Protein quality depends on the amino acid composition and bioavailability. It is reported that the content of most essential amino acids in sweet potato roots conform to the FAO values (Ravindran et al., 1995; Van Hal, 2000). Although protein content is low, the quality is superior to some other roots and tubers such as cassava, plantain, yam and giant swamp taro. Anti-nutrient factors such as trypsin inhibitors (protease inhibitor) have been reported in some varieties with variation in the inhibitor activity among cultivars (Dickey & Collins, 1984; Bradbury et al., 1985; Ravindran et al., 1995; Zhitian et al., 2002). The occurrence of these inhibitors has nutritional implications for human and animal feeding. The role of these inhibitors in plants is to protect from pests or pathogens, by inhibiting their digestive enzymes (Ryan, 1981). When consumed with foodstuffs, protease inhibitors inhibit proteolysis of protein in the intestine leading to mal-absorption of amino acids and hence reduction in growth. Protease inhibitors work by competing with the substrate to share the active site of the enzyme-binding site. It is important therefore to destroy or reduce sweet potato protease inhibitors to make the protein more bioavailable to consumers.
The breeding programmes in National Agriculture programmes in Uganda are focused on improving the yield of sweet potatoes and developing new varieties with high dry matter that are resistant to pests and diseases. However most of these newly developed varieties have not been investigated for nutrient composition, starch quality and nutritive value to date.

In this study, the biochemical and nutritional properties of three varieties (Spk 004, Nasport 005 and Tanzania) of sweet potato grown in Uganda before and after drying for increased preservation are reported.

3.2 Materials and Methods

3.2.1 Proximate composition

All determinations were carried out in triplicates. Moisture, nitrogen, crude fat and ash were determined according to the standard analytical methods (AOAC, 1995). Crude protein was calculated as N × 6.25.

3.2.2 Amino acid analysis

The amino acid composition was analysed using a Waters Pico-Tag workstation (Waters, Watford, UK). Hydrolysis of sweet potato protein was undertaken at 110°C for 48 h. Samples containing approximately 20 mg of protein in screw-capped Pyrex vials were hydrolysed with 6 N HCl (20 ml) at 110°C for 48 h. The hydrolysed samples and amino acid standards (20 μl) were dried under vacuum at 20°C using Pico-Tag workstation. All samples were derivatised with PITC (Phenyl isothiocyanate) according to the waters Pico-Tag method as described by Badii & Howell (2001).
3.2.2.1 *Derivatisation of amino acids with PITC*

Drying solution (20 μl) containing methanol/water/triethylamine (TEA) (2:2:1,v/v/v) was added to each vacuum-dried standard or sample in a tube. The derivatising reagent was made by mixing 50 μl PITC (this reagent was kept at -20°C under nitrogen to prevent degradation), 350 μl methanol (HPLC grade), 50 μl of TEA and 50 μl Milli-Q water (Millipore, Watford, UK). The derivatising reagent 20 μl was added to each tube, vortex-mixed, sealed and left at room temperature for 20 min. The reagent was then removed under vacuum. The derivatised samples were vacuum-dried and dissolved in 100 μl of sample buffer (eluent A, prepared by dissolving 19.0 g sodium acetate trihydrate in 1 l of Milli-Q water, followed by the addition of 0.5 ml TEA, adjusted to pH 6.4 and filtered; to 940 ml of this solution was added 60 ml of acetonitrile). The samples were analysed in quadriplicate by reverse phase HPLC together with amino acid standards according to the method described in the Waters Pico-Tag Amino acid Analysis Manual (Waters Chromatography Division, 1986).

3.2.3 *Total starch*

This was determined by the method of McCleary *et al.* (1997) using a megazyme kit ([www.megazyme.com](http://www.megazyme.com)).

3.2.3.1 *Principle*

This method involves the use of two enzymes, α-amylase and amyloglucosidase. Starch is partially hydrolysed and totally solubilised. The starch dextrins are quantitatively hydrolysed to glucose by amyloglucosidase.
3.2.3.2 Procedure

Sweet potato (dried sample) was milled to pass through a 0.5 mm screen. The samples (100 mg) were weighed in five replicates into a glass tube (16 x 120 mm). Aqueous ethanol 80% v/v (5 ml) was added and the tubes were incubated for 5 min at 80°C. The contents were mixed on the vortex mixer and another 5 ml of 80% aqueous ethanol was added. The tubes were centrifuged for 10 min at 1,000 x g. The supernatant was discarded and the pellet re-suspended in 10 ml of 80% aqueous ethanol and stirred on a vortex mixer. The mixture was centrifuged as above and the supernatant carefully poured off. Dimethyl sulphoxide (DMSO) 2 ml was added and the tubes stirred on the vortex mixer. The tubes were placed in a vigorously boiling water bath and removed after 5 min. Thermo stable α-amylase (300 units) in MOPS buffer (50mM, pH 7.0) 3 ml was added to each tube and vigorously stirred on a vortex mixer. The tubes were incubated in a boiling water bath for six minutes. During this period the tubes were stirred vigorously after every two minutes to ensure complete homogeneity of the slurry.

The tubes were placed in the water at 50°C and sodium acetate buffer (4 ml, 200 mM, pH 4.5), followed by amyloglucosidase (0.1 ml, 20 U) were added. The tubes were stirred on a vortex mixer and incubated for 30 min.

The entire contents of each tube were transferred to a 100 ml volumetric flask and the volume adjusted to 100 ml mark with distilled water. This mixture was mixed thoroughly and an aliquot of this solution was centrifuged at 1000 x g for 10 min. Duplicate aliquots (0.1 ml) of the supernatant were transferred to test- tubes, 3 ml of glucose oxidase peroxidase (GOPOD) reagent was added. Glucose controls and reagent blanks were also prepared. The tubes were incubated at 50°C for 20 min.
Glucose controls consisted of 0.1 ml of glucose standard solution (1mg/ml) and 3.0 ml of GOPOD reagent. Reagent blank solutions consisted of 0.1 ml of water and 3.0 ml of GOPOD reagent. The absorbance of each sample and Glucose control was read against the reagent blank at 510 nm.

**Calculation for starch**

\[
\text{Starch} = \frac{\Delta E \times F \times 1000 \times 1 \times 100 \times 162}{1000 \times W \times 180}
\]

\( \Delta E \) = Absorbance read against the reagent blank.

\( F \) = Conversion from absorbance to micrograms

\( W \) = Weight in milligrams of the flour analysed.

\( 162 \) = Adjustment from free glucose to anhydro glucose (as occurs in starch).

\( 180 \)

Starch % (dry weight, basis):

\[
= \frac{\text{Starch} \times 100}{(100 - \text{moisture} \%)}
\]

**3.2.4 Total sugars**

**3.2.4.1 Principle**

The total reducing sugar was determined by the modified anthrone method (Whistler et al., 1984), which involved extraction of the soluble sugars with hot ethanol (80%). The ethanolic extract was then evaporated to remove the ethanol. The residue was dissolved in distilled water and the soluble sugar was determined spectrophotometrically with anthrone reagent. Anthrone (9,10 dihydro-9-oxo anthracene) reacts with carbohydrates in concentrated sulphuric acid solutions to produce a characteristic blue-green colour, which was then measured on the spectrophotometer at 620 nm.
3.2.4.2 Procedure

Dried sweet potato samples (0.05g) were weighed into test tubes, 5 ml of distilled water added followed by 25 ml of hot ethanol (80%). This was stirred several times and centrifuged at 1200 x g for 10 min at 25°C. This was done three times to ensure that all the sugar was extracted. The ethanolic solution containing the soluble sugar was evaporated to remove the ethanol. The remaining soluble sugar was diluted to 1000 ml with distilled water. Diluted sample (2 ml) was mixed with anthrone reagent (10 ml) and heated for 15 min, cooled and the absorbance was measured at a wavelength of 620 nm. A standard curve was prepared using standard glucose of concentration 0.02% w/v in distilled water.

Fig 3.1: Glucose standard curve used in the determination of total sugars in sweet potato
3.2.5 Resistant starch

3.2.5.1 Principle

Resistant starch was determined by the method of McCleary et al., (1997), which was developed by the Megazyme Company (www.megazyme.com). The method involved incubating samples in a water bath (Grant instruments Ltd, Cambridge, UK) with pancreatic alpha-amylase and amyloglucosidase (AMG) (from Megazyme company, Ireland) for 16 h at 37 °C. Non-resistant starch was solubilised and hydrolysed to glucose by the combined action of the enzymes. The reaction was terminated by the addition of equal volume of industrial methylated spirit (IMS) and the resistant starch was recovered as a pellet on centrifugation. This pellet was suspended and washed twice with aqueous IMS (50% v/v) and all free liquid was removed by decantation. The resistant starch was dissolved by stirring with 2 M KOH in an ice water bath. The solution was neutralised with acetate buffer and the starch was quantitatively hydrolysed to glucose with AMG. Glucose was measured with glucose oxidase/peroxidase reagent (GOPOD) and this is a direct measure of the resistant starch content.

3.2.5.2 Procedure

Sweet potato samples (sun dried, freeze dried and the native) (100 mg) were accurately weighed into test tubes and gently tapped to ensure that the samples fell to the bottom. This was done in five replicates. Pancreatic α-amylase (10mg/ml) containing AMG (3 U/ml) was added to each tube. The test tubes were tightly capped and stirred on a vortex mixer and placed in shaking water bath. The tubes
were incubated at 37°C with continuous shaking (200 strokes/min for exactly 16 h). After 16 h the tubes were removed from the water bath and the contents were treated with 4.0 ml of IMS (99%v/v) with vigorous stirring on a vortex mixer. The tubes were centrifuged at 1000 × g (3000 rpm) for 10 min. The supernatants were carefully decanted and the pellets re-suspended in 2 ml of 50% IMS with vigorous stirring on a vortex mixer. A further 6 ml of 50% IMS was added, mixed and centrifuged at 1000g for 10 min. The supernatants were decanted and the pellet suspended in 6 ml 50% IMS and centrifuged once more. Finally the supernatants were carefully decanted and the tubes inverted on absorbent paper to drain excess liquid.

Magnetic stirrers were added to the tubes followed by 2 ml of 2 M KOH and the resistant starch was stirred for 20 min in an ice/water bath over a magnetic stirrer to be dissolved. Each tube was treated with 8 ml of 1.2 M sodium acetate buffer pH 3.8 with stirring. Amyloglucosidase (3300 U/ml) 0.1 ml was added, the contents mixed well and the tubes placed in the water bath at 50°C. The tubes were incubated for 30 min with intermittent mixing on a vortex mixer.

The contents of the tubes were quantitatively transferred to a 100 ml volumetric flask and the volume adjusted to the mark by adding distilled water using a wash bottle. Aliquot (5 ml) was centrifuged at 1000 × g for 10 min. Supernatants (0.1 ml) were transferred in duplicates to glass test tubes and 3 ml of GOPOD was added to each of the test tubes, incubated at 50°C for 20 min. After incubation at 50°C for 20 min, the absorbance of each solution was measured at 510 nm against a reagent blank. Glucose standards were prepared (in quadruplicates) by mixing 0.1 ml of
glucose (1 mg/ml) and 3.0 ml of GOPOD. The reagent blank was prepared by mixing 0.1 ml of 0.1 M sodium acetate buffer (pH 4.5) and 3.0 ml of GOPOD.

**Calculation for resistant starch (% on a dry weight basis)**

Resistant starch (g/100g sample) =

\[
\Delta E \times F \times 100/0.1 \times 1/1000 \times 100/W \times 162/180
\]

\[
= \Delta E \times F/W \times 90.
\]

For samples with less than 10% resistant starch

\[
= \Delta E \times F \times 10.3/0.1 \times 1/1000 \times 100/W \times 162/180
\]

\[
= \Delta E \times F/W \times 9.27.
\]

Where \( \Delta E \) = absorbance read against the reagent blank;

\( F \) = conversion from absorbance to micrograms;

\( 100/0.1 \) = Volume correction (0.1 ml taken from 100 ml)

\( 1/1000 \) = conversion from micrograms to milligrams;

\( W \) = Dry weight of sample analyzed

\( 162/180 \) = factor to convert from free glucose, as determined, to anhyro-glucose as occurs in starch;

\( 10.3/0.1 \) = volume correction for samples containing less than 10% resistant starch

**3.2.5.3 Resistant starch in boiled samples**

The above procedure was followed except that before hydrolysing the samples for 16 hrs 1 ml of water was added to 100 mg and boiled for 10 min at a temperature of 100°C. While heating, the samples were vigorously stirred to avoid formation of gelatinous lumps of starch. The rest of the procedure outlined above was followed.
3.2.6 Amylose and amylopectin determination

This was determined by the modified method of Gibson et al. (1997) using a Megazyme kit (www.megazyme.com).

3.2.6.1 Principle

Starch samples were completely dispersed by heating in dimethyl sulphoxide (DMSO). Lipids were removed by precipitating the starch. After dissolution of the precipitated sample in the acetate/salt solution, amylopectin was precipitated by the addition of Concanavalin A and removed by centrifugation. The amylose in the aliquot of the supernatant was enzymatically hydrolysed to glucose, which was analysed using glucose oxidase/peroxidase reagent. The total starch in a separate aliquot of the acetate/salt solution was similarly hydrolysed to glucose and measured colorimetrically by glucose oxidase/peroxidase. The concentration of amylose in the starch sample was estimated as the ratio of GOPOD absorbance at 510 nm of the supernatant of the Con A precipitated sample, to that of the total starch sample.

3.2.6.2 Procedure

Sweet potato starch and sun-dried, freeze dried samples (20 mg) were weighed in triplicates into 10 ml test tubes. DMSO (1 ml) was added to each tube while gently stirring at low speed on a vortex mixer. The tubes were capped and heated in a boiling water bath until the sample was completely dispersed. The contents were vigorously mixed at a high speed and placed in a boiling water bath for 15 min with intermittent high-speed stirring on a vortex mixer. The test tubes were stored at
room temperature for approximately 5 min and 2 ml of 95% ethanol with continuous stirring on a vortex mixer. A further 4.0 ml was of ethanol was added and the tubes were allowed to stand for 15 min. The mixture was centrifuged at $2000 \times g$ for 5 min and the supernatant discarded and tubes were drained on absorbent paper to ensure that all of the ethanol was drained. The pellet was used for subsequent amylose and starch determinations.

DMSO (1 ml) was added with gentle mixing to the starch pellet and the tubes placed in a boiling water bath for 15 min and the contents were mixed after every 5 min; this was to prevent the formation of gelatinous lamps. Concanavalin A Solvent (buffer 3) 2 ml was added and the contents were quantitatively transferred to a 25 ml volumetric flask. The solution was diluted to the volume mark with Con A solvent (solution 1). The diluted solution (1.0 ml) was transferred to a 2 ml microfuge tube and 0.5ml of con A solution (4 mg/ml) was added and capped, mixed gently and allowed to stand for 1 h at room temperature. The samples were then centrifuged at 20,000 x g for 10 min at 20°C. The supernatant (1 ml) was transferred to a 15 ml centrifuge tube and 3.0 ml of 100 mM sodium acetate buffer, pH 4.5 was added. The contents were mixed, placed in a boiling water bath for 5 min to denature the con A. The tubes were placed in a water bath at 40°C and allowed to equilibrate for 5 min. Amyloglucosidase/ amylase (0.1 ml) mixture was added and incubated for 30 min. The hydrolysate was centrifuged at 2000 x g for 5 min. To 1.0 ml of the supernatant 4 ml of GOPOD reagent was added, incubated for 20 min. The reagent blanks and the glucose controls were incubated concurrently.
Total starch was determined by mixing 0.5 ml of solution 1 with 4 ml of 100 mM sodium acetate buffer pH 4.5. Amyloglucosidase/amylase (0.1 ml) was added and the mixture was incubated for 30 min. The hydrolysates (1 ml) were transferred in duplicates to the test tubes and 4 ml of GOPOD added and the mixture was incubated for 20 min. The absorbance was read at 510 nm for each sample, total starch and the glucose controls against the reagent blank. The reagent blank was prepared by adding 1.0 ml of sodium acetate buffer to 4 ml of GOPOD reagent and incubating for 20 min.

**Calculation of amylose content (%)**

\[
\text{Amylose} \% = \frac{\text{Absorbance}_{\text{con A supernatant}} \times 6.15 \times 100}{\text{Absorbance}_{\text{total starch aliquot}} \times 9.2 \times 1}
\]

Where 6.15 and 9.2 were dilution factors for the Con A and total starch extracts respectively.

### 3.2.7 *In vitro* digestibility of starch

*In vitro* digestibility of starch in the flour was measured according to Singh *et al.* (1982) with some modification. Dried samples (50 mg) were weighed into test tubes and 1.0 ml of phosphate buffer pH 6.9 added. The suspension was boiled for 10 min to gelatinise the starch. During the boiling session, the samples were stirred every 3 min. to avoid formation of lumps. The solution was transferred to a water bath at 37°C and 0.5 ml of pancreatic amylase (20 mg of the amylase dissolved in 50 ml of the phosphate buffer pH 6.9). This was incubated for 2 h, the hydrolysate was diluted 10 times. To 5 ml of the diluted hydrolysate 2 ml of DNSA (Dinitrosalicylic
acid) was added and boiled for 5 min, distilled water was added to make the final volume of 25 ml. The absorbance of the solution was measured at 550 nm against a reagent blank, which consisted of buffer 1.0 ml, DNSA 2 ml and distilled water 22 ml (Bernfeld, 1955). Maltose in the samples before hydrolysis was determined as described above but the enzyme was omitted. The absorbance of maltose was calculated from the difference of absorbance of samples after hydrolysis and absorbance of samples without the enzyme. Maltose was used as a standard and the concentration of the samples were obtained from the standard curve prepared as shown below.

Starch digestibility (%) was expressed as;

\[
\text{Mg maltose liberated} \times 100
\]

Mg starch in flour sample

**Fig. 3.2: Standard curve of maltose for in vitro digestibility of starch determination**
3.2.8 Beta-carotene analysis

3.2.8.1 Sample extraction

Beta-carotene was evaluated by a modified HPLC method developed by Hart and Scott (1994). Duplicate samples (2 g) of sweet potato (freeze, sun and oven-dried) were extracted by 10 ml of tetrahydrofuran (TFH) and methanol (1:1 v/v, THF:MeOH) with 20 µg internal standard, β-apo-8'-carotenal. Solid magnesium carbonate 1 g was added to neutralize any organic acids. Carotenoids were extracted from food matrix by homogenizing for 3 min using omni mix homogenizer. The resulting suspension was filtered through a filter in a buchner funnel under vacuum. The flask and the homogeniser were washed with 10 ml THF/MeOH and the washing was filtered. The filter and buchner flask was washed by further aliquots of THF:MeOH. The combined filtrates were transferred to a separating funnel. Ten ml of petroleum ether (40-60° fraction, containing 0.1% BHT) and 10 ml of 10% sodium chloride were added and mixed by careful shaking. The lower THF/MeOH/aqueous phase was drawn off and the upper petroleum ether was transferred to a 250 ml evaporating flask. The THF/MeOH/aqueous phase was extracted two more times with 10 ml of aliquots of petroleum ether. The petroleum ether phases were combined in the flask and evaporated at 35°C in a rotary evaporator to dryness. The residue was redissolved to a final volume of 5 ml by a mobile phase (acetonitrile: methanol: dichloromethane) 75: 20: 5 v/v/v. The diluted extract was then filtered through a 0.45 µm PVDF syringe filter and 10 µl of the filtrate injected into the 50 µl HPLC loop.
3.2.8.2 HPLC system

The HPLC system comprised of a dual piston delivery pump (P2000 pumps), autosamplers and detectors (Thermofinnegan, UK), chromatography management software. The column system consisted of a 5-cm C18 ODS guard column connected to a 250 × 4.6 mm stainless steel column (internal diameter) packed with C18 reverse-phase material with particle size of 5 μm and a pore size of 30 nm (Phenomenex, UK). The mobile phase consisted of acetonitrile, methanol and dichloromethane (MeCN: MeOH: DCM) 75: 20: 5 v/v/v, containing 0.1% butylated hydroxytoluene (BHT) as an antioxidant and 0.05% triethylamine (TEA). The methanol contained 0.05 M ammonium acetate. All reagents were from Sigma (Poole, UK) HPLC grade except acetonitrile which was obtained from Fischer chemicals. The prepared mobile phase was degassed using ultrasonic agitation. The flow rate was 1.5 ml/min. Samples were injected via a syringe loading sample injector fitted with a 50 μl loop.

Peak responses were measured at 450 nm.

3.2.8.3 Internal standard

An internal standard (β- apo-8'-carotenal) was used to assess losses during the extraction procedures.

3.2.8.4 Identification and quantitation of carotenoids in sweet potato samples

The identification of β-carotenoids was carried out by comparing the retention times and absorption spectra with reference standards and also comparing with chromatograms obtained by other authors. Typical chromatograms obtained were as
Excel 2001 software. Six concentrations of the standards (0, 2, 4, 6, 8, 10 μg/ml) were used to prepare the standard curve of β-carotene. The regression equation was obtained and the β-carotene in the sample was calculated based on this equation. The final concentration of the sweet potato sample was expressed as ug/100g sample.

![chromatogram showing standard beta-carotene](image)

**Fig 3.3:** A chromatogram showing standard beta-carotene (a) 4 ug/ml and (b) 8 ug/ml and internal standard
3.2.9 Analysis of antinutrients

3.2.9.1 Principle

Trypsin inhibitor activity measurement was based on the method of Smith et al., (1980), which involved extraction of the inhibitor from the sample at pH 9.5 and mixing the unfiltered suspension with bovine trypsin. The activity of the remaining trypsin was measured by reacting it with benzoyl-DL-arginine-p-nitroanilide (BDH, Poole, UK), the p-nitroaniline released was measured at 410 nm. The amount of trypsin inhibited per unit weight of sample is calculated as follows;

\[
TIA = \frac{2.632 \times D \times A_1}{S}
\]

Where D is dilution factor, \( A_1 \) is the change in absorbance due to trypsin inhibition ml\(^{-1}\) diluted samples extract and S is the weight of the dried material used (g).

3.2.9.2 Reagents

Tris buffer (50 mM, pH 8.2), Tris hydroxymethyl amine (6.05 g) and CaCl\(_2\) \(\cdot\)2H\(_2\)O (2.94 g) obtained from Sigma Company were dissolved in 900 ml of deionised water. The pH was adjusted to 8.2 with HCl and checked after dilution to 1 litre.

*BAPNA substrate*

Benzoyl-DL-arginine-P-nitroanilide hydrochloride 40 mg (BDH, Poole) was completely dissolved in 1 ml DMSO and diluted to 100 ml with tris buffer previously warmed 37 °C. This reagent was kept at 37 °C while in use and freshly prepared.
Crystalline bovine trypsin (40 mg) was dissolved in 1 mM HCl and made up to 2 litres with the acid. The solution was stable for two weeks at 4 °C.

3.2.9.3 Sample preparation

Dry sweet potato cubes were finely powdered into a blender so as to pass through a 100-mesh sieve. A sample (1 g) dried material was weighed out (s). The sample was briefly shaken with 50 ml 10 mM NaOH; it was macerated for 30 seconds. The pH of the resulting slurry was adjusted to between 9.4-9.6 with 1 M NaOH or 1 M HCl. At this stage the solution was left at 4°C overnight. After extraction the suspension was shaken and diluted 4 times with distilled water so that 1 ml produced trypsin inhibition of between 40 and 60%.

3.2.9.4 Procedure

The following solutions were pipetted into a series of 10 ml tubes:

a) Reagent blank; 2 ml deionised water

b) Standard trypsin solution (2 ml), distilled water (2 ml)

c) Sample blanks: 1 ml diluted sample extract and 1 ml of distilled water

d) Diluted sample extract 1 ml, distilled water 1 ml and standard trypsin solution (2 ml)

After mixing and pre-heating to 37 °C for 10 min, 5 ml BAPNA solution was pipetted into each tube and mixed. After 10 min incubation at 37 °C, each tube received 1.0 ml acetic acid 30% v/v to stop the reaction. Standard trypsin 2.0 ml was then added to the reagent blank (a) and sample blank (c) tubes. After filtration the
absorbance of the clear solutions was measured at 410 nm; the colour was stable for several hours. The change in absorbance was $A_1$ due to trypsin inhibition ml$^{-1}$ diluted sample extract is $(A_b-A_a) - (A_d-A_c)$, where the subscripts refer to tubes (a)- (d) above.

3.3 Results and discussion

Table 3.1 shows that the moisture in sweet potato root ranged between 58.9 and 68% for raw sweet potato roots. Nsp and Spk variety had more moisture compared with Tz, this may explain why Tz variety is preferred in Uganda. The moisture content of the flours ranged from 8 to 12%. Moisture content of sweet potato flour is considered a quality characteristic because water can accelerate chemical or microbiological spoilage during storage. Starch ranged from 58 to 79%, protein from 2.7 to 4.9%, ash 1.7 to 3.4 % and lipid from 1.1 to 2.3% (dry weight basis). These values are in good agreement with those reported for other varieties from North America (Purcell et al., 1978), Taiwan (Lii & Chang, 1978), Nigeria (Onwueme, 1978), Papua New Guinea (Bradbury et al., 1985), Sri Lanka (Ravindran et al., 1995) and China (Zhitian et al., 2002).

There was no significant difference in the proteins observed among the three varieties. Neither freeze-drying nor sun-drying had any effect on the crude protein content. Sweet potato roots have high values of protein compared with other roots and tubers (cassava, yam and cocoyams) consumed in Uganda. However, this value is not sufficient to meet the protein requirements of a growing child (1.1g protein per kg weight per day); thus there is need to improve the protein content of tuber based products by blending with other food stuffs that are rich in protein such as
soya, fish, ground-nuts and sesame. Besides the quantity, the quality of protein used is important and it depends on bioavailability and the amino acid composition. Table 3.8 shows the amino acid content in the three Ugandan varieties. There were no significant differences observed in the amino acid among the three varieties. Aspartic acid was the highest while the limiting ones were the sulphur containing amino acids. The amino acid in these varieties were comparable with those reported for samples of cultivars from Sri lanka (Ravindran et al., 1995) and Papua New Guinea (Bradbury et al., 1985) On the whole, except for low methionine and cysteine the amino acid composition were comparable to FAO reference protein (Van Hal, 2000).

Starch was the predominant nutrient in the sweet potato. The average content was 70 g starch /100g of dry weight. Tanzania had the highest amount of starch (79 g/100g dry weight, or 32% fresh weight basis of raw sweet potato root). Differences were observed between the sun-dried and freeze-dried samples. The freeze-dried sweet potato flour had a significantly higher amount (P<0.05) of starch compared with the sun-dried samples. This is probably due to some of the starch being hydrolysed to simple sugars during the process of sun drying. Some researchers have reported that sweet potato has appreciable amounts of β and α-amylase enzymes (Hagenimana et al., 2001). It is possible that an increase in temperature during sun drying activated these enzymes to break down some of the starch into reducing sugars. This phenomenon also explains the high amounts of reducing sugars observed in sun-dried compared with freeze-dried flour samples.
Table 3.1: Proximate composition of sweet potato g/100g sample (fresh weight basis)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture</th>
<th>Ash</th>
<th>lipid</th>
<th>starch</th>
<th>protein</th>
<th>Total sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Native (Raw root)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nsp</td>
<td>67.97±1.23</td>
<td>1.84±0.12</td>
<td>0.82±0.05</td>
<td>24.62±0.04</td>
<td>1.70±0.18</td>
<td>5.63±0.25</td>
</tr>
<tr>
<td>Tz</td>
<td>58.92±0.36</td>
<td>2.54±0.3</td>
<td>0.42±0.00</td>
<td>32.52±2.83</td>
<td>1.09±0.01</td>
<td>4.29±0.10</td>
</tr>
<tr>
<td>Spk</td>
<td>62.23±0.04</td>
<td>2.23±0.03</td>
<td>1.06±0.43</td>
<td>27.0±1.06</td>
<td>1.57±0.03</td>
<td>4.84±0.07</td>
</tr>
<tr>
<td><strong>Freeze-dried flour</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nsp</td>
<td>8.50±0.8</td>
<td>2.86±0.16</td>
<td>1.96±0.23</td>
<td>66.56±1.12</td>
<td>3.73±0.21</td>
<td>14.75±0.15</td>
</tr>
<tr>
<td>Tz</td>
<td>8.61±1.7</td>
<td>2.66±0.47</td>
<td>1.77±0.47</td>
<td>68.6±1.01</td>
<td>3.30±0.28</td>
<td>7.36±0.77</td>
</tr>
<tr>
<td>Spk</td>
<td>10.3±0.0</td>
<td>3.39±0.5</td>
<td>2.28±0.40</td>
<td>66.67±2.06</td>
<td>3.88±0.05</td>
<td>9.09±0.69</td>
</tr>
<tr>
<td><strong>Sun-dried flour</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nsp</td>
<td>11.80±0.01</td>
<td>3.28±0.67</td>
<td>1.40±0.26</td>
<td>52.2±0.5</td>
<td>4.17±0.17</td>
<td>16.17±0.7</td>
</tr>
<tr>
<td>Tz</td>
<td>11.08±0.15</td>
<td>1.71±0.26</td>
<td>1.54±0.47</td>
<td>58.17±1.10</td>
<td>3.98±0.01</td>
<td>14.12±1.4</td>
</tr>
<tr>
<td>Spk</td>
<td>11.85±0.15</td>
<td>2.50±0.54</td>
<td>1.20±0.31</td>
<td>53.3±1.20</td>
<td>3.58±0.05</td>
<td>13.90±0.3</td>
</tr>
</tbody>
</table>

Values in this table are means of three replicates ± standard deviation

There was no noticeable difference observed between the amylose content in freeze-dried and sun-dried flour (Table 3.2). From these results it is noted that sweet potato is a rich source of starch; well-digested and absorbed starch is particularly suitable during early complementary feeding (weaning) to ensure adequate energy. Sweet potato is gluten-free, which makes it a good alternative raw material for baby food formulation especially for children that are intolerant to gluten. Weaning foods should provide sufficient energy and protein to cover the infant’s requirements (Walker, 1990). Satisfying energy needs is critical because if energy intake is insufficient then protein will be used as an energy source instead of being available for growth and maintenance. It is
generally recommended that increasing starch intake with age should be encouraged provided the total energy intake remains adequate (DOH, 1995).

**Table 3.2: Amylose in freeze-dried and sun dried sweet potato flour**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Freeze-dried</th>
<th>Sun-dried</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nsp</td>
<td>18.89±0.77</td>
<td>15.90±2.03</td>
</tr>
<tr>
<td>Tz</td>
<td>18.34 ± 3.18</td>
<td>14.35± 1.73</td>
</tr>
<tr>
<td>Spk</td>
<td>19.82 ± 2.11</td>
<td>20.06±2.13</td>
</tr>
</tbody>
</table>

Values in this table are means of three replicates ± standard deviation

**Table 3.3: Resistant starch in un-boiled sweet potato flour**

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Freeze-dried sample</th>
<th>Sun-dried sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nsp</td>
<td>19.48 ± 0.99</td>
<td>5.65 ± 0.1</td>
</tr>
<tr>
<td>Tz</td>
<td>29.13 ± 1.42</td>
<td>16.59 ± 1.9</td>
</tr>
<tr>
<td>Spk</td>
<td>17.05 ± 0.35</td>
<td>4.13 ± 0.33</td>
</tr>
</tbody>
</table>

Values in this are means of three replicates ± standard deviation

**Table 3.4: Resistant starch in boiled sweet potato flour**

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Freeze-dried sample</th>
<th>Sun-dried sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nsp</td>
<td>3.71 ± 0.66</td>
<td>3.39 ± 0.41</td>
</tr>
<tr>
<td>Tz</td>
<td>7.07 ± 0.54</td>
<td>3.72 ± 0.20</td>
</tr>
<tr>
<td>Spk</td>
<td>5.36 ± 0.65</td>
<td>4.59 ± 0.42</td>
</tr>
</tbody>
</table>

Values in this are means of three replicates ± standard deviation

Resistant starch is defined as starch and starch degradation products that escape digestion in the small intestine and become available for fermentation by the microflora in the large intestine (Ring et al., 1998). Resistant starch ranged from 4.1
± 0.66 to 29 ± 1.42 % in un-boiled samples (Table 3.3). The freeze-dried samples had significantly (p<0.05) higher amounts of resistant starch than the sun-dried samples (Table 3.3). Tanzania cultivar had the highest value of resistant starch (29.1± 1.42%) and Nsp (sun dried) cultivar had the lowest (3.4± 0.41%) whereas Spk (un-boiled) had 17 ± 0.35%. Among the un-boiled samples Spk starch was not as resistant to digestion as the Tz and Nsp. In general, the resistant starch in sweet potato was lower compared with native potato (77 %) and maize starch (42 %) (McCleary & Monaghan, 2002).

On boiling the sweet potato samples, the resistant starch was remarkably reduced (Tables 3.4). This is because once starch in water is heated, the semi crystalline structure which retards the digestion by the pancreatic amylase is disrupted and the polysaccharide chains assume a random conformation causing swelling of the granules. In this state starch is readily accessible to digestive enzymes. Sun-dried sweet potato flour had very low values of resistant starch; this is probably because most of the starch was converted to reducing sugars. It is important to select the varieties with low resistant starch for the preparation of baby food. High resistant starch increases the bulk of food and prevents the digestion of starch. On the other hand the resistant starch may have beneficial effects on the microflora in the colon. The resistant starch, which is not digested in the gut can find its way to the colon where it is metabolised by bacterially produced enzymes to provide energy for the growth of colonic bacteria (bifidobacteria and lactobacilli). The intestinal microflora improve resistance to infections and is involved in the neutralisation of toxins (Macfarlane & Cummings, 1991). During the metabolisation of carbohydrate via fermentation, the colonic pH is lowered and short-chain fatty acids such as
acetate, propionate and butyrate are released. Of these butyrate is implicated in promoting health. Butyrate is said to suppress the emergence of tumour cells and may decrease the proliferation of colonic mucosal cells (Johnson & Gee, 1996).

Table 3.5: *In-vitro* digestibility (IVDS) of starch in sun-dried and freeze-dried sweet potato flour

*In vitro* digestibility expressed as mg maltose released per 100 mg starch in the flour

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Freeze-dried sample</th>
<th>Sun dried Flour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nsp</td>
<td>63.9 ± 2.7</td>
<td>82.5 ± 1.9</td>
</tr>
<tr>
<td>Tz</td>
<td>55.7 ± 3.8</td>
<td>85.09 ± 2.9</td>
</tr>
<tr>
<td>Spk</td>
<td>60.5 ± 1.9</td>
<td>80.36 ± 3.6</td>
</tr>
</tbody>
</table>

Values are Means of three replicates ± standard deviation

The *in vitro* digestibility of starch (IVDS) ranged from 56% to 64% in freeze-dried flour and this was comparable to the range 47-59 % reported for the varieties from China (Zhitian et al., 2002). For the sun-dried sweet potato flour the IVDS ranged from 80 to 85%. There was significant difference (P<0.05) in the *in-vitro* digestibility of starch between Nsp and Tz; Spk and Tz varieties and between the sun-dried and freeze-dried samples (p<0.001). All sun-dried samples had higher IVDS values (80-85%) compared with freeze-dried samples (56-64%). Tanzania cultivar (freeze-dried) had the lowest *in vitro* digestibility (Table 3.5). The low digestibility of the freeze-dried samples could be due to poor accessibility of polymer chain in the network to the enzyme and high concentration of resistant starch (29 %). From the correlation graph (Fig. 3.4) it is observed that there is a
negative correlation between IVDS and the resistant starch. Thus reducing resistant starch increases IVDS (Fig. 3.4). This was apparent for Tz variety. In general, sweet potato flour especially when boiled, is not resistant to the pancreatic amylase breakdown as more than 50% starch could undergo breakdown to reducing sugars after 120 min. A high \textit{in vitro} enzymatic digestion implies good utilisation of dietary starches of sweet potato origin when consumed on its own or incorporated into food formulations. High digestible foods are recommended for infants. High IVDS is an indication that sweet potato starch will be well-utilized by the infants.

\textbf{Fig. 3.4}: The correlation of resistant starch and \textit{in vitro} digestibility of starch
They were significant differences observed in β-carotene content among the varieties of sweet potato. The orange varieties (Nsp and Spk) had higher β-carotene content (p<0.05) compared with Tz (yellow) variety (Table 3.6). As seen from the results 100 g of freeze-dried sweet potato (Nsp and Spk) can supply 100% of the daily requirement of vitamin A (retinol equivalents) to both children (1-10 yrs) and adults while Tz (yellow) variety can supply 46% and 36% RDA to children (1-10 yrs) and adults respectively. As already indicated by other authors, sweet potato is a good source of Vitamin A especially for developing countries that cannot afford expensive animal food sources for infant feeding (Hagenimana & Low, 2000; Hagenimana et al., 2001). Recently much attention has been focused on β-carotene as a physiological bioactive substance. For example, there are reports that carotene has antitumour activity (Peto et al., 1981; Murakosi et al., 1992) and antioxidant activity (Foote et al., 1970; Di Mascio et al., 1989). Therefore efficient utilisation of sweet potato roots should be encouraged, particularly the orange-fleshed varieties. From the results it is also evident that different drying methods affect the content of β-carotene. The freeze-dried samples (Nsp, 3115 µg/100g) contained higher levels of β-carotene (P<0.001) compared with sun-dried (Nsp, 880 µg/100g) and oven dried varieties (Nsp, 213 µg/100g). It is reported that during thermal processing trans-β-carotene is converted to the cis-isomer having lower provitamin A activity (Chandler & Schwartz, 1988).
Table 3.6: β-carotene content of sweet potato flour obtained from Nsp, Spk and Tz varieties

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Freeze-dried</th>
<th>Sun-dried</th>
<th>Oven-dried</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nsp</td>
<td>3115 ± 72</td>
<td>880 ± 23</td>
<td>213 ± 2.43</td>
</tr>
<tr>
<td>Spk</td>
<td>2915 ± 86</td>
<td>348 ± 5.0</td>
<td>142 ± 4.5</td>
</tr>
<tr>
<td>Tz</td>
<td>1095 ± 24</td>
<td>167 ± 0.0</td>
<td>128 ± 2.35</td>
</tr>
</tbody>
</table>

Values are means of three replicates ± standard deviation

3.3.2 Trypsin inhibitor

Analysis of trypsin inhibitors in the samples indicated that solar drying and oven-drying had little effect on the trypsin inhibitor activity in sweet potato flour. From the results, trypsin inhibitor could be generally regarded as too low to cause any concern nutritionally. There are reports that indicate that moist heat treatment reduces trypsin inhibitor activity (Ravindran et al., 1995).

It is important that sweet potato or its products should be boiled or cooked to increase the safety and quality of the product.

Table 3.7: Trypsin inhibitor levels in freeze, sun and oven dried sweet potato varieties (Nsp, Tz and Spk)

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Freeze-dried</th>
<th>Sun-dried</th>
<th>Oven-dried</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSp</td>
<td>2.77 ± 0.28</td>
<td>3.52 ± 0.18</td>
<td>2.37 ± 0.25</td>
</tr>
<tr>
<td>Tz</td>
<td>2.67 ± 0.43</td>
<td>3.35 ± 0.31</td>
<td>1.58 ± 0.06</td>
</tr>
<tr>
<td>Spk</td>
<td>2.85 ± 0.19</td>
<td>2.78 ± 0.22</td>
<td>1.43 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means of three replicates ± standard deviation
3.4 Conclusion

Sweet potato is a nutritious crop that can be used in many food products including baby food formulation. It is a good source of nutrients such as β-carotene, carbohydrates and also provides small amounts of protein. The in vitro digestibility of starch is high particularly in sun-dried samples indicating that it may be utilised efficiently. However sweet potato cannot be used in isolation since it contains low levels of protein and lipid, which are important for growth and tissue repair and brain development
Table 3.8: Amino acid content in sweet potato expressed as g/100g protein

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>NSP F</th>
<th>Tanz F</th>
<th>Spk F</th>
<th>Nsp s</th>
<th>Tanz S</th>
<th>Spk S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>10.97±0.6</td>
<td>12.94±0.05</td>
<td>12.80±0.61</td>
<td>10.24±0.70</td>
<td>11.12±1.01</td>
<td>9.57±0.57</td>
</tr>
<tr>
<td>Glu</td>
<td>8.97±0.58</td>
<td>8.52±0.21</td>
<td>9.52±0.70</td>
<td>6.87±0.54</td>
<td>6.63±0.61</td>
<td>6.41±0.20</td>
</tr>
<tr>
<td>h. pro</td>
<td>1.38±0.59</td>
<td>0.65±0.07</td>
<td>0.68±0.07</td>
<td>0.82±0.31</td>
<td>1.22±0.23</td>
<td>0.94±0.29</td>
</tr>
<tr>
<td>Ser</td>
<td>3.27±0.13</td>
<td>5.72±0.04</td>
<td>5.89±0.26</td>
<td>5.63±0.21</td>
<td>5.72±0.28</td>
<td>5.28±0.38</td>
</tr>
<tr>
<td>Gly</td>
<td>7.40±0.31</td>
<td>7.47±0.08</td>
<td>7.58±0.46</td>
<td>7.65±0.35</td>
<td>8.14±0.70</td>
<td>7.86±0.49</td>
</tr>
<tr>
<td>His</td>
<td>2.31±0.44</td>
<td>1.99±0.03</td>
<td>2.20±0.17</td>
<td>1.94±0.12</td>
<td>1.87±0.05</td>
<td>2.02±0.17</td>
</tr>
<tr>
<td>Arg</td>
<td>3.21±0.10</td>
<td>2.12±1.20</td>
<td>3.23±0.21</td>
<td>3.60±0.19</td>
<td>3.26±0.16</td>
<td>3.58±0.30</td>
</tr>
<tr>
<td>Thr</td>
<td>4.56±0.20</td>
<td>5.07±0.04</td>
<td>4.97±0.41</td>
<td>5.58±0.23</td>
<td>5.68±0.31</td>
<td>5.22±0.38</td>
</tr>
<tr>
<td>Ala</td>
<td>11.14±0.0</td>
<td>11.06±0.10</td>
<td>11.92±0.80</td>
<td>11.19±0.60</td>
<td>11.49±0.23</td>
<td>11.02±0.69</td>
</tr>
<tr>
<td>Pro</td>
<td>3.72±0.90</td>
<td>8.20±0.20</td>
<td>7.77±0.02</td>
<td>8.32±0.08</td>
<td>8.77±1.70</td>
<td>9.24±0.77</td>
</tr>
<tr>
<td>Tyr</td>
<td>5.56±1.50</td>
<td>3.51±0.18</td>
<td>4.37±0.02</td>
<td>3.60±0.06</td>
<td>4.36±0.91</td>
<td>4.46±0.71</td>
</tr>
<tr>
<td>Val</td>
<td>6.66±0.20</td>
<td>7.06±0.06</td>
<td>7.20±0.32</td>
<td>7.29±0.19</td>
<td>7.24±0.53</td>
<td>6.94±0.52</td>
</tr>
<tr>
<td>Met</td>
<td>1.89±0.20</td>
<td>1.50±0.03</td>
<td>2.07±0.18</td>
<td>1.82±0.14</td>
<td>1.68±0.14</td>
<td>1.98±0.17</td>
</tr>
<tr>
<td>Cys</td>
<td>0.86±0.30</td>
<td>0.47±0.00</td>
<td>0.49±0.09</td>
<td>0.44±0.14</td>
<td>0.39±0.13</td>
<td>0.37±0.05</td>
</tr>
<tr>
<td>Ileu</td>
<td>3.64±0.23</td>
<td>3.98±0.03</td>
<td>3.97±0.24</td>
<td>3.94±0.28</td>
<td>4.05±0.28</td>
<td>3.85±0.33</td>
</tr>
<tr>
<td>Leu</td>
<td>5.94±0.58</td>
<td>6.99±0.06</td>
<td>6.87±0.64</td>
<td>6.47±0.32</td>
<td>6.72±0.31</td>
<td>6.68±0.75</td>
</tr>
<tr>
<td>Phe</td>
<td>3.86±0.15</td>
<td>4.34±0.04</td>
<td>4.50±0.10</td>
<td>4.36±0.30</td>
<td>4.40±0.32</td>
<td>4.26±0.33</td>
</tr>
<tr>
<td>Trp</td>
<td>2.80±1.25</td>
<td>1.30±0.02</td>
<td>1.30±0.10</td>
<td>1.35±0.26</td>
<td>1.59±0.32</td>
<td>1.66±0.25</td>
</tr>
<tr>
<td>Lys</td>
<td>5.42±0.35</td>
<td>6.20±0.14</td>
<td>6.02±0.29</td>
<td>5.57±0.36</td>
<td>5.78±0.28</td>
<td>5.47±0.58</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = 4)

F= Freeze-dried
S= Sun-dried
4.0 CONTENT AND BIOAVAILABILITY OF ELEMENTS IN DRIED UGANDAN SWEET POTATO

4.1 Introduction

Sweet potato (Ipomoea batatas L. Lam) is a major source of food in Uganda (Bashaasha et al., 1995). Uganda is the second largest producer of sweet potato in Africa and third in the world (FAO, 2002). Sweet potato production in Uganda is on the increase due to the following factors: a decline of cassava production due to the infection by the cassava Mosaic virus; sweet potato stores well in the soil as a famine reserve crop and withstands extreme weather conditions and performs well in marginal soils. Furthermore, it plays an important role in providing household food security and generates income especially for rural areas (Bashaasha et al., 1995). There is a campaign to plant more sweet potatoes, especially the orange and the yellow-fleshed varieties as they are seen as a cheap source of vitamin A (Hagenimana & Low, 2000).

Despite the importance of sweet potato as a food crop there is little information available on the mineral content and bioavailability of Ugandan sweet potato varieties. However, for varieties grown elsewhere, it is reported that sweet potato is a good source of minerals such as K, Na, Ca, Mg and trace elements such as Fe, Cu, Zn (Monro et al., 1986 & Ohtsuka et al., 1984). In the present study the elemental content of three varieties of Ugandan sweet potato prepared by freeze, sun and oven-drying were determined, in order to potentially estimate the nutrient intake for infants who may consume this food as follow-on from breast-feeding.

Elements (Na, K, Ca, Mg, Cu, I, Mn, Fe, Ni, Zn, Se, Mo) are essential micronutrients, which play a vital role in the growth, health and development of...
humans. All body tissues and fluids contain varying quantities of elements, which are important factors in maintaining physiological processes, strengthening skeletal structures and preserving the vigour of the heart and brain as well as muscle and nervous system (Ward, 2002). Elements also help in maintaining the delicate water balance essential to proper functioning of mental and physical processes (Ward, 2002). It is therefore important that the diet should contain appropriate amount of essential elements. In addition the bioavailability of elements is important for meeting nutritional needs, especially in the infant growth period (DoH, 1995).

Bioavailability is the proportion of nutrient that is digested, absorbed and utilized in normal metabolism (Jackson, 1997). However, bioavailability measurement relies heavily upon the estimates of amounts absorbed and determines the usefulness of a particular diet. Bioavailability depends on the chemical form of the element, the nature of food ingested and the composition of the total diet and the health and the nutritional status of the individual (O'Dell, 1984) as well as the binding strength of the ligands in the lumen of the gastrointestinal tract (Fairweather-Tait, 1999).

### 4.1.1 Bioavailability methods

There are two main approaches for determining the bioavailability of elements. The first is the measurement of *in vivo* absorption directly in human subjects by employing either a chemical balance or a radio-isotopic technique. In chemical balance studies the quantity of nutrient (element) absorbed is measured by taking the difference between the total element ingested and that, which is excreted (Watzke, 1998). The weakness with this method is that it may underestimate the absorption since it does not account for the endogenous elements lost in faecal excretion.
In the radioisotope technique, a whole body counter measures the amount of element absorbed and retained in the body. This requires the element in the food to be biosynthetically labelled with a suitable isotope. Using a stable isotope technique, allows for a single nutrient in a whole diet to be followed (Fairweather-Tait, 1993). The in vivo methods, particularly the one involving the use of the extrinsic radioisotope tagging technique in human subjects, provide a reliable estimate of the true absorption (Narasinga, 1994). However in vivo methods are very expensive and complicated to perform, time consuming and require sophisticated equipment for measurements like whole body count. Therefore a second approach, an in vitro method, which was adopted in this study, includes an estimate of elemental solubility and dialysability. The in vitro digestion method used was developed by Miller et al. (1981). The method mimics the conditions to which the food is subjected in the gut, for example, the pepsin HCl digestion in the stomach at pH 1.5 followed by a pancreatic digestion at pH 7.0 in the duodenum. The pH is changed to the alkaline conditions of the intestine through the use of Pipes solution (Piperazine-N,N-bis 2-ethane-sulphonic acid). A major advantage of this method is that it can be used for the bioavailability assessment of any essential element. This method has been used in a study of Ca, Fe and Zn bioavailability and the results show good agreement with the in vivo prediction of their bioavailability, especially for Fe and Zn (Garcia, 1998; Schwartz et al., 1982).

The purpose of the present research was to quantify elements in three different varieties of sweet potato grown in Uganda and to assess the bioavailability in freeze-dried, sun-dried and oven-dried processed product by an in vitro digestion method.
4.2 Materials and methods

4.2.1 Materials

Materials used in this study are the same as indicated in methods section, chapter 2

4.2.1.1 Reagents for elemental analysis

Analar™ nitric acid 70% was used for the digestion of the samples, (BDH Laboratory Supplies, Poole, Dorset, UK). Dilutions were made using doubly distilled deionised water (DDW) obtained from an Elgastat UHQ water dispenser (Elga Ltd. High Wycombe). The reference material NIES Unpolished Rice Flour (No. 10-b) was obtained from the National Institute for Environmental Studies, Japan and used to establish the quality control data for all elemental analysis.

4.2.2 Sample preparation

Sweet potato sample 0.20 ± 0.01 g (freeze-dried, sun-dried or the oven-dried) was weighed into pre-acid washed borosilicate beakers that were then placed in a muffle furnace (GallenKamp, Royal Holloway Company, England) at 450°C for 24 hours. After cooling, the sample ash was removed from the muffle furnace and 1 ml of concentrated nitric acid was added (67%). The digested volume was made up to 10 ml by adding DDW and the solutions were stored in a propylene bottle in a refrigerator at 4°C until analysis. Digested sample solutions were measured using an inductively coupled mass spectrometer (ICP-MS) and flame atomic absorption spectrometer (FAAS).

4.2.3 Flame atomic absorption spectroscopy

The atoms in this case are volatilised in a flame and in this state the elements absorb electromagnetic radiation of a characteristic wavelength. Atomisers (nebulisers) are used to spray the test solution into the flame through which the light is passed. Within a particular concentration range, there exists a linear relationship
between the degree of absorption and concentration of the element present. This relationship is described by the Beer Lambert law as illustrated below;

\[ A = E \times C \times L \]

where \( A \) = Absorbance, \( E \) = Molar absorptivity, \( C \) = Concentration of the analyte and \( L \) = path length.

![Figure 4.1: Schematic layout of flame atomic absorption spectrometer](image)

**Table 4.1: FAAS parameters and linear calibration range**

<table>
<thead>
<tr>
<th>Element</th>
<th>( \lambda ) (nm)</th>
<th>Lamp current (MA)</th>
<th>Calibration-range (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>324.8</td>
<td>5</td>
<td>0.1 - 10</td>
</tr>
<tr>
<td>Zn</td>
<td>213.9</td>
<td>10</td>
<td>0.05 - 7.5</td>
</tr>
<tr>
<td>Pb</td>
<td>217.0</td>
<td>10</td>
<td>0.5 - 10</td>
</tr>
<tr>
<td>Na</td>
<td>589.0</td>
<td>8</td>
<td>0.1 - 5.0</td>
</tr>
<tr>
<td>Ca</td>
<td>422.7</td>
<td>6</td>
<td>0.1 - 5.0</td>
</tr>
<tr>
<td>Mg</td>
<td>285.2</td>
<td>6</td>
<td>0.1 - 2.0</td>
</tr>
<tr>
<td>Fe</td>
<td>248.3</td>
<td>15</td>
<td>0.1 - 10</td>
</tr>
</tbody>
</table>
4.2.4 Inductively coupled plasma mass spectrometry (ICP-MS)

In general, sample solutions are introduced to the plasma as an aerosol in a stream of argon gas. The argon plasma heats the aerosol to approximately 7000K where the droplets are consequently desolvated, atomised/vaporised and ionised before extraction into a series of high vacuum compartments. The positively charged ions are collected and focused into a quadrupole mass analyser, which separates the ions according to their mass-charge ratio. In the case of Finnigan MAT SOLA instrument which was used, the separated ions exit either to a Faraday cup detector or a channeltron electron multiplier detector.

The Faraday detector is a metallic plate maintained at a relatively high potential compared to the remainder of the instrument and this allows ions to be captured. From the quadrupole mass analyser the ions pass through a collimator slit and through one or more electrodes where they finally strike the Faraday cup. The current produced from these bombardments is converted to a potential and subsequently amplified and transmitted to a read-out device.
Table 4.2: Summary of settings on ICP-MS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incident power</td>
<td>1.5 KW</td>
</tr>
<tr>
<td>Coolant flow</td>
<td>15 dm³ min⁻¹</td>
</tr>
<tr>
<td>Intermediate flow</td>
<td>800 cm³ min⁻¹</td>
</tr>
<tr>
<td>Nebuliser pressure</td>
<td>2.2 bar</td>
</tr>
<tr>
<td>Spray chamber temperature</td>
<td>2 °C</td>
</tr>
<tr>
<td>Resolution</td>
<td>34</td>
</tr>
<tr>
<td>Pump speed</td>
<td>15 rpm</td>
</tr>
<tr>
<td>Focus</td>
<td>5.35</td>
</tr>
<tr>
<td>Filter</td>
<td>8.05</td>
</tr>
<tr>
<td>Multiplier voltage</td>
<td>6.00</td>
</tr>
<tr>
<td>Match</td>
<td>6.60</td>
</tr>
<tr>
<td>Interspace</td>
<td>0.10</td>
</tr>
<tr>
<td>Extraction</td>
<td>2.85</td>
</tr>
<tr>
<td>Pole bias</td>
<td>6.00</td>
</tr>
<tr>
<td>Discriminitor</td>
<td>0.60</td>
</tr>
<tr>
<td>Vacuum 1</td>
<td>2.9 × 10⁰ mbar</td>
</tr>
<tr>
<td>Vacuum 2</td>
<td>3.0 × 10⁻³ mbar</td>
</tr>
<tr>
<td>Vacuum 3</td>
<td>43.9 × 10⁻⁵ mbar</td>
</tr>
</tbody>
</table>
4.2.5 Instrumentation

Flame atomic absorption spectrometry measurements were performed on a Perkin Elmer 5000 Atomic absorption spectrophotometer (Perkin Elmer, High Wycombe, UK). The elements measured by this instrument were; Ca, Na, Mg, Zn, Fe, Ni and Cu. Table 4.1 shows the instrument parameters used. Standards containing 0.1 to 1.0 mg/l for Cu, Ni, Fe, Na and Zn; 0.1 to 3.0 mg/l for Ca and Mn; and 0.1 to 0.5 mg/l for Mg were prepared. All standards were prepared by the dilution of 10,000 mg/l stock standards (Aristar™, BDH Chemicals, Poole, Dorset, UK) using 1% nitric acid. Inductively coupled plasma mass spectrometry analysis of $^{65}$Cu, $^{66}$Zn, $^{112}$Cd, $^{53}$Co, $^{82}$Se, $^{76}$As, $^{53}$Cr, $^{206}$Pb, $^{27}$Al, and $^{55}$Mn utilised a Finnigan MAT SOLA instrument (Finnigan Mat, Hemel Hempstaed, UK) involving a V-groove nebuliser and Channeltron multiplier detector. Table 4.2 lists the ICP-MS instrument performance parameters. All measurements were carried out using a TMDA 54.2 certified reference material (trace elements in water obtained from National Water Research Institute, Canada) and calibration standards prepared from 10,000 or 1000 mg/L stock solutions. (Aristar™, BDH). Multi-element standards (Cu, $^{66}$Zn, $^{112}$Cd, $^{39}$Co, $^{76}$As, $^{53}$Cr, $^{206}$Pb, $^{27}$Al, and $^{55}$Mn) over the range 0 to 500 µg/l were prepared. All measurements were coupled with the on-line addition of a 100 µg/l of indium standard solution (BDH Chemicals, Poole, Dorset, UK) and was used to correct for instrument drift or matrix modification in the plasma.

The calibration curves were linear over the concentration ranges stated (obeys Beer Lambert law). The detection limits on the ICP-MS instrument for the following elements: Al, Fe, Cu, Zn, Se, Pb, Cd, Cr and Ni were as follows; 0.05, 0.02, 0.01, 0.002, 0.001, 0.0003, $6 \times 10^{-5}$, $3 \times 10^{-5}$ and 0.0003 respectively.
4.2.6 Bioavailability measurements

4.2.6.1 Chemicals and reagents

Pepsin (cat no. P-7000,) from the stomach mucosa, bile salts, HCl, sodium hydrogen carbonate and pancreatin (P-7545), porcine (B-8631) were purchased from Sigma-Aldrich Company Ltd, Poole, England. The pancreatin preparation consisted of a mixture of protease, amylase and lipase and was obtained from Sigma-Aldrich Company Ltd, Poole, England.

4.2.6.2 Procedure

An estimation of elemental bioavailability was carried out by the method of Miller et al., (1981). Sweet potato samples (oven, sun and freeze-dried) 0.5 ± 0.1 g were weighed into 250 ml propylene bottles in triplicate. Hydrochloric acid 0.01M (20 ml) was added followed by 1 ml of pepsin solution and adjusted to pH 2 using 6 M HCl. The mixture was incubated in a shaking water bath (Grant instruments Ltd, Cambridge, UK) for 2 h at 37 °C. Dialysis bags (simulating small intestines) containing 20 ml PIPES buffer (Sigma-Aldrich company Ltd, Poole,England) at pH 6.1 were immersed into the hydrolysate and incubated for a further 30 minutes. A Pancreatin-bile mixture (5.0 ml) was added and the incubation continued for a further 2 h. The dialysis bags were removed and rinsed by dipping in distilled deionised water. The dialysate solutions were emptied into pre-weighed polypropylene bottles and weighed. The elements in the dialysate were then measured by inductively coupled plasma mass spectrometer (\(^{27}\)Al, Ni, \(^{82}\)Se, \(^{56}\)Fe, \(^{53}\)Cr, \(^{66}\)Zn and \(^{55}\)Mn) and the flame atomic absorption spectrometer (Mg and Ca). The bioavailability or dialysability was expressed as the percentage of the amount of elements present in the sweet potato samples.
Dialyzability percentage is expressed as:

\[ \% = \left( \frac{D}{T} \right) \times 100 \]

Where \( D \) is elemental content in the dialysate and \( T \) is the total elemental content in the sweet potato sample (Garcia et al., 1998). To calculate the elemental content (mg/kg) of the dialysate solution (\( D \)) the intensity data was converted to a concentration by the linear regression data obtained from the respective calibration graph and then multiplied by the volume of the dialysate solutions and divided by the weight of the sample used.

4.3 RESULTS AND DISCUSSION

The results obtained for the reference material Nies Unpolished Rice Flour No10-b (Table 4.3) were in good agreement with the certified values except for Cr, Mn and Na. The relative standard deviation values of Nies rice were typically \( < \pm 10\% \) indicating a satisfactory level of precision for the replicate measurements.

Tables 4.4 and 4.5 summarise the elemental content (dry weight basis) of the three varieties of Ugandan sweet potato obtained by the FAAS and ICP-MS methods. The results in Tables 4 and 5 indicate that the main elements present in sweet potato are potassium, sodium, magnesium and calcium as well as appreciable amounts of trace elements such as iron and zinc. The values obtained were in good agreement with those reported in the literature (Table 1.5, chapter 1) for varieties from Nigeria (Nnam, 2001), Sri Lanka (Ravindran et al, 1995), South Pacific (Bradbury & Holloway, 1988), Tonga (Monro et al., 1986) and Peru (Collazos, 1974). The content of all the trace elements in sweet potato were with in the range of the typical concentration values of plant food stuffs; Al (3-140), Cr (0.01-14), Mn (1.3-90), Fe (6-130), Ni (0.06-5), Cu (0.08-9), Zn (1.2-45), Se (0.003-0.15), Cd (0.008-0.5) and
Pb (0.05-4) except for Se (Ward, 2002). Potassium and sodium elements were the most abundant values, which ranged from 865 to 1715 and 229 to 715 mg/kg (d.w) respectively. The elements in sweet potato are comparable with those in maize meal, which is a commonly used material for making baby food in Uganda (Table 4.6).

Zinc levels ranged from 14 to 40 mg/kg, which would cover 80% of the RDA as calculated from an intake of 100 g food per day. Zinc is especially important in physical growth, development of intelligence, learning ability, sexual maturity and behavioural control (Garrow et al., 2000).

Iron content ranged from 22 to 52 mg/kg, which would provide 60% of the RDA. Iron content in 100 g sweet potato is comparable with the amount in beef (3.7 mg/100g) and spinach (2.2 mg/100g), which are considered as high iron content foods (Anonymous, 1979). Iron deficiency is a cause for concern because it is essential to the oxygen carrying role of haemoglobin, also acts as oxygen reservoir in myoglobin and acts as a cofactor for enzymes such as xanthine oxidase, aconitase and amino acid hydroxylases. Its deficiency may lead to poor growth, developmental delay and poor immunity. Although sweet potato appears to be a good source of iron, and zinc the availability of iron is very low as shown in Tables 4.7, 4.8 and 4.9. Non-haem iron is less bioavailable than haem iron. It is important that diets used during weaning should provide adequate absorbable iron and other essential elements.
Table 4.3: Elemental content of certified NIES unpolished rice flour mg/kg dry weight

<table>
<thead>
<tr>
<th>Element (n=3)</th>
<th>Certified or reported value (mg/kg)</th>
<th>Measured Value (Mean ± SD)</th>
<th>RSD(CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FAAS/ ICP-MS</td>
<td>%</td>
</tr>
<tr>
<td>Al</td>
<td>2.0</td>
<td>1.40 ± 0.01</td>
<td>1.0</td>
</tr>
<tr>
<td>Ca</td>
<td>78</td>
<td>79.20 ± 2.50</td>
<td>3</td>
</tr>
<tr>
<td>Cd</td>
<td>0.32</td>
<td>0.19 ± 0.03</td>
<td>15</td>
</tr>
<tr>
<td>Cr</td>
<td>0.22</td>
<td>0.06 ± 0.001</td>
<td>1.6</td>
</tr>
<tr>
<td>Cu</td>
<td>3.30</td>
<td>3.75 ± 0.10</td>
<td>2.6</td>
</tr>
<tr>
<td>Fe</td>
<td>13.4</td>
<td>11.2 ± 1.60</td>
<td>17</td>
</tr>
<tr>
<td>K</td>
<td>2450</td>
<td>2405 ± 314</td>
<td>13</td>
</tr>
<tr>
<td>Mg</td>
<td>1310</td>
<td>1034 ± 24</td>
<td>2.3</td>
</tr>
<tr>
<td>Mn</td>
<td>31.5</td>
<td>54.30 ± 8.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Na</td>
<td>17.8</td>
<td>57 ± 2.5</td>
<td>4.3</td>
</tr>
<tr>
<td>Ni</td>
<td>0.39</td>
<td>0.22 ± 0.05</td>
<td>22</td>
</tr>
<tr>
<td>Pb</td>
<td>Nv</td>
<td>0.14 ± 0.01</td>
<td>7.1</td>
</tr>
<tr>
<td>Se</td>
<td>0.02</td>
<td>0.02 ± 0.003</td>
<td>15</td>
</tr>
<tr>
<td>Zn</td>
<td>22</td>
<td>24 ± 1.41</td>
<td>5.8</td>
</tr>
</tbody>
</table>

The oven-dried native sweet potato flour had a slightly lower elemental content compared with freeze-dried and sun-dried sweet potato flour (p< 0.05). This is probably due to losses during oven drying. Some of the elements particularly the
volatile ones may have been lost during oven-dry heating. The slight variation among the cultivars could be due to soil chemistry including the inhomogeneity of soil, pH, organic and moisture content. The toxic elements Pb and Cd were present in very minute amounts (0.6-1.8 mg/kg), which are considered not to be detrimental to the consumer. These values are within the range of typical concentrations of plant foods reported by Ward, 2002. These values are below the acceptable statutory limits recommended by WHO/FAO (1998).
Table 4.4: Elemental content (mean ± standard deviation, n = 3) of freeze-dried and sun-dried sweet potato samples (mg/kg, dry weight)

<table>
<thead>
<tr>
<th>Element</th>
<th>Freeze-dried varieties</th>
<th>Sun-dried varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nsp</td>
<td>Tz</td>
</tr>
<tr>
<td>Al</td>
<td>28.20 ± 0.10</td>
<td>29.75 ± 0.15</td>
</tr>
<tr>
<td>Ca</td>
<td>601 ± 8.30</td>
<td>476 ± 8.80</td>
</tr>
<tr>
<td>Cd</td>
<td>0.17 ± 0.04</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>Cr</td>
<td>1.55 ± 0.01</td>
<td>1.57 ± 0.04</td>
</tr>
<tr>
<td>Cu</td>
<td>3.36 ± 0.30</td>
<td>3.20 ± 0.60</td>
</tr>
<tr>
<td>Fe</td>
<td>35.90± 0.10</td>
<td>27.32± 1.21</td>
</tr>
<tr>
<td>K</td>
<td>1715 ±43</td>
<td>1110±10.21</td>
</tr>
<tr>
<td>Mg</td>
<td>761± 1.00</td>
<td>762± 2.10</td>
</tr>
<tr>
<td>Mn</td>
<td>29.52±0.70</td>
<td>46.42± 8.80</td>
</tr>
<tr>
<td>Na</td>
<td>621 ±6.11</td>
<td>665 ± 4.32</td>
</tr>
<tr>
<td>Ni</td>
<td>3.11 ± 0.21</td>
<td>3.55 ± 0.17</td>
</tr>
<tr>
<td>Pb</td>
<td>0.64 ± 0.01</td>
<td>0.69 ± 0.02</td>
</tr>
<tr>
<td>Se</td>
<td>3.73 ± 0.72</td>
<td>3.76 ± 0.06</td>
</tr>
<tr>
<td>Zn</td>
<td>39.41 ± 0.62</td>
<td>34.71 ± 1.71</td>
</tr>
</tbody>
</table>

* a value for one sample
4.5: Elemental content (mean ± standard deviation, \( n = 3 \)) in oven-dried sweet potato varieties from Uganda (mg/kg dry weight basis)

<table>
<thead>
<tr>
<th>Element</th>
<th>Nsp</th>
<th>Tz</th>
<th>Spk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>32.00 ± 0.31</td>
<td>28.41 ± 0.21</td>
<td>26.23 ± 0.11</td>
</tr>
<tr>
<td>Ca</td>
<td>385 ± 5.43</td>
<td>434 ± 2.00</td>
<td>707 ± 22.00</td>
</tr>
<tr>
<td>Cd</td>
<td>0.13 ± 0.02</td>
<td>0.09 ± 0.01</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>Cr</td>
<td>0.74 ± 0.04</td>
<td>0.74 ± 0.01</td>
<td>0.72 ± 0.03</td>
</tr>
<tr>
<td>Cu</td>
<td>5.43 ± 1.52</td>
<td>3.16 ± 0.20</td>
<td>8.51 ± 0.32</td>
</tr>
<tr>
<td>Fe</td>
<td>28.41 ± 1.51</td>
<td>22.71 ± 0.01</td>
<td>23.43 ± 1.21</td>
</tr>
<tr>
<td>K</td>
<td>920 ± 12.62</td>
<td>880 ± 6.70</td>
<td>865 ± 3.0</td>
</tr>
<tr>
<td>Mg</td>
<td>514 ± 8.21</td>
<td>564 ± 7.63</td>
<td>834 ± 4.20</td>
</tr>
<tr>
<td>Mn</td>
<td>15.23 ± 4.22</td>
<td>24.31 ± 0.38</td>
<td>22.45 ± 0.01</td>
</tr>
<tr>
<td>Na</td>
<td>229 ± 1.42</td>
<td>336 ± 5.44</td>
<td>274 ± 4.95</td>
</tr>
<tr>
<td>Ni</td>
<td>1.55 ± 0.35</td>
<td>2.97 ± 0.67</td>
<td>2.44 ± 0.08</td>
</tr>
<tr>
<td>Pb</td>
<td>1.33 ± 0.01</td>
<td>1.53 ± 0.46</td>
<td>1.81 ± 0.07</td>
</tr>
<tr>
<td>Se</td>
<td>1.83 ± 0.15</td>
<td>2.33 ± 0.24</td>
<td>1.93 ± 0.26</td>
</tr>
<tr>
<td>Zn</td>
<td>14.32 ± 0.01</td>
<td>28.34 ± 0.11</td>
<td>33.35 ± 7.00</td>
</tr>
</tbody>
</table>

Table 4.6: Some elements in sweet potato compared with maize flour mg/100 g dry weight and the recommended daily intake for 1-3 yr children

<table>
<thead>
<tr>
<th>Element (mg)</th>
<th>Mg</th>
<th>Fe</th>
<th>Zn</th>
<th>Cu</th>
<th>Ca</th>
<th>Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDA for 1-3 year old child</td>
<td>85</td>
<td>6.9</td>
<td>5.0</td>
<td>(0.7-1.0)*</td>
<td>350</td>
<td>15 µg</td>
</tr>
<tr>
<td>Sweetpotato (mg/100g)</td>
<td>76</td>
<td>4.6</td>
<td>3.5</td>
<td>0.32</td>
<td>60</td>
<td>0.38</td>
</tr>
<tr>
<td>Maize meal (mg/100g)</td>
<td>100</td>
<td>4.7</td>
<td>2.4</td>
<td>0.30</td>
<td>73.3</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Recommended reference values obtained from DOH, 1991
* obtained from Garrow et al., 2001
The bioavailability data of elements obtained by an *in vitro* digestion method are summarised in Table 4.7 to 4.9. The availability of elements was similar in all the three varieties. A difference was observed in those samples that were oven-dried and freeze-dried. There was a statistically higher (Student t-test, probability p<0.05) bioavailability of oven-dried compared with the freeze-dried samples, particularly for elements such as Zn, Fe, Ni and Cr. This is probably due to a reduction of resistant starch and fibre in the oven dried sweet potato. Resistant starch/fibre and phytate can potentially combine with these elements thereby preventing their absorption (Bosscher *et al.*, 2001; Rosalind, 1994). Heating at high temperatures may also induce some chemical changes within a substance, such as hydrolysis of protein and denaturation thereby leading to the decrease in protein-element binding. This decrease may reduce the number of ligands formed with some elements like iron and may be responsible for the observed increase in minerals bioavailability (Fig 4.2 e) (Hurrell, 1990 & Sarria *et al.*, 2000). The highest bioavailability values were found for Ca (36 to 74%), Zn (35 to 80%), Mg (7.6 to 57%) and Se (20 to 87%). Bioavailability is important because these elements are essential for the proper functioning of the body; Ca and Mg are required for bone formation and are important for muscular, neurological and endocrine systems. Zinc is necessary for stabilizing the structure of organic components and membranes, as a constituent of metalloenzymes (alcohol dehydrogenase, superoxide dismutase, DNA-polymerase and alkaline phosphatase) and proper functioning of immune system (Sandström, 1992). Selenium is an essential constituent of the glutathione peroxidase enzymes, which help in preventing oxidative damage of the cells.

Although the iron content of the three varieties was high, its bioavailability was less than 20%. Iron is most readily absorbed from foods rich in haem, particularly red
meat and meat products whereas non-haem iron present in plants is less well absorbed (DoH, 1995). To enhance the absorption of iron, ascorbic acid may be added to the food with non-heam iron. For toxic elements such as Al, As, Pb and Cd, the quantity and the bioavailability were low (<10%) implying that sweet potato is a potentially safe product. The low bioavailability of these toxic elements may be a protective mechanism against toxicity.

4.4 Conclusion

Sweet potato can generally serve as a source of minerals and trace elements (Na, K, Mg, Ca, Fe, Zn, Se and Cu). The in vitro digestion method on bioavailability shows that the elements such as Ca, Mn, Mg and Zn in sweet potato are moderately bioavailable (55, 51, 45 and 65 % respectively). Elements in oven- dried samples were more bioavailable suggesting use of mild heat treatment in the processing of sweet potato roots is beneficial. For essential elements that are less available for example iron, ascorbic acid may be used during processing to increase availability. Some potential sources of Vitamin C, which may supplement sweet potato meal, are the sweet potato leaves, spinach, fresh vegetables or other readily available fruits like oranges, lemons and tomatoes. It is suggested that further work using in vitro methods could be developed to include the gastric juices or enzymes associated with infant gut to provide a more accurate picture of the bioavailability of essential elements provided by sweet potato for the infant digestion system.
Table 4.7: Bioavailability(%) of mineral in three varieties of Freeze-dried sweet potato varieties

<table>
<thead>
<tr>
<th>Mineral</th>
<th>F Nsp</th>
<th>F Tz</th>
<th>F Spk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>1.93 ± 0.11</td>
<td>1.99 ± 0.11</td>
<td>1.74 ± 0.02</td>
</tr>
<tr>
<td>Mn</td>
<td>29.42 ± 1.55</td>
<td>16.75 ± 0.47</td>
<td>14.77 ± 0.80</td>
</tr>
<tr>
<td>Ni</td>
<td>13.50 ± 0.01</td>
<td>10.82 ± 0.52</td>
<td>14.33 ± 0.06</td>
</tr>
<tr>
<td>Zn</td>
<td>35.44 ± 6.01</td>
<td>42.08 ± 1.17</td>
<td>45.07 ± 2.20</td>
</tr>
<tr>
<td>Mg</td>
<td>21.56 ± 3.14</td>
<td>7.63 ± 1.89</td>
<td>42.75 ± 1.77</td>
</tr>
<tr>
<td>Ca</td>
<td>49.68 ± 0.40</td>
<td>58.67 ± 1.14</td>
<td>44.17 ± 1.60</td>
</tr>
<tr>
<td>Se</td>
<td>33.98 ± 9.25</td>
<td>20.26 ± 2.80</td>
<td>35.28 ± 14.5</td>
</tr>
<tr>
<td>Fe</td>
<td>8.21 ±0.51</td>
<td>11.41 ± 0.53</td>
<td>6.35 ± 0.45</td>
</tr>
<tr>
<td>As</td>
<td>4.12 ±0.23</td>
<td>7.23 ± 1.24</td>
<td>3.91 ± 0.39</td>
</tr>
<tr>
<td>Cr</td>
<td>4.57± 0.62</td>
<td>4.92 ± 0.63</td>
<td>4.39 ± 0.44</td>
</tr>
</tbody>
</table>
Table 4.8: Bioavailability (%) of minerals in three varieties of sun-dried sweet potato flour

<table>
<thead>
<tr>
<th>Mineral</th>
<th>S Nsp</th>
<th>S Tz</th>
<th>S Spk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>1.48 ± 0.06</td>
<td>2.86 ± 0.24</td>
<td>3.28 ± 0.06</td>
</tr>
<tr>
<td>Mn</td>
<td>51.06 ± 12.5</td>
<td>27.24 ± 1.47</td>
<td>55.46 ± 0.65</td>
</tr>
<tr>
<td>Ni</td>
<td>22.07 ± 0.21</td>
<td>15.54 ± 0.38</td>
<td>30.92 ± 2.11</td>
</tr>
<tr>
<td>Zn</td>
<td>55.75 ± 4.71</td>
<td>68.04 ± 0.66</td>
<td>82.99 ± 1.12</td>
</tr>
<tr>
<td>Mg</td>
<td>47.22 ± 1.26</td>
<td>22.68 ± 0.40</td>
<td>45.38 ± 0.45</td>
</tr>
<tr>
<td>Ca</td>
<td>36.38 ± 0.0</td>
<td>40.29 ± 0.50</td>
<td>42.11 ± 4.00</td>
</tr>
<tr>
<td>Se</td>
<td>55.80 ± 12.4</td>
<td>77.71 ± 9.54</td>
<td>73.04 ± 15.8</td>
</tr>
<tr>
<td>Fe</td>
<td>7.84 ± 0.78</td>
<td>13.19 ± 0.16</td>
<td>9.81 ± 0.02</td>
</tr>
<tr>
<td>As</td>
<td>4.51 ± 0.01</td>
<td>5.49 ± 0.22</td>
<td>6.53 ± 2.26</td>
</tr>
<tr>
<td>Cr</td>
<td>12.94 ± 6.93</td>
<td>5.54 ± 0.23</td>
<td>5.28 ± 0.76</td>
</tr>
</tbody>
</table>

Table 4.9: Bioavailability (%) of minerals in three varieties of oven-dried sweet potato

<table>
<thead>
<tr>
<th>Mineral</th>
<th>O Nsp</th>
<th>O Tz</th>
<th>O Spk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>2.87 ± 0.11</td>
<td>3.88 ± 0.25</td>
<td>3.52 ± 0.29</td>
</tr>
<tr>
<td>Mn</td>
<td>28.65 ± 0.31</td>
<td>76.28 ± 7.30</td>
<td>43.30 ± 2.10</td>
</tr>
<tr>
<td>Ni</td>
<td>57.32 ± 1.92</td>
<td>39.10 ± 2.97</td>
<td>43.75 ± 2.53</td>
</tr>
<tr>
<td>Zn</td>
<td>59.53 ± 4.62</td>
<td>80.16 ± 6.15</td>
<td>56.77 ± 2.71</td>
</tr>
<tr>
<td>Mg</td>
<td>57.00 ± 17.5</td>
<td>32.71 ± 1.97</td>
<td>32.61 ± 4.94</td>
</tr>
<tr>
<td>Ca</td>
<td>74.72 ± 12.0</td>
<td>55.47 ± 1.50</td>
<td>56.33 ± 10.00</td>
</tr>
<tr>
<td>Se</td>
<td>53.37 ± 6.27</td>
<td>86.16 ± 6.14</td>
<td>82.67 ± 17.6</td>
</tr>
<tr>
<td>Fe</td>
<td>13.35 ± 0.08</td>
<td>18.09 ± 0.76</td>
<td>15.3 ± 5.13</td>
</tr>
<tr>
<td>As</td>
<td>9.57 ± 0.61</td>
<td>4.15 ± 0.56</td>
<td>3.37 ± 0.12</td>
</tr>
<tr>
<td>Cr</td>
<td>5.55 ± 0.36</td>
<td>6.26 ± 0.0</td>
<td>6.52 ± 1.35</td>
</tr>
</tbody>
</table>
Fig. 4.2: Bioavailability of a) Al, b) Mn, c) Zn,  d) Ni, e) Fe in sweet potato varieties Nasport (Nsp), Tanzania (Tz) and Spk 004 (Spk) that were freeze-dried, sun-dried and oven-dried.
CHAPTER FIVE
5.0 ANALYSIS OF PHENOLIC COMPOUNDS IN UGANDAN SWEET POTATO VARIETIES (NSP, SPK AND TZ)

5.1 Introduction

Phenolic compounds are a class of low molecular weight secondary plant metabolites found in most land plants. These compounds are of great importance for food and drink since they are responsible for their organoleptic properties; some polyphenols in food add colour like anthocyanins, which can be purple, black or red (Goda et al., 1996; Alonso et al., 2003) and this can be desirable in red wines. In addition polyphenols affect the taste of the food like the pungency of chillies and astringent taste of beers. Polyphenols are the building blocks of lignin and dietary fibre, which is known to determine the texture and nutritional value of vegetable foods.

Phenolic substances such as flavonols, cinnamic acids, coumarins and caffeic acids or chlorogenic acids are believed to have antioxidant properties, which are suggested to play an important role in protecting food, cells and any organ from oxidative degeneration (Osawa, 1999; Tikkanen et al., 1998; Wiseman et al., 2000; Rice-Evans, 1998; Pratt, 1998). In model systems, antioxidants are able to scavenge free radicals and thereby prevent the free radicals from causing damage. Such properties may be important in processed foods, but whether such properties are retained when phenols are absorbed and metabolised is less clear.

Reports indicate that diets rich in phenolic compounds play a role in the prevention of various diseases associated with oxidative stress such as cancer, cardiovascular and neurodegenerative diseases (Hertog et al., 1993; Anderson et al., 1995; Manach et al., 2004; Nestle, 2004; Hang et al., 2004). In addition polyphenols, which constitute the
active substances found in many medicinal plants, modulate the activity of a wide range of enzymes and cell receptors (Middleton et al., 2000).

Because of the benefits these polyphenols contribute, isolation and identification of these substances in sweet potato is of considerable interest. As it has been mentioned in chapter one, sweet potato contains chlorogenic acids but due to limitations in methods at that time different phenolic compounds were not fully identified.

In the present study, high-pressure liquid chromatography coupled with mass spectrometry (HPLC-MS) was used in analysing sweet potato phenolics. HPLC—MS is a powerful technique in food analysis for the identification of compounds of interest and elucidation of their structures. HPLC is used for separating and quantitation of the compound of interest (phenolics). The separation of compound/analytes are based on the interaction of individual components with stationary and mobile phases. The chromatographic separation provides information about the retention time and photodiode array (PDA) detector can determine $\lambda$ max and full UV-VIS spectrum for analytes. Although this is important, it may not be sufficient to discriminate between closely related compounds. The retention times may be the same for many compounds for a particular set of HPLC conditions and accurate quantitation may be compromised by co-elution with other analytes. The separated compounds are then injected into the mass spectrometer where mass data of individual substances are provided according to their mass-charge ratio ($m/z$). These compounds can further be subjected to collision-induced dissociation leading into fragmentations. The information obtained together with their mass spectra are
important in the elucidation of the structures of a compound of interest and the pattern of fragmentation may allow isomers to be distinguished (Clifford et al., 2003)

5.2 Materials and methods

5.2.1 Materials used

The materials used in these study were freeze-dried, sun-dried, oven-dried and fresh sweet potatoes varieties; Nasport (Nsp), Spk004 (Spk) and Tanzania (Tz) from Uganda. Peels from Tz variety were used to assess the differences in quantity between the polyphenols in flesh and those in the peel.

5.2.1.1 Equipment

Freeze drier (Edwards Modulyo, UK), Soxhlet extraction system (Tecator Company Sweden), blender/homogeniser, nitrogen evaporator (N-Evap-111, Organomation Associates Inc, Berlin, Ma, USA), HPLC Column, Phenyl-hexyl, 150 mm × 3 mm, 5 μm (Phenomenex, UK), HPLC vails (Phenomenex, UK), HPLC-MS® Spectrometer (Thermofinnigan LC Deca, San Jose, CA, USA) with UV absorption detector, Micro centrifuge (13000 × g) (Fischer scientific Ltd, Loughborough, Leicestershire, UK), Rotary evaporator (Orme Scientific Ltd, UK).

5.2.1.2 Reagents

Carrez A: Zinc acetate. 2H₂O, 21.9 g in 100 ml of milli-Q water
Carrez B: Potassium Ferro cyanide, 10.6 g in 100 ml of milli-Q water.
HPLC grade methanol, 70% v/v aqueous methanol, acetonitrile and acetic acid.
All the chemicals were obtained from Sigma-Aldrich Company Ltd, Poole, England.
5.2.2 Method

Sweet potato (1 g) (oven, freeze, sun-dried) or dried peel was weighed into soxhlet extraction thimbles. The phenolic compounds present in sweet potato powder (flour) were extracted into 40 ml 70% v/v aqueous methanol using a soxhlet apparatus. Three extractions were carried out to ensure full extraction of the phenolic compounds present in sweet potato. The solvents containing the extract were cooled for a few minutes, and then filtered into a 100 ml volumetric flask using Whatman No.1 filter paper. The final volume was made up to 100 ml using 70% v/v aqueous methanol. To 100 ml extract, Carrez reagent A (4 ml) was added and vortexted for 2 min and left for one minute. Carrez B reagent (4 ml) was then added and vortexed for 20 seconds. These reagents were added to precipitate out the polysaccharides and proteins. The mixture was centrifuged at 2000 × g for 20 min. The supernatant was drawn off and stored in a cold room (5 °C) until HPLC—MS analysis.

5.2.2.1 Preparation of extracts for HPLC and MS analysis

Sweet potato phenolic extract (100 ml) was evaporated in the rotary evaporator to dryness. Methanol 10% v/v aqueous (2 ml) was added to the residue (phenolic compound). The solution was centrifuged at 13000× g to remove any particulates that would block the column. The supernatant was filtered via Durapore polyvinylidene difluoride filter (PVDF-D) (0.45 μm) (Millipore Company, UK) and 20 μl was injected into the HPLC—MS system for analysis.

5.2.2.2 HPLC—MS analysis

The HPLC—MS equipment (Thermofinnigan, San Jose, CA) comprised a Surveyor MS pump, an autosampler with a 20 μl loop and a PDA/ UV detector. For the sweet
potato analysis the UV detector was set to record at channel A = 324 nm, channel B = 280 nm and Channel C = 450 nm. The detector was interfaced with an LCQ deca XP plus mass spectrometer fitted with an electro spray interface (ESI) source (Thermofinnigan) and operating in zoom scan mode for accurate determination of parent ion $m/z$ and in data-dependent, MS$^n$ mode to obtain fragment ion $m/z$. The software for the control of the equipment and the acquisition and treatment of data was Xcalibar version 1.3. The phenolic separation was achieved on a 150 x 3 mm column containing Luna 5 µ phenyl hexyl packing (Phenomenex, Macclesfield, UK). The gradient temperature control (column oven control) was 30 °C. The chromatographic conditions were: flow rate of 0.3 ml/min, sample injection volume of 20 µl and mobile phases A (2 % aqueous acetonitrile, 0.5 % acetic acid, pH 2.68) and B (99.5 % acetonitrile, 0.5 % acetic acid). The gradient program was set as shown in Table 1 below. The run time of 110 minutes was set to elute all phenolic compounds that might be present in the sweet potato.

The interface conditions were negative ionisation mode, temperature of the capillary 350 °C, an ionisation voltage 3.5 KV, gas flow rate 80 arbitrary units and auxiliary gas flow, 10 (arbitrary units). The mass detection was performed in the base peak mode, for $m/z$ between 20 and 750.
Table 5.1: Gradient programme

<table>
<thead>
<tr>
<th>No.</th>
<th>Time (min)</th>
<th>Flow, ul/min</th>
<th>A%</th>
<th>B%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>300</td>
<td>96</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>300</td>
<td>67</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>95</td>
<td>300</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>300</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>105</td>
<td>300</td>
<td>96</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>110</td>
<td>300</td>
<td>96</td>
<td>4</td>
</tr>
</tbody>
</table>

The number of scan events was set as 5, which was necessary to fragment the parent ion into daughter ions to give structural information.

5.3 Results and discussion

The extracts were analysed by HPLC—MS in full scan mode for the identification of phenolic compounds. The examination of HPLC—MS chromatograms revealed the presence of several compounds, which were identified using hierarchical key (Appendix B) identification developed by Clifford et al., 2003. Some isomers were distinguished by comparing their retention time with similar compounds that have been previously studied under similar conditions. By means of this HPLC—MS method, several phenolic compounds were detected which included; three isomers of caffeoylquinic acid, three isomers of dicaffeoylquinic acid, one isomer of p-coumaroylquinic acid, one isomer of feruloylquinic acid, and six isomers of caffeoylferuloylquinic acid. Walter et al. (1979) and Thompson (1981) in their findings reported the presence of chlorogenic acid in sweet potato but due to the
limited methods used at that time they could not identify the different chlorogenic compounds present.

5.3.1 *p*-Coumaroylquinic acid

A very weak *5-p*-coumaroylquinic acid signal (Fig. 5.1) was detected in the sweet potato samples at retention time *ca* 27 min and was identified based on its parent ion at *m/z* 337 and MS² base peak *m/z* 191 (Fig. 5.2). The isomers 3 and 4-*p*-coumaroylquinic acids were not observed in any of sweet potato samples.

![Structure of 5-pCoQA](image)

Fig. 5.1: Structure of 5-pCoQA
5.3.2 Caffeoylquinic acids

Different isomers were detected in the full scan experiment showing \( m/z \) 353 at the retention times ca 13, 19 and 22 min (Fig. 5.4) for the freeze-dried extract of Tz variety and specimen structures are shown in Fig. 5.3; these isomers were identified as 3-caffeoylquinic (3-CQA), 5-caffeoylquinic (5-CQA) and 4-caffeoylquinic acid (4-
CQA), respectively. The three isomers were distinguished based mainly on the MS² fragments and MS³ fragments. For the 3-CQA and 5-CQA, the MS² fragment base peak was \( m/z \) 191, deprotonated quinic acid (ii and iii) and so they could only be distinguished based on the relative intensity of the secondary ion \( m/z \) 179. For 5-CQA the secondary ion at \( m/z \) 179 is very weak (< 5%) or undetectable while 3-CQA the relative intensity of the secondary ion at \( m/z \) 179 is about 50% or more. 4-CQA can be differentiated from other CQA isomers by its MS² fragmentation (Fig. 5.4). 4-CQA gives an MS² base peak at \( m/z \) 173 due to loss of caffeic acid and a water molecule while 3-CQA and 5-CQA gave MS² at \( m/z \) 191 (deprotonated quinic acid) as a result of loss of caffeic acid unit. The Tz flesh variety extract had similar isomers eluted at the same retention time and were identified as 4-CQA and 5-CQA. In this study CQA isomers eluted in the sequence 3, 5 and 4, which was consistent with the findings reported by Clifford et al., 2003. In freeze-dried Nsp extract one isomer 5CQA was detected at \( ca \) 17 min while in the oven-dried sample it was observed at \( ca \) 15 min.
Fig. 5.3: Specimen structures of CQA, Clifford et al., 2003
Fig. 5.4: MS^2 spectra for some carboxylic acids.
5.3.3 Feruloylquinic acids

Feruloylquinic acids particularly the 5-feruloylquinic acid was detected in all the sweet potato at ca 32 min. This was identified based on the parent ion at $m/z$ 367 and its MS$^2$ base peak at $m/z$ 191 (Fig. 5.6). The specimen structures are as shown in Fig. 5.5.

![Structures of feruloylquinic acid (4-FQA and 5-FQA).](image)

Fig. 5.5: Structures of feruloylquinic acid (4-FQA and 5-FQA).
Fig. 5.6: MS² Spectra for Feruloylquinic acid
Relative Abundance
5.3.4 Dicaffeoylquinic acid (diCQA)

The dicaffeoylquinic acid isomers were located by their parent \( m/z \) 515 and each isomer was further distinguished by its fragmentation. The isomers identified in freeze-dried Tz peel and whole root samples were 3,4-diCQA, 3,5-diCQA and 4,5-diCQA eluted at ca 52, 58 and 60 min respectively (Fig. 5.7). Again this identification was possible by use of hierarchical key (Appendix B) and comparison with similar compounds in other substances such as artichoke and coffee (Sanchez-Rabanda et al., 2003; Clifford et al., 2003) respectively. The fragmentation for 3,4 and 4,5-diCQA was similar to that observed in coffee bean extract (Clifford et al., 2003).

The two 3,4 and 4,5-diCQA gave the expected molecular ion \([\text{diCQA—H}^+]\) at \( m/z \) 515 (Fig. 5.8) and MS\(^2\) base peak 353 (Fig. 5.8). Consistent with hierarchical key, 3,4 and 4,5 diCQA gave expected MS\(^3\) and MS\(^4\) base peaks at \( m/z \) 173.5 and \( m/z \) 93.5 respectively. The distinction between 3,4 and 4,5 was based on the intensity of MS\(^2\) secondary ion at \( m/z \) 335. 3,4-diCQA has more intense MS\(^2\) 335 (>20%) compared with 4,5-diCQA isomer with less than 5%. 3,5-DiCQA had same molecular mass as 3,4 and 4,5 diCQA (516) but its MS\(^3\) base peak was different from those of 3,4 and 4,5-diCQA. The MS\(^3\) base peak for 3,5-diCQA was at \( m/z \) 191 while for 3,4 and 4,5-diCQA was at \( m/z \) 173. The former can be distinguished by the intensity of the MS\(^2\) secondary ion fragment at \( m/z \) 335 and MS\(^3\) fragment ion at \( m/z \) 179. Freeze dried Nsp variety extract contained 3,4 and 4,5-diCQA and oven dried Nsp sample contained only one isomer 3,5-diCQA detected at retention time 52 min. The same isomers were detected in the sun-dried Nsp extract. In general the diCQA were eluted between 52 and 59 min comparable with the diCQA from coffee extract.
Fig. 5.7: Specimen structures of dicafeoylquinic acid.
Fig. 5.8: MS, MS₂, MS₃, and MS₄ spectra for 3',5'-dicaffeoylquinic acid
Fig. 5.9: MS², MS³, and MS⁴ spectra for 4',5'-dicaffeoylquinic acid.
FIG. 5.10: MS, MS, MS spectra for 3,4-dichlorophenylacetic acid

(III) (MS,)

(III) (MS,)

(III) (MS,)

(III) (MS,)

Relative Abundance

m/z

180

120

60

0

180

120

60

0

180

120

60

0

m/z

m/z

m/z

Relative Abundance
5.3.5 Caffeoylferuloylquinic acids (CFQA)

Figure 5.11 shows specimen structures of caffeoylferuloylquinic acid. More isomers of CFQA were observed in Tz flesh and the peel samples than in the rest of the varieties. The isomers identified in Tz flesh and Tz peel included; 3F 4CQA, 3F 5CQA, 3C 5FQA, 4F 5CQA, 3C 4FQA and 4C 5FQA eluted at ca 63.5, 64.3, 64.7, 66.0, 67.6 and 69.3 min respectively. These phenolic compounds, like the rest, were identified based on their parent ion at $m/z$ 529 and MS$^2$ and MS$^3$ fragments as described in hierarchical key (Appendix B) and comparison with the isomers obtained from coffee. All these isomers had a parent ion at $m/z$ 529 so the distinction of isomers was based on the MS$^3$ and MS$^2$ secondary ions at $m/z$ 173 and 353 or 367 respectively (Fig. 5.12 to 5.14). 3-Caffeoyl-5-feruloylquinic acid was also observed at ca 66 min in Nsp freeze-dried extract and in Spk sun-dried extract. This is the first time that CFQA isomers have been detected or identified in sweet potato samples. These compounds were detected in coffee by Clifford (2003). It is interesting to note that although sweet potato is not botanically related to coffee it contains the CFQA that so far have not been reported elsewhere.

There were some differences observed among the cultivars. CFQA was not observed in sun dried and oven dried Nsp samples. In the Nsp variety, they were probably converted into other products or were simply too low to be detected or were probably destroyed during heating. In general, processing (sun and oven drying) affected the quantity of some of the phenols in sweet potato. Further work would be required to investigate the quantity of each of these isomers of the chlorogenic acids found in
sweet potato in fresh samples and processed (sun-dried, freeze-dried and oven dried) samples to assess the impact of processing on phenol content.

Fig. 5.11: Specimen structures of caffeoylferuloylquinic acid
Fig. 5.12: MS² spectra for caffeoylferuloylquinic acids ($m/z$ 529)
Fig. 5.13: MS3 spectra for caffeoylferuloylquinic acids (m/z 529)

Relative Abundance

4C' 5FQA (XI)

3C' 5FQA (X)
Fig. 5.14: MS spectra for caffeoylferuloylquininic acids (m/z 529)

RT: 67.56
Fig. 5.15: Spectra for MS$^3$ caffeoylferuloyquinic acid (m/z 529)

Relative Abundance

RT: 67.56

3C, 4FQA
CHAPTER SIX
6.0 PHYSICOCHEMICAL AND RHEOLOGICAL PROPERTIES OF UGANDAN SWEET POTATO STARCH AND FLOUR

6.1 Introduction

Starch is one of the most abundant food components, that is found in the leaves of green plants in the plastids, where it is synthesized. It is also synthesized in the amyloplasts of seeds, grains, roots and tubers of many plants, where it serves as the chemical storage form of the energy from the sun. Starch is stored within the plant cells in the form of water insoluble granules, which have shapes characteristic for each species. Starch is the predominant carbohydrate in all the major foods, for example rice, wheat, maize, beans, potatoes, cassava, sweet potatoes, cocoyams etc (Garrow et al., 2000).

Besides providing a major source of energy in food products, starch plays a crucial role in textural modification via a process of gelatinisation, which is the break up and partial dissolution of the starch granule upon heating in the presence of water (Thomas & Atwell, 1999). Starch is a valuable ingredient for the food industry and is being widely used as a thickener, a gelling agent, bulking agent and water retention agent (Singh et al., 2003).

Starch that is commonly used commercially in the world are corn and potato starch (Santacruz et al., 2002) and cassava in tropical Africa (Olayide, 2004). The high carbohydrate content of sweet potato and its wide availability in Uganda makes it a potential source of starch for both domestic and industrial uses in tropical Africa. The uses of sweet potato starch are primarily determined by its physicochemical properties, which have been extensively reviewed. Tian et al. (1991) indicated wide variations of sweet potato physicochemical properties due to differences in varieties, environment, climate and degree of maturity.
Noda et al. (1997) also found that planting seasons affected some of the characteristics of sweet potato starch such as gelatinisation temperature and enthalpy and amylose content of starch.

Considerable research has been devoted to the development of sweet potato products. Previous scientific reports on increasing the consumption of sweet potato emphasised the production of acceptable convenience products (Bouwkamp, 1985). This may be possible by using varieties with the right or acceptable physicochemical properties because these characteristics affect the textural quality of the products developed. Although the properties of some sweet potato starches and flour have been investigated (Noda et al., 1995 & Tian et al., 1991), the properties of Ugandan sweet potato varieties (Nsp, Tz, Spk) have not been studied to date. The aim of the present investigation was to study the physicochemical properties of flour and starch from Ugandan sweet potato varieties and to characterise the molecular structure of starch by Raman spectroscopy.

6.2 Material and methods

6.2.1 Materials

Sun-dried or freeze-dried flour and starch samples extracted from the three Ugandan sweet potato varieties (Tz, Nsp and Spk) were used in this study. Rice starch was obtained from Sigma-Aldrich Company Ltd, Poole, England.

6.2.2 Starch extraction

Medium sized roots (weight 200 g, length 20 cm and diameter 6 cm) of sweet potatoes (Nsp, Tz, Spk) were selected, peeled and chopped into small pieces. These were then homogenized in a waring blender with addition of 0.1 M sodium chloride solution containing mercuric chloride (0.01 M) to inhibit amylase activity. The suspension was
filtered through cheesecloth and the homogenate centrifuged immediately at 2000x g for 10 min. Crude impurities were removed by resuspending the starch in water and centrifuging at 2000 x g. This was repeated three times. The extracted starch was dried in an oven at a temperature of 40°C. The dried starch was kept in an airtight clean plastic container for further analysis.

6.2.3 Morphology
Granule morphology of sweet potato starch was examined by scanning electron microscopy. Starch samples were mounted on circular aluminium stubs with double sticky tape and then coated with 20 nm of gold and examined and photographed in Hitachi (S 570) scanning electron microscope at an accelerating potential of 15 KV.

6.2.4 Amylose content
This was determined by the Mcleary et al., 1997 method as described in chapter 3.

6.2.5 Solubility
Solubility studies were carried out according to the method of Dubois et al. (1956). Starch (20 mg) was dispersed in 10 ml distilled water and heated in calibrated tubes at 55, 65, 75, 85 and 95 °C for 30 min. The tubes were then cooled to room temperature and centrifuged at 3000 rpm for 10 min. The supernatant (1 ml) was diluted with distilled water (x10). One ml of the diluted supernatant was transferred into a screw capped test-tube and 1 ml of phenol solution was added. Five ml of concentrated sulphuric acid was added rapidly, the stream of acid being directed against the liquid surface rather than against the side of the test tube in order to get good mixing. The tubes were allowed to stand for 10 min in a water bath at 20°C before readings were taken. The absorbance of the characteristic yellow-orange colour was measured at 490 nm, using a
spectrophotometer. The blank was distilled water. The amount of sugar was determined with reference to a standard curve (Fig. 6.1).

The total starch dissolved in the supernatant was calculated using the equation below;

\[
\text{Solubility of starch}\% = \frac{\text{Weight of starch in supernatant}}{\text{Weight of starch on dry basis}} \times 100
\]

6.2.5.1 Preparation of standard curve

A series of six tubes containing from 0.0 to 1.0 ml of standard glucose (100 µg/ml) solution in steps of 0.2 ml were prepared in duplicate. The total volume was made up to 1.0 ml with distilled water. One ml phenol (5%) was added followed by 5 ml of concentrated sulphuric acid. The tubes were allowed to stand for 10 min in a water bath at 20 °C before readings were taken. The absorbance was measured at 490 nm against distilled water as the blank.

![Fig. 6.1: Standard curve for solubility](image)

\[
y = 0.0112x \\
R^2 = 0.9989
\]
6.2.6 Swelling Power
Swelling power of 0.1 g starch was determined using three to four replicates by modifying the method of Tsai et al. (1997). Starch was weighed into a centrifuge tube with a coated screw cap to which 10 ml distilled water was added. The tube was heated at 55, 65, 75, 85 and 95°C in a shaking water bath and centrifuged (8000 x g) for 20 min. The supernatant was poured out from the tube together with some cloudy solids. Only the materials adhered to the wall of centrifuge tube were considered as the sediment and weighed (Ws). The supernatant was dried to constant weight (W1) in an air oven at 100°C. The water-soluble index (WSI) and swelling power (SP) were calculated as follows:

\[
WSI = \frac{W1}{0.1} \times 100\%
\]

\[
SP = \frac{Ws}{0.1(100-WSI)} \text{g/g}
\]

6.2.7 Amylose leaching
Starch was treated exactly the same as in the solubility method above. After centrifugation 1 ml of the supernatant was withdrawn and its amylose content was determined by the Megazyme method described above.

6.2.8 Small deformation testing
The principle and procedure of the method is as described in chapter 2 section (2.2.5).
6.2.9 Differential scanning calorimetry (DSC)

6.2.9.1 Principle

Differential scanning calorimetry measures the difference in energy input into a substance and a reference material while the substance and the reference material are subjected to a controlled temperature programme. Any thermally induced changes for example protein denaturation, starch gelatinisation, and melting of fat are recorded as a differential heat flow displayed as a peak (Harwalkar, 1996). Enthalpy change of the process is obtained by integrating heat flow as a function of time or temperature. The thermogram provides several parameters that are useful in characterising a substance and its thermal behaviour such as gelatinisation temperature for starch, denaturation of proteins, the onset temperature determined at the point of intersection between baseline and the leading edge of the peak. Enthalpy change of transition can be calculated by integration of the area under the transition peak and is usually expressed as joules per gram.

6.2.9.2 Procedure

Thermal characteristics of isolated sweet potato starch and flour were studied by a Setaram DSC microcalorimeter at a scan rate of 0.1°C covering a temperature range from 5 to 100°C. Containers were filled with a solution of 3 g flour (sun-dried and freeze-dried) or starch for the three sweet potato varieties in 10 ml of distilled water (30%w/w). A reference container of distilled water was adjusted to within ± 0.1 g of the same weight. The onset temperature, peak temperature and heat of gelatinisation were determined by using a data analysis system (Setaram DSC version 3.3)
6.2.10 Raman Spectroscopy

6.2.10.1 Principle

Raman spectroscopy is used to monitor the vibrational motions of atoms in a molecule. It involves scattering of incident light and the information is obtained from the resulting changes in the frequency of the light. Raman scattering lies in inelastic collisions between molecules and photons, which are the particles of light making up the light beam. An inelastic collision means that there is an exchange of energy between the photon and the molecule with a consequent change in energy and hence wavelength of the photon (Carey, 1988). The transfer or acceptance of energy by the molecule must correspond to a difference $\Delta E$ between the energy levels of the molecule during the scattering. It also follows that the consequent change in frequency of the scattered photon reflects only this value of $\Delta E$ and is independent of the frequency of the exciting light. The energy level to which the oscillating molecule is raised to is called Virtual energy level (Campbell & Dwek, 1984). When a molecule loses its energy elastically by a direct scattering process, it can emit light at the same frequency as the irradiation referred to as Rayleigh scattering (Fig. 6.2). Alternatively, upon inelastic scattering a photon can lose energy by raising a molecule to an excited vibrational state or gain energy by inducing the reverse process, producing stokes or anti-stokes lines respectively as shown in Figure 6.1 (Li- chan, 1996). Raman scattering depends on the variation of polarisability of molecule ($\alpha$) during vibration and Raman intensity ($I_R$)
Fig. 6.2: Relationship between Rayleigh scattering, Raman stokes scattering and Raman anti-stokes scattering (Li-Chan, 1996).

6.2.10.2 Experimental aspect

The method of Raman studies is to obtain and assign frequencies associated with molecular vibrations of interest. Since Raman scattering can be weak, its detection requires the use of an intense monochromatic radiation provided by a laser, a high resolution monochromator and a highly efficient photodetector. The scattered photons of varying frequencies are resolved by a scanning monochromator and their intensities are plotted as a function of vibration frequency. The use of computer related capabilities such as multiple scanning, signal averaging and background subtracting and smoothing leads to significant enhancement in spectra quality.
6.2.10.3 Procedure

Sweet potato starch samples were placed in glass tubes and Raman spectra were recorded on a Perkin Elmer 2000 FT-Raman spectrometer (Beaconsfield, Buckinghamshire, UK). Spectral resolution was set at 4 cm\(^{-1}\) using a laser power of 1785 mW and the data presented were based on 64 co-added spectra. Frequency calibration of the instrument was performed using the sulphur line at 217 cm\(^{-1}\). The spectra were analysed using Grams 32 software (Galactic Industries Corp, Salem NH). The starch spectra obtained were baseline corrected and the major bands in the spectra were assigned by comparison with Raman spectra of sugars (glucose and starch), which have been reported in the literature (Corbett et al., 1991; Kizil et al., 2002 & Kim et al., 1989).

6.3 Results and Discussion

6.3.1 Morphology

The granule surface of the starch appeared to be smooth under the scanning electron microscope. The granules were spherical (Fig. 6.3 a to 6.3 c) and about 10 \(\mu\)m, which is within the reported range of 10.5 to 14.2 \(\mu\)m (Noda et al., 1992b & Madamba et al., 1975). It has been suggested that many types of starch granules have shapes that often resemble the type of plant material from which they are isolated (Jane et al., 1994). Sweet potato starch is one of those starches that do not fit this description. The granule is oval or spherical but the root is rod or snake shaped. In other tuber and root starches, oval and polygonal shapes have been reported for potato starch (Seog et al., 1987), round and polygonal for cocoyam (Xanthosoma sagittifolium) starch (Olayide, 2004) and a round shape for cassava starch (Moorthy, 1994). In this study no noticeable difference were
observed between the appearances of the starch obtained from the three different varieties of sweet potato (Fig. 6.3 a, b, c).
Fig. 6.3: Scanning electron micrographs of starch granules from a) Nasport (Nsp), (300 ×), b) Spk (300 ×) and c) Tanzania (Tz) (800 ×) starch

6.3.2 Amylose content, amylose leaching, solubility and swelling power

Amylose content of starches isolated from the three varieties of Ugandan sweet potato (Tz, Nsp and Spk) ranged between 24 and 27% (Table 6.1), which is in agreement with values reported by Zhang, (1999) & Hoover (2001). Nsp had the highest amylose content.

Table 6.1: Amylose content in isolated starch from the three cultivars of sweet potato from Uganda (N=3)

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Amylose%</th>
<th>Amylopectin%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nsp</td>
<td>27 ± 3.0</td>
<td>73 ± 3.0</td>
</tr>
<tr>
<td>Tz</td>
<td>24 ± 1.0</td>
<td>76 ± 1.0</td>
</tr>
<tr>
<td>Spk</td>
<td>24 ± 1.0</td>
<td>75.5 ± 1.0</td>
</tr>
</tbody>
</table>
The solubility and ability of starches from the varieties Nsp, Tz and Spk to swell in excess water is presented in Fig. 6.4, 6.5, amylose leaching results are shown in Table 6.2 respectively.

Table 6.2: Amylose leaching % determined in three cultivars of Ugandan sweet potato

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Temperature °C</th>
<th>55</th>
<th>65</th>
<th>75</th>
<th>85</th>
<th>95</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSP</td>
<td>0.0</td>
<td>0.0</td>
<td>0.41 ± 0.01</td>
<td>1.09 ± 0.04</td>
<td>1.95 ± 0.01</td>
<td>3.71 ± 0.05</td>
</tr>
<tr>
<td>TZ</td>
<td>0.0</td>
<td>0.0</td>
<td>0.72 ± 0.01</td>
<td>1.09 ± 0.02</td>
<td>2.54 ± 0.02</td>
<td>3.94 ± 0.00</td>
</tr>
<tr>
<td>SPK</td>
<td>0.0</td>
<td>0.0</td>
<td>0.71 ± 0.00</td>
<td>1.35 ± 0.01</td>
<td>2.93 ± 0.01</td>
<td>3.32 ± 0.01</td>
</tr>
</tbody>
</table>

Values reported in this table are means of three replicates ± SD.

Fig. 6.4: Swelling power of starch from three cultivars (Nsp, Tz and Spk) grown in Uganda
Initially below 55°C the swelling power, solubility and amylose leaching were very low; however, these properties increased linearly with temperature (Fig. 6.4, 6.5 and Table 6.2). There was a steep increase in solubility and swelling power between temperatures 65 and 75 °C, which is the gelatinisation temperature of sweet potato starch (Fig. 6.7 a, b, c) (Zhang, 1999). Swelling power has been reported to depend on water holding capacity of starch molecules by hydrogen bonding (Lee & Osman, 1991). Hydrogen bonds stabilizing the structure of the double helices in crystallites are broken during gelatinisation and are replaced by hydrogen bonds with water and swelling is regulated by the crystallinity of starch (Tester & Karkalas, 1996).

Amylose leaching in general was quite low compared to the values reported in the literature (9%-11%) (Garcia & Walter, 1998) probably due to a difference in cultivars studied and differences in the analytical methods used. The trend of increase of swelling,
solubility and amylose leaching was the same for all the starches obtained from the three cultivars probably because they all contain similar amounts of amylopectin and amylose (Table 6.1). Crystallites within the amylopectin molecules determine the onset of swelling and gelatinisation (Tester & Morrison 1990). A high proportion of long chain molecules are said to contribute to the increase in swelling (Sasaki & Matsuki, 1998). Sandhya & Bhattacharaya (1989) also indicated that low amylose content starch granules are less rigid and swell freely when heated compared with a higher amylose content, which inhibits swelling (Morrison et al., 1993; Tester & Morrison, 1990). All the three varieties yielded a maximum swelling power at 95°C.

6.3.3 Rheological characteristics

The small deformation rheological results of sweet potato starch and flour (10-30%) in distilled water are presented in Table 6.3 and representative graphs are shown in Fig. 6.6. Results indicated very low storage modulus (G') and loss modulus (G'') at 20 °C. This is because sweet potato starch is insoluble in cold water due to strong hydrogen bonds binding the chains together. As the temperature was increased the G' and G'' values increased and this behaviour can be ascribed to the progressive swelling and solubility of starch granules that occurs upon heating (Fig. 6.4 and 6.5). Between 65 and 75 °C there was a sharp rise of G' and G'' values as the medium changed to a system of swollen deformable particles suspended in a more viscous medium contributed by amylose leaching and starch gelatinization. Between 65 and 75 °C at which the consistency was observed to increase was the gelatinisation temperature (72 °C) of starch and sweet potato flour recorded in DSC tests (Fig. 6.7) and also reported by other researchers (Zhang, 1999; Hoover, 2001; Noda et al., 1996; Garcia et al., 1998). On cooling the G' and G'' values increased due to the re-association of hydrogen bonds between the
amylose and amylopectin molecules into an ordered structure with a gel-like consistency (Atwell, et al., 1988); the increase on cooling was higher at a higher flour or starch concentration (Fig 6.5 a, b and c).

There was a noticeable increase in both G' and G'' values with increase in concentration of sweet potato flour samples used (Table 6.3). G' and G'' values being highest for the 30% solutions. This concentration would not be suitable for baby food formulation since babies require soft food to swallow, as they do not have tough muscles and teeth to chew viscous products. Swelling capacity depends on the network density and polymer-solvent interaction. It is reported that rigidity of the swollen granule is related to starch concentration. The total available surface for the interaction among swollen granules in lower concentration (Tz 10% Fig 6.6 a) systems were smaller than in higher concentration systems. Consequently, both G' and G'' of lower concentrations were lower than those of higher concentrations during heating (Cheng et al., 1997).

In general sweet potato flour had lower (G' 208 Pa, G'' 54.5 Pa, Tz) compared to the extracted starch (G' 31375 Pa, G' 7275 Pa, Tz starch). This may be due to the presence of other substances such as lipids, proteins and fibers, which could have competed for water making less water available to the starch in the flour thereby reducing its swelling behaviour.
Table 6.3: Small deformation analysis showing the elastic modulus (G') values and G'' (loss modulus) for freeze-dried and starch and sun-dried flour at 20°C (beginning of the heating cycle) 90 and 20 °C (end of the cooling cycle)

<table>
<thead>
<tr>
<th></th>
<th>Temperature (°C)</th>
<th>Concentration</th>
<th>Tz</th>
<th>Nsp</th>
<th>Spk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Freeze-dried flour</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>10% G' (Pa)</td>
<td>9.2 ± 1.5</td>
<td>27 ± 4.0</td>
<td>52 ± 6.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G'' (Pa)</td>
<td>6.8 ± 1.4</td>
<td>64.7 ± 16</td>
<td>23 ± 4.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 % G' (Pa)</td>
<td>31.4 ± 4.3</td>
<td>240 ± 21</td>
<td>16 ± 4.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G'' (Pa)</td>
<td>12.5 ± 1.5</td>
<td>90 ± 5.2</td>
<td>5.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30% G' (Pa)</td>
<td>544 ± 32</td>
<td>866 ± 68</td>
<td>25 ± 3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G'' (Pa)</td>
<td>126 ± 4.0</td>
<td>533 ± 34</td>
<td>8.8 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>10% G' (Pa)</td>
<td>208 ± 91</td>
<td>1999 ± 30</td>
<td>1084 ± 38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G'' (Pa)</td>
<td>54.5 ± 5.6</td>
<td>737 ± 28</td>
<td>470 ± 82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 % G' (Pa)</td>
<td>342 ± 43</td>
<td>4415 ± 55</td>
<td>2455 ± 218</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G'' (Pa)</td>
<td>80 ± 9.5</td>
<td>740 ± 6.0</td>
<td>2603 ± 74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30% G' (Pa)</td>
<td>28018 ± 3920</td>
<td>14745 ± 936</td>
<td>8154 ± 35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G'' (Pa)</td>
<td>17958 ± 765</td>
<td>4104 ± 163</td>
<td>2689 ± 23</td>
</tr>
<tr>
<td></td>
<td>20 end of cycle</td>
<td>10% G' (Pa)</td>
<td>817 ± 10.3</td>
<td>2196 ± 20</td>
<td>20174 ± 314.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G'' (Pa)</td>
<td>299 ± 0.5</td>
<td>133 ± 12</td>
<td>3590 ± 30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 % G' (Pa)</td>
<td>950 ± 25</td>
<td>7020 ± 235</td>
<td>6722 ± 74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G'' (Pa)</td>
<td>149 ± 11</td>
<td>2550 ± 171</td>
<td>865 ± 74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30% G' (Pa)</td>
<td>69430 ± 646</td>
<td>77675 ± 903</td>
<td>27573 ± 23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G'' (Pa)</td>
<td>13146 ± 459</td>
<td>17840 ± 1056</td>
<td>90043 ± 35</td>
</tr>
<tr>
<td></td>
<td>Starch</td>
<td>20</td>
<td>10% G' (Pa)</td>
<td>1136 ± 22</td>
<td>2574 ± 6.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G'' (Pa)</td>
<td>682 ± 63</td>
<td>756 ± 36</td>
<td>1.6 ± 0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30% G' (Pa)</td>
<td>1228 ± 23</td>
<td>2495 ± 34</td>
<td>8222 ± 192</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G'' (Pa)</td>
<td>567 ± 52</td>
<td>860 ± 60</td>
<td>185 ± 14.4</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>10% G' (Pa)</td>
<td>31375 ± 43</td>
<td>6515 ± 58</td>
<td>11744 ± 530</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G'' (Pa)</td>
<td>7275 ± 187</td>
<td>3060 ± 34</td>
<td>4099 ± 720</td>
</tr>
</tbody>
</table>
Continuation of Table 6.3

<table>
<thead>
<tr>
<th>Starch</th>
<th>Temperature (°C)</th>
<th>Concentration</th>
<th>Tz</th>
<th>Nsp</th>
<th>Spk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30% G' (Pa)</td>
<td>G''(Pa)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
<td>12150 ± 195</td>
<td>3778 ± 33</td>
<td>14659 ± 840</td>
<td>21707 ± 332</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3778 ± 33</td>
<td></td>
<td>5034 ± 188</td>
<td>4480 ± 478</td>
</tr>
<tr>
<td>20 end of cycle</td>
<td>10% G' (Pa)</td>
<td>82586 ± 531</td>
<td>10827 ± 667</td>
<td>6899 ± 52</td>
<td>48157 ± 844</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10827 ± 667</td>
<td></td>
<td>392 ± 7.5</td>
<td>16257 ± 294</td>
</tr>
<tr>
<td></td>
<td>30% G' (Pa)</td>
<td>130780 ± 5220</td>
<td>70200 ± 1800</td>
<td>114589 ± 5000</td>
<td>453398 ± 8593</td>
</tr>
<tr>
<td></td>
<td></td>
<td>130780 ± 5220</td>
<td></td>
<td>49688 ± 922</td>
<td>98231 ± 596</td>
</tr>
<tr>
<td>Sun-dried Flour</td>
<td>20</td>
<td>4330 ± 110</td>
<td>1366 ± 66</td>
<td>2076 ± 64</td>
<td>2101 ± 64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4330 ± 110</td>
<td></td>
<td>841 ± 41</td>
<td>849 ± 49</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>4553 ± 27</td>
<td>3595 ± 102</td>
<td>8078 ± 43</td>
<td>9382 ± 43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4553 ± 27</td>
<td></td>
<td>2804 ± 19</td>
<td>4114 ± 89</td>
</tr>
<tr>
<td></td>
<td>20 end of cycle</td>
<td>6411 ± 49</td>
<td>1150 ± 15</td>
<td>3772 ± 92</td>
<td>6282 ± 158</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6411 ± 49</td>
<td></td>
<td>456 ± 3.5</td>
<td>1311 ± 11</td>
</tr>
</tbody>
</table>
6.3.4 Thermal properties of sweet potato flour and starch

Table 6.4 shows the thermal properties of starch and sweet potato flour (30%) determined by DSC. The representative thermograms are shown in Fig. 6.6. There was no significant difference in gelatinisation temperature and enthalpy change observed between extracted starch and the flours. The results indicate that the number of double helices in the amorphous and crystalline domain (double helices formed between outer branches of amylopectin) that unravel and melt during gelatinisation are similar in all the starches of the three sweet potato varieties. The values of peak temperature referred to gelatinisation temperature ranged between 67 and 72 °C, which is consistent with reported results (Zhang, 1999; Noda et al., 1996; Garcia et al., 1998). The enthalpy change values are reported to be influenced by the structure of the crystalline region and the presence of short amylopectin chain (Hoover, 2001).
Table 6.4: Onset and gelatinisation temperature and enthalpy change for freeze-dried, sun-dried flour and sweet potato starch from the Nasport (Nsp), Tanzania (Tz) and Spk cultivars

<table>
<thead>
<tr>
<th>Sample</th>
<th>Onset temp.</th>
<th>Peak temp.</th>
<th>Enthalpy change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T^o$</td>
<td>$T_p$</td>
<td>$J/\text{g sample}$</td>
</tr>
<tr>
<td>Freeze-dried flour</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nsp</td>
<td>66.56±0.97</td>
<td>69.95±0.03</td>
<td>1.32 ± 0.44</td>
</tr>
<tr>
<td>Tz</td>
<td>67.92±2.19</td>
<td>71.39±1.52</td>
<td>0.94±0.30</td>
</tr>
<tr>
<td>Spk</td>
<td>68.58±0.37</td>
<td>71.00±0.94</td>
<td>0.937±0.15</td>
</tr>
<tr>
<td>Sun-dried flour</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nsp</td>
<td>68.69±0.005</td>
<td>72.13±0.15</td>
<td>0.871±0.005</td>
</tr>
<tr>
<td>Tz</td>
<td>68.74±0.39</td>
<td>71.62±0.14</td>
<td>0.989±0.02</td>
</tr>
<tr>
<td>Spk</td>
<td>67.62±0.03</td>
<td>71.60±0.13</td>
<td>0.854±0.03</td>
</tr>
<tr>
<td>Starch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nsp</td>
<td>62.39±0.42</td>
<td>67.33±0.318</td>
<td>1.517±0.06</td>
</tr>
<tr>
<td>Tz</td>
<td>67.93±0.67</td>
<td>71.12±1.050</td>
<td>0.837±0.02</td>
</tr>
<tr>
<td>Spk</td>
<td>66.36±0.703</td>
<td>69.26±0.16</td>
<td>1.457±0.06</td>
</tr>
</tbody>
</table>
c)

Fig. 6.7: DSC thermograms for a) Spk starch, b) Tz starch, c) Nsp starch 30% (w/w) in distilled water.

6.3.5 Raman spectroscopy of sweet potato and rice starch

The Raman spectra of sweet potato starch and rice starch are shown in Fig 6.8 a, b and the band assignments of the spectra are presented in Table 6.5. The Raman spectra of the starches from sweet potato and rice exhibited similar bands; originating mainly from the vibrational modes of amylose and amylopectin (Dupuy and Laureyns, 2002). However there were some differences observed between sweet potato and rice starch bands; for example the O—H stretch region (3000-3600 cm⁻¹) in sweet potato was higher than that in rice starch. This could be probably due to a high content of water molecule in sweet potato starch. The bands at 615cm⁻¹ in rice was very weak (1.95) (Fig 6.8 a and Table 6.5 b) compared to the one in the sweet potato (Spk) starch (2.65). The bands 616 represent the skeletal mode of the pyranose ring (Corbett et al., 1991; Kizil et al., 2002).
Differences in this region may be as a result of change in the orientation of the glycosidic linkages between the D-glucose residues. There were also differences in the location and intensity of the glycosidic linkage band. The glycosidic linkage band in rice starch was at 933 cm$^{-1}$ while that of sweet potato starch was at 945 cm$^{-1}$ and the intensity was lower in rice starch than in sweet potato starch (Table 6.5 b). The reason for this difference may be attributed to the $\alpha$-1, 6 linkage of the amylopectin that could have shifted the band in sweet potato to the higher wavenumber. Sweet potato has a higher content of amylopectin compared with the rice starch and this could account for the higher intensity observed at band 944 cm$^{-1}$. The finger print region 800-1600 cm$^{-1}$ of sweet potato is identical to rice starch because spectra of starch and other polysaccharides are said to originate mainly from the vibrational state of their monomers the glucose units (Kizil et al., 2002), which dominate the spectral region 1600-800 cm$^{-1}$.
Fig. 6.8: Spectra of (a) rice and (b) sweet potato starch (Spk) respectively between 3600 and 100 cm$^{-1}$
## Table 6.5 a: Band assignments for the starches

<table>
<thead>
<tr>
<th>Wave number cm$^{-1}$</th>
<th>Raman Band assignment</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>3000-3600</td>
<td>O—H stretching</td>
<td>Kizil et al, 2002</td>
</tr>
<tr>
<td>2934, 2913</td>
<td>C—H stretching</td>
<td>Corbett et al., 1991</td>
</tr>
<tr>
<td>1461</td>
<td>CH$_2$ bending</td>
<td></td>
</tr>
<tr>
<td>1389</td>
<td>C—H and C—O—H deformation</td>
<td>Santha et al., 1990</td>
</tr>
<tr>
<td>1349</td>
<td>C—O—H bending and CH$_2$ twist</td>
<td>Corbett et al., 1991</td>
</tr>
<tr>
<td>1264</td>
<td>CH$_2$OH side chain</td>
<td></td>
</tr>
<tr>
<td>1127</td>
<td>C—O stretching, C—O—H</td>
<td></td>
</tr>
<tr>
<td>1110</td>
<td>C—O</td>
<td>Kizil et al., 2002</td>
</tr>
<tr>
<td>1083</td>
<td>C—O—H</td>
<td></td>
</tr>
<tr>
<td>1052</td>
<td>C—H bend</td>
<td></td>
</tr>
<tr>
<td>941</td>
<td>Skeletal Vibrations of α-1,4 glycosidic linkage</td>
<td>Corbett et al., 1991</td>
</tr>
<tr>
<td>866</td>
<td>CH$_2$ deformation</td>
<td>Santha, 1990</td>
</tr>
<tr>
<td>770</td>
<td>CO side chain</td>
<td>Corbett et al.,</td>
</tr>
<tr>
<td>616, 568, 478,</td>
<td>Skeletal modes of</td>
<td>Cael et al., 1975</td>
</tr>
<tr>
<td>441, 410, 203,</td>
<td>pyranose ring</td>
<td></td>
</tr>
<tr>
<td>194</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6.5 b: Intensity values at selected regions of the F-T Raman spectra of rice and sweet potato starch

<table>
<thead>
<tr>
<th>Peak assignment</th>
<th>Rice starch</th>
<th>Sweet potato starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>O—H stretching</td>
<td>1.05 ± 0.01 (3383)</td>
<td>1.43 ± 0.13 (3383)</td>
</tr>
<tr>
<td>C—H stretching</td>
<td>6.58 ± 0.21 (2922)</td>
<td>8.92 ± 0.99 (2923)</td>
</tr>
<tr>
<td>CH₂ bending</td>
<td>3.57 ± 0.8 (1462)</td>
<td>5.20 ± 0.62 (1463)</td>
</tr>
<tr>
<td>C—O stretching, C—O—H</td>
<td>5.12 ± 0.94 (1136)</td>
<td>6.88 ± 0.98 (0.98)</td>
</tr>
<tr>
<td>α-1,4 glycosidic linkage</td>
<td>4.10 ± 0.95 (933)</td>
<td>5.42 ± 0.89 (944)</td>
</tr>
<tr>
<td>Skeletal modes</td>
<td>1.95 ±0.00 (615)</td>
<td>2.68 ± 0.01 (615)</td>
</tr>
<tr>
<td>of pyranose ring</td>
<td>14.18 ± 0.86 (477)</td>
<td>18.82 ± 1.70 (478)</td>
</tr>
</tbody>
</table>

Figures in parenthesis are wavenumbers cm⁻¹.
6.4 Conclusion

This study showed some differences among the sweet potato varieties in relation to rheological characteristics. For example, the viscosity of Tz and Spk on heating and cooling was not very high compared with that of Nsp variety. These two varieties would be more useful in baby food formulation. Sweet potato flour and the starch exhibited different rheological characteristics. The G' values for starch were higher than for flour; this could be attributed to the presence of other components that could have competed for water thereby affecting the swelling behaviour of the starch in the flour. The results also reveal that rheology depends on the solubility and swelling of starch in water which are also dependant on temperature. Characterisation of sweet potato starch can be achieved by Raman spectroscopy. The skeletal region (below 950 cm\(^{-1}\)) may be useful in the differentiation of sweet potato starch from other starches.
CHAPTER SEVEN
7.0 PHYSICOCHEMICAL AND RHEOLOGICAL PROPERTIES OF SWEET POTATO FLOUR AND PROTEINS IN MIXED SYSTEMS

7.1 Introduction

The structure, rheological and physicochemical properties (flow, stability, texture, mouthfeel and taste) of many processed and convenience foods are determined to a large extent by the behaviour of the macromolecular proteins, polysaccharides and lipids components. Foods are always multicomponent systems and it is therefore of great interest to consider the properties of these mixtures in addition to the properties of the separate components (Tolstoguzov, 1986; Howell, 1992).

When two different polymers are mixed, they will interact with each other either by separating (segregative or antagonistic effects) or by combining together (associative or synergistic effects) (Rao, 1999; Zasypkin et al., 1997; Howell, 2000). These properties depend on the compatibility of the components in the system.

Segregative effects occur when the two biopolymers mixed are structurally incompatible and when there is a net repulsion between the biopolymers resulting in two phases, each being enriched with one of the two biopolymers (Ledward, 1993; Schmitt et al., 1998; Comfort & Howell, 2002). Phase separation occurs under conditions such as high ionic strength and specific pH conditions with respect to the isoelectric point, which inhibit interactions between macromolecules of different types (Doublier et al., 2000). Phase separation can also exist between macromolecules that are dissimilar in structure just like in a busy cocktail party chairs are separated from visitors to allow freedom for the visitors to interact (Comfort & Howell, 2002); the macromolecules are in different phases due to limited
thermodynamic compatibility of biopolymers in aqueous media (Ledward, 1993; Kruif & Tuinier, 2001). The presence of charged groups on the polymers often has a major influence on the phase behaviour. In particular, if one biopolymer is charged, the molecules start to interact and order, leading to suppression of phase separation (Piculell et al., 1995).

Associative or synergistic effect in mixed polymers occur when two components are structurally compatible with each other (Howell & Lawrie, 1985; Howell, 1995; Ngarize et al., 2004; Ravindran et al., 2004) that results in an effect which is greater than that produced by each macromolecule in isolation. The effect manifests itself in greater viscosity, cohesiveness or gel strength values for instance protein-protein between plasma proteins and egg albumen (Howell & Lawrie, 1985) or whey-egg albumen mixtures (Ngarize et al., 2004). Alternatively there may be protein-polysaccharide interaction such as those between lysozyme-alginrate mixture (Howell et al., 1998).

Protein-polysaccharide interactions play an important role in the structure and stability of many processed foods. Some studies indicate that gelling and functional properties of food proteins are modified in the presence of other biopolymers such as polysaccharides and lipids as a result of interaction (Howell, 2000; Yufei et al, 2003). Protein-polysaccharide interaction could be a simple and safe way of modifying the functional properties of proteins. For example, soya protein and polysaccharide (non-starch) mixture have been used in the manufacture of imitation cheeses (Yang et al., 1983) and some studies have shown that the addition of polysaccharide increases the water binding capacity of soya protein (Sanchez et al., 1995) and also improves
textural capacity of tofu (Karim et al., 1999). Similarly, addition of different starches to fish protein resulted in desirable gelling and textural properties of surimi products (Wu et al., 1985; Groove & Barbosa, 1996); the mechanism of interaction between starch and fish protein is proposed to be due to swelling and water uptake of starch during gelatinisation (Lee & Kim, 1985); the starch granules expand to a considerable degree and distribute through the fish protein network. Expansion of the starch granules then causes pressure on the protein matrix and this together with moisture withdrawal from the surrounding protein results in a firmer and more cohesive gel (Lee & Kim, 1985; Aguilera & Rojas, 1996). Cheow et al. (2001) found that the viscoelastic properties of keropok (fish cracker/snack in south eastern Asia) increased with increasing fish protein to cassava starch ratio due to the formation of a fish protein network in the dough.

Although sweet potato can provide energy in the diet, it is lacking in proteins and lipids (Nandutu & Howell 2004). Therefore the rheological properties and application of sweet potato in combination with other proteins is important for producing nutritionally balanced food products. In this study the effect of mixtures of sweet potato flour and soya and fish proteins on the rheological and thermal properties was investigated.
7.2 Methods and materials

7.2.1 Materials

Sweet potato Tanzania variety (Tz) was obtained from Uganda. The sweet potato was freeze-dried and milled into a powder. Tilapia fish (*Oreochromis niloticus*) was obtained from a local supermarket, Guildford and used on the day of purchase. Soya protein isolate (SUPRO 760 IP Non-GM) was obtained from the Solae Company and had the following composition, moisture (5.5%), protein dry basis (86%) and fat (1%).

7.2.1.1 Sample preparation

Soya isolate and sweet potato (sp) powders were weighed into a 20 ml beaker and an appropriate quantity of distilled water added. The mixture was subjected to high-speed homogenisation for 3 min to ensure thorough mixing between soya protein and sweet potato flour. Sp and soya protein isolate were mixed in the following ratios; (20% soya: 0 sp, 15 soya: 5% sp, 10% soya: 10% sp, 5% soya: 15% sp, 0 soya: 20% sp, 10% soya: 0 sp, 6% soya: 4 sp, 4% soya: 6% sp and 10% sp) in water. The fish and sp or soya were mixed in the following ratio (20% fish: 0 sp, 15% fish: 5 sp, 10% fish: 10% sp, 5% fish: 15% sp) in water and homogenised at 4°C. These samples were used for both rheological and differential scanning calorimetry studies as described below.

7.2.2 Rheological measurements

Samples prepared as described above were tested on a Rheometrics controlled stress rheometer 200 using a 40 mm parallel plate geometry with a gap of 1 or 2.0 mm.
Silicone oil was applied to prevent evaporation during heating. Stress sweep in the range 1 to 100 Pa was first conducted at room temperature (25°C) at a frequency of 1.0 rad s\(^{-1}\) to determine the linear viscoelastic region, necessary for the subsequent frequency and temperature sweep. The frequency sweep in the range of 0.1 to 100 rad s\(^{-1}\) was conducted at stress 1.0 Pa at room temperature. The temperature of the Peltier plate was programmed to ramp at a rate 1 °C min \(^{-1}\) from 20 to 90°C and then cooled to 20 °C at the same rate. A frequency of 1 rad s\(^{-1}\) was used and the applied stress was 1.0 Pa. The G' and G'' values were noted at 20 °C before heating, at 90 °C and 20 °C after cooling.

7.2.3 Differential scanning calorimetry

The differential scanning calorimetry (DSC) measurements were carried out on (Setaram) DSC instrument using samples prepared as described above. The samples were weighed (0.8 g) into stainless steel containers, sealed and heated from 20 to 100 °C at a rate of 0.1° C/min. Distilled water adjusted to within ±0.1 g of the same weight as the sample was used as a reference. The onset temperature, peak temperature and enthalpy of gelatinisation or denaturation were determined by using a data analysis system (Setaram DSC 3.3).

7.2.4 Phase contrast microscopy

7.2.4.1 Principle
In phase contrast microscopy samples are not stained in order to distinguish their characteristics. The method is based on the observation that changes in the phase of emergent light are caused by either diffraction or by changes in refractive index of the material within the specimen or even by differences in the thickness of specimen. At the point of focus the converging light rays show interferences resulting in either
increases or decreases in the amplitude of the resultant wave, which the eye detects as differences in brightness.

7.2.4.2 Materials
The samples used in this study included sweet potato 20%; mixture of sweet potato flour and soya isolate (20%) mixed in the ratio of 1:1 and a mixture of sweet potato flour and fish (20%) in the ratio 1:1.

7.2.4.3 Procedure
A small drop (4 µl) of the prepared samples above were placed on a microscopic slide and covered with a cover slip. The slides were examined at ×100 using a phase contrast microscope connected to a digital camera.

7.3 Results and discussion
7.3.1 Small deformation studies
The rheological results are summarised in Table 7.1 and some representative temperature sweeps are shown in Fig 7.1. There was an increase of both $G'$ (elastic or storage modulus) and $G''$ (loss modulus) on heating sweet potato suspension (20%) in water. The initial value of elastic modulus ($G'$) was low (263 ± 38 Pa) indicating that sweet potato flour does not interact with water at room temperature. At higher temperature, the $G'$ gradually increased and a significant increase was observed at 71°C, the gelatinisation temperature of starch. Most starches are known to gelatinise between 60 and 70 °C and sweet potato starch is reported to gelatinise between 68-72 °C (Garcia et al., 1998). The swelling power and the solubility index of starch
increased on heating following a further increase in $G'$ and $G''$ on cooling as starch precipitated and retrograded (Banks & Greenwood, 1975).

Soya protein isolate (20%) dispersions heated to 90 °C showed a steady decrease in $G'$ with heating to about 70 °C followed by an increase in $G'$ upon further heating to 90 °C. Elastic modulus ($G''$) of soya protein in water was higher than $G''$ throughout heating suggesting solid-like behaviour that did not change substantially with temperature; thus indicated there was little tendency for protein molecules to undergo cross-linking on heating. The soya protein isolate showed one major peak at denaturation temperature 83°C (Fig. 7.2 f) which corresponds to denaturation temperature of 11S fraction (Hermansson, 1978). However, on cooling there was an increase in $G'$ due to cross-linking by hydrophobic and other non-covalent interactions.

On heating fish paste, $G'$ and $G''$ increased significantly. This is due to hydration and swelling of fish proteins. For both proteins there was an increase in $G'$ values on cooling as unfolded molecules underwent cross-linking due to hydrophobic and covalent interactions.

Mixtures of sp (5%) and 15% soya or 8% sp and 12 % soya behaved similarly to pure soya protein isolate in water, indicating that sweet potato flour did not have any effect on rheological properties of soya protein isolate. This was probably because the protein network was formed before that of starch and the leaking out of amylose from the starch granules might have been hindered by the aggregating protein molecules, thus reducing the swelling of the starch (Muhrbeck & Eliasson, 1991). On increasing
the concentration of starch to 15%, the $G'$ and $G''$ increased with temperature behaving similarly to pure sweet potato flour in water. A 1:1 mixture of soya protein isolate and sweet potato behaved differently from the above. There was an initial decrease of $G'$ with increasing temperature until at about 80°C when the $G'$ began to increase. There was a further increase on cooling leading to a formation of a rigid gel whose $G'$ value was higher than that of soya protein isolate and sweet potato flour on their own after cooling cycle. This may be due to phase separation of the two components whereby each phase is concentrated and results in a higher $G'$ values compared with each component measured in isolation. This could also be due to combined effect of both soya gelation and starch gelatinisation and might be as a result of unfolding soya protein molecules penetrating the amylose aggregates during heating (Muhrbeck & Eliasson, 1991). In this way the mixed systems form a network that is stronger compared to the pure systems.

For the mixed system of fish and soya, (1:1) the $G'$ and $G''$ were high initially and reduced steadily on increasing the temperature. On cooling, $G'$ increased gently to almost the initial value. Overall there was a small change of $G'$ on both heating and cooling. The gels formed at the end of the cycle are quite weak. This could be useful in making products such as infant food because babies require soft food for easy swallowing or to avoid choking. Furukawa et al. (1982) reported a synergistic interaction between fish and soya and a similar case was reported between egg-white and soya protein (Jao, 1980). This observation was contrary to what was observed in this study. The result in this study is in agreement with findings by Howell (1992) that soya has the tendency of reducing gel strength of plasma and egg proteins. The tendency for this kind of interaction may be due to differences in molecular weight
and structure (Howell, 1995) and probably processing and extracting soya proteins
could have significantly altered interactive behaviour.

Table 7.1: Small deformation analysis showing the elastic modulus (G') values
for sweet potato (sp) in water and mixtures of sp with soya or fish proteins at
20°C (beginning of the heating cycle), 90°C and 20°C (end of the cooling cycle)

<table>
<thead>
<tr>
<th>Samples</th>
<th>G' (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20°C beginning of</td>
</tr>
<tr>
<td></td>
<td>heating cycle.</td>
</tr>
<tr>
<td>Sweet potato 20%</td>
<td></td>
</tr>
<tr>
<td>20% soya</td>
<td>263 ± 38</td>
</tr>
<tr>
<td>5% sp + 15% soya</td>
<td>2285 ± 203</td>
</tr>
<tr>
<td>8% sp + 12% soya</td>
<td>2729 ± 596</td>
</tr>
<tr>
<td>10% sp + 10% soya</td>
<td>2075 ± 62</td>
</tr>
<tr>
<td>15% sp + 5% soya</td>
<td>709 ± 180</td>
</tr>
<tr>
<td>Fish 20%</td>
<td>38 ± 2</td>
</tr>
<tr>
<td>10% fish + 10% soya</td>
<td>262 ± 15</td>
</tr>
<tr>
<td>5% fish + 15% soya</td>
<td>845 ± 66</td>
</tr>
<tr>
<td>10% sp + 10% fish</td>
<td>1504 ± 332</td>
</tr>
<tr>
<td></td>
<td>76 ± 16</td>
</tr>
</tbody>
</table>
Mixtures of sweet potato flour and fish indicated some association on heating particularly at the 1:1 ratio (20%). The $G'$ increased on heating and a further increase was observed on cooling. The $G'$ value for the mixture was higher than $G'$ for the individual samples (fish or sweet potato) on their own. It is possible that during heating the starch granules expanded as a result of imbibing water from the surrounding and distributed through the fish protein network which caused pressure on the protein matrix and this together with moisture withdrawal from the surrounding protein resulted in a more cohesive gel (Lee & Kim, 1985; Cheow et al., 2001).
Fig 7.1: Temperature sweep of mixtures of (a) sweet potato and soya protein isolate (20%) (b) sweet potato and fish (20%) and (c) soya protein and fish (20%) in distilled water. All the mixtures were in the ratio of 1:1.

DSC results obtained for the sweet potato flour in water and the mixtures with soya and fish proteins are given in Table 7.2 and representative thermograms are shown in Fig.7.2. Sweet potato flour indicated a cooperative gelatinisation temperature at 71°C
and was similar and dominant in all the mixed systems (soya + sp and sp + fish) and the presence of soya or fish protein did not affect the starch thermal properties. A mixture of 1:1 (10% sp and 10% fish) gave two transitions; a low peak at 49 °C and a sharp cooperative transition at 72 °C representing denaturation temperature of fish proteins (Badii & Howell, 2002) and gelatinisation temperature of starch respectively (Fig 2c). This behaviour indicated that the gelatinisation of starch was unaffected by the presence of fish proteins. Increasing starch concentration resulted into a thermogram with only one peak at a temperature of 71°C (Table 7.2) obscuring the contribution of the denaturation peak of fish protein or soya protein. The DSC results indicate that sweet potato starch and fish or soya proteins act independently of each other during processing In contrast, the mixed system of fish (10% w/w) and soya (10% w/w) protein indicated a reduced denaturation temperature (44°C) and the enthalpy compared with those of fish alone (Fig. 7.2e) suggesting that soya protein might have had influence on the thermal properties of fish.
### 7.3.2 Differential scanning calorimetry

**Table 7.2: Thermodynamic properties of sweet potato flour and mixtures with soya and fish proteins by differential scanning calorimetry**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Onset temperature °C</th>
<th>Peak temperature °C</th>
<th>Enthalpy change (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweet potato (sp) 20%</td>
<td>68.86</td>
<td>71.64</td>
<td>0.686</td>
</tr>
<tr>
<td>6% sp + 4% soya</td>
<td>70.09</td>
<td>71.74</td>
<td>0.195</td>
</tr>
<tr>
<td>6% soya + 4% sp</td>
<td>67.68</td>
<td>70.57</td>
<td>0.137</td>
</tr>
<tr>
<td>10% soya + 10% sp</td>
<td>67.91</td>
<td>70.78</td>
<td>0.292</td>
</tr>
<tr>
<td>Fish 20%</td>
<td>47.85</td>
<td>50.00</td>
<td>0.086</td>
</tr>
<tr>
<td>5% fish + 15% sp</td>
<td>68.20</td>
<td>71.27</td>
<td>0.606</td>
</tr>
<tr>
<td>10% fish +10% sp peak 1</td>
<td>48.17</td>
<td>49.20</td>
<td>0.022</td>
</tr>
<tr>
<td>,,</td>
<td>70.14</td>
<td>72.70</td>
<td>0.460</td>
</tr>
<tr>
<td>6% soya + 4% fish</td>
<td>41.39</td>
<td>44.69</td>
<td>0.025</td>
</tr>
<tr>
<td>20% soya</td>
<td>77.92</td>
<td>82.34</td>
<td>0.048</td>
</tr>
</tbody>
</table>

All values are mean of duplicates.
Fig. 7.2: DSC thermograms for a) 10% sweet potato flour, b) 20% fish, c) mixture of sweet potato and fish (10%sp:10% fish), d) a mixture of sweet potato and soya (10% : 10%) and e) a mixture of soya and fish (10%: 10%), f) soya isolate 20% in distilled water. In Fig 7.2 c 1 refes to the transition for fish proteins and 2 refers to the gelatinisation peak for sweet potato.
7.3.3 Phase contrast microscopy

The phase contrast micrographs of sweet potato starch and protein mixed systems are shown in the Fig 7.3. The photomicrographs of mixed systems (sp + soya, sp and fish) demonstrated separated phases, which is in agreement with findings of other researchers (Tolgostoguv, 1985; Braudo et al., 1986; Howell et al., 2000). They reported that most protein-polysaccharide systems tend to form separated solutions when mixed at neutral pH. Soya proteins tended to aggregate as did the starch granules (Fig. 7.3 b and c). The tendency for each polymer to self-associate has been reported by Comfort & Howell (2001).
Fig. 7.3: Phase contrast microscopy (x100) of (a) sweet potato flour 5% (b) mixture of sweet potato and soya (1:1) and (c) mixture of fish and sweet potato flour (1:1) ratio.
7.4 Conclusion

The mixtures of soya and fish, and soya and sweet potato separated in two phases as shown by phase contrast microscopy and DSC results. This incompatibility may be due to differences in the molecular weight of the macromolecules. Theoretically interactions are most likely between polymers of similar molecular weight and most likely between polymers, which have a similar affinity for the solvent in this case water. This behaviour of phase separation may be useful in the formulation of soft products such as infant food.
CHAPTER EIGHT
8.0 NUTRITIONAL AND RHEOLOGICAL PROPERTIES OF SWEET POTATO BASED INFANT FOOD AND ITS PRESERVATION USING ANTIOXIDANTS

8.1 Introduction

The weaning period is the most critical one in the life of an infant and pre-school children. During this period (3-6 months to 3 yrs) breast milk no longer meets the nutritional demands of the child. In response to this problem, weaning foods are usually given to infants (Morgan et al., 1984; DoH, 1995). In Uganda the mothers wean their infants mainly on cereal-based foods; these are often bulky and have low energy and nutrient density with a high antinutrient activity (phytic acid and fibre). Besides this problem, cereals are in high demand for alcohol production, as food for the adults and as source of income (cash crop); this has made cereals very expensive and exacerbated malnutrition. To reduce the incidence of malnutrition, cereal substitutes of raw material have to be used such as tubers or roots. Sweet potato was selected in this study as a potential raw material for infant food formulation because; it is adaptable to diverse environment, has high yields, is available all year round; it is cheap to grow and is a very good source of carbohydrate and β-carotene (Nandutu & Howell, 2004). Sweet potato incorporated with legumes or fish and oil can produce a nutritious product, provided it is protected from spoilage.

Foods especially fatty fish and oils often go rancid as a result of lipid oxidation. Lipid oxidation results in products such as aldehydes, hydroxides, hydrocarbons, which contribute to off flavours, bad taste, change in texture and the nutritive value of the food (Braddock et al., 1995; Fernandez et al., 1997; McCarthy et al., 2001; Bekhit et al., 2003) which may be toxic. To stabilise the lipid containing food
product, antioxidants are often used to reduce fat oxidation (Frankel, 1993; Karpiriska et al., 2001). Antioxidants work by scavenging the active forms of oxygen involved in the initiation step of oxidation, or can break the oxidative chain reaction by reacting with the fatty acid peroxy radicals such as (ROO\(^*\)), to form stable antioxidant radicals, which are insufficiently reactive to continue the chain reaction.

\[
AH + ROO^* \rightarrow ROOH + A^*
\]

They appear to enter the termination chain by reacting with lipid radical or with each other

\[
A^* + A^* \rightarrow A-A \quad \text{or}
\]

\[
A^* + ROO^* \rightarrow ROO-A
\]

Where, \(A^*\) is antioxidant radical, \(ROO^*\) is lipid peroxy radical

The antioxidants can be synthetic or natural. Synthetic antioxidants include propyl gallate, butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroxyquinone (BHTQ). Consumers are concerned about the health risks related to the consumption of some of these synthetic antioxidants, as some laboratory studies with animals have hinted at carcinogenicity of BHA and BHT (Attmann et al., 1986 & Powell et al., 1986). Although the issue is far from being resolved there is growing pressure for their withdrawal from use. Hence research is now focusing on the use of natural antioxidants such as vitamin E, ascorbic acid and other plant phenolic substances (Ahn et al., 1993).
8.1.1 Vitamin E (Tocopherol)

Vitamin E is a group of derivatives of 6-hydroxychroman carrying an isoprenoid side chain synthesized by plants. These compounds fall into two classes: tocopherols and tocotrienols. The isoprenoid side chain in the tocotrienols is unsaturated while in the tocopherols the chain is saturated (Fig 8.1). Each class comprises the four vitamers, which differ in the number and position of methyl groups on the chromanol ring as shown in the Fig. 8.1 (Garrow et al., 2000). Numerous studies have demonstrated tocopherol as an effective antioxidant in food and biological systems. It is believed that α-tocopherol is an extremely efficient inhibitor of free radical chain reactions (Qureshi et al., 2000). Alpha-tocopherol is a low molecular weight lipid soluble primary chain breaking antioxidant in free radical chain reactions. It can convert the lipid radicals to more stable products (Kishowar et al., 2004) thus extending the shelf life of the food product. Tocopherols through their chromanol moiety can donate a phenolic hydrogen to a lipid peroxy radical to form a resonance stabilized chromanoxyl radical which in turn reacts with other radicals to form stable adducts and therefore terminates the free radical chain reaction (Kamal-Edin & Appelqvist, 1996).
Fig. 8.1: Structure of (a) tocopherol and (b) tocotrienols adapted from Garrow et al., 2000

### 8.1.2 L-Ascorbic acid (Vitamin C)

Ascorbic acid is found in fruits and vegetables. Vitamin C can act as an antioxidant by scavenging free radicals resulting in the formation of low energy ascorbate radicals, which can be reversibly reduced to ascorbic acid or oxidized to form dehydroascorbic acid (Bendich et al., 1986). In addition to reacting directly with aqueous peroxyl free radicals, ascorbic acid is involved in the regeneration of \( \alpha \)-tocopherol by donating an electron to tocopheroyl radical restoring antioxidant potential to it. Because of its strong reducing potential, ascorbic acid can catalyse the reduction of transition metals, which in turn can react with lipid peroxides to
form radicals (Buettner, 1993). Hence ascorbic acid can exhibit pro-oxidative activity in the presence of transition metals (Cu$^{2+}$ and Fe$^{3+}$).

\[
\begin{align*}
\text{Fe}^{3+} + \text{ascorbate} & \rightarrow \text{Fe}^{2+} + \text{ascorbate} ' \\
\text{Cu}^{2+} + \text{ascorbate} & \rightarrow \text{Cu}^{+} + \text{ascorbate}' \\
\text{Fe}^{2+} + \text{ROOH} & \rightarrow \text{ROO}' + \text{Fe}^{3+} \\
\text{Cu}^{+} + \text{ROOH} & \rightarrow \text{ROO}' + \text{Cu}^{2+}
\end{align*}
\]

8.1.3 Citric acid

Citric acid is a metal chelator in food. Chelators, which exhibit antioxidative properties inhibit metal catalysed reactions by preventing metal redox cycling; occupying of all metal coordinate sites; formation of insoluble metal complexes or steric hinderance of interaction between metals and lipids (Decker, 1998). Citric acid is water and lipid soluble thus allowing it to inactivate metals in the lipid phase. Combination of chelators and antioxidant will result in increased inhibition of lipid oxidation. Chelators can also decrease the metal-reducing activity of antioxidant such as ascorbate thereby increasing the antioxidant activity of ascorbate (Nawar, 1996).

8.1.4 Measurement of lipid oxidation

Several chemical or sensory techniques are used to monitor oxidation of foods to predict their shelf life stability. Of all the methods in use, the measurement of primary (peroxides) and secondary oxidation products (aldehydes, hydrocarbons and ketones) are the most common. In this study a combination of peroxide value, TBARS and hexanal measurements were used to provide a fuller picture of the progress of oxidation.
8.1.5 Peroxide value

Peroxide value is used to measure the primary oxidation products of lipids (hydroperoxides). The method is based on the iodometric titration, which measures the iodine produced from potassium iodide by the peroxide present in the food or oil.

8.1.6 Thiobarbituric acid (TBA) test

TBA is the most widely used method for measuring the extent of lipid oxidation in foods (Harsret, 2004; Gomes et al., 2003; Raharjo & Sofos, 1993). The TBA method measures the level of aldehyde particularly malondialdehyde, which is formed as a result of oxidation of polyunsaturated fatty acids (PUFA). Thiobarbituric acid reacts with malondialdehyde to give a red chromogen, which is determined by HPLC or by spectrophotometry at wavelength 532-535nm. However, this method is not specific for MDA and reaction with a wide variety of other products may contribute to the increase in absorbance. For instance TBA reacts with other aldehydes such as 2,4 alkadienals; proteins, Maillard browning products, urea and sugars to form red chromogen (Guillen-Scans & Guzman-Chozas, 1998). The usefulness of the TBA test in practice outweighs its nonspecificity. In this study a number of modifications were made to overcome some of the limitations of this method. The TBARS were monitored by the HPLC to separate the MDA-TBA compound from other interfering substances. Before heating, BHA was added to inhibit oxidation during heating. The aims of this study were to formulate a dried infant food product using sweet potato; to investigate the nutritional and rheological properties of the product
and to study the effects of antioxidants (Vitamin E and C) on lipid oxidation in the product on storage.

8.2 Materials and Methods

8.2.1 Formulation of weaning food

The formulation of weaning food was carried out using the Microdiet programme 2000, a nutrition database, which provides information for a list of foods for over sixty nutrients as well as energy ratio, amino acid and fatty acid values. The composition of the food in the Microdiet programme is based on the McCance and Widdowsons (4th and 5th edn., 2000). Different recipes were prepared with freeze-dried sweet potato, fish (Tilapia skinned fillets), sunflower oil, milk and water to make a number of products (100) g. Each recipe was adjusted using the Microdiet 2000 programme to achieve a product that would comply with FAO/WHO guidelines for infant foods (Table 8.4).

The ingredients were weighed and placed in an omni-mixer, homogeniser (Camlab Ltd, Waterbury, C.T, U.S.A) and blended into a soft paste. The paste was transferred to a clean pyrex dish and cooked in a microwave oven on full power for six minutes and mixed after every two minutes to ensure no lumps were formed. Prior to cooking, antioxidants were added particularly to the recipes that contained fish since it was reported by Badii & Howell (2002) that about 50% of the antioxidants are destroyed on cooking. Besides, fish deteriorates so fast that it is necessary to add the antioxidant before cooking to avoid oxidation of fish oil. Two recipes of the weaning food were prepared as indicated in Table 8.1. For each recipe four samples weighing 1 kg each were prepared.
Table 8.1: Recipes for baby food prepared according to the Microdiet program

<table>
<thead>
<tr>
<th>Recipe A</th>
<th>weight (g)</th>
<th>Recipe B</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweet potato</td>
<td>128</td>
<td>Sweet potato</td>
<td>80</td>
</tr>
<tr>
<td>Soya flour</td>
<td>85</td>
<td>Soya flour</td>
<td>41</td>
</tr>
<tr>
<td>Tilapia</td>
<td>40</td>
<td>Skimmed dry milk</td>
<td>41</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>15</td>
<td>Sunflower oil</td>
<td>28</td>
</tr>
<tr>
<td>Water</td>
<td>732</td>
<td>Water</td>
<td>810</td>
</tr>
</tbody>
</table>

To each of the samples (1 kg) of recipe A, was added antioxidants as follows:

Table 8.2: Antioxidants added to recipe A

<table>
<thead>
<tr>
<th>Sample</th>
<th>Vit C (mg/kg)</th>
<th>Vit E (mg/kg)</th>
<th>Citric acid (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ii</td>
<td>200</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>Iii</td>
<td>350</td>
<td>350</td>
<td>100</td>
</tr>
<tr>
<td>iv</td>
<td>500</td>
<td>500</td>
<td>100</td>
</tr>
</tbody>
</table>

For recipe B, the antioxidants added are as follows;
Table 8.3: Antioxidants added to recipe B

<table>
<thead>
<tr>
<th>Sample</th>
<th>Vit C (mg/kg)</th>
<th>Vit E (mg/kg)</th>
<th>Citri acid (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>III</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>IV</td>
<td>250</td>
<td>250</td>
<td>100</td>
</tr>
</tbody>
</table>

After cooking, the samples were left to cool and antioxidants were added as above. The cooled samples were then frozen at -80 °C and freeze-dried. The freeze-dried samples were ground into a fine powder and stored into sealed polyethylene bags at room temperature (22°C) to simulate the conditions of storage in developing countries. Some of the samples without antioxidants were used for proximate compositional analysis of the food, *in vitro* digestibility and rheological studies. The composition and *in vitro* digestibility were determined as described in chapter 3.

Table 8.4: Macronutrient energy guidelines for infant food

<table>
<thead>
<tr>
<th>FAO/WHO guidelines (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat-energy ≤ 40</td>
</tr>
<tr>
<td>Protein-energy ≥ 10</td>
</tr>
<tr>
<td>Carbohydrate-energy ≤ 40</td>
</tr>
</tbody>
</table>

Adapted from FAO/WHO Codex Alimentarius: Codex standard, 2003
8.2.2 Rheological measurements

Food samples (formulated and commercial) were tested on rheometrics controlled stress rheometer 200 using a 40 mm parallel plate geometry with a gap of 1.0. Silicone oil was applied to prevent evaporation during heating. The temperature of the peltier plate was programmed to ramp at a rate 1 °C min$^{-1}$ from 20 to 90°C and then cooled to 20°C at the same rate. A frequency of 1 rad s$^{-1}$ was used and the applied stress was 1.0 Pa. The G' and G'' values were noted at 20 °C before heating, at 90 °C and 20 °C after cooling.

8.2.3 Measurement of lipid oxidation

8.2.3.1 Lipid extraction from freeze-dried infant Food

Lipids were extracted from the formulated infant food by the Bligh & Dyer (1959) method as modified by Saeed & Howell (1999). Portions of the food (20 g) were homogenised for 2 minutes with a mixture of 20 ml chloroform and 40 ml methanol. A further 20 ml of chloroform were added and the mixture was homogenised for 1 min. An additional 20 ml of water was added followed by 20 ml 1% NaCl and homogenised again for 1 min. The final mixture was then centrifuged at 3000 × g for 10 min, the upper layer was pipetted off. The lower layer and the residue were mixed and the filtered into a clean flask. This chloroformic extract was used for PV and TBARS analysis. A portion of this filtrate (5 ml) was removed and placed in a beaker and chloroform evaporated off; the solution left in the beaker was the extracted lipid. The weight of lipid in 5 ml solution was measured and this value was used in the calculation of the peroxide value.
8.2.3.2 Peroxide value determination

Reagents used

The reagents included sodium thiosulphate, starch, potassium iodide, glacial acetic acid and chloroform, which were purchased from Sigma-Aldrich Company Ltd, Poole, England.

Procedure

A chloroformic layer (5 ml), obtained during lipid extraction was evaporated under oxygen free nitrogen gas in triplicate. Chloroform (10 ml) was added to the evaporated oil and washed into a 250 ml conical flask. Acetic acid (15 ml) and freshly prepared saturated potassium iodide solution was added. The mixture was stoppered and swirled gently for about a minute. Distilled water 75 ml was added followed by 1 ml starch (1%) indicator solution. This solution was then titrated against 0.002 N sodium thiosulphate solution until the blue-black colour disappeared. A blank titration was also carried out. The peroxide value was calculated as follows:

\[ PV = \frac{(PV \text{ titre} - PV \text{ blank}) \times T \times 1000}{\text{Weight of fat used}} \]

Where PV titre = ml of sodium thiosulphate solution used in sample

PV Blank = ml of sodium thiosulphate used in blank

T = concentration of thiosulphate solution
8.2.3.3 Thiobarbituric acid reactive substances (TBARS)

Reagents

Tetraethoxypropane, BHA 0.2% in ethanol, HCl 25% v/v, thiobarbituric acid 1% in 50 mM sodium hydroxide were purchased from Sigma-Aldrich company Ltd, Poole, England.

Procedure

Standard solutions of TEP were prepared to give the dilutions containing the equivalent of 10, 1, 0.8, 0.6, 0.4, and 0.2 μg/ml malondialdehyde. To 1 ml of each of the standard solutions was added: 100 μl 0.2% BHA, 500 μl of 25% HCl, 500 μl 1% TBA and vortexed for 20 seconds. Chloroformic extract (200 μl) was pipetted into a 10 ml screw cap tube. BHA (100 μl), 150 μl of 25% HCl, 150 μl of 1% TBA and 750 μl of distilled water were added and vortexed for 20 seconds.

All the samples and standards were incubated in a water bath at 80 °C for 30 min and cooled down to 20 °C. Butanol (2 ml) was added to all the samples and standards and vortexed for 20 seconds. The mixture was transferred to Beckman J6 centrifuge tubes and centrifuged at 3000 × g for 10 min. The pink layer 1 ml was pipetted into 10 ml clean screw cap tubes. The tubes were placed in a water bath at 37 °C and dried under oxygen free nitrogen. Dried samples and standards were reconstituted using 1 ml of 50% water and 50% acetonitrile and vortexed for 20 seconds and transferred to eppendorf tubes and centrifuged at 13000 × g for 10 min.
**HPLC analysis**

The supernatant was transferred to HPLC vails and 100 µl injected onto HPLC (Spectra system AS 3000) for analysis. The pink chromogen separation was achieved on a 150 × 3 mm column containing Luna 5 µ phenyl hexyl C18 packing (Phenomenex, Macclesfield, UK). The gradient temperature control (column oven control) was 30 °C. The chromatographic conditions were: flow rate of 0.3 ml/ml and mobile phases A (2 % aqueous acetonitrile, 0.5 % acetic acid, pH 2.68) and B (99.5 % acetonitrile, 0.5 % acetic acid). The gradient programme was set as shown in Table 8.3 below. The run time of 35 minutes was set.

**Table 8.5: General gradient used in the analysis of TBARS by HPLC**

<table>
<thead>
<tr>
<th>Time</th>
<th>Solvent A %</th>
<th>Solvent B %</th>
<th>Flow rate ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>96</td>
<td>4</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>20</td>
<td>0.3</td>
</tr>
<tr>
<td>30</td>
<td>40</td>
<td>60</td>
<td>0.3</td>
</tr>
<tr>
<td>32</td>
<td>96</td>
<td>4</td>
<td>0.3</td>
</tr>
<tr>
<td>35</td>
<td>96</td>
<td>4</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Quantification of the HPLC data was undertaken by Microsoft Excel 2001 software. Six concentrations of the standards (0, 0.2, 0.4, 0.6, 0.8, 1.0 µg/ml) were used to prepare the standard curve of TEP. The regression equation was obtained and the MDA in the sample was calculated based on this equation while taking account of all the dilutions made. The final concentration of the MDA in the food sample was expressed in µg/g sample.
8.2.3.3 *Hexanal determination*

The hexanal method was kindly supplied by Masterfoods plc (Masterfoods, Central Nutrition and Microbiological Laboratories, UK).

*Standard preparation*

Stock hexanal (0.1 g) was weighed into 100 ml volumetric flask with cyclohexane.

Stock isobutyl acetate (0.1 g) was weighed into 100 ml volumetric flask with cyclohexane. Internal standard: 2 ml of stock isobutyl acetate (IBA) was pipetted into 100 ml volumetric flask and was made to 100 ml mark with cyclohexane.

Working standard: 2 ml of both stock solutions hexanal and isobutyl acetate were pipetted into a 100 ml volumetric flask and to volume by adding cyclohexane.

*Preparation of sample*

The sample (10 g) in triplicates was weighed into 500 ml round bottomed flask and saturated sodium chloride solution (150 ml) was added followed by spatula of antibumping granules. An internal standard, isobutyl acetate (2 ml) was pipetted into this mixture and the flask was placed on a heating mantle. A Clavenger trap was fitted and switched on. The mixture was refluxed on the heating mantle for 15 min and left to cool for 15 min. Using a pipette the cyclohexane layer was transferred from Clavenger to a vial and was injected onto the Gas chromatograph. The chromatographic conditions were: the run time was 17.60 and injection volume 4 μl. The retention times for isobutyl acetate was approximately 6.2 min and for hexanal 7 min.
The concentration of hexanal was calculated as follows:

\[
\text{Sample ratio} \times \text{Hexanal standard concentration} \times \frac{\text{Internal standard amount}}{\text{Average standard weight of sample}}
\]

\[
\text{Sample ratio} = \frac{\text{Peak area of hexanal}}{\text{Peak area of IBA}}
\]

Average standard ratio = average ratio of calibration working standard run.

Internal standard amount = 2 ml.

8.3 Results and discussion

8.3.1 Proximate composition and rheology of the sweetpotato based infant food

The results of proximate composition of sweet potato based baby food (recipe A) formulated by the Microdiet program are presented in Table 8.6. The results indicate that the food contained carbohydrate 66%, protein 20.4%, ash 3.2% and fat 2.2%, which compared quite well with that of commercial baby food cerelac used in Uganda. From the age of 5 or 6 months infants grow and develop very fast and breast milk is not enough to meet their nutritional demands and infants therefore require an adequate diet that can meet their nutrient needs. The results indicate that the baby foods produced are nutritionally adequate and suitable for infant weaning or supplementation feeding. The quantity of carbohydrate, protein and fat in both recipes was sufficient to meet the requirements of the weaning infant for growth and development. Besides the \textit{in vitro} digestibility of starch in the foods indicated that the carbohydrates may be well utilised. It is important to have an easily or readily digested carbohydrate to avoid using protein as a source of energy.
Table 8.6: Proximate composition of sweet potato based food and *in vitro* digestibility of starch

<table>
<thead>
<tr>
<th>composition</th>
<th>g/100g dry weight basis recipe A</th>
<th>g/100g dry weight basis recipe B</th>
<th>Commercial Food cerelac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>8.0 ± 0.4</td>
<td>8.4 ± 0.6</td>
<td>2.5</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>66.0 ± 0.2</td>
<td>58 ± 1.4</td>
<td>69.5</td>
</tr>
<tr>
<td>Protein</td>
<td>20.4 ± 0.11</td>
<td>28.0 ± 0.4</td>
<td>15.0</td>
</tr>
<tr>
<td>Fat</td>
<td>2.0 ± 0.1</td>
<td>3.4 ± 0.5</td>
<td>9.0</td>
</tr>
<tr>
<td>Ash</td>
<td>3.2 ± 0.8</td>
<td>2.0 ± 0.0</td>
<td>2.6</td>
</tr>
<tr>
<td><em>In vitro</em> digestibility</td>
<td>69.5 ± 0.2</td>
<td>60.0 ± 2.7</td>
<td>64.9 ± 1.9</td>
</tr>
</tbody>
</table>

An infant needs a gradual transition from fluid to solid foods over the weaning period to allow development of feeding skills (Walker, 1990). Thus the viscosity or consistency of a baby food is critical and was measured by rheology of the product. The oscillation technique is used to determine the $G'$ which is a measure of the elastic energy stored and $G''$ which is a measure of the energy dissipated due to viscous behaviour. The viscoelastic properties of the formulated products are presented in Table 8.7 and the representative figures are indicated in Fig 8.2. The $G'$ and $G''$ values of baby food A (sweet potato based baby food with fish) and B (a baby food without fish) were measured at 20 °C at the beginning of the temperature sweep, at 90 °C and at 20 °C on cooling. The $G'$ values for both recipes were low before heating and increased slightly on heating probably due to the swelling effect of carbohydrate and the water binding capacity of amylose and amylopectin in the
samples and also due to the denaturation of fish proteins and soya proteins. After heating and cooling G’ values increased further but the values were not high and the sample was still soft enough for infant feeding. Addition of soya, fish, milk and fat to sweet potato flour led to a reduction of viscoelasticity values. Fat reduced the viscosity through the formation of insoluble complexes with amylose molecules or through the formation of a fat layer around the starch granules (Kikafunda et al., 1997); this reduces water absorption of the starch granules during the cooking process, thus reducing viscosity of the food. The smaller increase in G’ may indicate a form of phase separation between sweet potato flour and proteins soya and fish (Samant, 1993). This may have been due to incompatibility of the interacting molecules above due to differences in charge or molecular weight (Howell, 1992). The rheological characteristics of the two formulations were comparable to a commercial Heinz baby food (Table 8.6) obtained from local supermarket. The G’ and G’’ values of Ugandan commercial infant food product cerelac were very low compared with the formulated product. This was probably due to high content of fat as indicated in Table 8.6. On the whole the formulated food was within the accepted consistency (< 500 Pa) after heating and cooling.
Table 8.7: Small deformation analysis showing the elastic modulus (G') values and G'' (loss modulus) for sweet potato based food, Heinz baby food and Cerelac at 20°C (beginning of the heating cycle) 90 and 20 °C (end of the cooling cycle)

<table>
<thead>
<tr>
<th>Viscoelasticity</th>
<th>Temperature °C</th>
<th>Recipe A</th>
<th>Recipe B</th>
<th>Heinz baby food</th>
<th>Cerelac</th>
</tr>
</thead>
<tbody>
<tr>
<td>G' (Pa)</td>
<td>20</td>
<td>113 ± 39</td>
<td>91 ± 8</td>
<td>434 ± 28.6</td>
<td>10.1 ± 1.1</td>
</tr>
<tr>
<td>G'' (Pa)</td>
<td></td>
<td>71.2 ± 21</td>
<td>205 ± 8.0</td>
<td>208 ± 7.5</td>
<td>7.7 ± 0.6</td>
</tr>
<tr>
<td>G' (Pa)</td>
<td>90</td>
<td>166 ± 3.2</td>
<td>242 ± 8.2</td>
<td>183 ± 23</td>
<td>3.8 ± 0.8</td>
</tr>
<tr>
<td>G'' (Pa)</td>
<td></td>
<td>136 ± 18</td>
<td>53 ± 2.4</td>
<td>77.3 ± 18</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>G' (Pa)</td>
<td>20</td>
<td>564 ± 9.1</td>
<td>492 ± 9.4</td>
<td>346 ± 32.3</td>
<td>21.0 ± 0.9</td>
</tr>
<tr>
<td>G'' (Pa)</td>
<td></td>
<td>196 ± 33</td>
<td>118 ± 20</td>
<td>121 ± 56</td>
<td>25.4 ± 3.6</td>
</tr>
</tbody>
</table>

(a)
b)

c

Temperature °C

$G', G''$ (Pa)

Temperature °C

$G', G''$ (Pa)
Fig. 8.2: Rheological analysis of a) baby food with fish (recipe A), b) Baby food without fish (recipe B), c) Cerelac and d) Heinz baby food using a temperature sweep.

8.3.2 Effects of antioxidants on freeze-dried weaning foods during storage

Food quality deteriorates on storage due to oxidative processes induced by atmospheric oxygen. In this study oxidation was monitored by measuring the primary (peroxides) and secondary products (aldehydes such as malonaldehyde and hexanal) using peroxide value, TBARS and gas chromatography techniques respectively. Two recipes A and B prepared as indicated in Table 8.1 were studied to monitor the changes of lipids on storage. The results for peroxide value (PV), TBARS and hexanal for baby food A are shown in Fig. 8.3 a-c and for baby food B in Fig. 8.4 a and b. The peroxide value for the untreated baby food with fish (Recipe A) increased throughout the storage period. The increase was higher compared to samples with antioxidants (Fig. 8.3 a and b). There was a significant difference in peroxide values between the food with antioxidants particularly concentrations (350; 350; 100 mg/kg), (500; 500; 100 mg/kg) (vitamin E + C + citric acid) and food without any antioxidants (P<0.001). There was a general increase of hexanal values
in all the samples on storage but the ones stored with antioxidants increased slowly (Fig. 8.3b). The increase of hexanal was as a result of peroxides break down to produce aldehyde such as malondialdehyde and hexanals. The TBARS values increased until upto 12 weeks and started to fall for baby food samples with fish (Fig. 8.3c). The decrease observed in this food was probably due interactions between malondialdehyde and proteins (Shahidi, 1997; Babji et al., 1998).

The antioxidants with high concentration were more effective in reducing the lipid oxidation (Fig 8.3) in the product containing fish (recipe A), suggesting that the antioxidant activities are concentration dependent. The optimum levels of antioxidants required to stabilise the lipids in the baby food (Recipe A) is 350-500 mg/kg Vit E, C and 100 mg citric acid, which is comparable with findings reported by Huang et al., 1995; Yoshida et al., 1993. These researchers reported that the optimum concentration of α-tocopherols required for stabilising the soya oil and palm oil was 200 and 500 mg/kg respectively. Badii & Howell (2002) reported the beneficial effect of using vitamin E, C and Citric acid (250 mg/kg, 250 mg/kg and 100 mg/kg respectively) for stabilising fish fillets and dried fish products. Alpha tocopherol is considered to be chain-breaking antioxidant in free radical chain reactions, which can convert lipid radicals to more stable products, thus extending the shelf life of a food product containing edible oils. Tocopherols’ antioxidant activity can be enhanced when combined with ascorbic acid and citric acid. Ascorbic acid is involved in the regeneration of α-tocopherol by donating an electron to tocopheroyl radical restoring antioxidant potential to it.
**Graph a)**

- **Y-axis:** Peroxide value meq/kg fat
- **X-axis:** Number of weeks of storage

- **1.** 0 (untreated)
- **2.** 200 (Vit E, C + 100 mg Citric acid)
- **3.** 350 (Vit E, C + 100 mg citric acid)
- **4.** 500 (Vit E, C + 100 mg citric acid)

**Graph b)**

- **Y-axis:** Hexanal concentration ug/g
- **X-axis:** Number of weeks in storage

- **1.** 0 (untreated)
- **2.** 200 (Vit E, C + 100 mg Citric acid)
- **3.** 350 (Vit E, C + 100 mg citric acid)
- **4.** 500 (Vit E, C + 100 mg citric acid)
Lipid oxidation in the recipe B (baby food without fish) was not inhibited by the addition of antioxidants as observed in the product containing fish. In all the samples there was an increase of peroxides, malondialdehyde and hexanal on storage. Surprisingly the control samples without antioxidants were less oxidized than samples with antioxidants. It is possible the product contained its own natural antioxidants that protected it leading to lower levels of peroxides as indicated in Fig. 8.4. The high concentration of antioxidants exacerbated the problem of lipid oxidation. There was a steep increase in peroxide and hexanal values on adding high level of antioxidants, a response quite opposite to that of baby food with fish. Fish lipids generated enough free radicals, which were counteracted by the antioxidants. However the initial free radical production in the recipe without antioxidant was low to begin with.
It seems antioxidants in the recipe B without fish acted as pro-oxidants. It has been reported that Vitamin C can act as an antioxidant or pro-oxidant depending on the concentration, presence of metal ions and the tocopherol content (Schaefer et al., 1995). It is also reported that ascorbic acid at low concentrations (20-100 mg/l) acted as pro-oxidant while at high concentration higher than 500 mg/l acted as antioxidant (Clarkson, 1995). The level of ascorbic acid added to recipe B ranged between 50 and 250 mg/kg. It is also noted that α-tocopherol is capable of becoming a pro-oxidant in fats (Liu et al., 1991). Therefore antioxidants must be added with sufficient recognition of the pro-oxidant and antioxidants behaviour in the matrix to be protected.
b)

Fig. 8.4 a) Peroxide, b) hexanal values obtained from freeze-dried baby food without fish (recipe B) stored at 22 °C for 16 weeks.

8.4 Conclusion

Sweet potato based infant food developed in this study may be a potential weaning food because of its nutritional values and viscoelastic properties, which compared well with commercial baby foods. The shelf life of the formulated food containing fish may be increased by use of antioxidants vitamin E, C and citric acid. However antioxidants were not needed in the products without fish. It is important that the levels of antioxidants are chosen carefully to avoid pro-oxidation. In general there is a need to investigate the appropriate combination of antioxidants that can inhibit oxidation of fat in different food samples.
CHAPTER NINE
9.0 GENERAL DISCUSSION AND CONCLUSIONS

Sweet potato is one of the major root crops widely grown in Uganda and used mainly as a security crop because it withstands extreme weather conditions. Despite its wide production its processing is remarkably limited. The main objective of this study was to investigate the nutritional, biochemical, physico-chemical properties of three sweet potato varieties including Nasport (Nsp), Tanzania (Tz) and Spk004 (Spk), to assess rheological and physicochemical properties of sweet potato starch and mixtures of starch and protein (soya and fish), and to develop a sweet potato based infant food and assess its nutritional and rheological properties.

9.1 Nutritional and biochemical properties of Ugandan sweet potato varieties

Chemical composition and nutritional properties of Ugandan sweet potato varieties, Tanzania (Tz), Nasport 005 (Nsp) and Spk 004 (Spk) were analysed. Starch was the most abundant nutrient in sweet potato and ranged from 58 to 79 g/100g flour on a dry weight basis; Tanzania variety had the highest value. Resistant starch values ranged between 17 and 29%; these values were lower in sun-dried samples compared with freeze-dried ones and were reduced significantly on boiling. In addition, the in vitro digestibility was high, particularly in the sun-dried samples (80-85%), indicating sweet potato carbohydrates may be utilised efficiently. All sweet potato varieties contained low levels of lipids (1.1-2.3 g/100g) and proteins (2.7-5.0 g/100g). The protein content in sweet potato is higher compared with that found in other roots and tubers (cassava, yam and coco yam). However, the amount of protein in sweet potato may not be able to meet the nutritional requirements of 6-36 months old infant. Amino acid analysis indicated that aspartic acid and glycine were the most abundant amino acids while the limiting ones were the sulphur
containing amino acids, cysteine and methionine. To improve the quality of sweet potato protein there is a need to blend it with other plant proteins such as soya, sesame or animal protein to provide the full range of essential amino acids. Trypsin inhibitors in many plant materials can reduce protein availability. The trypsin inhibitor levels ranged from 1.4 to 3.5 mg/g. These levels are too low to pose any problems to consumers. The orange-fleshed varieties NSP and Spk contained high levels of β-carotene (provitamin A) indicating the potential usefulness of sweet potato as a good source of vitamin A in tropical regions. However, it is a sad fact that Xerophthalmia is common in some parts of Uganda where orange sweet potato roots are available but are not used in weaning foods. Beta-carotene levels were reduced in sun-dried and oven-dried samples by about 70% and 92% respectively.

The content and the bioavailability of elements in three different varieties of sweet potato grown in Uganda were evaluated. Flame atomic absorption spectrometry and Inductively coupled plasma-mass spectrometry methods were used in the measurement of the levels of the elements in the sweet potato and the bioavailability of the extraction dialysate. The main elements observed in sweet potato were K, Na, Mg and Ca with an average of 1190, 408, 698 and 576 mg/kg, dry weight respectively. Sweet potato can also serve as a moderate source of trace elements such as iron (37 mg/kg) and Zn (40 mg/kg). These minerals are important micronutrients necessary for growth, development and also play a vital role in general health; they strengthen skeletal structures (Mg and Ca) and also help in maintaining the delicate water balance essential to the proper functioning of physical processes (Na and K). Some of these minerals act as carriers of oxygen to the tissues and as transport medium for electrons within the cells (iron). A daily intake of a 100
g of sweet potato would contribute 89, 67, 70, 46 and >100 % of elements Mg, Ca, Zn, Fe and Se respectively of the recommended daily allowance for 1-3 year old children. It is important that the minerals consumed are made available to meet the nutritional needs of the individual. The *in vitro* digestion method, which mimics the physiological condition to which food is subjected in the gut, was used to predict the bioavailability of the elements in sweet potato. Most of the elements from the freeze-dried samples were less available (for example the bioavailability of Zn, Mg, Ca, Fe in freeze dried Nsp samples were 35, 22, 50 and 8 % respectively) compared with the oven-dried samples (57, 57, 75 and 13%). The content of some important toxic elements were very low (Cd 0.1 mg/kg, and Pb 1.00 mg/kg) making sweet potato a potentially safe food to use.

### 9.2 Phenolic compounds in sweet potato

Phenolic compounds were analysed because of the benefits they are considered to offer including antioxidant ability and the prevention of some cancer and cardiovascular diseases. The combination technique of HPLC and mass spectrometry made it possible to identify a number of phenolic compounds present in sweet potato some of which have not been identified before. The main phenolic substances present in Ugandan sweet potato included caffeoylquinic acid, dicaffeoylquinic acid, 5p-comouroylquinic acid, feruloylquinic acid and caffeoylferuloylquinic acids. CFQA (6 isomers) were identified for the first time in sweet potato and surprisingly, similar CFQA were reported in coffee (Clifford *et al.*, 2003). Phenols in sweet potato are known to bind with non-haem iron and thus inhibit iron absorption (Craig, 1994); this depends on the quantity of polyphenols and availability in the body. This problem can be overcome by taking vitamin C and
citric acid that facilitate iron absorption or by consuming foods of animal origin. Processing affected the quality of phenolics in sweet potato, for example CQA were not detected in sun dried Spk and CFQA was not observed in oven dried Nsp samples probably due to conversion to other products, destruction or because the quantity was too small to be detected.

9.3 Physicochemical and rheological properties of sweet potato starch, flour and mixed systems

The results in chapter 3 indicated that carbohydrate particularly starch is the main constituent of sweet potato. To fully utilise this starch, it is important that its characteristics are studied. Starch is the main source of energy in the diet and is used in the food industry as a thickening, gelling and bulking agent to impart texture to diverse foodstuffs such as soups, sauces, dairy and baking products. The rheological properties of starch depend on a number of factors including solubility in water, swelling capacity and amylose and amyllopectin ratio. Results showed the amylopectin and amylose in sweet potato varieties ranged between 73-76% and 24-27% respectively. Swelling and solubility of sweet potato starch increased with an increase in temperature and a steep increase in swelling and solubility was observed at the gelatinisation temperature. The storage and elastic modulus values for starch and sweet potato flour in water increased on heating but the extent to which the extracted starch increased was higher compared with that of flour. This behaviour may be explained by the fact that sweet potato flour contains other constituents such as proteins and lipids that limit starch swelling and compete for water making water less available to the starch. Gelatinisation temperature values for extracted starch and sweet potato flour were similar indicating other constituents did not influence this property of starch.
A study of the physicochemical characteristics of starch in mixed systems was necessary in order to understand the effects of (e.g. protein) ingredients in isolation and in combination in the formulated product (Chapter 7). Besides their nutritional role, these macromolecules have the ability to modify the structure of a food and thus affect the texture, for example a mixture of sweet potato and soya protein isolate led to low $G'$ and $G''$ values. On increasing soya or fish protein in a mixture the mixed system showed the behaviour of the main constituent: thus a mixture of 5% starch and 15% w/w soya in water behaved similarly to pure soya protein isolate in water indicating sweet potato flour did not have any effect on the rheological properties of soya protein isolate. Conversely, when sweet potato flour was increased to 15% and the soya isolate reduced to 5% the rheological characteristics observed were similar to those shown by the sweet potato flour in water. This pattern has been reported previously for soya and whey protein (Comfort & Howell, 2002). The blending of sweet potato flour and soya in water led to soft mixtures on heating and cooling indicating that they would be useful in baby food formulation and production of soups, sauces and dressings.

The rheological properties of fish in combination with soya proteins indicated phase separation and lowering of the viscoelastic properties; this property has been used in the design of low fat products. They can also be used in the development of infant foods, which need to be soft. A combination of fish and sweet potato particularly at 1:1 ratio (10% sweet potato and 10% fish in water) resulted in the mixture with higher $G'$ and $G''$ values on both heating and cooling compared with the individual components measured in isolation; this showed a synergistic interaction. Phase
contrast microscopy showed the mixtures were phase separated. Upon heating, the increase in gelation properties resulted from presence of two highly concentrated phases.

9.4 Nutritional, rheological and preservation properties of sweet potato based infant food
Using the Microdiet program sweet potato based infant food was developed whose nutritional attributes were adequate for complementary feeding. The energy contribution by the macronutrients such carbohydrate, fat and protein were achieved as required by the WHO/FAO guidelines (Table 8.4). The nutritional properties of the formulated food were comparable with commercial Heinz baby food and cerelac, a Ugandan baby food.

Since an infant needs a gradual transition from fluid to solid foods over the weaning period, it is necessary that solid foods be introduced gradually starting with soft ones. In all cases nutritional quality should not be compromised. The product formulated in this study had low viscoelasticity values on both heating and cooling. Addition of other ingredients such as fish, soya milk and oil led to lowering of $G'$ and $G''$ values probably due to phase separation or due to the fat in the formulae, which may have reduced the viscoelasticity by formation of fat layer around the starch granules thus reducing water absorption of the starch during cooking. In addition the lipid also minimises protein interactions thus lowering $G'$ values (Howell, 2000). The rheological characteristics of the sweet potato based infant foods formulated were comparable to the commercial Heinz baby food and its $G'$ value on cooling was $< 500$ Pa.
As a way of preserving the formulated dried food, antioxidants were added to the product to inhibit the lipid oxidation. Lipid oxidation is the main cause of food deterioration particularly in products containing fish lipids. The level of oxidation in this study was monitored by measurement of peroxides and the breakdown products such as aldehydes (malondialdehyde and hexanal). Two products were monitored which included recipe A with fish and recipe B without fish (Table 8.1).

In recipe A, containing fish the antioxidants especially with the concentration 350, 500 (Vit C + E) mg/kg and 100 mg/kg citric acid had a significant (P<0.001) effect on the reduction of lipid oxidation products compared to the untreated products. The sample without fish responded differently. The untreated product without fish had lower peroxide values and aldehydes compared with those treated with antioxidants. The antioxidants in this case acted as pro-oxidants. In order to avoid pro-oxidation a correct combination of antioxidant are needed to prevent spoilage.

9.5 Conclusions

- Sweet potato is a good source of carbohydrates, β-carotene, and minerals especially K, Mg, Na and Fe. The minerals in sweet potato are moderately bioavailable. Sweet potato carbohydrate can be used efficiently judging by the in vitro digestibility values. The orange varieties (Spk and Nsp) are a good source of β-carotene.

- Phenolic compounds identified in sweet potato included; CQA, FQA, p-CoQA and CFQA. This was the first time CFQA isomers were detected in sweet potato.
• Physicochemical characteristics indicate that sweet potato varieties Tz and Spk would be more useful in infant food formulation since their viscosity on heating and cooling were not high compared with Nsp variety.

• Characterisation of sweet potato starch can be achieved by Raman spectroscopy.

• Sweet potato can be a potential raw material for weaning food formulation due to its nutritional attributes. However sweet potato cannot be used in isolation since it contains low levels of protein and lipid.

• Addition of soya protein to sweet potato led to reduction of viscoelasticity values indicating that this product would be useful in formulation of infant food and low fat products whose consistency is required to be low.

• Processing affected the nutritional properties of sweet potato varieties. β-carotene was significantly reduced by oven drying and sun-drying. The bioavailability of sweet potato minerals was higher in oven-dried samples than in freeze-dried samples.

• The rheological and nutritional characteristics of two recipes incorporating sweet potato, fish, sunflower oil, soya, skimmed milk and water using the Micro diet programme, were comparable to commercial baby foods. The formulations complied with nutritional requirements for infant foods as stated by the FAO and WHO.
• Lipid oxidation of products especially those containing fish can be reduced by the use of antioxidants Vit E, C and citric acid 350: 350: 100 mg/kg. However, antioxidant levels need to be assessed carefully as they can lead to prooxidation.

9.6 Further work

• To study the sensory properties of the infant food formulation and compare with commercial products in order to assess the acceptability of the new product.

• To carry out microbiological studies to assess the levels of microbes in the product to ensure that the developed food is safe.

• To quantify the levels of phenols in sweet potato

• To carry out bioavailability studies on β-carotene to assess the levels that are actually available to the body.

• To investigate the formation of acrylamide in processed sweet potato.
REFERENCES


Powell, C. J., Connelly, J. C., Jones, S. M., Grasso, P. & Bridges, J. W. (1986). Epatic responses to the administration of high doses of BHT to the rat: Their relevance to epatocarcinogenicity. Food and Chemical Toxicology, 24, 1131-1143.


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APPENDICES
APPENDICES

Appendix A

a)

b)
c) 

\[ y = 0.1422x + 0.018 \]
\[ R^2 = 0.9591 \]

Absorbance at 213 nm vs. Zinc Concentration (ppm)

---

d) 

\[ y = 0.3482x \]
\[ R^2 = 0.9888 \]

Absorbance at 205 nm vs. Magnesium Concentration (ppm)
Fig. 1: Standard curves for a) Ca, b) Na, c) Zn, d) Mg, e) Cu, f) Fe used in determining minerals by FAAS method.
# Appendix B

A hierarchical key for the identification by LC–MS\(^n\) of caffeoylquinic acids and dicaffeoylquinic acids including those substituted at position 1 (M.N. Clifford, personal communication)

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Identification</th>
<th>Action</th>
</tr>
</thead>
</table>
| 1 Parent ion \(m/z\) 337.5  
Parent ion \(m/z\) 353.5  
Parent ion \(m/z\) 367.5  
Parent ion \(m/z\) 515.5  
Parent ion \(m/z\) 529.5 | \(p\)-Coumaroylquinic acids  
Caffeoylquinic acids  
Feruloylquinic acids  
Dicaffeoylquinic acids  
Caffeoylferuloylquinic acids | Go to reference (5)  
Go to 2  
Go to reference (5)  
Go to 3  
Go to reference (5) |
| 2 MS\(^2\) base peak \(m/z\) 191.5, and relatively intense (ca 50% base peak) secondary ion at \(m/z\) 179.5  
MS\(^2\) base peak \(m/z\) 173.5  
MS\(^2\) base peak \(m/z\) 191.5, and weak or undetectable (<5% base peak) secondary ion at \(m/z\) 179.5 | 3-Caffeoylquinic acid (II)  
4-Caffeoylquinic acid (IV)  
5-Caffeoylquinic acid (III) or 1-Caffeoylquinic acid (I) | Go to 4  
Distinguish by retention time on reverse phase packing |
| 3 MS\(^3\) base peak \(m/z\) 173.5  
MS\(^2\) base peak \(m/z\) 191.5 | 4-Acyl dicaffeoylquinic acids  
Dicaffeoylquinic acids NOT substituted at position 4 | Go to 4  
Go to 5 |
| 4 Strong (>50% base peak) MS\(^1\) fragment ions at \(m/z\) 299.5 and \(m/z\) 203.5  
Weak (ca 15%) MS\(^1\) fragment ion at \(m/z\) 335.5 and strong MS\(^2\) (>50% base peak) fragment ion at \(m/z\) 179.5  
MS\(^1\) fragment ion at \(m/z\) 335.5 undetectable, strong MS\(^2\) (>50% of base peak) fragment ion at \(m/z\) 179.5 | 1,4-Dicaffeoylquinic acid (VI)  
3,4-Dicaffeoylquinic acid  
(VIII)  
4,5-Dicaffeoylquinic acid (X) | |
| 5 MS\(^1\) fragment ion at \(m/z\) 335.5 (>30% of base peak) and strong MS\(^2\) (>50% of base peak) fragment ion at \(m/z\) 179.5  
Weak MS\(^1\) fragment ion at \(m/z\) 335.5 (<10% of base peak) and weak MS\(^2\) fragment ion at \(m/z\) 179.5 (<10% of base peak)  
MS\(^1\) fragment ion at \(m/z\) 335.5 undetectable, strong MS\(^2\) fragment ion at \(m/z\) 179.5 (<50% of base peak) | 1,3-Dicaffeoylquinic acid (V)  
1,5-Dicaffeoylquinic acid  
(VII)  
3,5-Dicaffeoylquinic acid (IX) | |

---

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A hierarchical key for the identification by LC–MS" of mono- and di-acyl-CGA not substituted at position 1 (From Clifford et al., 2003).

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Identification</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Parent ion m/z 337.5.</td>
<td>pCoQA</td>
<td>Go to 2</td>
</tr>
<tr>
<td>Parent ion m/z 353.5.</td>
<td>CQA</td>
<td>Go to 3</td>
</tr>
<tr>
<td>Parent ion m/z 367.5.</td>
<td>FQA</td>
<td>Go to 4</td>
</tr>
<tr>
<td>Parent ion m/z 515.5.</td>
<td>DiCQA</td>
<td>Go to 5</td>
</tr>
<tr>
<td>Parent ion m/z 529.5.</td>
<td>CFQA</td>
<td>Go to 6</td>
</tr>
<tr>
<td>2 MS2 base peak m/z 163.5.</td>
<td>3-pCoQA</td>
<td></td>
</tr>
<tr>
<td>MS2 base peak m/z 173.5.</td>
<td>4-pCoQA</td>
<td></td>
</tr>
<tr>
<td>MS2 base peak m/z 191.5.</td>
<td>5-pCoQA</td>
<td></td>
</tr>
<tr>
<td>3 MS2 base peak m/z 191.5, and relatively intense (ca 50% base peak) secondary ion at m/z 179.5.</td>
<td>3-CQA</td>
<td></td>
</tr>
<tr>
<td>MS2 base peak m/z 173.5.</td>
<td>4-CQA</td>
<td></td>
</tr>
<tr>
<td>MS2 base peak m/z 191.5, and weak or undetectable (&lt;5% base peak) secondary ion at m/z 179.5.</td>
<td>5-CQA</td>
<td></td>
</tr>
<tr>
<td>4 MS2 base peak m/z 193.5.</td>
<td>3-FQA</td>
<td></td>
</tr>
<tr>
<td>MS2 base peak m/z 173.5.</td>
<td>4-FQA</td>
<td></td>
</tr>
<tr>
<td>MS2 base peak m/z 191.5.</td>
<td>5-FQA</td>
<td></td>
</tr>
<tr>
<td>5 MS2 base peak m/z 353.5, MS3 base peak m/z 173.5, and comparatively intense (ca 20% of base peak) secondary ion at m/z 353.5.</td>
<td>3,4-diCQA</td>
<td></td>
</tr>
<tr>
<td>MS2 base peak m/z 353.5 and MS3 base peak m/z 191.5.</td>
<td>3,5-diCQA</td>
<td></td>
</tr>
<tr>
<td>MS2 base peak m/z 353.5, MS3 base peak m/z 173.5</td>
<td>4,5-diCQA</td>
<td></td>
</tr>
<tr>
<td>6 MS3 base peak m/z 173.5.</td>
<td>Vic-CFQA</td>
<td>Go to 7</td>
</tr>
<tr>
<td>MS3 base peak not m/z 173.5.</td>
<td>3,5CFQA</td>
<td>Go to 10</td>
</tr>
<tr>
<td>7 MS2 secondary ions at m/z 335.5 or m/z 349.5 with intensities not less than ca 40% of base peak.</td>
<td>3,4CFQA</td>
<td>Go to 8</td>
</tr>
<tr>
<td>MS2 secondary ions at m/z 335.5 or m/z 349.5 with intensities not more than ca 20% of base peak.</td>
<td>4,5CFQA</td>
<td>Go to 9</td>
</tr>
<tr>
<td>8 MS2 base peak at m/z 367.0 and MS3 secondary ion at m/z 193.5.</td>
<td>3C,4FQA</td>
<td></td>
</tr>
<tr>
<td>MS2 base peak at m/z 353.5 or m/z 367.0 with m/z 353.5 of near identical intensity, and MS3 secondary ion at m/z 179.5.</td>
<td>3F,4CQA</td>
<td></td>
</tr>
<tr>
<td>9 MS2 base peak at m/z 367.0 and an intense (&gt;50% of base peak) MS3 secondary ion at m/z 193.5</td>
<td>4F,5CQA</td>
<td></td>
</tr>
<tr>
<td>MS2 base peak at m/z 353.5 and an intense (&gt;50% of base peak) MS3 secondary ion at m/z 179.5.</td>
<td>4C,5FQA</td>
<td></td>
</tr>
<tr>
<td>10 MS2 base peak at m/z 367.0 and MS3 base peak at m/z 193.5.</td>
<td>3F,5CQA</td>
<td></td>
</tr>
<tr>
<td>MS2 base peak at m/z 353.5 and an intense (&gt;50% of base peak) MS3 secondary ion at m/z 179.5.</td>
<td>3C,5FQA</td>
<td></td>
</tr>
</tbody>
</table>
Product Description

SUPRO® 760 IP Non-GM is an isolated soy protein that provides excellent emulsification, emulsion stability properties and has excellent nutritional characteristics.

SUPRO 760 IP Non-GM is backed by The Solae Company's "Quality: Our Guarantee" Program. This program is based on sound quality principles that guarantee the consistency, safety, and performance of our products.

This product is produced in accordance with an Identity Preservation (IP) program that consists of 1) a non-GM soybean source of origin, 2) documented identity preservation at each stage of processing, 3) independent, third-party certification of conformance to defined IP procedures, and 4) a maximum of 1% adventitious residual GM DNA as tested by The Solae Company's process testing protocols.

Nutrition

The protein in SUPRO 760 IP Non-GM is an isolated soy protein with a P.D.C.A.A.S. (Protein Digestibility Corrected Amino Acid Score) equal to 1.0, the highest possible score. It is equivalent in protein quality to milk or egg protein. It meets or exceeds the Essential Amino Acid Requirements of Children and Adults.

Ingredient Statement

• Isolated Soy Protein

Product Analysis

This data is not intended to be considered or used as a specification. It is compiled from limited data sources, and is presented to provide estimated values to the prospective customer. Should a guaranteed specification be required, please contact your account manager.

Chemical Analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Max</th>
<th>Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (N x 6.25, Dry Basis)</td>
<td></td>
<td>90.0%</td>
</tr>
<tr>
<td>Protein (N x 6.25, As Is)</td>
<td></td>
<td>85.5%</td>
</tr>
<tr>
<td>Fat, Free (PE Extract)</td>
<td>Max: 1.0%</td>
<td></td>
</tr>
<tr>
<td>Fat, Total (Acid Hydrolysis)</td>
<td>Max: 6.0%</td>
<td></td>
</tr>
</tbody>
</table>

Microbiological Analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Max</th>
<th>Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Plate Count</td>
<td>10,000/g</td>
<td></td>
</tr>
<tr>
<td>Salmonella (by test)</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>E. Coli (by test)</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Yeast-Mold</td>
<td>100/g</td>
<td></td>
</tr>
</tbody>
</table>

NFPA Guidelines for Microbial Spores:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Max</th>
<th>Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Thermophilic Aerobic Spores</td>
<td>125/10g</td>
<td></td>
</tr>
<tr>
<td>Thermophilic Aerobic Flat Sour Spores</td>
<td>50/10g</td>
<td></td>
</tr>
<tr>
<td>Thermophilic Anaerobic H2S Positive Spores</td>
<td>5/10g</td>
<td></td>
</tr>
<tr>
<td>Thermophilic Anaerobic H2S Negative Spores</td>
<td>+3/6 tubes</td>
<td></td>
</tr>
</tbody>
</table>

Issue Date: 9/03 Supercedes all previous data sheets on this product

The information contained herein is, to the best of our knowledge, correct. It is the user's responsibility to comply with appropriate government standards and requirements. No warranties, expressed or implied, are made. The information contained herein should not be construed as permission for violation of patent rights.