PHYSICOCHEMICAL PROPERTIES OF STARCHES OF SOME TROPICAL TUBERS

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CERTIFICATION

This is to certify that this project work was carried out by **OLADEBEYE ABRAHAM OLASUPO** in the Department of Chemistry of the Federal University of Technology, Akure and that this project has not been submitted elsewhere for the award of a degree, diploma or certificate.

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DEDICATION

This project work is dedicated to the Almighty God, the Alpha and Omega of my success and to my wife, Ronke and my son, Ayomikun.

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I give glory to the Almighty God, who availed me the privilege to run this programme successfully. He owns all I was, all I am and all I will be.

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ABSTRACT

The starches of corms and cormels of red and white cocoyam (*Colocasia esculenta*) varieties, cassava (*Manihot esculenta*), sweet potato (*Ipomea batata*), bitter yam (*Dioscorea dumentorum*) and water yam (*Dioscorea alata*) were extracted. Their proximate compositions, physicochemical properties, mineral compositions and antinutrient contents were analyzed. The results of the proximate analysis ($p<0.05$) showed that crude protein ranged from 2.09±0.01% in the corm of red cocoyam (RCCM) to 1.17±0.01% in the cormels of white cocoyam (WCCL). The highest crude fibre value (0.75±0.02%) was observed in sweet potato (SPOTA) followed by that of water yam (WYAM), which was 0.74±0.01%. The value of moisture content of SPOTA (8.72±0.03%) made it the most microbial stable starch of all the samples analyzed. The ash contents of the samples varied from 1.64±0.01 to 1.98±0.02% while that of fat contents for the samples ranged between 0.33±0.01 and 0.52±0.01%. The starch content was highest and lowest in cassava (CASS) and RCCM respectively while the sugar content was highest and lowest in SPOTA and WCCM respectively. The results of physicochemical properties revealed that the initial pH of the starches ranged from 5.60 to 6.33. The level of acidity of the starch samples decreased in the order BYAM>WCCM (corms of white cocoyam)>WYAM>SPOTA>WCCL >RCCL (cormels of red cocoyam)>CASS. The darkest starch sample was WCCL while the lightest was CASS. The results of bulk density ranged from 0.70 g/ml to 0.76 g/ml. The values obtained were higher than that of a commercial starch, Starch 1500 used in the pharmaceuticals as tablet binder. The increasing order of water absorption capacities obtained was RCCL<BYAM<RCCM<SPOTA< WCCL<CASS<WYAM<WCCM. The gel strengths of three starches: WCCL, RCCL and WYAM were found to be appreciably high (15.00%). RCCM exhibited the highest swelling power of 11.21 g/g while the highest solubility was found in RCCL. All the starches are a good source of energy with values ranging from 328.8 to 335.7 kcal. The results of pasting properties revealed that CASS

had the lowest ability to retrograde after cooling its paste followed by SPOTA. The most viscous starch paste was that of WYAM with paste stability of 119.58 RVU. From the results, it was generally observed that the higher the retrogradation, the lower the stability. The gelatinization temperatures were between 60.45 and 62.15^{0}C. The results of paste clarity showed that after freezing for three days, the most transparent starch paste was CASS. All the starch samples were good sources of minerals. The predominant mineral was potassium and its peak value was exhibited by SPOTA (3102.00±0.50 mg/kg), which incidentally had the highest value of phosphorus (1012.00±0.50 mg/kg) among all the samples. The peak value of iron (21.00± 0.02 mg/kg) was in WCCM. The values of zinc were low with the highest value as 4.50±0.02 mg/kg in RCCL. Other heavy metals such as Hg, Pb, Cd, Cu, Mn, As and Ni were not detected in all the starch samples. The results of antinutrients in the starches showed that the level of trypsin inhibitor ranged from 0.09 to 0.13±0.01 mg/100g. Free cyanides were also detected in all the samples at micro quantity. The range of values obtained for cyanide content was lower than the recommended value. The highest value of cyanide content was 21.03±0.02 µg/100g (≈ 0.21±0.02 mg/kg) in CASS and the least was 7.31±0.01 µg/100g (≈ 0.07±0.01 mg/kg) in RCCL.

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LIST OF ABBREVIATIONS

In this thesis, the following abbreviations, whose meanings are given below, are used to represent the starch samples worked on:

WCCM-White cocoyam corms

WCCL-White cocoyam cormels

RCCM-Red cocoyam corms

RCCL-Red cocoyam cormels

CASS-Cassava

SPOTA-Sweet potato

BYAM-Bitter yam

WYAM-Water yam

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 ROOTS AND TUBERS

Food has been defined as anything eaten or drunk, which can be absorbed by the body as source of energy, building, regulating or protective material (Mudambi and Rujagopal, 1987). The components of food that are needed by the body, which are referred to as nutrients, include water, proteins, fats and oils, carbohydrates, minerals and vitamins (Mudambi and Rujagopal 1987).

According to Oke (1990), roots and tubers are the plants that store edible material in subterranean roots, corms or tubers. They belong to the class of foods that basically provides energy in the human diet in form of carbohydrates. Examples of root crops are cassava, yam, cocoyam and potato. The fact that root crops are mainly starch has led to the disparagement of the protein content, which is low compared to cereals.

Most of the root crops that are high in protein are not fully utilized, because of food taboos (Ogbeinde, 1974; Ojofeitimi and Tanimowo, 1980; Oke and Ojofeitimi 1980), believing that they are meant for the poor (Oke, 1990).

The main nutritional value of roots and tubers lies in their potential ability to provide one of the cheapest sources of dietary energy, in form of carbohydrates. This energy is about one-third of that of an equivalent weight of grain, such as rice or wheat, because tubers have high water content. However, the high yields of most root crops ensure an energy output per hectare per day, which is considerably higher than that of grains.

Malnutrition is often the outcome of either an insufficient food intake or poor utilization of food by the body, or both simultaneously. Recent surveys show that very few people in tropical countries suffer from a simple protein deficiency. The most prevalent deficiency is protein-energy, in which an overall energy deficiency forces the metabolism to utilize the limited intake of protein as

a source of energy. This is an area in which root crops could play a more significant role as additional sources of dietary energy and protein. Increasing the consumption of root crops could help save the much-needed protein provided essentially by other foods such as cereals and legumes. Traditionally, in Africa, root crops such as cassava are eaten with a soup or stew made of fish, meat or vegetables, providing an excellent protein supplement to cassava meal (Collins and Walter, 1982).

1.1.1 COCOYAM *(Colocasia esculenta)*

Colocasia esculenta is an ancient tuber of the Araceae family (Plucknett 1983, Chandra 1984), which originated from South-East Asia, possibly, India (Onwueme, 1978 and Wang, 1983), where it has been cultivated for more than 2000 years. It has both red and white varieties. Now, it is a plant grown widespread throughout the tropics.

Colocasia esculenta is an herbaceous bush of 1-2m high that is built by leaves originating from a starchy corm (the edible portion). The leave lamina is up to 1 m in length and has the shape of a heart, resembling elephant ears.

As a crop of the tropical rainforest, *Colocasia esculenta* prefers very high precipitation of 2500mm per year and a temperature of 25–27°C. The crop is mature after a cultivation period of 8-10 months. Yield is very variable, ranging normally from 7 to 30 t/ha. Cocoyam (*Colocasia esculenta)* grows on all soil types but deep, well-drained loams are preferred. Planting of vegetative propagules is labour intensive with plant populations ranging from 10,000 to 30,000 plants/ha.

Colocasia esculenta has a large central corm, which is spherical or short, ovoid to cylindrical and measures 10–15 cm in diameter and 20-30cm in length. The weight of the tuber may be between 1 and 2.5 kg. There are edible cormels attached to the central corm. The upper part of the corm tissue is soft (leaf tissue),

but the skin on the lower portion can be quite hard and thick (1-2 mm). The colour of the flesh is normally white, but can also be yellow, cream or violet. Overall, the brown skin is uneven with ridges and small pits.

Cocoyam (*Colocasia esculenta)* can be processed into several food and feeds products and industrial inputs, similar to that of potatoes in Western world. The processes include boiling, roasting, frying in oil, pasting, milling and conversion into 'fufu', soup thickeners, flour for baking, chips, beverages powder, porridge and specialty food for gastro-intestinal disorders (Onwueme, 1978; Hussain et. al., 1984; Ihekoronye and Ngoddy, 1985)

Tuber processing is aimed at obtaining products that are stable in terms of longitivity, nutrition and palatability. However, it has been reported that the consistent palatability problems (bitter and astringent taste and scratchiness in mouth and throat) associated with cocoyams have hindered the realization of its potential (Greenwill, 1947; Irvine, 1960; Carpenter and Steinke, 1963 and Hussain et. al., 1984). The causes of these anti-nutritional and off-taste problems have been identified as calcium oxalate crystals (raphides) and other acidic and proteinaceous principles (Hussain et. al., 1984). The high content of calcium oxalate crystals, about 780 mg per 100 g in some species of cocoyam, Colocasia and Xanthosoma, has been implicated in the acridity or irritation caused by cocoyam. Oxalate also tends to precipitate calcium and makes it unavailable for use by the body. Oke (1967) has given an extensive review of the role of oxalate in nutrition including the possibility of oxalaurea and kidney stones.

Proteolytic enzymes as in snake venoms can also cause acridity. Attempts have been made to isolate such enzymes from taro, *Colocasia esculenta*, and the principal component has been called "taroin" by Pena et al. (1984).

However, studies aimed at eliminating these limitations in cocoyam and its products have included those on baking (Moy et. al., 1979 and Ezedinma, 1987),

peeling, grating, cooking, anaerobic fermentation (Carpenter and Steinke, 1985) and soaking in water.

1.1.2 CASSAVA *(Manihot esculenta)*

Cassava was taken to India by the Portuguese in the seventeenth century. In about 1850, it was transported directly from Brazil to Java, Singapore and Malaya. Missionaries and travelers introduced cassava to the South Pacific territories during the first half of the nineteenth century, but its importance varies from island to island. . The Portuguese first introduced it into the Congo basin as early as 1558. It then spread rapidly through Angola, Zaire, Congo and Gabon and later to West Africa. There was a separate introduction to the east coast of Africa and to Madagascar in the eighteenth century by Portuguese and Arab traders, after which it rapidly became a dietary staple throughout many lowland tropical areas (Jones, 1959). The cultivation of cassava in Africa increased during the nineteenth and twentieth centuries as a result of encouragement by administrative authorities, which recognized its value as a famine relief crop.

At present, cassava is grown throughout tropical and subtropical areas approximating 30°N and 30°S of the equator and up to an altitude of 1500 metres (Oke, 1990)

Cassava is a perennial woody shrub, grown as an annual crop, with an erect stem branching in some varieties only at the base. But, in others, branching is such, that widely spreading branches are formed. Varieties used for production of staple food such as bread, cassava rice (a traditional food in Philippine), ‘gari’ and ‘eba’ are 2-3m high, but some varieties are up to 5m. *M. esculenta* produces enlarged tuberous roots that contain about 32% carbohydrates and very little protein. Secondary thickening produces these roots and their number per plant is about 5 to 10.

Cassava is a tropical root crop frequently described as bitter or sweet. 8-10 months of cultivation period is required for sweet cultivars and 18–36 months for bitter cultivars to produce crops. Traditionally, it is grown in a climate providing 1000–2000mm precipitation per year, but can be grown in extremes of rainfall with minimum of 500mm precipitation per year. Cassava needs high temperature for high yields and does not tolerate freezing conditions. It is grown in altitudes up to 1400m above sea level. *M. esculenta* tolerates a wide range of soil pH, from 4.0 to 8.0, and can also be grown on poor soils with low nutrient supply. Cassava is most productive in full sun (Medlicott, 1980).

The tuber is enclosed by a periderm, which is thick and rough in many varieties but may also be thin and smooth. The skin, which is 1 mm thick, is brown-yellow to brown-red or white-pink colour. Tuber flesh colour is usually white, with exception of a few clones having yellow-fleshed roots. Cassava contains 30-40% dry weight, of which starch and sugar are the predominant components. The tuber contains significant amount of vitamin C, about 35mg per 100g fresh weight (Onwueme, 1978).

1.1.3 SWEET POTATO (*Ipomoea batata*)

Ipomoea batata plants belong to the convolvulaceae family, (they are commonly misnamed yams, especially at thanksgiving time, when it is usually the sweet potato that is eaten). Many varieties of sweet potatoes (Ipomoea) are grown, with a purple or tan outside and with a white, orange, or purple meat (Radley, 1968). Nutritionists favor the orange meat variety because of its higher carotene content (Standal, 1979). Originating in South America, its production has spread throughout the tropics and it is a staple food in countries of Africa and the South Pacific (Noda et. al., 1995; Swinkels, 1985; Takahata et. al., 1995; Thomas and Atwell, 1999).

Sweet potatoes are a critical source of carbohydrates in much of the tropics and subtropics, especially in Africa and Latin America (FAO, 1998). The sweet potato tuber is eaten raw, boiled, steamed, baked, fried, mashed, dried, or fermented. In addition, it is used as a source of starch (Facciola, 1998). Only potato and sweet potato are grown to any extent in the United States and of these two, sweet potatoes has the greatest potential for increased usage and consumption (Collins, 1993).

The range of variability in sweet potatoes is so great that many different phenotypes can be made available for special product development depending on the characteristics needed. Often it is difficult to determine, even through trial and error, what the best characteristics are for particular products. Value-added products such as French fries, chips, and flakes have been developed from sweet potatoes, but none has been successfully marketed for any length of time (Collins, 1993). Much effort has been devoted to sweet potato fries. However, consumer comments often refer to the sweetness, texture, and oil content as problems.

Sweet potato contains raffinose, one of the sugars responsible for flatulence. Three of the sugars which occur in plant tissues, raffinose, stachyose and verbascose are not digested in the upper digestive tract, and so are fermented by colon bacteria to yield the flatus gases, hydrogen and carbon dioxide. The level of raffinose present depends on the cultivar. In some parts of Africa the cultivars used are considered too sweet and cause flatulence (Palmer, 1982).

In response to injury, or exposure to infectious agents, in reaction to physiological stimulation or on exposure of wounded tissue to fungal contamination, sweet potato will produce certain metabolites. Some of these compounds, especially the furano-terpenoids are known to be toxic (Uritani, 1967). Fungal contamination of sweet potato tubers by Ceratocystis fimbriata and several Fusarium species leads to the production of ipomeamarone, a hepatoxin,

while other metabolites like 4-ipomeanol are pulmonary toxins. Baking destroys only 40 percent of these toxins. Catalano et al. (1977) reported that peeling blemished or diseased sweet potatoes from 3 to 10 mm beyond the infested area is sufficient to remove most of the toxin.

1.1.4 BITTER YAM (*Dioscorea dumetorum*)

The edible, mature, cultivated yam does not contain any toxic principles. However, bitter principles tend to accumulate in immature tuber tissues of *Dioscorea rotundata* and *D. cayenensis*. They may be polyphenols or tannin-like compounds (Coursey, 1983). Wild forms of D. dumetorum do contain bitter principles, and hence are referred to as bitter yam. Bitter yams are not normally eaten except at times of food scarcity. They are usually detoxified by soaking in a vessel of salt water, in cold or hot fresh water or in a stream. The bitter principle has been identified as the alkaloid dihydrodioscorine while that of the Malayan species, *D. hispida*, is dioscorine (Bevan and Hirst, 1958). These are water-soluble alkaloids, which, on ingestion, produce severe and distressing symptoms (Coursey, 1967). Severe cases of alkaloid intoxication may prove fatal. There is no report of alkaloids in cultivated varieties of *D. dumentorum.*

1.1.5 WATER YAM (*Dioscorea alata*)

Water yam (*D. alata*), which is referred to as greater yam or Asiatic yam is believed to have originated and first cultivated in Southeast Asia, but now widely grown throughout the humid tropics. It requires at least sixty inches of rain per year, and is the highest yielding of the yam crops. Tubers can weigh up to 130 pounds and take ten months to a year to mature, and keeps well for five or six months after harvest. The fingered yam is a cultivar of water yam, which has finger-like protuberances, dark brown skin and white flesh that is sometimes tinged with pink. The greater yam is thought to have reached Madagascar by

1000 CE and by the 16th century, Portuguese and Spanish traders had taken it to West Africa and the New World. Christopher Columbus knew it as a staple food regularly used for ships' supplies because it was easy to handle and could be stored for months without deteriorating.

There are literally hundreds of different forms producing tubers, with an average weight of eight to twenty-two pounds, although massive specimens are not uncommon. One is recorded as having a weight of 136 pounds (http://www.innvista.com/health/foods/default.htm).

1.2 TOXICITY IN FOODS

Many plants and animals that man uses for food contain as natural constituents chemical substances known to have toxic properties. By experimentation man has learned to avoid dangerous exposure to the natural chemical components of his foods. Although acute poisoning is usually avoided, the public health significance associated with naturally-occurring toxicants is usually in the realm of chronic toxicity. A number of possible cause and effect relationships of this kind exist, but usually they only point to the presence of a toxicant. This is true because the intake is too small to cause severe effects, and because chronic effects are difficult to identify. Recognition of factors in the environment that may affect public health is basic to the eventual control of those factors. Some naturally-occurring toxicants have already been identified and are listed under the categories as antienzymes or inhibitors, carcinogens, cholinesterase inhibitors and cyanide (as in cassava) (Committee on Food Protection, 1973).

1.2.1 ANTINUTRIENTS

Many foods of plant origin contain substances, which interfere with the assimilation of nutrients contained in them. Some of the anti-nutritional factors

like tannins, phytates, oxalates and trypsin inhibitors interfere with the utilization of other nutrients like proteins and minerals like iron, calcium, iodine, zinc.

Tannins are a common name applied to a group of chemicals compounds found in plants, seed coats, tamarind, tumeric, bajra and raji, and in certain vegetables and fruits. They are both amorphous and crystalline with variable composition. Some, called condensed tannins, are phenols of moderately complex structure, and others are esters of glucose or some other sugar with one or more trihydroxybenzoic acids. The empirical formula $C_{14}H_{14}O_{11}$, often given for common tannin, is only an average (Microsoft Encarta Encyclopedia, 2005). Tannins hinder absorption of iron, damage the mucosal lining of the gastrointestinal tract, alter excretion of cations, and reduce availability of protein by increasing excretion of proteins and essential amino acids (Reddy and Pierson 1994)

Seed proteins such as cereal grains, legumes, and oil seeds are important sources of dietary protein in many areas of the world but some of them contain enzyme inhibitors. Perhaps the best known and most extensively studied of these toxic factors in seeds used as food are the trypsin inhibitors. Trypsin is an enzyme involved in protein digestion and trypsin inhibitors can result in a decreased availability of protein (Dack, 1956). However, under conditions of controlled processing, the antitryptic factor in the seeds can be partially or completely altered and the nutritional value improved.

Trypsin inhibitors, which for the most part are completely destroyed by heat, have been reported in wheat flour, soybeans, lima beans, mung beans, peanuts, oats, buckwheat, barley, sweet potatoes, garden peas, corn and white potatoes. Chymotrypsin is another enzyme involved in protein digestion and a chymotrypsin inhibitor has been found in potatoes.

Lin et al. (1985) have established that sweet potato shows trypsin inhibitor activity (TIA) ranging from 90 percent inhibition in some varieties to 20 percent

in others. There is a significant correlation between the trypsin inhibitor content and the protein content of the sweet potato variety. Heating to 90°C for several minutes inactivates trypsin inhibitors. Lawrence and Walker (1976) have implicated TIA in sweet potato as a contributory factor in the disease enteritis necroticans. This seems doubtful since sweet potato is not usually eaten raw and the activity of the trypsin inhibitor present is destroyed by heat.

1.2.2 CARCINOGENS

In the past three decades a large number and variety of synthetic chemical carcinogens have been discovered, and the increasing use of chemicals in the modern world has caused much concern about their hazard to man. Until recently little attention has been given to the possibility that various forms of life might produce agents, which give rise to tumors in other living systems.

Today we are aware of a small but increasing number of such agents. Some of these agents could find their way into human food, and indeed this has occurred in some instances. Aflotoxin, a type of mycotoxin, is produced by the organism, Aspergillus flavus. The fungus usually occurs on peanuts, corn, and wheat that have been improperly stored and where the temperature and humidity favor its growth (Dack, 1956).

The carcinogenicity of aflatoxins and their sporadic occurrence in food have given rise to considerable concern. Some toxin appears in milk from cows fed toxic meals, and trace amounts of aflatoxins have been reported in some peanut butters. Commercial peanut butter producers carefully monitor their peanuts and have instruments that can detect aflotoxin at one-half part per billion. Peanut oils are free of the toxin because the alkaline treatment used in processing destroys the toxins. Toxic corn has also been reported, and in the United States there have been cases of livestock toxicity from moldy feed (Committee on Food Protection, 1973).

1.2.3 CHOLINESTERASE INHIBITORS

The cholinesterases represent a group of enzymes that are of great significance in both the physiological and economic sense. Their principal characteristic appears to be control of the conduction of nerve impulses, a function that makes them of unique importance to the lives of both higher and lower animals. The inhibition of cholinesterases is one of man's more powerful chemical weapons against insect enemies (Liener, 1980). Two of the general classes of synthetic organic insecticides, the organophosphates and the carbamates, owe their effectiveness to this mechanism.

Study of the chemistry and toxicology of natural cholinesterase inhibitors such as glycoalkaloids, is important, for they represent a real hazard under certain conditions. Several instances of poisoning have been attributed to human consumption of "green" potatoes high in solanine. The toxic properties of green potatoes are not due to the green color, however, for the greening is due to chlorophyll which often accompanies the synthesis of the toxic sub- stance solanine, a glycoalkaloid. Glycoalkaloid synthesis increases when the tuber is exposed to stress conditions such as light, cutting, or bruising. Potato varieties differ widely in their glycoalkaloid content and some varieties have had to be taken off the market because their glycoalkaloid content exceeded the level considered safe for human consumption; a glycoalkaloid level above 20 milligrams per 100 grams of fresh weight is considered unsafe. The fruit of eggplant and the root and leaves of tomatoes also contain cholinesterase inhibitors.

1.2.4 CASSAVA TOXICITY

The main toxic principle, which occurs in varying amounts in all parts of the cassava plant is a chemical compound called linamarin (Nartey, 1981). It often coexists with its methyl homologue called methyl-linamarin or lotaustralin.

Linamarin is a cyanogenic glycoside, which is converted to toxic hydrocyanic acid or prussic acid when it comes into contact with linamarase, an enzyme that is released when the cells of cassava roots are ruptured. Otherwise linamarin is a rather stable compound, which is not changed by boiling the cassava. If it is absorbed from the gut to the blood as the intact glycoside it is probably excreted unchanged in the urine without causing any harm to the organism (Philbrick, 1977). However, ingested linamarin can liberate cyanide in the gut during digestion.

Hydrocyanic acid or HCN is a volatile compound. It evaporates rapidly in the air at temperatures over 28 C and dissolves readily in water. It may easily be lost during transport, storage and analysis of specimens. The normal range of cyanogen content of cassava tubers falls between 15 and 400 mg HCN/kg fresh weight (Coursey, 1973). The concentration varies greatly between varieties and also with environmental and cultural conditions. The concentration of the cyanogenic glycosides increases from the centre of the tuber outwards (Bruijn, 1973). Generally, the cyanide content is substantially higher in the cassava peel. Bitterness is not necessarily a reliable indicator of cyanide content.

Traditional processing and cooking methods for cassava can, if efficiently carried out, reduce the cyanide content to non-toxic levels. An efficient processing method will release the enzyme linamarase by disintegrating the microstructure of the cassava root. On bringing this enzyme into contact with linamarin the glucoside is converted into hydrogen cyanide. The liberated cyanide will dissolve in the water when fermentation is effected by prolonged soaking, and will evaporate when the fermented cassava is dried. Sun drying fresh cassava pieces for short periods is an inefficient detoxification process. Cyanide will not be completely liberated and the enzyme will be destroyed during drying. Sun drying processing techniques reduces only 60 to 70 percent of the total cyanide content in the first two months of preservation. Cyanide residues

can be quite high in the dry tubers, from 30 to 100 mg/kg (Casadei, 1988). Simple boiling of fresh root pieces is not always reliable since the cyanide may be only partially liberated, and only part of the linamarin may be extracted in the cooking water. The reduction of cyanides depends on whether the product is placed in cold water (27°C) or directly into boiling water (100°C). After 30 minutes cooking, the remaining cyanides are, in the first case, 8 percent of the initial value, and in the second case about 30 percent (Essers, 1986).

Various authors have suggested different minimal levels for toxicity. Rosling (1987) was of the opinion that an intake of over 20 mg per 100 g of cassava is toxic, while Bolhuis (1954) set the toxic level at an intake of 50 to 60 mg daily for a European adult.

1.2.5 HEAVY METALS TOXICITY

Heavy metals are chemical elements with a specific gravity that is at least 5 times the specific gravity of water. The specific gravity of water is 1 at 4°C (39°F). Simply stated, specific gravity is a measure of density of a given amount of a solid substance when it is compared to an equal amount of water. Some well-known toxic metallic elements with a specific gravity that is 5 or more times that of water are arsenic, 5.7; cadmium, 8.65; iron, 7.9; lead, 11.34; and mercury, 13.546 (Lide, 1992). Others are antimony, bismuth, cerium, chromium, cobalt, copper, gallium, gold, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium, and zinc (Glanze, 1996).

Interestingly, small amounts of these elements are common in our environment and diet and are actually necessary for good health, but large amounts of any of them may cause acute or chronic toxicity (poisoning).

Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Heavy metals may enter the human body through food, water, air, or absorption through the skin when they come in

contact with humans in agriculture and in manufacturing, pharmaceutical, industrial, or residential settings. Industrial exposure accounts for a common route of exposure for adults. Ingestion is the most common route of exposure in children (Roberts, 1999). Children may develop toxic levels from the normal hand-to-mouth activity of small children who come in contact with contaminated soil or by actually eating objects that are not food (dirt or paint chips) (Dupler, 2001). Less common routes of exposure of heavy metals are during a radiological procedure, from inappropriate dosing or monitoring during intravenous nutrition, from a broken thermometer (Smith et al., 1997), or from a suicide or homicide attempt (Lupton et al., 1985).

Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs. Long-term exposure may result in slowly progressing physical, muscular, and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy, and multiple sclerosis. Allergies are not uncommon and repeated long-term contact with some metals or their compounds may even cause cancer (International Occupational Safety and Health Information Centre, 1999).

1.3 STARCHES IN FOODS

Starch, the food reserve homopolysaccharide of plants (Malcolm, 1990), occurs widely in nature and is the most commonly used (Whistler and Paschall, 1965, 1967). This is partly because of the wide range of functional properties it can provide in its various natural and modified forms, and partly because of its low cost relative to alternatives (Sanderson, 1981).

In the raw food, starch is located in discrete granules, which have structural characteristics of the foodstuff in question (Percival. 1970). The starch

granule has been the subject of a considerable amount of network, and the arrangement of the glucose units within the granule itself is highly ordered.

In freshly extracted granules, the original centre generally appears solid, or with a minute black point; but if the starch is dry, the center appears hollow, and sometimes it is even occupied by air with some starch grains containing a large cavity. If alcohol is applied to the fresh grains, the extraction of water, likewise, produces a hollow in the central point of growth, and in all these cases, cracks typically run out toward the surface. The lines in the starch granules are the boundaries of concentric superimposed layers. Sometimes these lines are very distinct and faint. Quite often, more distinct lines appear at intervals in the series of the same granule, and in these cases, a thin vacancy, or in the dried granules a stratum of air seems to exist between the layers. The specific gravity of starch is 1.53, and its chemical composition is $C_6H_{I0}0_5$, or a multiple of this formula (Satin, 1999; Swinkels, 1985; Thomas and Atwell, 1999). Because of their composition, starch and products derived from starch are used to modify the physical properties of many foods.

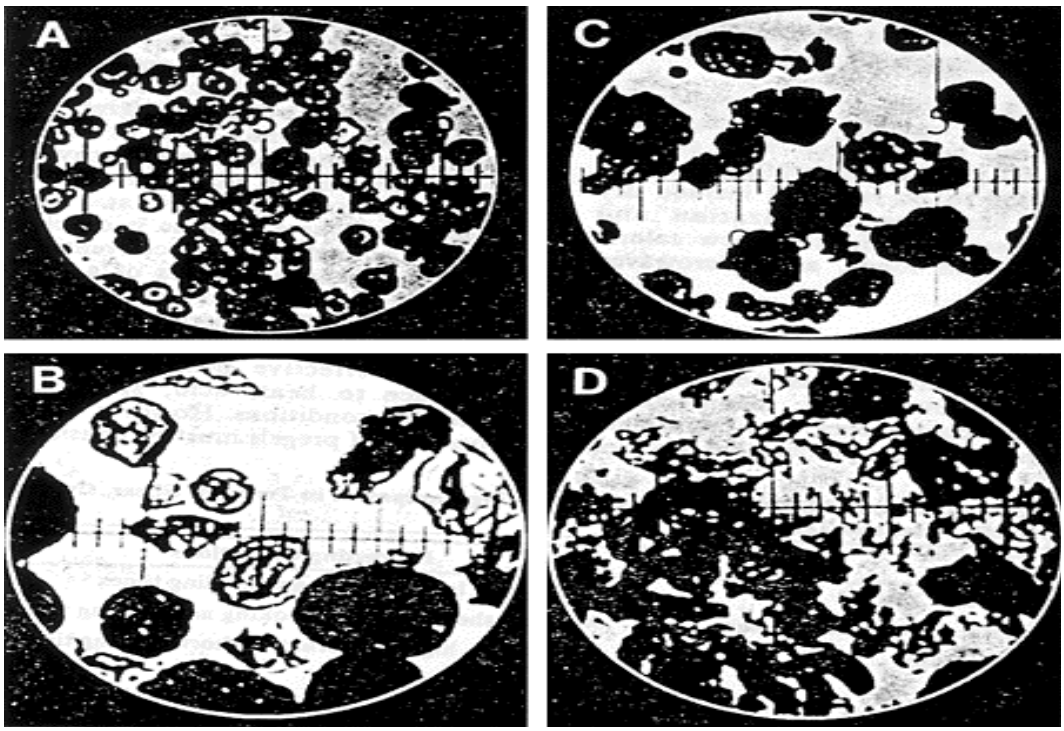

FIGURE 1.1: **STARCH GRANULES PHOTOGRAPHS** (Meyer, 1982).

Uncooked starch when viewed under a microscope shows small intact granules, 10-20 microns in diameter (Fig. 2.1A). If observed under polarized light, birefringent maltase crosses would be exhibited due to the crystalline structure of the granule. If added to water, the starch would settle. When properly cooked (for applications requiring maximum viscosity), the majority (approximately 80%) of starch granules are highly swollen with small percentages (10% each) of uncooked and ruptured granules (Fig. 2.1B). The granules would not exhibit birefringence under polarized light since the crystalline nature has been disrupted. Such granules provide excellent viscosity and textural characteristics, and the starch cook can show good clarity, excellent sheen, a heavy-bodied short texture, and good stability (Atwell et. al., 1988). In applications where highly swollen granules are desired, the food processor must aim for just the right cook. Either undercooked or overcooked starch may lead to poor functionality.

When starch is undercooked, the granules are slightly swollen and highly intact (Fig. 2.1C). Slurry of such starch would be low in viscosity, have poor clarity, a starchy taste, and poor stability. To improve the cook might involve using a less crosslinked starch or increasing the cooking time and temperature (Atwell et. al., 1988).

If the starch is overcooked, the majority of the granules are ruptured with a large percentage of fragments (Fig. 2.1D). Even though this cook will have good clarity, it is cohesive and usually lower in viscosity than desired.

Starch contains two components that contribute to its molecular structure-amylose, a linear molecule, which consists of several glucose units linked together via $\alpha(1\rightarrow4)$ glycosidic linkage, and amylopectin, a non linear molecule, consisting of several glucose units linked together by $\alpha(1\rightarrow4)$ glycosidic linkage, but having an $\alpha(1\rightarrow6)$ glycosidic linkage at selected sites, thus generating a branch point (Whistler et. al., 1984) While amylose has average molecular

weight of 1.0 x 10^6, present in about 20% by weight and is soluble in water, amylopectin has average molecular weight of 5.0 x 10^6, present in about 80% by weight of the starch and is insoluble in water (Ihekoronye and Ngoddy, 1985).

1.3.1 AMYLOSE AND AMYLOPECTIN

Amylose and amylopectin do not exist free in nature, but as components of discrete, semi crystalline aggregates called starch granules. The size, shape, and structure of these granules vary substantially among botanical sources (Thomas and Atwell, 1999). Starch, from any source, exits in the form of white granules of varied size and form; these granules are organized structures, although their existence in relation to that of the cell is transitory (Thomas and Atwell, 1999). They are the first formed products of assimilation, insoluble in the ordinary cell-sap of the plants containing them, through a process of organization analogous to that by which the development of the cell itself is effected. When these minute granules acquire appreciable dimensions, concentric lines may be observed, more or less distinctly in different cases; a relevant example is the granules of the potato-starch. These lines increase in number with an increase in size, and in many cases, become eccentrical from the preponderating growth of one side of the granule (Delpeuch et. al., 1978; Swinkels, 1985; Thomas and Atwell, 1999 and Whistler, 1964).

The structural differences between these two polymers contribute to significant differences in the starch properties and functionality (Radley, 1968; Radley, 1976; Satin, 1999; Takahata, et. al., 1995; Thomas and Atwell, 1999 and Whistler, 1967).

The starch properties depend upon their amylose and amylopectin contents (Peterson and Johnson, 1978). Amylose holds the starch granules intact as they swell and gelatinize. Crystalline areas of the starch are partially dependent on the amylose, which reinforces the granule network (Numfor et. al., 1995).

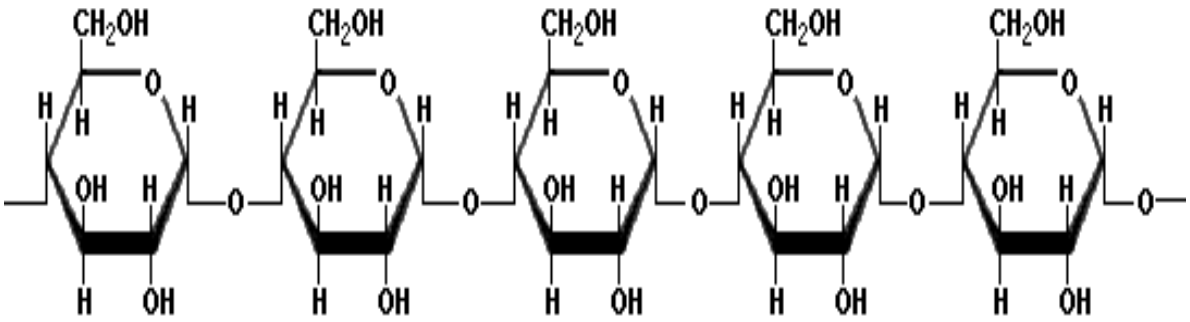

FIGURE 1.2: STRUCTURAL PART OF AMYLOSE CHAIN

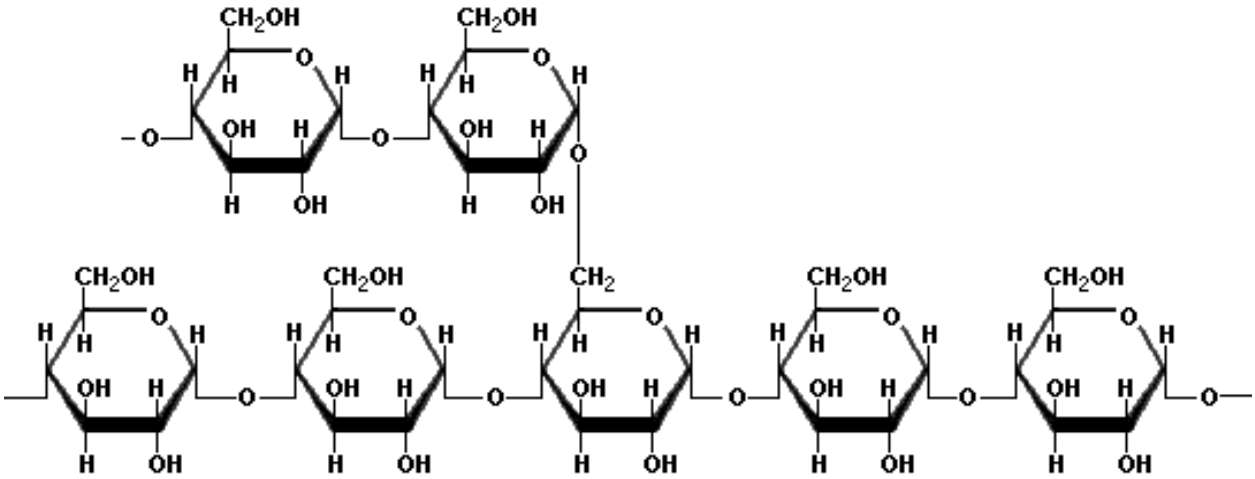

FIGURE 1.3: SRUCTURAL PART OF AMYLOPECTIN

1.3.2 CLASSIFICATION OF STARCHES

1.3.2.1 CLASSIFICATION OF STARCH BASED ON COOKING

deNoord (1970) had classified cooked starches as either cereal starch or roots and tubers starch, and deMan (1976) reported another class of cooked starch as waxy starch.

1.3.2.1.1 CEREAL STARCHES

In cereals (e.g. maize, wheat, rice and sorghum), the moisture content of the grain is low, and the starch granules are embedded in a hard, proteinaceous matrix, which requires preliminary softening before starch extraction (Ihekoronye and Ngoddy, 1985). The cereal starch granules have a low swelling power and

show only moderate peak viscosities on heating when compared to other starches (Sanderson, 1981). This is due to the fact that starches from cereals have more amylose (Ihekoronye and Ngoddy, 1985). On cooling, cereal starch pastes set to opaque gels (deMan, 1976).

1.3.2.1.2 ROOT AND TUBER STARCHES

Unlike cereals, root and tuber crops, such as yams, cassava, potatoes, cocoyam, have much high moisture content. However, no preliminary softening process is required for extracting the starch granules, since they are relatively loosely bound in the cellular plant structure (Ihekoronye and Ngoddy, 1985). Highly viscous long-bodied clear pastes are formed, resulting in only weak gels on cooling (deMan, 1976).

1.3.2.1.3 WAXY STARCHES

Waxy rice and corn form heavily bodied clear stringy pastes. The pastes have low tendency for gel formation (deMan, 1976). High amylose starch (corn) requires high temperatures for gelatinization and gives short bodied paste, which forms a very firm opaque gel on cooling (deMan, 1976).

1.3.2.2 CLASSIFICATION OF STARCH BASED ON MODIFICATION

As with waxy corn, other native starches such as those from dent corn, potato, and tapioca have inherent disadvantages that would make them unsuitable for food systems and processing. These include a narrow peak viscosity range, undesirable textures, and poor stability and processing tolerance (Atwell et. al., 1988). In many cases the native starch would make an uneconomical, poor quality product (Atwell et. al., 1988). However, by appropriate structural modification, these undesirable properties can be turned into desirable ones. Such

modified products can provide the needed controlled peak viscosity, improved tolerance to rigorous processing conditions, desirable texture, and prolonged stability.

1.3.2.2.1 DERIVATIVES OF MODIFIED STARCHES AND USES

Davidson (1967) had reported the following modified starch derivatives:

Acid Conversion

Starches modified through acid conversion have amylopectin units greater than amylose and form gels on cooling, have decreased hot paste viscosity and decreased gelatinization temperature. They are used as gum candies.

Hydroxyethyl Starches

Starches chemically modified with hydroxyethyl are characterized with reduced gelatinization temperature, increased rate of swelling and low tendency of pastes and gels to retrograde.

Hydroxypropyl Starches

Propylene oxide is used as the modifying agent in producing hydroxypropyl starches. They are used as salad dressings, pie fillings and thickenings.

Starch Phosphates

These modified starches are characterized with low gelatinization temperature, swelling in cold water, increased paste viscosity and decreased retrogradation.

Starch Acetates

These form fibers similar to those of cellulose acetates, but they are not as strong as the latter. They possess low gelatinization temperature and ability to resist retrogradation after pasting and cooling.

The modifications listed above make starch an important functional ingredient for numerous food systems. Starch can be utilized in numerous

possible functional application areas, including adhesion, antistaling, binding, clouding, dusting, emulsion stabilization, encapsulation, flowing aid, foam strengthening, gelling, glazing, moisture retention, molding, shaping, stabilizing and thickening. For example, starch can be used on fried fish where it binds the breading to the fish piece, in processed meats where starch binds the juices, in orange soda where it provides emulsion stability, in candy where starch provides structure, and in numerous other applications where starch is used as a thickener.

1.3.3 STARCH PASTES AND GELS

1.3.3.1 STARCH PASTES

A starch paste is the viscous mixture of gelatinized starch and liquid (Miller et. al., 1973). It is a colloidal sol. Under the microscope, a starch paste appears as granules swollen until they touch each other (see Fig. 1.1). Such fully gelatinized granules look like gelatinous sacs filled with an aqueous solution of starch (Griswold, 1970).

The explanation of the structure of sol (or paste) has been extended to cover the formation of lumps in starches (Meyer and Gibbons, 1951). When starch granules are heated in water, the molecules of low molecular weight are dissolved from the surface of the granules, leaving those of high molecular weight in swollen, elastic meshes. As water penetrates the granules through these meshes and dissolves amylose molecules of low molecular weight, the granules swell, making the paste viscosity high (Griswold, 1970). Then, as more molecules are dissolved and diffuse out, granules shrink, because their osmotic pressure has been lowered and the viscosity of the starch decreases.

In many instances, the starch paste is a transient process, as in a good viscous starch paste; the starch fraction may begin to undergo retrogradation (gelation) if all conditions are acceptable (Miller et. al., 1973).

1.3.3.2 STARCH GELS

The structure of starch gels is sometimes described as an interlacing network formed by amylose, amylopectin and water molecules held together by intermolecular bonds (Griswold, 1970), giving rise to three-dimensional macro molecules (Sanderson, 1981). The same or different molecular species may be involved in such network (Rees and Welsh, 1977).

Paste formation usually precedes gel formation, since starch paste is considered a paste as long as no gelation has occurred (Miller et. al., 1973). In other words, starch gel is the rigid structure, which is formed when cooked starch paste is cooled and stored at room temperature. It is usually associated with native starches (containing amylose) and acid-thinned starches.

1.4 NUTRITIONAL SIGNIFICANCE OF FOOD STARCH

1.4.1 PROXIMATE COMPOSITION

1.4.1.1 PROTEINS IN FOOD

Proteins are important constituents of foods for a number of different reasons. They are a major source of energy, as well as containing essential amino-acids, such as lysine, tryptophan, methionine, leucine, isoleucine and valine, which are essential to human health, but which the body cannot synthesize. Proteins have a major effect on the structure and sensory quality of numerous foods in fresh and processed conditions, for example, on the consistency and texture of meat and meat products, milk and cheese, pasta and bread.

Food quality most often depends on the structural and physicochemical quality of the protein components. Some of these functional properties are: hydration, solubility, viscosity, gel formation, texture, dough formation, emulsification, foaming, aroma binding and interaction with other food components (FAO/WHO, 1990). Crude protein is the total amount of protein in

the food, but it says nothing about its digestibility. The quality of the protein is very important.

In practice, most biological methods for evaluating protein quality are, in fact, evaluating nitrogen but are expressed as crude protein (N x 6.25). Nitrogen in foods not only comes from amino acids in protein, but also exists in additional forms that may or may not be used as a part of the total nitrogen economy of humans and animals. The nitrogen content of proteins in foods can vary between 150 and 180 g/kg 115-18 per cent), depending on the amino acids they comprise.

In addition, purines, pyridines, free amino acids, vitamins, creatine, creatinine, and amino sugars can all contribute to the total nitrogen present. In meat, a portion of the nitrogen occurs as free amino acids and peptides; fish may contain these and volatile base nitrogen and methyl-amino compounds (Udy, 1956). Marine elasmobranchs may also contain urea. Half of the nitrogen of the potato may not be in the form of protein (Neuberger and Sanger, 1942), and even in human milk as much as 50 per cent of the total nitrogen may be urea nitrogen (Erickson et. al., 1963). Nitrogen analysis of a food is usually much more precise than the nutritional significance that can be attached to it, because the nutritional significance of much of the non-amino acid and non-peptide nitrogen is unclear.

The protein content of foodstuffs is conventionally estimated from the nitrogen content determined by the Kjeldahl technique. Munro and Fleck (1969) have proposed numerous modifications of the original procedure of the Association of Official Analytical Chemists (AOAC, 1975).

1.4.1.2 MOISTURE CONTENT

Moisture content is one of the most commonly measured properties of food materials. It is important to food scientists for a number of different reasons:

Legal and Labeling Requirements: There are legal limits to the maximum or minimum amount of water that must be present in certain types of food.

Economic: The cost of many foods depends on the amount of water they contain - water is an inexpensive ingredient, and manufacturers often try to incorporate as much as possible in a food, without exceeding some maximum legal requirement.
Microbial Stability: The propensity of microorganisms to grow in foods depends on their water content. For this reason many foods are dried below some critical moisture content.
Food Quality: The texture, taste, appearance and stability of foods depend on the amount of water they contain.
Food Processing Operations: Knowledge of the moisture content is often necessary to predict the behavior of foods during processing, e.g. mixing, drying, flow through a pipe or packaging.

In principle, the moisture content of a food can therefore be determined accurately by measuring the number or mass of water molecules present in a known mass of sample. It is not possible to directly measure the number of water molecules present in a sample because of the huge number of molecules involved.

1.4.1.3 CRUDE FIBRE

Crude fibre is indigestible carbohydrate that adds bulk to the diet. It is the organic residue after food sample has been treated under standardized conditions with light petroleum, boiling tetraoxosulphate (vi) acid, boiling sodium hydroxide and dilute hydrochloric acid (Pearson et. al., 1981). Sources of fiber include plant hulls, such as oat bran, or vegetable material such as beet pulp. There should be 4% or less fiber in foods intended for ferrets (http://www.peteducation.com/ article.cfm?cls=11&cat=1296&articleid=563).

1.4.1.4 ASH CONTENT

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food. Analytical techniques for providing information about the total mineral content are based on the fact that the minerals (the "analyte") can be distinguished from all the other components (the "matrix") within a food in some measurable way.

The most widely used methods are based on the fact that heating does not destroy minerals, and that they have a low volatility compared to other food components. The three main types of analytical procedure used to determine the ash content of foods is based on this principle: dry ashing, wet ashing and low temperature plasma dry ashing. The method chosen for a particular analysis depends on the reason for carrying out the analysis, the type of food analyzed and the equipment available.

Ashing may also be used as the first step in preparing samples for analysis of specific minerals, by atomic spectroscopy or the various traditional methods described below. Ash contents of fresh foods rarely exceed 5%, although some processed foods can have ash contents as high as 12%, e.g., dried beef (Anderson, 1996). Ash content is very important because it contains mostly the essential and non-essential minerals and, in fact, it is a good evaluation of the nutritive quality of food (Pearson et. al., 1981; http://www.peteducation.com/article.cfm?cls=11&cat=1296&articleid=563).

1.4.1.5 FAT CONTENT

Fats are esters of alcohol with aliphatic fatty acids and normally occur in animal and vegetable tissue as a mixture of several pure fats plus free fatty acids. The most common of such fats are palmitin, the ester of palmitic acid; stearin, the ester of stearic acid; and olein, the ester of oleic acid. These pure chemical

compounds are contained in different proportions in various natural fats and oils, and they determine the physical characteristics of each of these substances (Microsoft Encarta Encyclopedia, 2005). Fats are a good source of energy and serve as the body osmoregulator (Pearson et. al., 1981).

1.4.2 PHYSICOCHEMICAL PROPERTIES OF FOOD

Oshodi and Ekperigin (1989) have defined functional properties of food as the intrinsic physicochemical characteristics, which may affect the behaviour of food system during processing and storage. Such physicochemical properties are gelatinisation temperature, retrogradation, viscosity, stability, clarity, pH, solubility, swelling power and so on. Functionality is the key to marketing starches in the wide range of food and non-food applications, if pre-treated to eliminate possible toxicity (Oladebeye, 2003).

1.4.2.1 PASTING PROPERTIES

The use of Brabender Amylographs in characterizing the pasting properties (that is, gelatinization temperature, viscosity, retrogradation and stability) has been studied (Mazurs et. al., 1957; Georing and Shurb, 1967 and Lii et. al., 1982), and the use of Rapid Visco Analyzer (RVA) as an alternative to Brabender Amylographs had been confirmed by Thiewes and Steeneken (1997).

RVA was designed with the objectives of providing simple test by incorporating precise temperature and speed control, and use of paddle sensor to ensure maintenance of homogeneous sample as appropriate for starch-based samples (IITA, 1995).

The commonly measured parameters with RVA method are shown in figure 2.4.

1.4.2.1.1 GELATINIZATION TEMPERATURE

Gelatinization, according to Califano and Anon (1990), is a transition from an ordered state to a disordered state of the starch granules when the starch is heated in an aqueous medium. The transition is evident by the sudden swelling of the starch granules at a critical gelatinization temperature; the point at which the optical birefringence disappears, indicating loss of crystallinity (deMan, 1976) and it occurs as soon as the pasting temperature is reached (IITA, 1995).

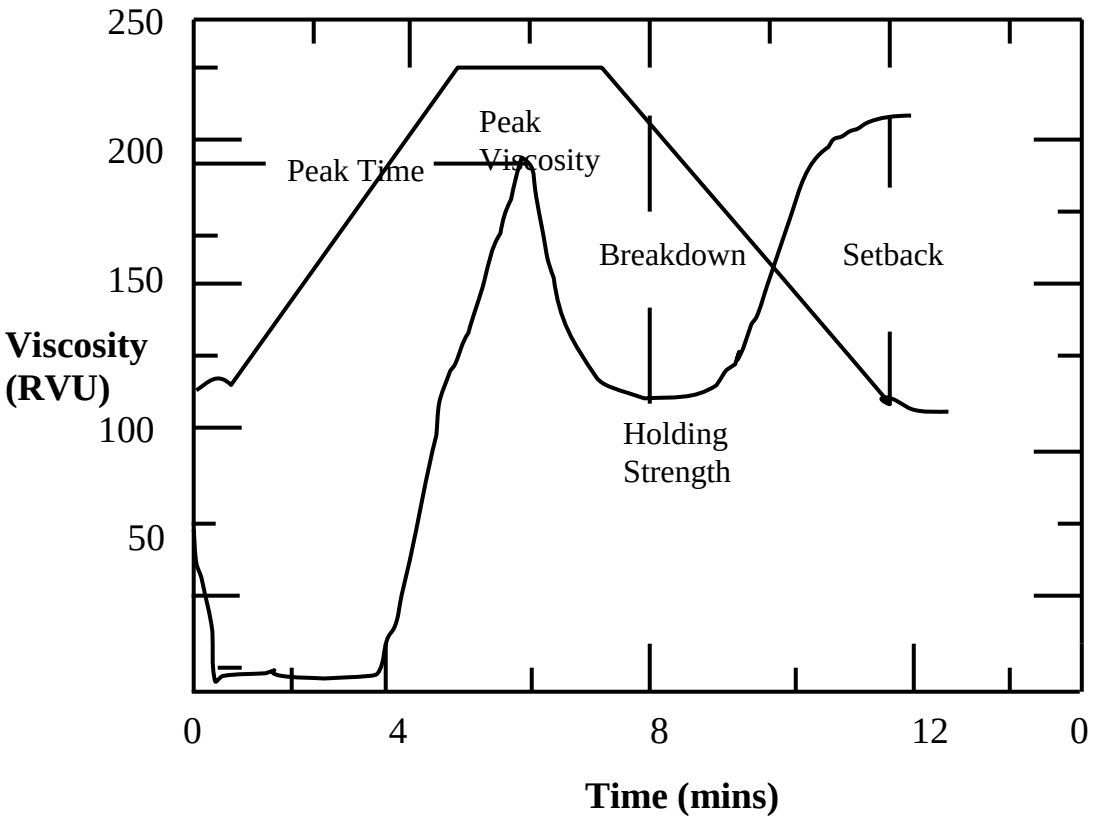

FIGURE 1.4: TYPICAL RVA PASTING CURVE

Miller et. al. (1973) has defined gelatinization temperature as the temperature at which a particular starch gelatinizes to give a starch paste. This depends on the starch type, the environment and several other factors. Normally, chemical stabilization reduces the gelatinization temperature and the presence of high solids increases it.

Ihekoronye and Ngoddy (1985) have found out and stated that it is at the gelatinization temperature of a starch that some of the smaller molecules of amylose become disentangled and are leached out into the surrounding water. However, if gelatinization occurs under reduced water content or in the absence of water (Blanshard and Mitcheil, 1979), it results in pyrolysis, although at very high temperature of about 220^{o}C (Akintayo, 1999).

1.4.2.1.2 PASTE VISCOSITY

The gelatinization of a starch could be measured in terms of swelling changes, solubility and viscosity (Miller et. al., 1973; Collison, 1968; Doublier, 1981; Zobel, 1984; Langton and Hermansson, 1989; Launay et. al., 1966 and Radosta et. al., 1991)

Viscosity or thickness (Miller et. al., 1973) is the term used to describe the resistance of liquids to shear agitation or flow and it occurs when the starch paste is heated above its gelatinization temperature (deMan, 1976), and on cooling, the paste becomes more viscous, especially, when a gel is formed (Griswold, 1970).

Kramer and Twigg (1970) had shown the importance of viscosity in characterizing and selecting starch for food uses, which includes the functions of viscosity as an index of consistency; as a quality control tool on the raw products; as a measure of a constitution of polymers; as a useful criterion of disaggregation or depolymerization, usually found in the initial stages of hydrolysis of protein, starch and pectin, and as an index of the amount of ingredient, which should be added to the composition of a product.

1.4.2.1.3 RETROGRADATION

Retrogradation, also known as “set back”, has been described by Lewandowicz et. al., (2002) as the realignment of the glucose polymers,

primarily amylose, resulting in the syneresis phenomenon with possible exudation of liquid and a fall in viscosity (Alais et. al., 1999).

Retrogradation phenomenon is associated with amylose and the tendency of amylose to retrograde is increased by molecular weight reduction to a chain length of around 100 anhydroglucose units (Lewandowicz et. al., 2002) and amylopectin shows less tendency to retrograde. Ihekoronye and Ngoddy (1985) had reported that when aqueous starch solutions are allowed to stand under aseptic conditions, they become opalescent, increasingly cloudy, increasingly resistant to enzyme action and lower in viscosity as a result of retrogradation.

Furthermore, retrogradation of starch has some important consequences in various areas such as a cake not risen well, cream separated, bread hardened (gone stale) without being dry, runny pastes and glues (Alais et. al., 1999). deMan (1979) also ascribed the staling of bread to retrogradation, which gives the bread its elastic and tender crumb structure. Staling is the hardening of the crumb upon storage.

1.4.2.1.4 PASTE STABILITY

As suggested by O'Dell (1979), the first step to stabilize starch in a food system is to remove its amylose units. Paste stability of a starch has been defined by Lewandowicz et. al., (2002) as the resistance of the starch to viscosity breakdown when shear stress is highly applied.

In RVA studies, the stability of a starch paste is the measure of its "breakdown", which occurs during the "hold" period of the test, whereby the sample is subjected to a period of constant high temperature (usually $95^{o}C$) and mechanical shear stress. Hence, the granules are disrupted and amylose molecules will generally leach out into the solution, and will be aligned in the direction of the shear (IITA, 1995).

1.4.2.1.5 PASTE CLARITY

Clarity is a term used to indicate the degree of transparency in starch pastes and syrups and, its measurement is based on light transmission. (Lewandowicz et. al., 2002).

When starch is first mixed with water, the suspension is cloudy and milky in appearance, but becomes clearer on heating (Griswold, 1970). Perera and Hoover (1999) had reported that the clarity of a starch paste can be determined by measuring its absorbance (or transmittance) value at 640nm every 24 hours by storing it for a couple of days at 4^{o}C.

Based on Beer-Lambert's law, the clearer the suspension, the lower the absorbance and the higher the transmittance.

Mathematically,

$A = Ecb = -\log_{10}T$

and

$I_o = I_a + I_t$

Where

A = Absorbance; E = Molar absorptivity; c =molar concentration; b= cell length; T =transmittance; I_o =incident intensity; I_a =absorbed intensity and I_t =transmitted intensity.

1.4.2.2 GEL STRENGTH

This is the resistance of a starch gel to deformation under specific conditions (Lewandowicz et. al., 2002). In this research work, the gel strength of a starch is determined by modifying the procedure of least gelation concentration (Coffman and Garcia, 1977), and the gel strength is taken to be the highest least gelation concentration.

1.4.2.3 SWELLING POWER

When an aqueous suspension of starch is heated above its gelatinization temperature, the granules will undergo irreversible swelling and the degree at which they swell is a function of the starch type and the presence of any physical or chemical modification.

The swelling power of a starch paste is the measure of its hydration capacity and it is expressed as the ratio of the weight of the centrifuged swollen granules to the weight of the original dry starch used to make the paste (Lewandowicz et. al., 2002). According to Lee and Osman (1991), the swelling power of starch depends on the water holding capacity of the starch molecules by hydrogen bonding. Hydrogen bonds stabilizing the structure of the double helices in crystallites are broken during gelatinization and are replaced by hydrogen bongs with water, and swelling is regulated by the crystallinity of the starch (Tester et. al., 1996). However, the crystalline region of starches with a higher portion of long chain of amylopectin is stabilized due to longer double helices and may form more hydrogen bonds with water when they are heated in excess water than those starches with shorter chains of amylopectin (Sasaki and Matsuki, 1998).

deMan (1976) had stated that the swelling of starch granules can be inhibited in the presence of fatty acids, presumably, through the formation of insoluble complexes with the linear fraction, that is, amylose.

1.4.2.4 SOLUBILITY

The hydrogen bonds are established between chains of starch granules by the hydroxyl groups, thus influencing both the physical resistance and solubility of the molecules. They allow the formation of quite compact masses, which have a certain degree of crystallinity, which is to say, a regular spatial structure, Alais et. al. (1999) has stated that these bonds can be broken through heating; in this

way the solubility is raised and the crystallinity reduced. However, solubilization occurs by assuming that a part of the amylose units is involved in the micelles network while the rest is free from entanglement and preferentially solubilized (Purshottam et. al., 1990).

The solubility of a starch, therefore, as described by Purshottam et. al., (1990), is expressed as the percentage by weight of the starch sample that is dissolved molecularly after heating in water between 65 and 95^{o}C for about 30 minutes. It is expressed as g/100g of the sample at dry weight basis (Akintayo, 1999).

1.4.2.5 WATER ABSORPTION CAPACITY (WAC)

Water absorption capacity (WAC) is defined as the water absorbed by the protein content of the particulate matter of a food substance after equilibration against water vapour at a known relative humidity (Mellon et. al., 1947). It generally depends on starch and protein contents and particle size. Fine particle size (425 pm) was associated with higher water absorption than coarse particle size regardless of variety of plant (Kulkarni et. al., 1988).

1.4.2.6 pH AND ACIDITY

pH means the potential of hydrogen. It is the measure of acidity or alkalinity of a substance. A pH scale is the scale that shows the variance of the pH values from 0 to 14. pH values less than 7.0 indicate acidic substances; pH value of 7.0 indicates a neutral substance and pH values greater than 7.0 indicate alkaline substances. The pH values of most foods range generally from neutral to acid (Ihekoronye and Ngoddy, 1985). The combination of high temperature and low pH encourages the denaturation of protein with concomitant loss of water holding capacity.

1.4.2.7 COLOUR OF FOODS

Colour is an important quality of many foods. It is a quality attribute, which together with flavour and texture plays a significant role in the acceptability of food (Ihekoronye and Ngoddy, 1985).

Basically, colour cannot be studied without considering the human sensory system. Accordingly, colour can be defined as a psychological interpretation of a physiological response by the eye and brain to the physical stimulus of light radiation at different wavelength. The human eye, however, is limited when it comes to high resolution of colours.

In spectrophotometric measurement of colour, three attributes are considered namely: hue, L value and chroma. Hue is the quality by which one colour is distinguished from another, as red from yellow, green, blue or purple; L value is the measure of lightness from completely opaque to completely transparent and chroma is the measure of strength and weakness of a colour (Bakker and Arnold, 1993).

1.4.2.8 BULK DENSITY

The bulk density of a powder or particulate product is expressed as the weight of a unit volume of the powder including the pores and inter-particle voids. The bulk density may be measured on the loose unpacked powder or on the powder after tapping under defined conditions. The bulk density is generally affected by the particle size Bulk density is important for determining packaging requirements, material handling, and application in wet processing in the food industry (Kulkarni et. al., 1988).

1.4.2.9 ENERGY VALUE

Energy has been defined as the ability to do work. The primary source of energy is the sun and gets into the food chain or web through the green plant

during photosynthesis. Energy can neither be created nor destroyed, but can be converted from one form to another.

The measurement of the energy released when nutrient of food is oxidized is very important in nutrition. The energy value of a food can be assessed by burning the food in oxygen and measuring the amount of heat energy produced by means of bomb calorimeter (Ihekoronye and Ngoddy, 1985).

1.4.3 MINERAL COMPOSITIONS OF FOODS

Mineral, also known as inorganic matter of food that remains after the burning off of the organic matter, is a determinant of the quality of food, health, well-being and safety of the consumer (Olaofe et. al., 1987). It is, therefore, imperative to have adequate knowledge of the mineral composition of food.

Minerals in food can be grouped into macromolecules and micromolecules (or trace metals). The macromolecules, which include Ca, P, Mg, K and Na are required in amount greater than 100mg/day while micromolecules are needed in lower amount than 50ppm/day e.g. Fe, Cu, Mn, Cr, Co, Pd, As, Zn, Hg, Cd and Ni.

Trace elements are classified based on their effect on health into the following categories:

1. *Essential nutritive minerals:* These are minerals essential for the metabolic processes taking place in the body, although they may be harmful if taken in excess/. Examples are Na, K, Ca, Cu, Mg, Fe, Co, I, Mn and Zn.

2. *The non-nutritive, non-toxic minerals:* These are minerals with no harmful effect except when present in amount exceeding 100ppm. Examples are Al, B, Cr, Ni and Sn.

3. *The non-nutritive, toxic minerals:* These are minerals with toxic effect even when the diet contains less than 100ppm. Examples are As, Sb, Cd, F, Pb, Hg and Se.

One of the factors influencing the uptake of macro and microelements in plants is the composition of the soil on which the plant is grown, indicating the variation of the ash and mineral contents of the agricultural products owing to the planting location.

1.5 NUTRITIONAL VALUE OF STARCHES OF ROOTS AND TUBERS

Starch granules usually contain 10-20% (w/w) moisture and small amounts of proteins, fatty materials, phosphorous, and traces of mineral elements and inorganic salts (Swinkels, 1985). For example, tuber starches contain covalently bound phosphates (Thomas and Atwell, 1999). The phosphorous in the cereal starches is mainly present as phospholipids.

The root starches (e.g., tapioca) contain a very low amount of phosphorous compounds. Potato starch is the only commercial starch that contains an appreciable amount of bound phosphate ester groups. The ester phosphate groups are bound to the C-6 position of glucose units of amylopectin molecules in potato starch. The number of phosphate groups in potato starch ranges from 1 phosphate group per 200 to 400 glucose units (Radley, 1968; Swinkels, 1985; Takahata et. al., 1995). The phosphate substituent confers on potato starch amylopectin the properties of a polyelectrolyte when dispersed into aqueous solutions. The mutual repulsion of the charged group forces the molecule to expand. The phosphate group can be considered an ion-exchanging group (Radley, 1968 and Swinkels, 1985). Generally, potato starch shows a higher paste viscosity than other starches when compared at similar conditions. This may be explained by the influence of the phosphate groups in potato starch. A higher phosphate content in potato starch results in a higher viscosity. The root and waxy starches also tend to have a higher paste viscosity (Swinkels, 1985).

When starch is added to products as an ingredient, however, it is the functional properties of the starch that are usually important, not the calories. Starch is the main thickener in gravies, sauces, and puddings. It absorbs water, and becomes a gel when cooked. As the starch swells up with water, the amylose leaches out, and the amylopectin forms the gel. Some starches have higher amylopectin content, and make better gels than those containing lots of amylose.

As a thickener (as opposed to a gel), it is the amylose that has the main function. The long water-soluble chains increase the viscosity, and that viscosity doesn't change much with temperature.

Amylose chains tend to curl up into helices (spirals) with the hydrophobic parts inside. This allows them to trap oils and fats inside the helix, as well as aroma molecules. Because starches are so good at absorbing water and bulking up, they are important in the "mouth feel" of many food products, and are used as fat substitutes. Not all of the starch in a food ends up being digested. The starch that is not absorbed by the body is called "resistant starch", and is consider dietary fiber. It is also a source of nutrition for intestinal flora, which make important vitamins (and intestinal gas). Starches are added to processed meats (lunch meats, hot dogs, sausages, etc.) as a filler, binder, moisture retainer, and fat substitute. They are added to soups, sauces and gravies as thickeners. They are used in extruded cereals and snacks to hold the shape of the material.

1.6 OVERVIEW OF PHARMACEUTICAL STARCH

Historically, the majority of formulations in the development of new drug products contained native corn (maize) starch, which was used as both a binder and disintegrant. As a binder, the starch was converted to a paste before adding it to the wet granulation. As a disintegrant, it was added dry to the powder blend. Both methods were used frequently because the starch lost much of its disintegration properties when it gelatinized in the preparation of the starch paste.

As a dry addition to a granulation, the native corn starch did not flow well and was not very compressible (Cunningham, 2001).

Before now, polymers such as Povidone (PVP) are preferred as binders for wet granulated products. When hydrated, these binders produce viscous, tacky solutions. The tackiness holds the individual granules together. However, polymer binders can also lead to processing difficulties such as rapid over-granulation. Over time, they occasionally lead to tablet hardening and a decrease in dissolution performance. When polymer binders are chosen, the addition of strong disintegrants, such as super disintegrants, is typically required, because a balance must be maintained between the binding and the disintegration properties of a formulation. These materials surpass native corn starch in their ability to wick moisture and swell. However, super disintegrants are considerably more expensive and can have a negative effect on product stability as well as the film coating appearance of the finished product.

An alternative to native corn starch or polymers for wet granulations is pregelatinized starch. This is a starch that has been previously gelatinized and dried to powder form. Functionally, pregelatinized starches are split into two groups, fully pregelatinized and partially pregelatinized (Cunningham, 2001).

Fully pregelatinized starches are being used as binders in wet granulated formulations. But, due to the gelatinization, much of the disintegration properties are lost. Partially pregelatinized starches on the other hand, have a mixture of properties of both native and fully gelatinized starches. This makes them useful as both a binder and a disintegrant in wet granulated formulations. High shear wet granulation is also well suited for the use of partially pregelatinized starches. Partially pre-gelatinized starches can be hydrated with cold water to produce viscous slurries or, alternatively, can be added directly to the granulator bowl and water can be utilized to granulate (Leach et. al., 1959).

In recent years, formulators have begun to look past the traditional uses of pregelatinized starch as binder, disintegrant and filler. Pregelatinized starch has revealed new applications to enhance drug stability by preferentially binding moisture, and to control release rates for developing modified-delivery dosages.

Pregelatinized starch is defined as starch that has been chemically and/or mechanically processed to rupture all or part of the granules in the presence of water and subsequently dried. Some types of pregelatinized starch may be modified to render them compressible and flowable in character. The monograph does not specify the level of gelatinization or modification of the starch or differentiate between fully and partially pregelatinized starches. Many commercially available pregelatinized starch products meet monograph requirements, but differ in levels of modification and functionality (Cunningham, 2001).

Brookfield viscosity testing determines starch variations and provides insight into functional and physical-particle size, density and morphology—differences among starches with varying levels of modification. For instance, fully pregelatinized starch is extremely soluble in cold water, eliminating the need to prepare heated starch pastes for wet granulation applications. By eliminating this pre-solubilization step, the starch can be added directly to granulation equipment with other actives and excipients. Water can then be used as the granulation fluid. Partially pregelatinized starch (PPS) contains soluble (gelatinized) and insoluble fractions. In most cases, the insoluble fraction comprises intact starch grains.

The larger particle size of the more granular pregelatinized starch imparts better flow properties than native starch. PPS contains unmodified and modified starch, so can be used in wet granulation applications as a cold-water binder and still retain high-disintegrant functionality for immediate-release dosage forms. PPS also has been used as a disintegrant and powder flow aid in direct-

compression applications, improving content uniformity of low-dose actives. In order to ensure the homogeneity of small amounts of potent actives within a large quantity of excipients or diluents, blending techniques such as geometric dilution are often employed.

The primary diluent, or pre-blend carrier, is mixed in equal proportion to the active. Then, twice the volume of excipient is added and the mixing continued. This process is repeated until all the diluent is used. In one direct-compression application, Ahmed and Shah (2000) evaluated various excipients as pre-blend carriers or diluents to enhance uniformity and reduce the segregation potential of a micronized, low dose (0.07% w/w) active. The researchers mixed the drug substance with lactose, microcrystalline cellulose or PPS and subjected each blend to vibration at constant amplitude. The blend containing PPS as the pre-blend excipient yielded the most uniform results and superior content uniformity, with a mean drug content of 99% and relative standard deviation of 2%. Researchers speculated that PPS' adhesive characteristics, pregelatinized nature and inherent moisture content could have contributed to blend homogeneity. While the moisture content of starch is higher than other direct-compression excipients, the water activity—or equilibrium relative humidity (ERH)—is lower. Thus, formulations containing starch can equilibrate more slowly when exposed to high humidity. PPS might also enhance drug stability by preferentially binding moisture and decreasing the rate at which the ERH equilibrates with the environment (Heidemann and Jarosz, 1991).

The potential of PPS to bind moisture has practical applications in formulating moisture-sensitive actives. Super disintegrants, while advantageous in some formulations, have a high propensity for moisture uptake compared to PPS and should not be used at higher than their recommended levels. For example, one study examined the effect of PPS on the stability of aspirin 81mg

tablets combined with microcrystalline cellulose and two hydrophilic super disintegrants (Cunningham et. al., 2001).

Leach et. al. (1959) claimed that PPS has a limited obstructive gel layer on the surface of the tablet. This would indicate that PPS is not suitable for sustained-release applications. However, in combination with other polymers, PPS can be a viable excipient. The cellulose ether derivative, hypromellose, is commonly used in controlled-release tablets. The drug release rate from the HPMC (hydroxypropyl methylcellulose) matrix depends on the type and amount of other excipients used in the formulation. Including PPS in HPMC sustained-release tablet formulations can result in slower drug release compared to other commonly used fillers, such as lactose and microcrystalline cellulose (Levina et. al., 2001). The effect seen with PPS is not just a spatial one resulting from the presence of insoluble or partially soluble fillers, which may change the physical permeability characteristics of the polymer gel. When used in combination with HPMC, PPS actively contributes to drug release kinetics. This contribution might be imparted via interaction between PPS and HPMC or the filler actively forming an integral structure within the HPMC gel layer.

1.7 OBJECTIVE OF THIS RESEARCH WORK

This research work is aimed at providing ample information and data on the suitability of starches of other roots and tubers for pharmaceutical applications other than the commonly used corn starch, which has suffered some setbacks owing to its inability to flow well and compress easily. Eight different starches were selected for this research work, which are the corms and cormels of each of the red and white varieties of cocoyam (*Colocasia* esculenta), cassava (*Manihot* esculenta), sweet potato (*Ipomea* batata), bitter yam (*Dioscorea dumentorum*) and water yam (*Dioscorea alata*). To get this done, there is need to consider the nutritional values of the selected starches in terms of their proximate

compositions such as moisture content, crude protein, crude fibre, fat content, ash content, sugar and starch content. Other nutritional values to consider are physicochemical properties (such as gelatinization temperature, viscosity, retrogradation, stability, gel strength, swelling power, solubility, paste clarity, bulk density, water absorption capacity, pH and acidity) and mineral composition. The levels of toxicity of the selected starches are to be examined to guarantee their safety to human health.

The selection of other starches with cassava starch is to emphasize the underutility of roots and tubers. Cassava has become a “hot cake” raw material in many industries at the expense of other roots and tubers, leading to its expensiveness. Economically, breakthrough in the use of other starches as substituents for cassava starch is another aim of this research work.

In addition, this research work is presented to find alternative materials for polymers used in the pharmaceutical industries as fillers, disintegrants and binders for tablets. These proposed alternative materials are agricultural products that are at our disposal and relatively cheap.

CHAPTER TWO

2.0 EXPERIMENTAL

2.1 MATERIALS

The tubers of sweet potato (*Ipomea batata*), bitter yam (*Dioscorea dumetorum*), water yam (*Dioscorea alata*) and the corms and the cormels of both the white and red varieties of cocoyam (*Colocasia esculenta)* were purchased at Oja-Oba market, Akure South Local Government, Akure, Ondo State, and fresh cassava *(Manihot esculenta)* tubers were harvested at the farm of the Federal Polytechnic, Ado-Ekiti, Ekiti State. All the reagents used in Chemistry and FST Laboratories, FUTA were supplied by Paschal Scientific, Akure and those used in Food Technology Unit, IITA were manufactured and supplied by Fisher Chemicals Ltd., England.

2.2 SAMPLE PREPARATION

The starches of the selected roots and tubers were isolated following the processes shown in the flow chart below:

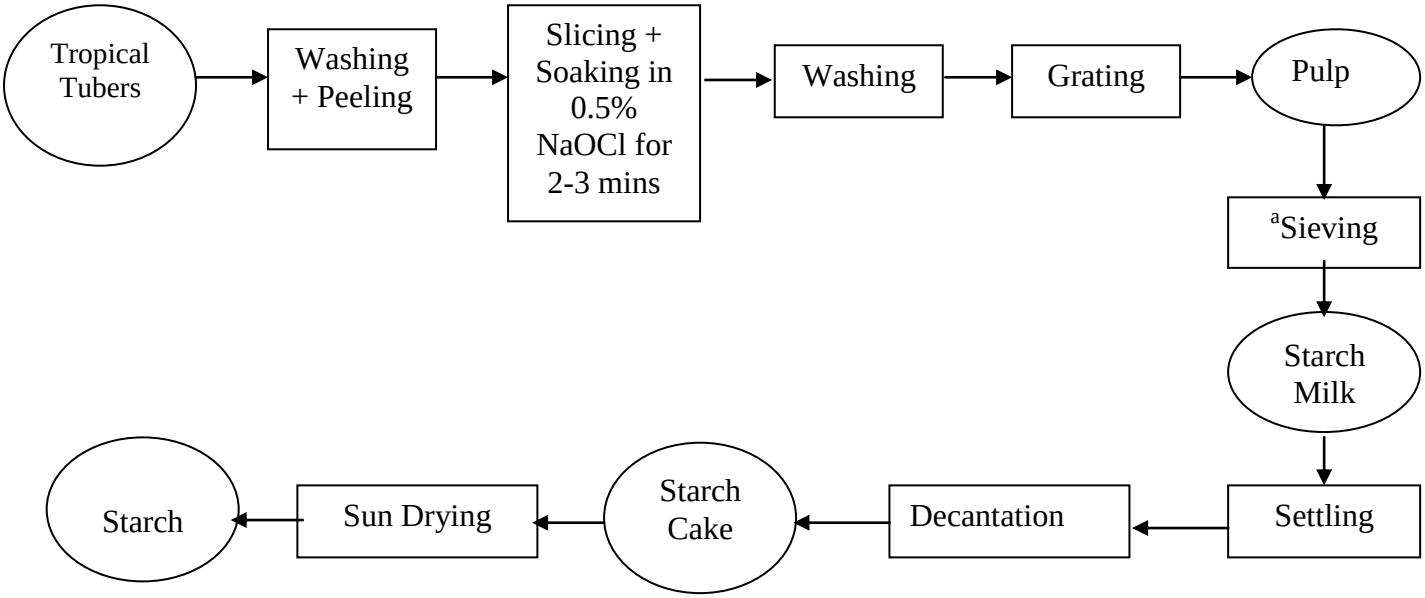

FIGURE 2.1: FLOW CHART OF STARCH PRODUCTION FROM ROOTS AND TUBERS (Asiedu, 1989)

[a]Muslin bag was used to sieve

The starch samples were coded as follows:

White cocoyam corms starch	-	WCCM
White cocoyam cormels starch	-	WCCL
Red cocoyam corms starch	-	RCCM
Red cocoyam cormels starch	-	RCCL
Cassava starch	-	CASS
Sweet potato starch	-	SPOTA
Bitter yam starch	-	BYAM
Water yam starch	-	WYAM

2.3 PROXIMATE COMPOSITIONS OF STARCH SAMPLES

2.3.1 DETERMINATION OF MOISTURE CONTENT

The air-oven method was used to determine the moisture content of the starch samples. The crucibles were first washed, dried in the oven, allowed to cool in the desiccator and the weight noted (W_1). Each of the starch samples was put in the crucibles and both the crucible and the sample were weighed as W_2. The crucibles containing the samples were then dried in the oven at 105^{o}C for 3 hours. The crucibles were taken out, allowed to cool in the desiccator and weight noted. The process of drying, cooling and weighing continued until constant weights were obtained (W_3) (AOAC, 1975),

The percentage moisture was calculated as follows:

$$\% \text{ Moisture content} = \frac{\text{Weight lost}}{\text{Weight of sample}} \times 100$$

$$\% \text{ Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

2.3.2 DETERMINATION OF ASH CONTENT

Each of the samples was put in a preweighed clean and dried crucibles (W_1) and the weight of the crucible and the samples was noted (W_2). The

crucibles were then transferred into a muffle furnace at 550^{o}C for 6 hours to burn off the organic matter until grey/white ash was obtained. The crucibles were then removed, allowed to cool in the desiccator and the weights of the crucibles and the ash contents were noted (W_3) (AOAC, 1990).

$$\% \text{ Ash} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

$$\% \text{ Ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

2.3.3 DETERMINATION OF CRUDE PROTEIN

Microkjeldahl method was used to determine the crude protein content of the starch samples (AOAC, 1990). A known weight of the sample was measured (W_1) into a Kjeldahl flask and a tablet of Kjeldahl catalyst (selenium catalyst) was added to the sample along with 25cm^3 of conc. H_2SO_4. The flask was gently placed in the digestor housed by a fume cupboard. The heating was continued until a clear solution was obtained. The clear solution was cooled, poured into a 100cm^3 volumetric flask and made up to the mark with distilled water. 10cm^3 of the resulting mixture was measured into the distillation unit and 5cm^3 of 2% boric acid was pipetted into a 100ml conical flask with 2 drops of mixed indicator and was placed at the receiving end of the distillator. The conical flask was placed in such a way that the delivery tube was dipped completely into the boric acid inside the conical flask. 40% NaOH was added to the sample and distillation continued until alkaline condition was attained until alkaline condition was attained. As soon as the alkaline condition was reached, the red colour changed to yellow, indicating excess NaOH. The liberated ammonia was trapped in the boric acid solution and about 50cm^3 of the solution was collected into the conical flask. The solution in the flask was titrated with 0.01M HCl until the first permanent colour change was observed. The same procedure was followed for the blank

$$\% \text{ Crude Protein} = \frac{\text{Molarity of HCl x 0.0014x Titre value x } D_f \text{ x } C_f \text{ x } 100}{\text{Weight of sample}}$$

where,

D_f = Dilution factor and C_f = Conversion factor = 6.25 (for starch)

2.3.4 DETERMINATION OF CRUDE FAT

The fat contents of the samples were determined in a soxhlet extractor (Pearson et. al., 1981). A known weight of each of the samples was weighed (W_1) into a preweighed filter paper (W_2), folded neatly and the total weight noted (W_3). The filter papers, holding the samples were then inserted into the extraction unit and extraction was carried out with petroleum ether (b.pt. 40-60^{o}C) for 5 hours. After the extraction, the filter papers were removed, dried in the oven at 100^{o}C for about 30 minutes, allowed to cool in the dessicator and the weights noted (W_4).

$$\% \text{ Crude fat} = \frac{\text{Loss in weight}}{\text{Weight of sample}} \text{ x } 100$$

$$\% \text{ Crude fat} = \frac{W_3 - W_4}{W_1} \text{ x } 100$$

2.3.5 DETERMINATION OF CRUDE FIBRE

200ml freshly prepared 1.25% H_2SO_4 were added to 2g of the residue after fat extraction and quickly brought to boiling. Boiling and refluxing was done for 30 minutes in a fume cupboard housing the digestion apparatus. The mixture was filtered through Whatman filter paper by gravity. The beaker holding the mixture was rinsed with distilled water and the residue on the paper equally washed with distilled water until the filtrate was free of acid. To the residue, which was transferred quantitatively into a digestion flask, 200ml freshly prepared 1.25% NaOH were added and the mixture quickly brought to boiling. Boiling and refluxing continued for 30 minutes. The mixture was filtered using Whatman

filter paper. The beaker was rinsed with distilled water and the residue equally washed with distilled water until the filtrate was free of alkali. The resulting residue was washed with methylated spirit and subsequently with petroleum ether three times each in small quantity. The solvents were allowed to drain properly and the residue was transferred into a crucible, which had been previously ignited at 600^0C and cooled in a dessicator. The crucible and the residue were dried to a constant weight at 105^0C (weight A). The organic matter was burnt off by igniting in Muffle furnace for 6 hours at 600^0C. The residue obtained was cooled in a dessicator and weighed (weight B). The loss in weight during incineration represented the weight of crude fibre in the sample (AOAC, 1990).

$$\% \text{ Crude Fibre} = \frac{(\text{weight A} - \text{weight B})}{\text{weight of sample}} \times 100$$

2.3.6 DETERMINATION OF FREE SUGARS

0.020g of the sample was weighed into a centrifuge tube and wetted with 1.0ml of 95% ethanol before the addition of 2.0ml distilled water. 2.0ml of hot 95% ethanol was added and centrifuged for 10 minutes at 2000rpm. The supernatant was decanted into test tubes and made up to 20ml. To develop colour for spectrophotometric analysis, 0.02ml of the aliquot was pipetted and 0.80ml of distilled water was added followed by the addition of 0.5ml of 5% phenol and the mixture mixed thoroughly in a vortex. 2.5ml of conc. H_2SO_4 was further added and the mixture vortexed. The mixture was allowed to cool and the absorbance taken at 490nm using UV 1601 Spectrophotometer. Standard calibration curve for was prepared by weighing 0.01g of D-glucose into 100ml volumetric flask and was made up to 100ml mark with distilled water (stock solution). 0, 0.1, 0.2, 0.3, 0.4ml of the stock solution into test tubes were pipetted and were made up to 1.0ml with distilled water, corresponding to 0, 10, 20, 30, 40µg/ml. Then 0.5ml of 5% phenol was added to each of the serial solutions and was vortexed (mixed

thoroughly). 2.5ml conc. H_2SO_4 was added to each solution and vortexed. The mixture was allowed to cool. The absorbance was read at 490nm using UV 1601 Spectrophotometer (McReady, 1970).

$$\% \text{ Sugar} = \frac{(A-I) \times D_f \times V \times 100}{B \times W \times 10^6}$$

where,

A = absorbance

I = intercept

D_f = dilution factor

V = volume of aliquot

B = slope of the standard curve

W = weight of the sample

2.3.7 DETERMINATION OF STARCH CONTENT

To the residue from sugar analysis, 7.5ml of perchloric acid for hydrolysis and was filtered. The hydrolysate (filtrate) was allowed to stand for 1 hour before it was diluted with 17.0ml distilled water and filtered. 1.0ml distilled water was added and the solution mixed thoroughly in a vortex. Colour development was done by pipetting 0.01ml of the aliquot with the addition of 0.5ml of 5% phenol and the mixture vortexed. 2.5ml of conc. H_2SO_4 was added and the mixture vortexed. The mixture was allowed to cool and the absorbance taken at 490nm using UV 1601 Spectrophotometer (McReady, 1970).

$$\% \text{ Starch} = \frac{(A-I) \times D_f \times V \times 100 \times 0.9}{B \times W \times 10^6}$$

where,

A = absorbance

I = intercept

D_f = dilution factor

V = volume of aliquot

B = slope of the standard curve

W = weight of the sample

2.3.8 CARBOHYDRATE BY DIFFERENCE

The carbohydrate contents of the samples were determined by difference.

% Carbohydrate = 100% - (Sum of the percentages of moisture content, ash, fat, crude fibre and crude protein)

2.4 PHYSICOCHEMICAL PROPERTIES OF THE STARCHES

2.4.1 DETERMINATION OF PASTING PROPERTIES

Rapid Visco Analyzer (RVA) model 3D (Newport Scientific, Narrabeen, Australia) was used to determine the values of gelatinization temperature, paste viscosity, paste stability and retrogradation of each of the starch samples. 0.01g of each sample was weighed into the weighing vessel prior transfer into the test canister. 25.0ml of water was dispensed into a new canister and the sample was transferred onto the water surface in the canister. The paddle was placed into the canister and the blade was jogged vigorously through the sample up and down 10 times. The paddle was placed into the canister and the assembly was firmly inserted into the paddle coupling so that the paddle is properly centered. Then, the measurement cycle was initiated by pressing the motor tower of the instrument. The recorded RVA paste curve that appeared on the screen of the computer connected to the instrument was printed out to have the hard copy (IITA, 1995).

2.4.2 DETERMINATION OF PASTE CLARITY

Paste clarities of the samples were determined using the procedure described by Perera and Hoover (1999). 2% aqueous suspension of each of the samples was heated in a boiling water bath for 1 hour with constant stirring. The

heated suspensions were cooled for 1 hour to room temperature and the samples stored for 3 days at 4^0C in a refrigerator. The clarity of each sample was determined every 24 hour by measuring the transmittance at 540 nm against water blank using Ultra Violet (UV) 1601 spectrophotometer. The transmittances obtained with change in time were recorded.

2.4.3 pH DETERMINATION

The pH meter was switched on and left to warm for about 30 minutes, the electrode was then rinsed with distilled water and cleaned with a clean soft tissue paper, and standardized with buffer 4 solution, the electrode was rinsed and cleaned again and standardized with buffer 10 solution, the electrode was rinsed and cleaned again. 5 g of the sample was thoroughly mixed with 25ml of distilled water for 5 minutes at 30^0C and allowed to stay for 1 minute before measuring the pH using an electronic pH meter.

2.4.4 DETERMINATION OF ACIDITY

Total acidity was assayed by agitating 10% (w/v) aqueous suspension of the sample at ambient temperature (20 ± 2^0C) for 30mins and then centrifuges at 15000 rpm for 15 mins. 50ml of the supernatant was titrated with 0.1M NaOH in the presence of 1% phenolphthalein-alcohol solution to faint persistent pink colour. The results were measured in moles of acid per gram of dry weight of starch (AOAC, 1990).

2.4.5 COLOUR ANALYSIS

The method of Bakker and Arnold (1993) was adapted. A recording spectrophotometer model PU8740 was used with glass cells of 2 mm path length to obtain the absorption spectra from which L*a*b* values were calculated, using illuminant D65 and a10^0 observer. The L* value was measured as the lightness

from completely opaque (0) to completely transparent (100), a* was measured as the redness and b* the yellowness. Hue angle (H) was calculated from $H = \tan^{-1}(b^*/a^*)$ and chroma (C) from $C = [(a^*)^2 + (b^*)^2]^{0.5}$.

2.4.6 DETERMINATION OF SOLUBILITY AND SWELLING POWER

1.0g of starch sample was weighed, transferred into a clean, dried test tube and weighed as W_1. The starch was then dispersed in 40ml of distilled water at 90^{o}C. The resultant slurry was centrifuged at 1500 rpm for 5 minutes. The supernatant was decanted and dried to a constant weight in a hot-air oven at 100^{o}C. The residue was then weighed. The solubility was calculated as g/100g of starch on dry weight basis. The residue obtained after centrifugation with the water it retained was weighed as W_2 (Leech et. al., 1959).

$$\% \text{ Swelling power} = \frac{\text{Weight of centrifuged starch granules}}{\text{Weight of sample}} \times 100$$

2.4.7 DETERMINATION OF GEL STRENGTH

The modified method of Coffman and Garcia (1977) for the determination of least gelation concentration of starches was also modified to determine the gel strengths of the starch samples as that concentration when the sample gel did not fall down when the test tube was inverted. 2-20% suspensions of each of the samples were prepared with distilled water. 10ml of each suspension was put in a test tube and heated for 1 hour on boiling water bath, followed by a rapid cooling in a bath of cold water. The test tubes were further cooled at 4^{o}C for 2 hours. The concentration at which the gels did not fall down from the inverted test tubes were taken as the gel strengths of the starch samples

2.4.8 DETERMINATION OF BULK DENSITY

Bulk density of the starch samples was determined using Wang and Kinsella (1976) method. A known weight of the sample was measured into 25ml

graduated measuring cylinder. The sample was packed by tapping the cylinder gently on the bench top ten times from a height of 5cm. The volume of the sample after tapping was recorded.

$$\text{Bulk Density (g/ml)} = \frac{\text{weight of sample}}{\text{volume of sample after tapping}}$$

2.4.9 DETERMINATION OF WATER ABSORPTION CAPACITY

Water absorption capacity was determined using the centrifugal method. 1.0g of the sample was mixed with 10ml of distilled water in a 50ml centrifuge tube. The content was stirred for 3 minutes using a mechanical stirrer. Then the content was centrifuged for 30 minutes at 5000 rpm. At the end of the centrifuging, the content was allowed to stabilize and the supernatant was carefully decanted into a graduated measuring cylinder.

$$\%\ \text{Water Absorption Capacity} = \frac{\text{Weight of water absorbed}}{\text{Weight of sample}} \times 100$$

2.4.10 ENERGY VALUE DETERMINATION

The method of Fisher and Bender (1979) was adapted in determining the energy values of the starch samples. 0.1g of the starch sample was ignited electrically in a Ballistic Bomb Calorimeter (Gallerkamp CBB-330-010L) and burned in an excess of oxygen with the recommended oxygen pressure of 25atm. The maximum temperature rise of the bomb was measured with the thermocouple and galvanometer system. The rise in temperature obtained was compared with that of benzoic acid to determine the calorific value of the sample.

2.4.11 DETERMINATION OF AMYLOSE AND AMYLOPECTIN CONTENTS

2.0% flour suspension was filtered to remove insoluble residues, and the pH was adjusted to 6.3 with a phosphate buffer. The solution was stirred in a

boiling water bath for 2hrs to disperse the starch molecules. Thereafter, n-Butyl alcohol was added (20%, v/v), and the solution was stirred at 100^{o}C for 1hr, followed by cooling to room temperature over a period of 24-36 h. Amylose butyl alcohol complex crystals was formed and precipitated during cooling, and was separated by filtration and dried at 30±2^{0}C for 48 hours (Song and Jane, 2000).

$$\% \text{ Amylose content} = \frac{Dried\ crystals}{Weight\ of\ sample} \times 100$$

The amylopectin content was determined as follows:

% Amylopectin content = 100% - % Amylose content

2.5 MINERAL ANALYSIS

2 g of the sample was ignited in a Muffle furnace for 6 hours at 550^{0}C and the resulting ash was cooled in a desiccator after which 0.1M HCI solution was added to break up the ash. It was then filtered through acid washed Whatman number 43 filter paper into 100ml volumetric flask, and diluted to 100ml with the distilled water. The solution was analyzed for metals using an atomic absorption spectrometer with different hollow cathode lamps for calcium, sodium, potassium, iron, zinc, magnesium, mercury, lead, cadmium, copper, manganese, chromium, nickel and arsenic (Pearson et. al., 1981). The phosphorus content for each sample was determined by pipetting 5cm^3 of the ash solution into a 100cm^3 volumetric flask and made up to the mark with distilled water. 4cm^3 of ammonium molybdate was added and shaken followed by the addition of 0.7cm^3 of 2% tin (ii) chloride solution and shaken. The solution was analyzed for phosphorus using a colorimeter using a red filter (Ceirwyn, 1995).

2.6 ANALYSIS OF ANTINUTRIENTS

2.6.1 DETERMINATION OF CYANIDE CONTENT

The standard used was acetone cyanohydrin with molecular weight of 85.10, density of 0.93 and purity of 99%. This was prepared by dissolving 64.5 µL of acetone cyanohydrin in 10ml of 0.1M orthophosphoric acid to make a stock solution of 6.0mg/ml. Cyanohydrin stock solution was diluted with 0.1M orthophosphoric acid in the ratio 1:250. From the diluted solution of cyanohydrin, 0.025, 0.050, 0.075 and 0.100ml were pipetted into marked test tubes using automatic pipette. The volume of cyanohydrin in the tube was topped up with 0.100ml orthophosphoric acid to give corresponding concentrations of 6.00, 12.00, 18.00 and 24.00µg/ml. Then 0.5ml of 0.1M phosphate buffer followed by the addition of 0.6ml of 0.2M NaOH was added to each of the tubes. The colour was developed for spcetrophotometric analysis by adding 0.8ml of the colour reagent to each of the tubes. The colour reagent was prepared by dissolving 3.5 g NaOH in 200ml water and 7.0 g 1,3-dimethylbarbaturic acid and 5.7 g isonicotonic acid. The mixture was stirred and the pH adjusted between 7.0 and 8.0. The tubes were allowed to stand at room temperature for 10 minutes for colour development. Absorbance was read at 605nm against a blank prepared with the aid of UV 1601 Spectrophotometer. 30g of the starch sample was homogenized in 250ml of 0.1M orthophosphoric acid and then centrifuged. The supernatant was extracted. 0.1 ml of the supernatant was pipetted and 10 ml of 0.1M phosphate buffer was added to serve as the stock solution. The sample stock solution was mixed with 0.1M phosphate buffer pH 6.0 in the ratio 1:10 respectively. From the diluted sample solution, 0.025, 0.050, 0.075 and 1.00ml were pipetted using automatic pipette. The volume of the sample in each tube was topped up with 0.1M phosphate buffer pH 6.0 to give corresponding concentrations of 25, 50, 75 and 100 µg/ml. The standards were run for

spectrophotometric analysis against each of the samples after colour development had taken place (Essers, et.al., 1993).

$$\text{Cyanogenic Potential (CNP) (mg/kg or ppm)} = \frac{A \times I \times 0.0033 \times 250 \times 0.3176}{W \times 0.0088}$$

where,

A = Absorbance

I = Intercept of the standard curve

W = Weight of sample

2.6.2 DETERMINATION OF TANNIN CONTENT

0.5 g of the defatted sample was extracted with 5ml methanol for 20 minutes and centrifuged for 10 minutes at 3000 rpm to remove the grains. The mixture was filtered and the extract (filtrate) assayed for tannin. 1ml of the extract was pipetted into 100ml volumetric flask containing 75ml water and 5ml Folin-Doris reagent added. (Folin-Dorin reagent was prepared by adding 100g sodium tungstate, 20 g phosphomolybdic acid and 50ml of 0.10M orthophosphoric acid to 750ml distilled water and refluxed for 2 hours. The solution was allowed to cool and made up to 1 litre with distilled water.) To the mixture, 10ml of 3.30M Na_2CO_3 was added and the mixture shaken before diluting to 100ml with distilled water. 1ml aliquot was taken and the absorbance measured at 760 nm using UV 1601Spectrophotometer. Standard curve was prepared by pipetting 0, 2, 4, 6, 8, 10ml aliquot of the standard tannic acid solution into 100ml volumetric flask containing 75ml distilled water. 5ml Folin-Doris reagent and 10ml of 3.30M Na_2CO_3 were added. The mixture was shaken well and made up to the mark with distilled water. The absorbance was taken at 760 nm using UV 1601 spectrophotometer (Hoff and Singleton, 1977).

$$\%\text{Tannins} = \frac{10 \times A \times I}{10,000 \times S \times W} \times 100$$

where, A = Absorbance

I = Intercept

S = Slope

W = Weight of sample

2.6.3 DETERMINATION OF TRYPSIN INHIBITOR

A continuous spectrophotometric determination rate method was used to analyse the trypsin inhibitor. 0.067M sodium phosphate buffer was prepared and adjusted to pH 7.6 at 25^0C with 1.0M NaOH. 50ml of 0.25mM of N-α-benzoyl-L-arginine ethyl ester solution (BAEE) was also prepared. Several volumes 9.50, 9.40, 9.35, 9.30, 9.25mL of 0.001M HCl were pipetted into 100ml measuring cylinders and 0.10, 0.15, 0.20, 0.25, 0.30mL of 0.005 mg/L of freshly prepared trypsin enzyme solution were added respectively followed by addition of 0.50mL of 0.001mg/L freshly prepared trypsin inhibitor solution was added to each of the cylinders. This was called mixture A. Another mixture of solutions was obtained by adding 0.10mL of 0.001M HCl to 3.00mL each of BAEE already pipetted into five 100ml measuring cylinders. The second mixture obtained was called mixture B. Then mixtures A and B were further mixed by inversion and equilibrated at 25^0C. The resulting solution from each cylinder was pipetted into 1mm cuvette for spectrophotometric determination. The absorbance was monitored at 253nm until constant (Joselyn, 1970).

$$\text{Trypsin Activity (mg enzyme)} = \frac{(\Delta A_{253nm/min}\ \text{Test} - \Delta A_{253nm/min}\ \text{Blank})(d_f)(10.0)}{(0.001)(0.10)(0.5)}$$

where,

d_f = Dilution factor

0.001 = Change in A253nm/minute per unit of Trypsin at pH 7.6 at 25^0C in a 3.2 ml reaction mix

0.10 = Volume (in milliliters) enzyme used

10.0 = Total volume in milliliters of assay

0.5 = Volume (in milliliters) of enzyme used

CHAPTER THREE

3.0 RESULTS AND DISCUSSION

3.1 PROXIMATE COMPOSITION

3.1.1 Moisture Content

Table 3.1 shows the values of moisture content of the starches of white cocoyam corms (WCCM), white cocoyam cormels (WCCL), red cocoyam corms (RCCM), red cocoyam cormels (RCCL), cassava (CASS), sweet potato (SPOTA), bitter yam (BYAM) and water yam (WYAM). The values of moisture content range from 8.72±0.03% to 10.10±0.04%, indicating the lowest value for SPOTA and the highest for WCCL respectively. The low value of moisture content of SPOTA suggests its high microbial stability. Oladebeye (2003) has reported moisture content values between 10.59±0.17% and 16.45±0.17% for the starches of cocoyam (*Colocasia esculenta*). Adequate and accurate knowledge of the moisture contents of these samples will determine the amount of water to be incorporated during processing and industrial application of these samples.

3.1.2 Crude Protein

From Table 3.1, the crude protein of the starches range from 1.18±0.01% to 2.09±0.01% with CASS exhibiting the lowest crude protein and RCCM the highest. Low value of crude protein for CASS has been reported by Oladebeye (2003). The value of crude protein of RCCM reported by Oladebeye (2003) was 0.60±0.00%. That the values of crude protein of WCCM, RCCM, RCCL and WYAM are higher than that of CASS is in agreement with the reports earlier made by Oyenuga (1968), Oke (1967, 1972) and Miller et. al., (1952) that the values of digestible true protein of cocoyam, yellow yam and water yam are higher than those of white yam and cassava.

3.1.3 Ash Content

The values of ash content of the starches are contained in Table 3.1. The highest value is 1.98±0.02% in RCCL and the lowest is 1.64±0.01% in SPOTA.

Table 3.1: Proximate Compositions of the Starch Samples

Sample	Moisture Content (%)	Crude Protein (%)	Ash Content (%)	Fat Content (%)	Crude Fibre (%)	Free Sugar Content (%)	Starch Content (%)	Carbohy-drate By Difference (%)
WCCM	9.36±0.02^{c}	1.87±0.01^{d}	1.88±0.02^{e}	0.52±0.01^{f}	0.46±0.01^{b}	3.87±0.01^{a}	81.39±0.05^{d}	85.91±0.03^{B}
WCCL	10.10±0.10^{e}	1.17±0.01^{a}	1.82±0.01^{d}	0.43±0.01^{e}	0.50±0.01^{c}	4.80±0.01^{d}	80.59±0.04^{b}	85.98±0.10^{B}
RCCM	9.59±0.04^{d}	2.09±0.01^{e}	1.72±0.02^{b}	0.41±0.01^{d}	0.58±0.01^{d}	5.28±0.02^{g}	80.18±0.06^{a}	85.61±0.05^{a}
RCCL	9.02±0.07^{b}	1.41±0.01^{b}	1.98±0.02^{f}	0.40±0.01^{cd}	0.50±0.01^{c}	5.03±0.01^{e}	81.39±0.03^{d}	86.69±0.10^{d}
CASS	9.01±0.05^{b}	1.18±0.01^{a}	1.83±0.01^{d}	0.37±0.01^{b}	0.41±0.01^{a}	5.01±0.03^{e}	82.05±0.05^{f}	87.20±0.05^{f}
SPOTA	8.72±0.03^{a}	1.63±0.01^{c}	1.64±0.01^{a}	0.39±0.01^{c}	0.75±0.02^{g}	5.46±0.02^{c}	81.39±0.04^{d}	86.90±0.03^{e}
BYAM	9.33±0.07^{c}	1.87±0.01^{d}	1.75±0.02^{c}	0.33±0.01^{a}	0.69±0.02^{e}	4.15±0.02^{b}	81.79±0.04^{e}	86.03±0.19^{b}
WYAM	9.09±0.02^{b}	1.64±0.02^{c}	1.84±0.02^{d}	0.39±0.01^{c}	0.74±0.01^{f}	5.16±0.02^{f}	80.99±0.02$_{c}$	86.30±0.05^{c}

Results are the means of triplicate determinations ± standard deviation. The analysis of variance (ANOVA) is $p<0.05$ for all the samples (see the Appendix).
WCCM-White cocoyam corms
WCCL-White cocoyam cormels
RCCM-Red cocoyam corms
RCCL-Red cocoyam cormels
CASS-Cassava
SPOTA-Sweet potato
BYAM-Bitter yam
WYAM-Water yam

The range of values of ash content obtained are lower than those reported by Oyenuga (1968) and Ojofeitimi and Tanimowo (1980). The ash content of food is the indication of the level of availability of minerals. These starches may serve as a source of minerals in diets. The ash content of starch is typically less than 0.5% of the dry matter (Thomas and Atwell, 1999). However, the considerably high values of ash content obtained for the sample studied may suggest the high level of availability of minerals in the samples.

3.1.4 Fat Content

From Table 3.1, the fat contents of the starches vary from 0.33±0.01% in BYAM to 0.52±0.01 in WCCM. The value obtained for WCCL is next to that of WCCM. The earlier research work reported by Oladebeye (2003) showed that the fat content of WCCM (0.57±0.01%) was higher than that of WCCL (0.61±0.05%). Both SPOTA and WYAM, however, exhibit the same value of fat content (0.39±0.01%), which is appreciably higher than the value reported by Ojofeitimi and Tanimowo (1980) for WYAM. The relatively high fat content of SPOTA may probably contribute to the palatability of its tuber.

3.1.5 Crude Fibre

From Table 3.1, the results of crude fibre of the starches are between 0.41±0.01% (CASS) and 0.75±0.20% (SPOTA). SPOTA has been identified as a significant source of dietary fibre (Collins and Walter, 1982). Some epidemiological evidence suggests that increased fibre consumption may contribute to the reduction in the incidence of certain diseases such as diabetes, coronary heart disease, colon cancer and various digestive disorders (Augustin et. al., 1978).

3.1.6 Free Sugar Content

The sugar contents of the starches analyzed are shown in Table 3.1. The highest value is 5.46±0.02% in SPOTA and the lowest is 3.87±0.01% in WCCM. The high value of sugar content of SPOTA may probably provide information on

its sweetness. The sugar contents of these starches may have corresponding effect on the level of toxins in their tubers.

3.1.7 Starch Content

The starch contents of the starches range from 80.18±0.06% (RCCM) to 82.05±0.05% (CASS). Next in value of starch content to that of CASS is 81.79±0.04% in BYAM. The starch content of SPOTA (81.39±0.04%) is almost the same with that of sweet potato variety (81.3%) reported by CIAD and SAAS (1995). The starch contents of WCCM, RCCL and SPOTA are conspicuously the same (81.39). The high values of starch content of the samples analyzed are the evidence of the preponderance of amylose and amylopectin contents, which are the index of some functional properties of starches such as viscosity, retrogradation, and solubility and gelatinization temperature among others.

3.2 PHYSICOCHEMICAL PROPERTIES

3.2.1 pH and Acidity

Table 3.2 shows the pH and acidity values of the starches analyzed. While the pH values range from 5.60 (CASS) to 6.33 (WYAM), the total acidity vary from 0.35 (CASS) to 0.56 (BYAM). From the results, it is evident that the level of acidity of the starches decreases in the order BYAM>WCCM>WYAM> SPOTA>WCCL>RCCM>RCCL>CASS. Ihekoronye and Ngoddy (1985) have reported that the pH values of most foods range generally from neutral to acidic, and that some micro-organisms of significance in foods such as yeasts and lactic acid bacteria, grow best over the range pH 3.0-6.0. These ranges of values of pH and acidity imply that the proteins in the samples are not denatured and that enzyme reactions are activated (Ihekoronye and Ngoddy, 1985).

3.2.2 Colour

From Table 3.2, the L-values (lightness) of the starches are between 68.10 (WCCL) and 73.48 (WCCM). The higher the value of L, the more transparent the

Table 3.2: Physicochemical Properties of the Starch Samples

Sample	pH	Acidity	Colour			Bulk Density (g/ml)	Water Absorp-tion Capacity (%)	Gel Strength (%)	Amylose (%)	Amylop-ectin (%)	Swelling Power (g/g)	Solub-ility (%)	Energy Value (kcal)
			Hue Angle	L	Chroma								
WCCM	5.65	0.55	77.74	73.48	16.25	0.72	92.74	10.00	19.21	80.79	9.86	8.44	331.9
WCCL	5.75	0.48	75.81	68.10	14.40	0.75	88.23	15.00	25.15	74.85	8.03	7.56	328.8
RCCM	6.10	0.41	78.09	72.26	14.92	0.71	84.41	10.00	16.50	83.50	11.21	8.40	332.5
RCCL	5.83	0.38	77.88	73.42	15.96	0.74	82.74	15.00	18.93	81.07	10.78	8.65	333.3
CASS	5.60	0.35	77.92	73.63	16.24	0.70	89.25	10.00	24.52	75.48	9.64	7.44	334.5
SPOTA	5.73	0.51	77.31	72.98	16.06	0.76	84.91	8.00	19.20	80.80	10.23	8.16	335.7
BYAM	6.10	0.56	77.91	69.83	16.14	0.75	83.23	10.00	23.65	76.35	9.45	7.35	332.7
WYAM	6.33	0.53	78.25	70.67	16.01	0.70	89.40	15.00	21.78	78.22	9.56	7.22	333.1

colour. The hue angle, which depicts the brownness of colour, is found to be highest in WYAM (78.25) and lowest in WCCL (75.81). From the results of colour analysis, WCCL is the darkest starch (lowest L-value) and while WYAM is the most brownish starch. The chroma value, which is the measure of bias between redness and blueness varies from 14.40 (WCCL) to 16.25 (WCCM) (Baker and Arnold, 1993). Colour of a product is an important quality parameter, which has a direct influence on the acceptability of the product (Ihekoronye and Ngoddy, 1985). The effect of colour on the acceptability of starch-based products depends on the product itself and on consumer preference.

3.2.3 Bulk Density

From Table 3.2, the bulk density values range from 0.70 g/ml to 0.76 g/ml. the values obtained are in the same range with what was reported for *Colocasia esculenta* flour (0.77±0.01) by Njintang (2003). Since high particle size results in low bulk density, this adduces for the higher value of bulk density of BYAM (0.75 g/ml) than that of WYAM (0.70 g/ml) as BYAM has lower granule size (1-4 µm) than WYAM (5-50 µm) (Coursey, 1967; Emiola and Delarosa, 1981). The values obtained are greater than that of starch 1500 used as a binder and a disintegrant in high shear wet granulation (Levina et. al., 2001). The high values of the samples studied imply that these samples may be suitable as drug binders and disintegrants in pharmaceuticals.

3.2.4 Water Absorption Capacity (WAC)

Table 3.2 shows the values of WAC of the starches. The order of increase in water absorption capacity of the starches is RCCL<BYAM<RCCM<SPOTA< WCCL<CASS<WYAM<WCCM. Both WCCM and RCCM have higher WAC values than their corresponding WCCL and RCCL respectively. The difference may be as a result of variation in the values of their starch content, protein content and granular particle size.

3.2.5 Gel Strength

The gel strength values of the starches are contained in Table 3.2. The highest value is 15.00% in WCCL, RCCL and WYAM and the lowest is 8.00% in SPOTA. The values obtained are in agreement with those reported by Oladebeye (2003) for *Colocasia esculenta* (8.00-16.00%). However, the value of gel strength of SPOTA is about 0.5 times those of WCCL, RCCL and WYAM. In paper industry, starches are required to enhance the strength of tissues (Satin, 1999); outstanding gel strength values of WCCL, RCCL, and WYAM could make them serve as a binder in the industry. In addition, high values of gel strength of WCCL, RCCL, and WYAM may be adduced to their appreciably high values of amylose content compared to the units of amylopectin content (table 3.2).

3.2.6 Amylose and Amylopectin Contents

From Table 3.2, the amylose contents of the samples range between 16.50% in RCCM and 25.15% in WCCL while their amylopectin contents range from 74.85% in WCCL and 83.50% in RCCM. The differences in amylose and amylopectin contents contribute to significant differences in the starch or flour properties and functionality (Peterson and Johnson, 1978; Radley, 1968; Radley, 1976; Satin, 1999; Takahata, et. al., 1995; Thomas and Atwell, 1999 and Whistler, 1967). When starch or flour is added to products as an ingredient, however, it is the functional properties of the starch of flour that are usually important, not the calories.

3.2.7 Swelling Power

Table 3.2 shows the values of swelling power of the starches, which range from 8.03g/g in WCCL to 11.21g/g in RCCM. The value obtained for RCCM is in agreement with that reported by Oladebeye (2003) for RCCM. This high value may be adduced to high protein value of the starch. It appears that the higher the amylopectin content, the higher the value of swelling power. This is with the

view that the crystalline network of the starches with a higher portion of the long chain of amylopectin is stabilized due to longer double helices and may form more hydrogen bonds with water when they are heated in excess water than those starches with shorter chains of amylopectin (Sasaki and Matsuki, 1998). Tester et. al. (1996) has established that swelling of a starch is regulated by its crystallinity, which is a function of amylopectin chain (Sander et al,1990), the high swelling power of RCCM compared to other starches is hence, justified. Swelling mechanism has been widely reported to influence the disintegration of tablets (Wan and Prasad, 1989; Ferran et. al., 1996); increase in swelling, which is associated with increase in starch concentration, results in decrease in the time of disintegration of tablets (Alebiowu and Itiola, 2003; Bi et. al.1999; Iwuagwu and Onyekweli, 2002). This is with the view that more solid bridges are formed, which makes the annihilation of inter-particle force more difficult (Luangtanan-Anan and Fell 1990).

3.2.8 Solubility

From Table 3.2, the values of solubility of the starches vary from 7.22% (WYAM) to 8.65% (RCCL). The solubility values of WCCM (8.44%) and RCCL (8.65) are comparably higher than those of WCCL (7.56%) and RCCM (8.40%) respectively. This suggests that part of the linear amylose, capable of undergoing solubilization compared to those that form micelles network (Purshottan et. al, 1990), are higher in both WCCM and RCCL than WCCL and RCCM respectively. Solubility of native potato starch as well as of its fraction has been reported to be higher than corn starch (Nowotna et. al., 2000).

3.2.9 Energy Value

Table 3.2 shows the energy values of the starches ranging from 328.8 Kcal for WCCL and 335.7 kcal for SPOTA. The values obtained are lower than that reported for maize grains (365 kcal) by Cordain (1999). Energy consumption by the body is related both to heat energy associated with basic body functions and

temperature, and to the mechanical energy associated with the movement of organs and limbs (Osborne and Voogt, 1978). Wu (1980) found that the net energy of the sweet potato was only 79% that of maize, while Noblet *et al.* (1990) showed that net energy was the same. A considerable amount of energy of about 1.0 kcal per kg of body weight per hour is needed by the human body when at rest. All the samples are good sources of energy.

3.3 PASTING PROPERTIES

3.3.1 Gelatinization Temperature

The values of gel temperature are presented in Table 3.3. The highest value is 62.15^0C (WCCL) and the lowest 60.45^0C (CASS). The value obtained for WCCL may suggest high proportion of amylose chains in the starch, which are involved in the crystalline network of the starch with the view that high thermal energy was required to dissociate the network.

3.3.2 Viscosity

Table 3.3 shows the respective values of paste viscosity of the starches, which range between 471.08 RVU and 112.25 RVU. The highest value is obtained for WYAM and the lowest for CASS. That SPOTA has higher paste viscosity than those of WCCM, WCCL, RCCM, RCCL, CASS and BYAM is in agreement with the earlier report of Swinkels (1985) that sweet potato has higher value of viscosity than other roots and tubers. This may be explained by the influence of phosphate groups in potato starch. A higher phosphate content in potato starch results in a high viscosity. The phosphate substituent confers on potato starch amylopectin the properties of a polyelectrolyte when dispersed in aqueous solution thereby acting as an ion-exchange group (Swinkels, 1985; Radley, 1968). It is note worthy that the higher the paste viscosity the higher the final viscosity and vice versa (Table 3.3). The values of final viscosity reported

by Rasper and Coursey (1967) for *Dioscorea dumentorum* (bitter yam) was 25.00 BU, which is much lower than the one obtained for BYAM here (225.72 RVU).

Table 3.3: Pasting Properties of the Starch Samples

Sample	Gel. Temp. (^{0}C)	Paste Viscosity (RVU)	Final Viscosity (RVU)	Retrog-radation (RVU)	Paste Stability (RVU)	Pasting Time (mins)
WCCM	61.55	304.67	265.50	72.83	112.00	5.08
WCCL	62.15	273.58	246.33	68.33	95.58	4.79
RCCM	61.25	241.08	216.00	61.50	86.58	4.99
RCCL	61.25	244.33	245.17	86.83	86.00	4.99
CASS	60.45	112.25	57.58	18.67	73.00	3.97
SPOTA	61.60	405.92	234.95	47.17	220.33	4.37
BYAM	61.95	329.50	225.72	78.25	181.83	5.18
WYAM	61.10	471.08	400.08	48.58	119.58	5.38

3.3.3 Retrogradation

From Table 3.3, the value of retrogradation is highest in RCCL (86.83 RVU) and lowest in CASS (18.67 RVU). Earlier, low value of retrogradation has been reported by Oladebeye (2003) for CASS as 13.00 RVU. Retrogradation is an index of texture and acceptability of starch-containing products (Ward, 1984). The decreasing order of retrogradation for the sample is RCCL>BYAM> WCCM >WCCL.RCCM>WYAM>SPOTA>CASS. deMan (1976) has reported that the

worst phenomenon of retrogradation or setback of any pastry product is enhanced during freezing. Retrogradation is a phenomenon responsible for cake not risen well, cream separated, bread staling, running paste and glue (Alais et. al., 1999). In other words, high value of retrogradation indicates high tendency of starch, as an ingredient in starch-based products, to reduce the shelf-life of the products (Atwell et. al., 1988). From the values of retrogradation obtained for the samples studied, CASS possesses the lowest value of retrogradation (18.67RVU), indicating that the starch of CASS, if incorporated as an ingredient in a starch-based product, is capable of imparting textural and freeze-thaw stability, thus prolonging the shelf-life of the product (Atwell et. al., 1988).

3.3.4 Paste Stability

Table 3.3 shows the values of paste stability. The highest stability is 220.33 RVU in SPOTA followed by 181.83 RVU in BYAM. The least paste stability is 73.00 RVU in CASS, which incidentally exhibits the least value of retrogradation. However, it is note worthy to emphasize that a general trend of relationship can be established between retrogradation and stability. WCCM, WCCL, RCCM, CASS, SPOTA, BYAM and WYAM show a reciprocal relationship between paste stability and retrogradation. It appears that retrogradation is inversely proportional to paste stability. SPOTA, BYAM, WYAM and WCCM will take priority in pharmaceutical application where resistance to breakdown, shear stress or stability is of significance.

3.3.5 Pasting Time

The values of pasting time contained in Table 3.3 show a range between 3.97 and 5.38 mins. The least value is in CASS and the highest in WYAM. This difference could be responsible for differences in the values of gel temperature, paste viscosity, retrogradation and stability.

3.3.6 Paste Clarity

The paste clarities of the starches increase gradually with time of storage (Table 3.4). CASS exhibits the highest %transmittance of 28.50% at the end of the third day of storage. This suggests a correlation between the colour and the paste clarity of a starch. CASS is observed to be the starch with highest values of lightness and paste clarity.

Table 3.4: Paste Clarity of the Starch Samples Measured as %Transmittance at 540 nm

Sample	Time (Hrs)			
	0	**24**	**48**	**72**
[a]T_{WCCM}	16.80	20.90	23.80	27.32
T_{WCCL}	16.02	15.90	21.41	24.56
T_{RCCM}	17.50	18.43	19.09	21.40
T_{RCCL}	16.10	16.45	18.60	22.35
T_{CASS}	19.55	21.03	26.40	28.50
T_{SPOTA}	15.95	16.11	17.69	18.32
T_{BYAM}	17.25	19.20	21.86	25.09
T_{WYAM}	16.96	21.15	24.05	27.60

[a]T-%Transmittance

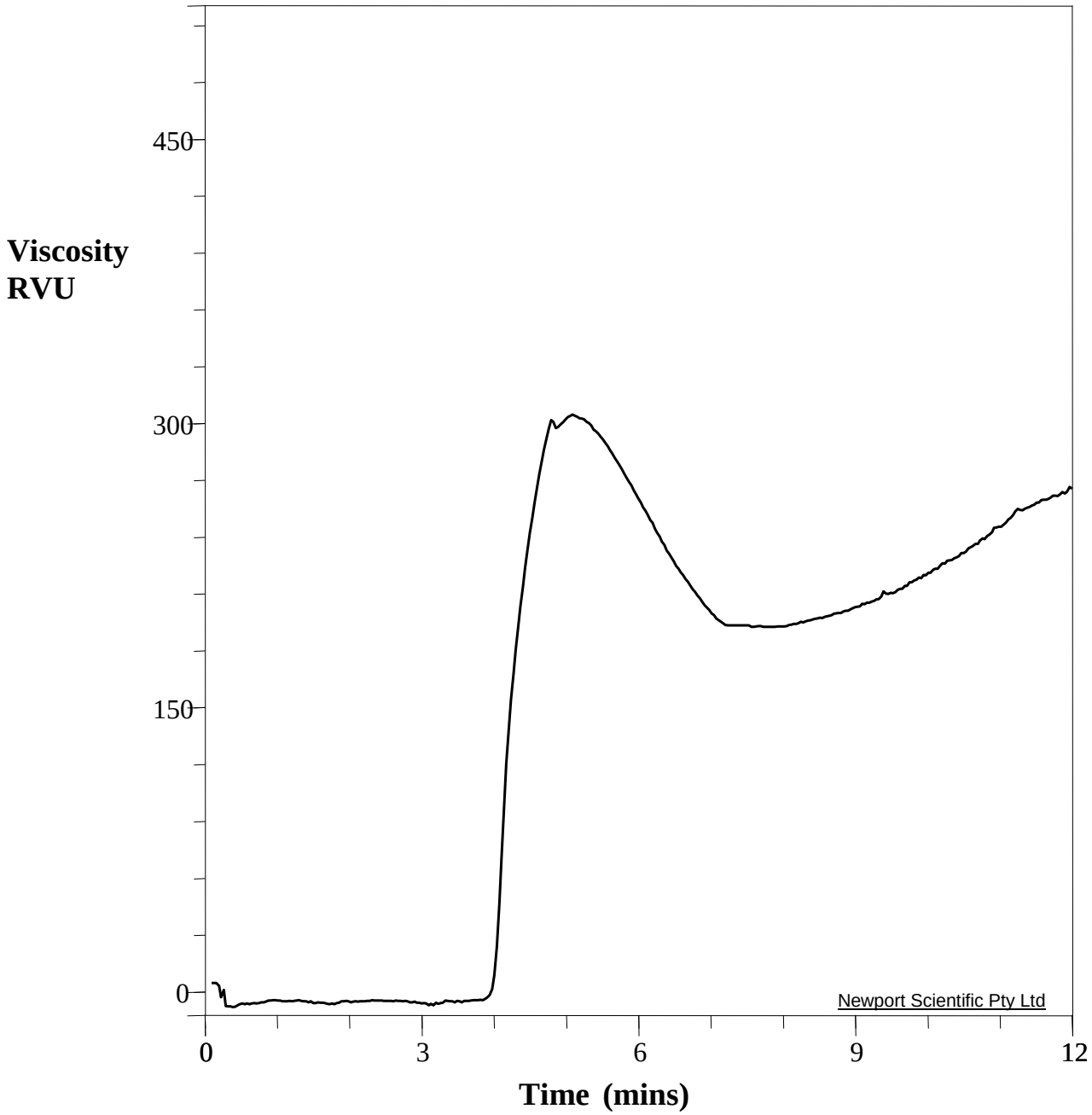

FIGURE 3.1: RVA PASTING CURVE OF WCCM

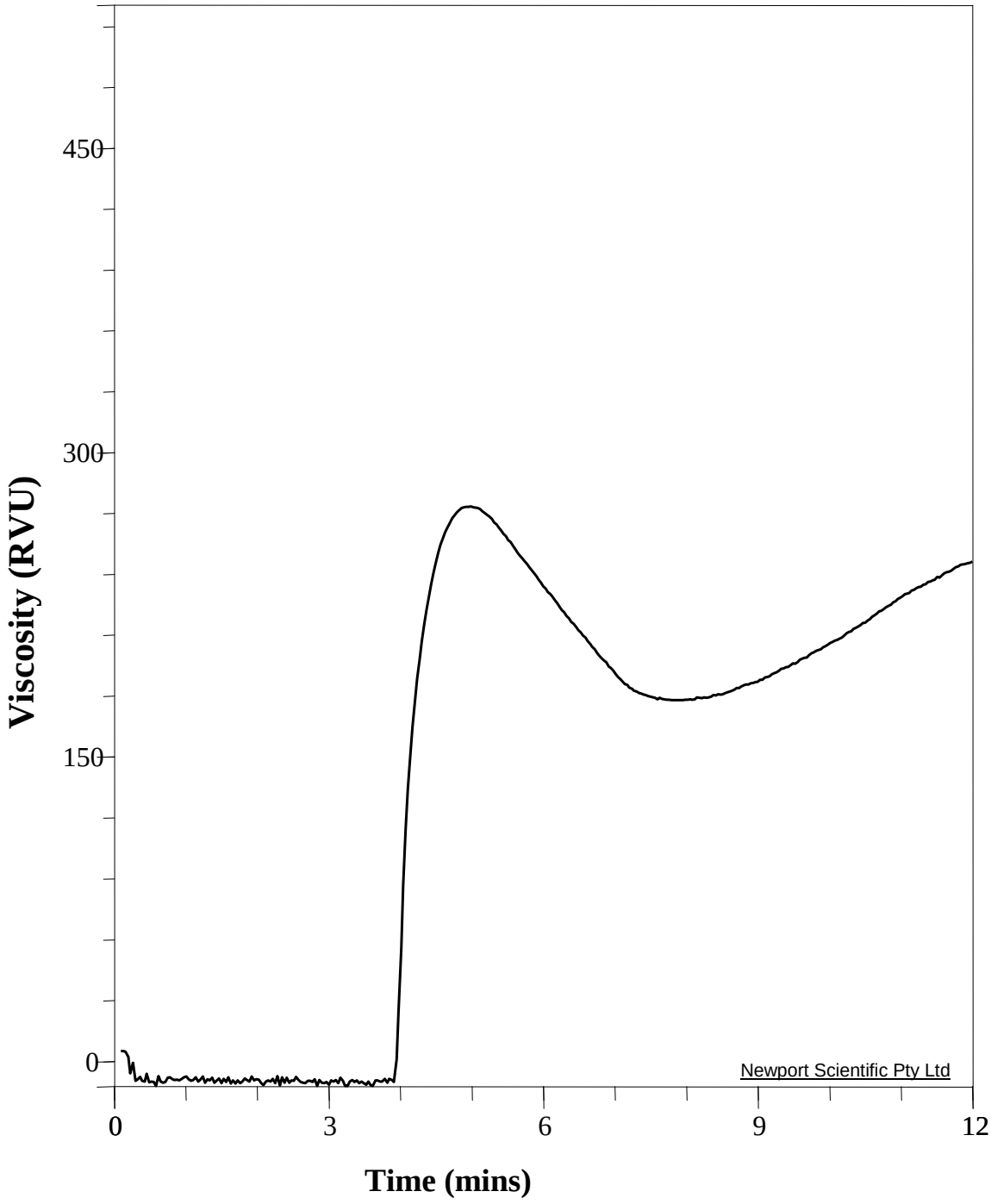

FIGURE 3.2: RVA PASTING CURVE OF WCCL

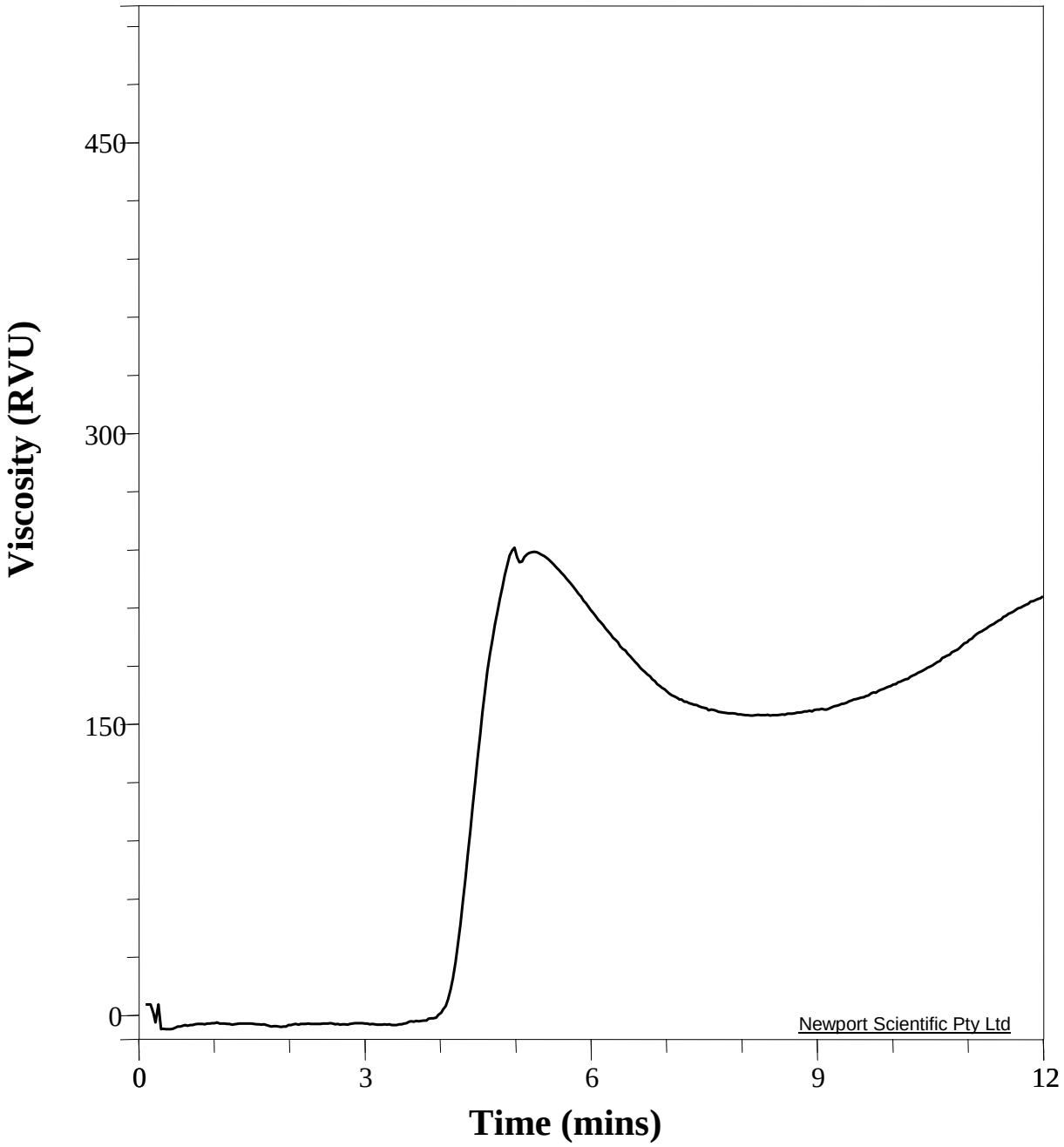

FIGURE 3.3: RVA PASTING CURVE OF RCCM

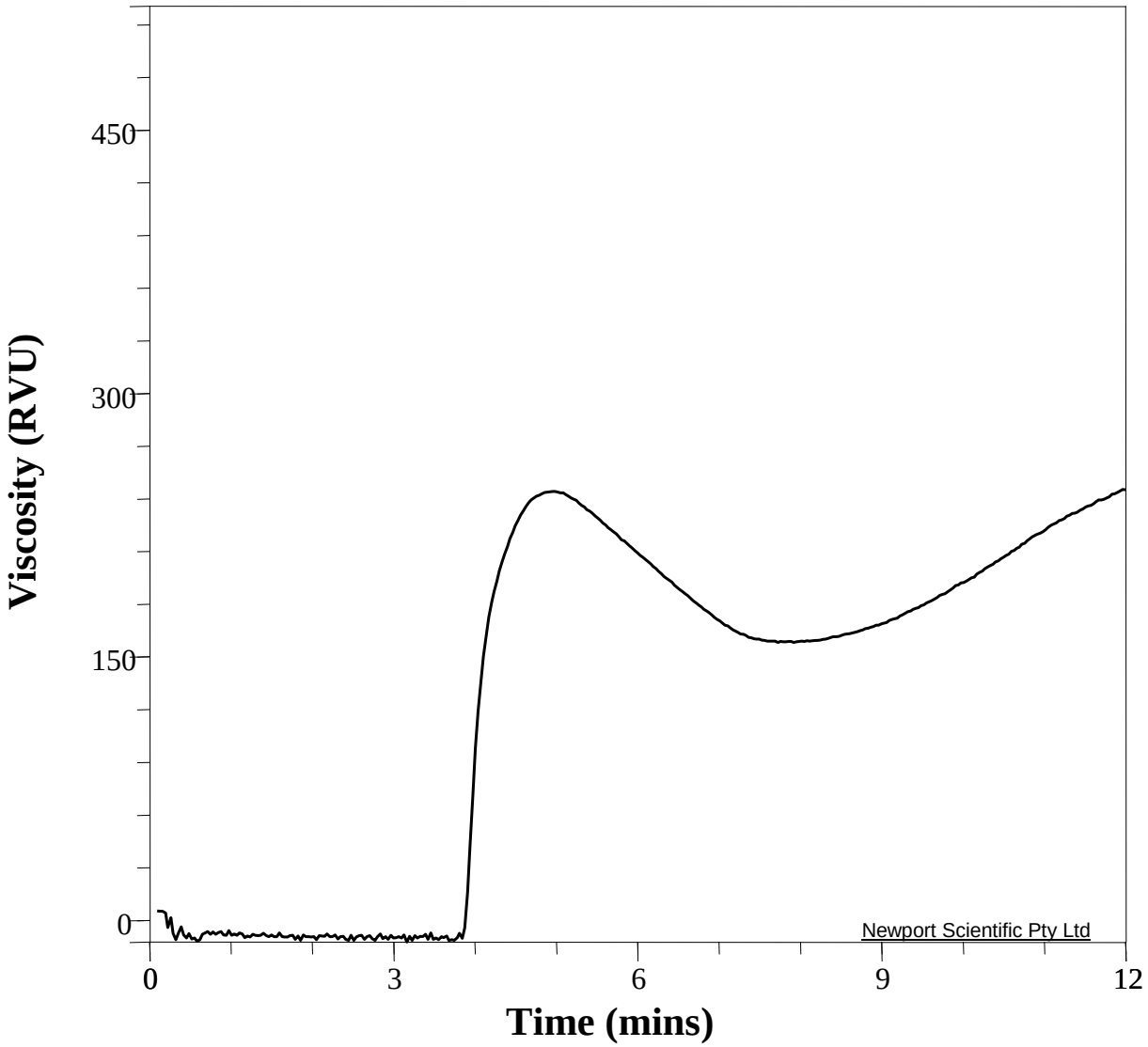

FIGURE 3.4: RVA PASTING CURVE OF RCCL

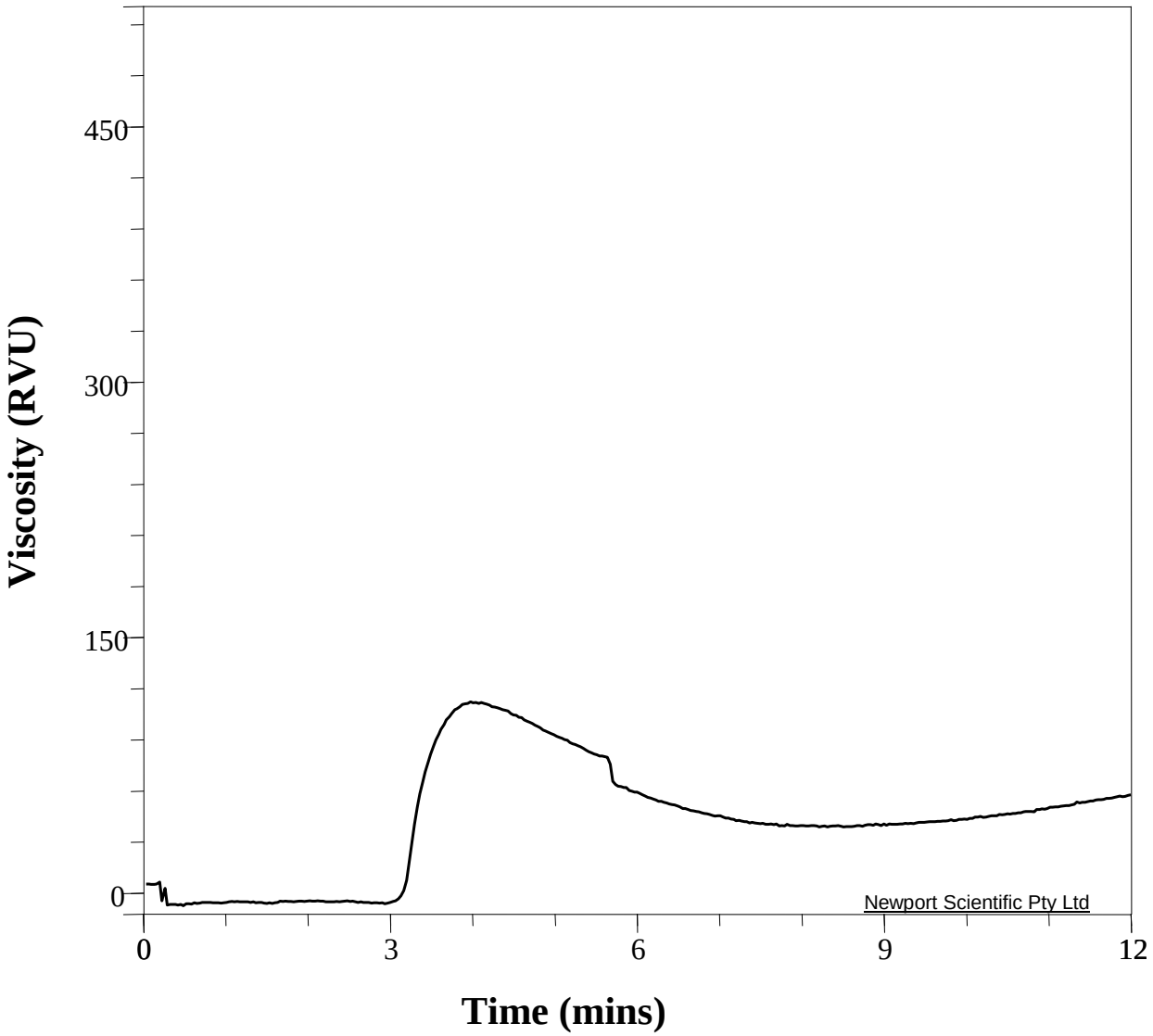

FIGURE 3.5: RVA PASTING CURVE OF CASS

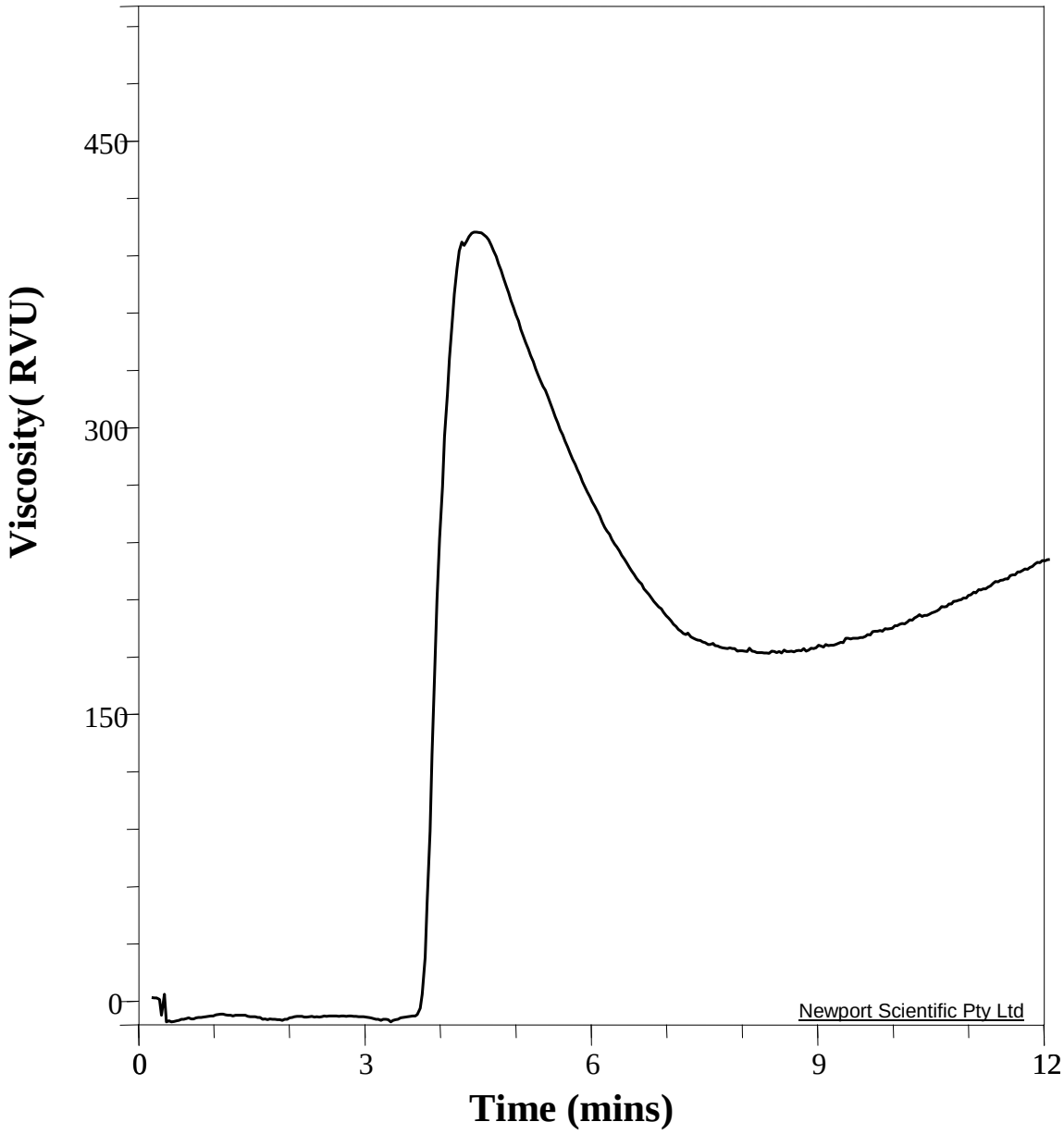

FIGURE 3.6: RVA PASTING CURVE OF SPOTA

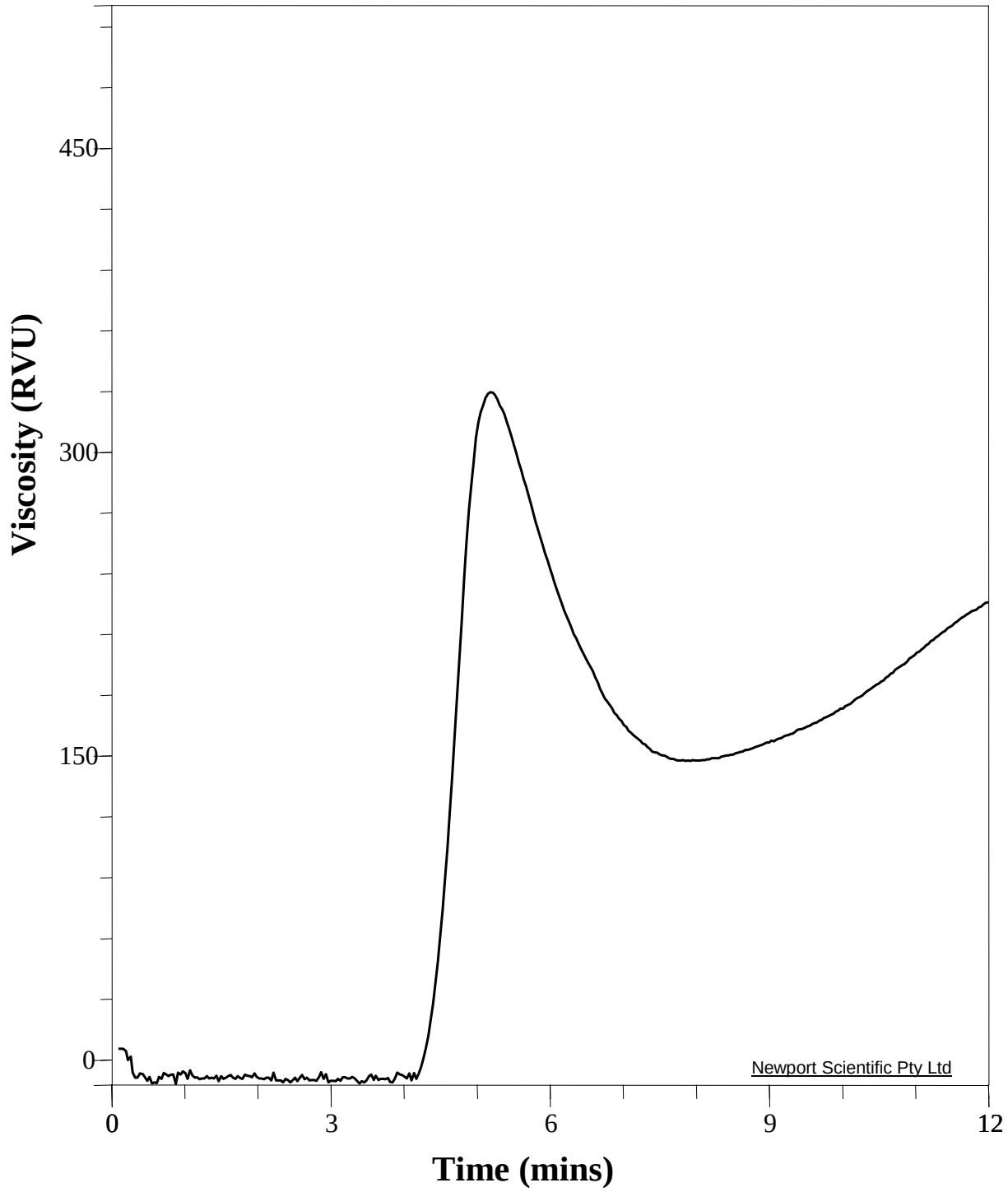

FIGURE 3.7: RVA PASTING CURVE OF BYAM

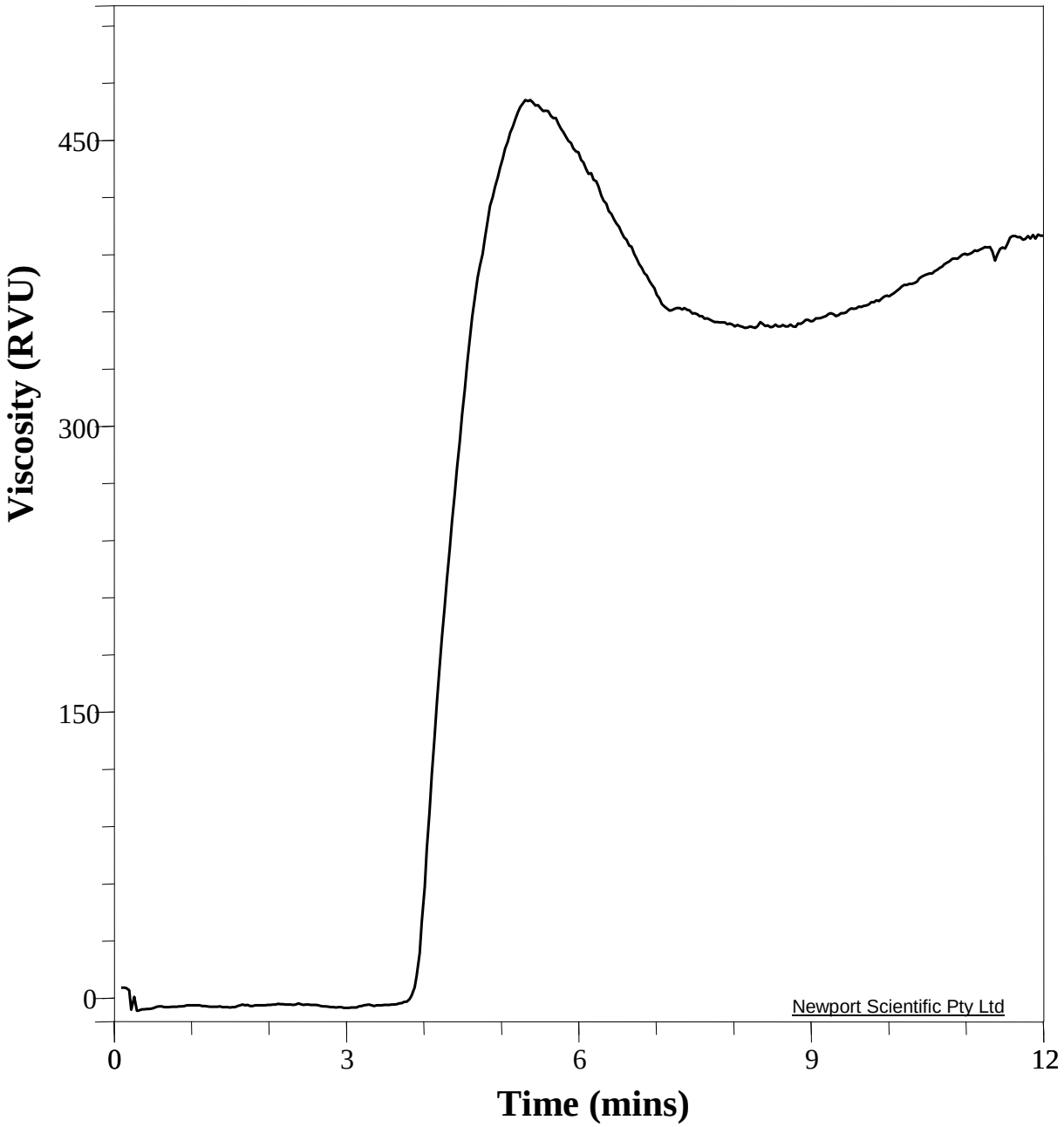

FIGURE 3.8: RVA PASTING CURVE OF WYAM

3.4 Mineral Compositions

Table 3.5 shows the mineral compositions of the starches analyzed. Potassium is the most abundant mineral in all the starches with the peak value (3102.00±0.50 mg/kg) in SPOTA and the least (2774.00±0.30 mg/kg) in CASS. These values are in full agreement with the report by Olaofe and Sanni (1988) that potassium is the predominant mineral in some Nigerian agricultural products. The values of sodium tend to be low in all the starches. High potassium and low sodium contents have been reported (Villareal, 1982). High potassium to sodium ratio may be an additional benefit in the diet of patients with high blood pressure, who have to restrict their sodium intake (Meneely and Battarble, 1976). In addition, high K/Na ratio serves as maintenance of a correct osmotic equilibrium and fluid pH in the body, which is essential for the movement of metabolites across cell membranes and around the body (Lake and Waterworth, 1980). However, high potassium foods are usually omitted in the diet of people with renal failure (McCay et. al., 1975). The values of Ca, Fe, Na, P, K and Zn obtained are higher than those obtained for corn grains as reported by (Cordain, 1999)

The values of calcium range from 433.30±0.12 mg/kg in CASS to 187.4±0.20 mg/kg in BYAM. The values of calcium in WCCM, WCCL, RCCM and RCCL are lower than that of CASS. This may be as a result of possible formation of calcium oxalate complexes, which reduces, if not totally prevent, the availability of calcium to the body. Calcium plays a significant role in the strengthening of body tissues and bones. Ca, P and Vitamin D combine to avoid rickets in children and osteomalacia (adult rickets) (Chesworth, 1992.).

The values of iron are relatively low for all the starches. The highest value is 21.00±0.02 mg/kg in WCCM and the lowest is 1.10±0.01 mg/kg in CASS. In addition to their deleterious influences on calcium metabolism, iron deficiency is

Table 3.5: Mineral Compositions of the Starch Samples (mg/kg)

Sample	Minerals													
	Ca	Fe	Na	P	K	Zn	Hg	Pb	Cd	Cu	Mn	Cr	As	Ni
WCCM	201.00±0.14^{d}	21.00±0.02^{e}	72.20±0.05^{d}	462.00±0.20^{f}	3033.00±0.50^{g}	3.50±0.02^{d}	ND	ND	ND	ND	ND	ND	ND	ND
WCCL	210.30±0.08^{e}	1.80±0.02^{b}	68.80±0.05^{a}	431.00±0.20^{b}	2989.00±0.50^{e}	2.90±0.02^{b}	ND	ND	ND	ND	ND	ND	ND	ND
RCCM	197.30±0.04^{c}	2.20±0.02^{d}	71.00±0.05^{c}	443.30±0.20^{e}	3015.00±0.70^{f}	3.50±0.01^{d}	ND	ND	ND	ND	ND	ND	ND	ND
RCCL	188.90±0.10^{b}	2.00±0.02^{c}	69.50±0.05^{b}	442.90±0.20^{e}	2954.00±0.20^{d}	4.50±0.02^{g}	ND	ND	ND	ND	ND	ND	ND	ND
CASS	433.30±0.12^{h}	1.10±0.01^{a}	103.40±0.06^{g}	434.40±0.40^{c}	2774.00±0.50^{a}	4.20±0.02^{f}	ND	ND	ND	ND	ND	ND	ND	ND
SPOTA	221.00±0.10^{g}	1.80±0.02^{b}	73.30±0.02^{f}	1012.00±0.50^{g}	3102.00±0.50^{h}	3.80±0.03^{e}	ND	ND	ND	ND	ND	ND	ND	ND
BYAM	187.40±0.20^{a}	2.20±0.02^{d}	68.80±0.10^{a}	427.50±0.30^{0a}	2881.00±0.50^{c}	3.20±0.01^{c}	ND	ND	ND	ND	ND	ND	ND	ND
WYAM	220.60±0.20^{f}	1.80±0.01^{b}	72.80±0.02^{e}	441.60±0.40^{d}	2855.00±0.70^{b}	2.70±0.02^{a}	ND	ND	ND	ND	ND	ND	ND	ND

Results are the means of triplicate determinations ± standard deviation. The analysis of variance (ANOVA) is $p<0.05$ for all the samples (see the Appendix).
ND-Not Detected

known to reduce work capacity and productivity in adults, increase the severity and incidence of infection, and increase maternal and prenatal mortality (Scrimshaw, 1991). Perhaps the most serious effect of iron deficiency is the often irreversible impairment of a child's bearing ability (Scrimshaw, 1991) and appetite (Pollit, 1993)

The value of phosphorus is highest in SPOTA (1012.00±0.50 mg/kg) and lowest in BYAM (427.50±0.30 mg/kg) Phosphorus is always found with calcium in the body both contributing to the supporting structure of the body. It is an essential component of nucleic acids and nucleoproteins, which are responsible for cell division, reproduction and transmission of dietary traits (Hegsted, 1973).

The values of zinc in all the starches are lower than 10.00 mg/kg. The peak value is 4.50±0.02 mg/kg in RCCL and the lowest is 2.7±0.02 mg/kg in WYAM. Zinc is an important component of more than 50 enzymes (Bender, 1992). The RDA (recommended dietary allowance) of zinc has been reported to be 15mg/kg for an adult (NRC, 1989). Sandstead (1995) has reported the deficiency of zinc as a public problem. However, by supplementing roots and tubers with other foods to ensure balanced diet may alleviate the adverse effect of low contribution of zinc by them.

Copper and manganese are of significance in diet. They are not detected in the starch samples analyzed. The reason for their absence may partly be adduced to possible low concentration of these minerals in the soil on which they were cultivated. Nevertheless, deficiency in copper has been reported as of little concern since it is widely distributed in other types of food, but failure to absorb it may lead to chronic diseases (Clifford, 1971).

The results of mineral analysis indicate absence of heavy metals whose presence is detrimental to the health status of both man and animals.

3.5 Antinutrient Content

The values of trypsin inhibitor, tannins and cyanide content are shown in Table 3.6. The peak value of trypsin inhibitor is 0.35±0.01 mg/100g in RCCM and BYAM, and the least is 0.17±0.01 mg/100g in WCCL, CASS and WYAM, indicating that trypsin inhibitor activity (TIA) in either RCCM or BYAM is more than twice in WCCL, CASS and WYAM. Trypsin inhibitors are believed to cause growth inhibitors by interfering with digestion, causing pancreatic hypertrophy and metabolic disturbance of sulphur amino acid utilization (Reddy and Pierson, 1994). Although, these inhibitors tend to be stable to heat, numerous reports have shown that trypsin inhibitor and other forms of protease inhibitors such as chymotrypsin inhibitors and amylase inhibitor levels are reduced during fermentation (Chaven and Kadam, 1989.Reddy and Pierson, 1994)

The values of tannin in the starches analyzed vary from 0.09±0.01 mg/100g in RCCL to 0.13±0.01 mg/100g in CASS (Table 3.6) Tannin- protein complexes can cause inactivation of digestive enzymes and reduces protein digestibility by interaction of protein substrate with ionizable iron (Salunkhe et. al., 1990) This could be responsible for low crude protein of CASS as a result of high tannin content (See Table 3.1). The presence of tannin in food can, therefore, lowers feed efficiency, depress growth, decrease iron absorption, damage the mucosal lining of the gastrointestinal tract and increase excretion of proteins and essential amino acids (Reddy and Pierson, 1994). Dehulling, cooking and fermentation can reduce the tannin content of foods.

From Table 3.6, the cyanide contents of the starches range from 7.31±0.01 μg/100g (≈ 0.073±0.01 mg/kg) in RCCL to 21.03±0.02 μg (≈ 0.21±0.02 mg/kg) in CASS. This implies that CASS is about three times richer in free cyanide than RCCL. Oke (1969) has reported 1.0 mg/kg of live weight as lethal dose of cyanide. Thus, if the cyanide content is equal to or exceeds this value, the root is considered toxic. The reduction in the value of cyanide content in CASS may be

as a result of washing, peeling and sieving, which are some of the processing factors that lower the level of toxicity of cyanide in cassava (Meuser and Smolnik, 1980). Manipueira, the technical name of the water compound of cassava root extracted in the pressing of the ground mass in flour processing, contains most of the cyanogenic glucoside. If it is disposed into water body, it results in pollution (O'Brien et. al., 1979), increasing the COD (chemical oxygen demand) to about 2500 mg O_2/litre, which corresponds to pollution produced by 460 persons/day (O'Brien et. al., 1979).

Table 3.6: Anti-nutrients and Cyanide Content of the Starch Samples

Sample	Anti-nutrient (mg/100g)		Cyanide Content (µg/100g)
	Trypsin Inhibitor	Tannin Content	
WCCM	0.26 ± 0.01^{b}	0.12 ± 0.01^{cd}	9.84 ± 0.02^{g}
WCCL	0.17 ± 0.01^{a}	0.11 ± 0.01^{bc}	8.92 ± 0.02^{f}
RCCM	0.35 ± 0.01^{c}	0.10 ± 0.01^{ab}	7.45 ± 0.01^{b}
RCCL	0.26 ± 0.01^{b}	0.09 ± 0.01^{a}	7.31 ± 0.01^{a}
CASS	0.17 ± 0.01^{a}	0.13 ± 0.01^{d}	21.03 ± 0.02^{h}
SPOTA	0.26 ± 0.01^{b}	0.11 ± 0.01^{bc}	8.51 ± 0.02^{e}
BYAM	0.35 ± 0.01^{c}	0.10 ± 0.01^{ab}	8.28 ± 0.02^{d}
WYAM	0.17 ± 0.01^{a}	0.12 ± 0.01^{cd}	7.93 ± 0.01^{c}

CHAPTER FOUR

4.0 CONCLUSION AND RECOMMENDATION

Studies on the proximate composition of the starch samples have revealed that the lowest moisture content is in sweet potato. This gives it an edge above other starches analyzed as the most microbially stable starch during processing. The highest value of crude protein is exhibited by the corms of red cocoyam.

Though roots and tubers cannot contribute to the RDA (recommended dietary allowance) of protein, when supplemented by a variety of other foods such as cereals, their protein contribution can be maximized. The starch content was at its peak in cassava.

Studies on the colour of the samples revealed that the lightest starch sample was cassava. This was also supported by the highest clarity of cassava after 3 days of freezing.

Bitter yam showed an outstanding bulk density compared to the commercial starch called starch 1500 used as a binder in tablets. While the bulk density value of sweet potato was higher than that of yam, an opposite trend was observed for the two samples in term of water absorption capacity.

The results of gel strength of the samples showed that the trio of cormels of white cocoyam, cormels of red cocoyam and water yam may find application in paper industry (Satin, 1999), in pharmaceutical industry, where high shear stress, which is synonymous to gel strength, is required.

The swelling powers of the corms of both cocoyam varieties were higher than those of the cormels with the starch of corms of red cocoyam exhibiting the highest value. Sweet potato had higher swelling power than both bitter yam and water yam. Since the higher the swelling power, the lower the disintegration time (Bi et.al, 1999; Iwuagwu and Onyekweli, 2002); the starches of the corms and cormels of red cocoyam, sweet potato, corms of white cocoyam, cassava can be

incorporated as alternative disintegrant in tablet formulation owing to their appreciably high values of swelling power.

The results of solubility showed that the corms of white cocoyam and cormels of red cocoyam are richer in free linear amylose units that undergo solubilization than the cormels of white cocoyam and corms of red cocoyam respectively.

All the starches showed considerably high values of energy, which are more than enough for what the body weight needs for a day.

Studies on the pasting properties revealed that the gelatinization temperature of the samples were in the range 60.45-62.15^{0}C. Within these gelatinization temperatures, the peak viscosity was obtained in water yam followed by that of sweet potato. Cassava exhibited the lowest retrogradation value. The starch with the highest stability was sweet potato. The value of stability of each starch sample was higher than the corresponding retrogradation value, indicating an inverse proportionality between the two parameters.

All the starch samples were abundant in potassium, phosphorus and calcium. The value obtained for calcium, iron, sodium, phosphorus and potassium were higher than those of corn starch.

All the starch samples had low contents of trypsin inhibitor, tannins and cyanide. Hence, they are non-toxic.

Further studies on the effects of salt concentration and pH should be carried out on the samples. This is to enable more insight into the utilization of the pharmaceutical potentials of these samples.

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APPENDIX

Descriptives

Parameter	Sample	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Moisture Content	WCCM	3	9.3600	1.732E-02	1.000E-02	9.3170	9.4030	9.35	9.38
	WCCL	3	10.1000	1.000E-01	5.774E-02	9.8516	10.3484	10.00	10.20
	RCCM	3	9.5900	4.359E-02	2.517E-02	9.4817	9.6983	9.54	9.62
	RCCL	3	9.0200	7.211E-02	4.163E-02	8.8409	9.1991	8.96	9.10
	CASS	3	9.0100	5.000E-02	2.887E-02	8.8858	9.1342	8.96	9.06
	SPOTA	3	8.7200	3.464E-02	2.000E-02	8.6339	8.8061	8.70	8.76
	BYAM	3	9.3300	7.211E-02	4.163E-02	9.1509	9.5091	9.25	9.39
	WYAM	3	9.0900	1.732E-02	1.000E-02	9.0470	9.1330	9.07	9.10
	Total	24	9.2775	.4094	8.358E-02	9.1046	9.4504	8.70	10.20
Crude Protein	WCCM	3	1.8700	1.000E-02	5.774E-03	1.8452	1.8948	1.86	1.88
	WCCL	3	1.1633	1.155E-02	6.667E-03	1.1346	1.1920	1.15	1.17
	RCCM	3	2.0900	1.000E-02	5.774E-03	2.0652	2.1148	2.08	2.10
	RCCL	3	1.4100	2.000E-02	1.155E-02	1.3603	1.4597	1.39	1.43
	CASS	3	1.1767	1.155E-02	6.667E-03	1.1480	1.2054	1.17	1.19
	SPOTA	3	1.6300	1.000E-02	5.774E-03	1.6052	1.6548	1.62	1.64
	BYAM	3	1.8700	1.000E-02	5.774E-03	1.8452	1.8948	1.86	1.88
	WYAM	3	1.6400	2.000E-02	1.155E-02	1.5903	1.6897	1.62	1.66
	Total	24	1.6063	.3220	6.573E-02	1.4703	1.7422	1.15	2.10
Fat Content	WCCM	3	.5033	5.774E-03	3.333E-03	.4890	.5177	.50	.51
	WCCL	3	.4300	1.000E-02	5.774E-03	.4052	.4548	.42	.44
	RCCM	3	.4100	1.000E-02	5.774E-03	.3852	.4348	.40	.42
	RCCL	3	.4000	1.000E-02	5.774E-03	.3752	.4248	.39	.41
	CASS	3	.3700	1.000E-02	5.774E-03	.3452	.3948	.36	.38
	SPOTA	3	.3900	1.000E-02	5.774E-03	.3652	.4148	.38	.40
	BYAM	3	.3300	1.000E-02	5.774E-03	.3052	.3548	.32	.34
	WYAM	3	.3900	1.000E-02	5.774E-03	.3652	.4148	.38	.40
	Total	24	.4029	4.859E-02	9.918E-03	.3824	.4234	.32	.51
Crude Fibre	WCCM	3	.4600	1.000E-02	5.774E-03	.4352	.4848	.45	.47
	WCCL	3	.5000	1.000E-02	5.774E-03	.4752	.5248	.49	.51
	RCCM	3	.5800	1.000E-02	5.774E-03	.5552	.6048	.57	.59
	RCCL	3	.5000	1.000E-02	5.774E-03	.4752	.5248	.49	.51
	CASS	3	.4100	1.000E-02	5.774E-03	.3852	.4348	.40	.42
	SPOTA	3	.7767	1.528E-02	8.819E-03	.7387	.8146	.76	.79
	BYAM	3	.6900	1.732E-02	1.000E-02	.6470	.7330	.67	.70
	WYAM	3	.7400	1.000E-02	5.774E-03	.7152	.7648	.73	.75
	Total	24	.5821	.1318	2.691E-02	.5264	.6377	.40	.79
Ash Content	WCCM	3	1.8833	1.528E-02	8.819E-03	1.8454	1.9213	1.87	1.90
	WCCL	3	1.8200	1.000E-02	5.774E-03	1.7952	1.8448	1.81	1.83
	RCCM	3	1.7200	1.732E-02	1.000E-02	1.6770	1.7630	1.70	1.73
	RCCL	3	1.9800	2.000E-02	1.155E-02	1.9303	2.0297	1.96	2.00
	CASS	3	1.8300	1.000E-02	5.774E-03	1.8052	1.8548	1.82	1.84
	SPOTA	3	1.6400	1.000E-02	5.774E-03	1.6152	1.6648	1.63	1.65
	BYAM	3	1.7500	2.000E-02	1.155E-02	1.7003	1.7997	1.73	1.77
	WYAM	3	1.8400	2.000E-02	1.155E-02	1.7903	1.8897	1.82	1.86
	Total	24	1.8079	.1006	2.054E-02	1.7654	1.8504	1.63	2.00
Sugar Content	WCCM	3	3.8700	2.000E-02	1.155E-02	3.8203	3.9197	3.85	3.89
	WCCL	3	4.8000	1.000E-02	5.774E-03	4.7752	4.8248	4.79	4.81
	RCCM	3	5.2800	2.000E-02	1.155E-02	5.2303	5.3297	5.26	5.30
	RCCL	3	5.0300	1.000E-02	5.774E-03	5.0052	5.0548	5.02	5.04
	CASS	3	5.0100	3.464E-02	2.000E-02	4.9239	5.0961	4.97	5.03
	SPOTA	3	4.4600	2.000E-02	1.155E-02	4.4103	4.5097	4.44	4.48
	BYAM	3	4.1500	2.000E-02	1.155E-02	4.1003	4.1997	4.13	4.17

	WYAM	3	5.1600	2.000E-02	1.155E-02	5.1103	5.2097	5.14	5.18
	Total	24	4.7200	.4860	9.921E-02	4.5148	4.9252	3.85	5.30
Starch	WCCM	3	81.3900	5.196E-02	3.000E-02	81.2609	81.5191	81.36	81.45
Content	WCCL	3	80.5900	3.606E-02	2.082E-02	80.5004	80.6796	80.55	80.62
	RCCM	3	80.1800	5.568E-02	3.215E-02	80.0417	80.3183	80.12	80.23
	RCCL	3	81.3900	3.000E-02	1.732E-02	81.3155	81.4645	81.36	81.42
	CASS	3	82.0500	4.583E-02	2.646E-02	81.9362	82.1638	82.01	82.10
	SPOTA	3	81.3900	3.606E-02	2.082E-02	81.3004	81.4796	81.35	81.42
	BYAM	3	81.7900	4.583E-02	2.646E-02	81.6762	81.9038	81.74	81.83
	WYAM	3	80.9567	7.506E-02	4.333E-02	80.7702	81.1431	80.87	81.00
	Total	24	81.2171	.5891	.1203	80.9683	81.4659	80.12	82.10
Carbohydrate	WCCM	3	85.9233	3.055E-02	1.764E-02	85.8474	85.9992	85.89	85.95
By	WCCL	3	85.9867	9.504E-02	5.487E-02	85.7506	86.2228	85.89	86.08
Differemce	RCCM	3	85.6100	5.292E-02	3.055E-02	85.4786	85.7414	85.57	85.67
	RCCL	3	86.6900	.1015	5.859E-02	86.4379	86.9421	86.58	86.78
	CASS	3	87.2033	4.726E-02	2.728E-02	87.0859	87.3207	87.15	87.24
	SPOTA	3	86.8433	3.055E-02	1.764E-02	86.7674	86.9192	86.81	86.87
	BYAM	3	86.0300	.1054	6.083E-02	85.7683	86.2917	85.93	86.14
	WYAM	3	86.2833	4.726E-02	2.728E-02	86.1659	86.4007	86.23	86.32
	Total	24	86.3212	.5203	.1062	86.1015	86.5410	85.57	87.24

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Moisture Content	Between Groups	3.803	7	.543	163.379	.000
	Within Groups	5.320E-02	16	3.325E-03		
	Total	3.856	23			
Crude Protein	Between Groups	2.382	7	.340	1856.282	.000
	Within Groups	2.933E-03	16	1.833E-04		
	Total	2.385	23			
Fat Content	Between Groups	5.283E-02	7	7.547E-03	82.331	.000
	Within Groups	1.467E-03	16	9.167E-05		
	Total	5.430E-02	23			
Crude Fibre	Between Groups	.397	7	5.676E-02	400.668	.000
	Within Groups	2.267E-03	16	1.417E-04		
	Total	.400	23			
Ash Content	Between Groups	.229	7	3.268E-02	128.560	.000
	Within Groups	4.067E-03	16	2.542E-04		
	Total	.233	23			
Sugar Content	Between Groups	5.426	7	.775	1824.000	.000
	Within Groups	6.800E-03	16	4.250E-04		
	Total	5.433	23			
Starch Content	Between Groups	7.945	7	1.135	474.554	.000
	Within Groups	3.827E-02	16	2.392E-03		
	Total	7.983	23			
Carbohydrate by Difference	Between Groups	6.147	7	.878	177.556	.000
	Within Groups	7.913E-02	16	4.946E-03		
	Total	6.226	23			

HOMOGENEOUS SUBSETS

MOISTURE CONTENT

			Subset for alpha = .05				
	Sample	N	a	b	c	d	e
Duncan[a]	SPOTA	3	8.7200				
	CASS	3		9.0100			
	RCCL	3		9.0200			
	WYAM	3		9.0900			

	BYAM	3			9.3300		
	WCCM	3			9.3600		
	RCCM	3				9.5900	
	WCCL	3					10.1000
	Sig.		1.000	.126	.533	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

CRUDE PROTEIN

	Sample	N	Subset for alpha = .05				
			a	b	c	d	e
Duncan[a]	WCCL	3	1.1633				
	CASS	3	1.1767				
	RCCL	3		1.4100			
	SPOTA	3			1.6300		
	WYAM	3			1.6400		
	WCCM	3				1.8700	
	BYAM	3				1.8700	
	RCCL	3					2.0900
	Sig.		.245	1.000	.379	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

FAT CONTENT

	Sample	N	Subset for alpha = .05					
			a	b	c	d	e	f
Duncan[a]	BYAM	3	.3300					
	CASS	3		.3700				
	SPOTA	3			.3900			
	WYAM	3			.3900			
	RCCL	3			.4000	.4000		
	RCCM	3				.4100		
	WCCL	3					.4300	
	WCCM	3						.5033
	Sig.		1.000	1.000	.243	.219	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

CRUDE FIBRE

	Sample	N	Subset for alpha = .05						
Duncan[a]			a	b	c	d	e	f	g
	CASS	3	.4100						
	WCCM	3		.4600					
	WCCL	3			.5000				
	RCCL	3			.5000				
	RCCM	3				.5800			
	BYAM	3					.6900		
	WAYM	3						.7400	
	SPOTA	3							.7767
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

ASH CONTENT

	Sample	N	Subset for alpha = .05					
			a	b	c	d	e	f
Duncan[a]	SPOTA	3	1.6400					
	RCCM	3		1.7200				
	BYAM	3			1.7500			
	WCCL	3				1.8200		
	CASS	3				1.8300		
	WYAM	3				1.8400		
	WCCM	3					1.8833	
	RCCL	3						1.9800
	Sig.		1.000	1.000	1.000	.164	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

SUGAR CONTENT

	Sample	N	Subset for alpha = .05						
			a	b	c	d	e	f	g
Duncan[a]	WCCM	3	3.8700						
	BYAM	3		4.1500					
	SPOTA	3			4.4600				
	WCCL	3				4.8000			
	CAS	3					5.0100		
	RCCL	3					5.0300		
	WYAM	3						5.1600	
	RCCM	3							5.2800
	Sig.		1.000	1.000	1.000	1.000	.252	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

STARCH CONTENT

	Sample	N	Subset for alpha = .05					
			a	b	c	d	e	f
Duncan[a]	RCCM	3	80.1800					
	WCCL	3		80.5900				
	WYAM	3			80.9567			
	WCCM	3				81.3900		
	RCCL	3				81.3900		
	SPOTA	3				81.3900		
	BYAM	3					81.7900	
	CASS	3						82.0500
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

CARBOHYDRATE BY DIFFERENCE

	Sample		Subset for alpha = .05					
		N	a	b	c	d	e	f
Duncan[a]	RCCM	3	85.6100					
	WCCM	3		85.9233				
	WCCL	3		85.9867				
	BYAM	3		86.0300				
	WYAM	3			86.2833			
	RCCL	3				86.6900		
	SPOTA	3					86.8433	
	CASS	3						87.2033
	Sig.		1.000	.096	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

Descriptives

Parameter	Sample	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Calcium Content	WCCM	3	201.0000	.1400	8.083E-02	200.6522	201.3478	200.86	201.14
	WCCL	3	210.3000	8.000E-02	4.619E-02	210.1013	210.4987	210.22	210.38
	RCCM	3	197.3000	4.000E-02	2.309E-02	197.2006	197.3994	197.26	197.34
	RCCL	3	188.9000	1.000E-01	5.774E-02	188.6516	189.1484	188.80	189.00
	CASS	3	433.3000	.1200	6.928E-02	433.0019	433.5981	433.18	433.42
	SPOTA	3	221.0000	.1000	5.774E-02	220.7516	221.2484	220.90	221.10
	BYAM	3	187.4000	.2000	.1155	186.9032	187.8968	187.20	187.60
	WYAM	3	220.6000	.2000	.1155	220.1032	221.0968	220.40	220.80
	Total	24	232.4750	78.5081	16.0254	199.3239	265.6261	187.20	433.42
Iron Content	WCCM	3	21.0000	2.000E-02	1.155E-02	20.9503	21.0497	20.98	21.02
	WCCL	3	1.8000	2.000E-02	1.155E-02	1.7503	1.8497	1.78	1.82
	RCCM	3	2.2000	2.000E-02	1.155E-02	2.1503	2.2497	2.18	2.22
	RCCL	3	2.0000	2.000E-02	1.155E-02	1.9503	2.0497	1.98	2.02
	CASS	3	1.1000	1.000E-02	5.774E-03	1.0752	1.1248	1.09	1.11
	SPOTA	3	1.8000	2.000E-02	1.155E-02	1.7503	1.8497	1.78	1.82
	BYAM	3	2.2000	2.000E-02	1.155E-02	2.1503	2.2497	2.18	2.22
	WYAM	3	1.8000	1.000E-02	5.774E-03	1.7752	1.8248	1.79	1.81
	Total	24	4.2375	6.4803	1.3228	1.5011	6.9739	1.09	21.02
Sodium Content	WCCM	3	72.2000	5.000E-02	2.887E-02	72.0758	72.3242	72.15	72.25
	WCCL	3	68.8000	5.000E-02	2.887E-02	68.6758	68.9242	68.75	68.85
	RCCM	3	71.0000	5.000E-02	2.887E-02	70.8758	71.1242	70.95	71.05
	RCCL	3	69.5000	5.000E-02	2.887E-02	69.3758	69.6242	69.45	69.55
	CASS	3	103.4000	6.000E-02	3.464E-02	103.2510	103.5490	103.34	103.46
	SPOTA	3	73.3000	2.000E-02	1.155E-02	73.2503	73.3497	73.28	73.32
	BYAM	3	68.8000	.1000	5.774E-02	68.5516	69.0484	68.70	68.90
	WYAM	3	72.8000	6.000E-02	3.464E-02	72.6510	72.9490	72.74	72.86
	Total	24	74.9750	11.1039	2.2666	70.2862	79.6638	68.70	103.46
Phosphorus Content	WCCM	3	462.0000	.2000	.1155	461.5032	462.4968	461.80	462.20
	WCCL	3	431.0000	.2000	.1155	430.5032	431.4968	430.80	431.20
	RCCM	3	443.3000	.2000	.1155	442.8032	443.7968	443.10	443.50
	RCCL	3	442.9000	.2000	.1155	442.4032	443.3968	442.70	443.10
	CASS	3	434.4000	.4000	.2309	433.4063	435.3937	434.00	434.80
	SPOTA	3	1012.0000	.5000	.2887	1010.7579	1013.2421	1011.50	1012.50
	BYAM	3	427.5000	.3000	.1732	426.7548	428.2452	427.20	427.80
	WYAM	3	441.6000	.4000	.2309	440.6063	442.5937	441.20	442.00
	Total	24	511.8375	193.3708	39.4716	430.1842	593.4908	427.20	1012.50
Potassium Content	WCCM	3	3033.0000	.5000	.2887	3031.7579	3034.2421	3032.50	3033.50
	WCCL	3	2989.0000	.5000	.2887	2987.7579	2990.2421	2988.50	2989.50
	RCCM	3	3015.0000	.7000	.4041	3013.2611	3016.7389	3014.30	3015.70
	RCCL	3	2954.0000	.2000	.1155	2953.5032	2954.4968	2953.80	2954.20
	CASS	3	2774.0000	.5000	.2887	2772.7579	2775.2421	2773.50	2774.50
	SPOTA	3	3102.0000	.5000	.2887	3100.7579	3103.2421	3101.50	3102.50
	BYAM	3	2881.0000	.5000	.2887	2879.7579	2882.2421	2880.50	2881.50
	WYAM	3	2855.0000	.7000	.4041	2853.2611	2856.7389	2854.30	2855.70
	Total	24	2950.3750	102.4806	20.9188	2907.1012	2993.6488	2773.50	3102.50
Zinc Content	WCCM	3	3.5000	2.000E-02	1.155E-02	3.4503	3.5497	3.48	3.52
	WCCL	3	2.9000	2.000E-02	1.155E-02	2.8503	2.9497	2.88	2.92
	RCCM	3	3.5000	1.000E-02	5.774E-03	3.4752	3.5248	3.49	3.51
	RCCL	3	4.5000	2.000E-02	1.155E-02	4.4503	4.5497	4.48	4.52
	CASS	3	4.2000	2.000E-02	1.155E-02	4.1503	4.2497	4.18	4.22

SPOTA	3	3.8000	3.000E-02	1.732E-02	3.7255	3.8745	3.77	3.83
BYAM	3	3.2000	1.000E-02	5.774E-03	3.1752	3.2248	3.19	3.21
WYAM	3	2.7000	2.000E-02	1.155E-02	2.6503	2.7497	2.68	2.72
Total	24	3.5375	.5891	.1203	3.2887	3.7863	2.68	4.52

ANOVA

Parameter		Sum of Squares	df	Mean Square	F	Sig.
Calcium Content	Between Groups	141760.785	7	20251.541	1140931.871	.000
	Within Groups	.284	16	1.775E-02		
	Total	141761.069	23			
Iron Content	Between Groups	965.876	7	137.982	424560.989	.000
	Within Groups	5.200E-03	16	3.250E-04		
	Total	965.881	23			
Sodium Content	Between Groups	2835.765	7	405.109	117422.981	.000
	Within Groups	5.520E-02	16	3.450E-03		
	Total	2835.820	23			
Phosphorus Content	Between Groups	860020.376	7	122860.054	1198634.671	.000
	Within Groups	1.640	16	.103		
	Total	860022.016	23			
POTASSIU	Between Groups	241547.625	7	34506.804	121609.880	.000
	Within Groups	4.540	16	.284		
	Total	241552.165	23			
Zinc Content	Between Groups	7.976	7	1.139	2940.553	.000
	Within Groups	6.200E-03	16	3.875E-04		
	Total	7.982	23			

HOMOGENEOUS SUBSETS

CALCIUM CONTENT

	Sample	N	Subset for alpha = .05							
			a	b	c	d	e	f	g	h
Duncan[a]	BYAM	3	187.4000							
	RCCL	3		188.9000						
	RCCM	3			197.3000					
	WCCM	3				201.0000				
	WCCL	3					210.3000			
	WYAM	3						220.6000		
	SPOTA	3							221.0000	
	CASS	3								433.3000
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

IRON CONTENT

	Sample	N	Subset for alpha = .05				
			a	b	c	d	e
Duncan[a]	CASS	3	1.1000				
	WCCL	3		1.8000			
	SPOTA	3		1.8000			
	WYAM	3		1.8000			

	RCCL	3			2.0000			
	RCCM	3				2.2000		
	BYAM	3				2.2000		
	WCCM	3					21.0000	
	Sig.		1.000	1.000	1.000	1.000	1.000	

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

SODIUM CONTENT

	Sample	N	Subset for alpha = .05						
			a	b	c	d	e	f	g
Duncan[a]	WCCL	3	68.8000						
	BYAM	3	68.8000						
	RCCL	3		69.5000					
	RCCM	3			71.0000				
	WCCM	3				72.2000			
	WYAM	3					72.8000		
	SPOTA	3						73.3000	
	CASS	3							103.4000
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

PHOSPHORUS CONTENT

	Sample	N	Subset for alpha = .05						
			a	b	c	d	e	f	g
Duncan[a]	BYAM	3	427.5000						
	WCCL	3		431.0000					
	CASS	3			434.4000				
	WYAM	3				441.6000			
	RCCL	3					442.9000		
	RCCM	3					443.3000		
	WCCM	3						462.0000	
	SPOTA	3							1012.0000
	Sig.		1.000	1.000	1.000	1.000	.146	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

POTASSIUM CONTENT

	Sample	N	Subset for alpha = .05							
			a	b	c	d	e	f	g	h
Duncan[a]	CASS	3	2774.0000							
	WYAM	3		2855.0000						
	BYAM	3			2881.0000					
	RCCL	3				2954.0000				
	WCCL	3					2989.0000			
	RCCM	3						3015.0000		
	WCCM	3							3033.0000	
	SPOTA	3								3102.0000
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

ZINC CONTENT

	Sample	N	Subset for alpha = .05						
			a	b	c	d	e	f	g
Duncan[a]	WYAM	3	2.7000						
	WCCL	3		2.9000					
	BYAM	3			3.2000				
	WCCM	3				3.5000			

RCCM	3				3.5000			
SPOTA	3					3.8000		
CASS	3						4.2000	
RCCL	3							4.5000
Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

Descriptives

Parameter	Sample	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Trypsin Inhibitor	WCCM	3	.2600	1.000E-02	5.774E-03	.2352	.2848	.25	.27
	WCCL	3	.1700	1.000E-02	5.774E-03	.1452	.1948	.16	.18
	RCCM	3	.3500	1.000E-02	5.774E-03	.3252	.3748	.34	.36
	RCCL	3	.2600	1.000E-02	5.774E-03	.2352	.2848	.25	.27
	CASS	3	.1700	1.000E-02	5.774E-03	.1452	.1948	.16	.18
	SPOTA	3	.2600	1.000E-02	5.774E-03	.2352	.2848	.25	.27
	BYAM	3	.3500	1.000E-02	5.774E-03	.3252	.3748	.34	.36
	WYAM	3	.1700	1.000E-02	5.774E-03	.1452	.1948	.16	.18
	Total	24	.2488	7.225E-02	1.475E-02	.2182	.2793	.16	.36
Tannin Content	WCCM	3	.12000	1.0000E-02	5.7735E-03	9.5159E-02	.14484	.110	.130
	WCCL	3	.11000	1.0000E-02	5.7735E-03	8.5159E-02	.13484	.100	.120
	RCCM	3	1.0000E-01	1.0000E-02	5.7735E-03	7.5159E-02	.12484	.090	.110
	RCCL	3	9.0000E-02	5.0000E-03	2.8868E-03	7.7579E-02	.10242	.085	.095
	CASS	3	.13000	1.0000E-02	5.7735E-03	.10516	.15484	.120	.140
	SPOTA	3	.11000	1.0000E-02	5.7735E-03	8.5159E-02	.13484	.100	.120
	BYAM	3	.10000	1.0000E-02	5.7735E-03	7.5159E-02	.12484	.090	.110
	WYAM	3	.12000	1.0000E-02	5.7735E-03	9.5159E-02	.14484	.110	.130
	Total	24	.11000	1.4818E-02	3.0247E-03	.10374	.11626	.085	.140
Cyanide Content	WCCM	3	9.8400	2.000E-02	1.155E-02	9.7903	9.8897	9.82	9.86
	WCCL	3	8.9200	2.000E-02	1.155E-02	8.8703	8.9697	8.90	8.94
	RCCM	3	7.4500	1.000E-02	5.774E-03	7.4252	7.4748	7.44	7.46
	RCCL	3	7.3100	1.000E-02	5.774E-03	7.2852	7.3348	7.30	7.32
	CASS	3	21.0300	2.000E-02	1.155E-02	20.9803	21.0797	21.01	21.05
	SPOTA	3	8.5100	2.000E-02	1.155E-02	8.4603	8.5597	8.49	8.53
	BYAM	3	8.2800	2.000E-02	1.155E-02	8.2303	8.3297	8.26	8.30
	WYAM	3	7.9300	1.000E-02	5.774E-03	7.9052	7.9548	7.92	7.94
	Total	24	9.9088	4.3637	.8907	8.0661	11.7514	7.30	21.05

ANOVA

Parameter		Sum of Squares	df	Mean Square	F	Sig.
Trypsin Inhibitor	Between Groups	.118	7	1.692E-02	169.232	.000
	Within Groups	1.600E-03	16	1.000E-04		
	Total	.120	23			
Tannin Content	Between Groups	3.600E-03	7	5.143E-04	5.675	.002
	Within Groups	1.450E-03	16	9.063E-05		
	Total	5.050E-03	23			
Cyanide Content	Between Groups	437.965	7	62.566	217622.292	.000
	Within Groups	4.600E-03	16	2.875E-04		
	Total	437.969	23			

HOMOGENEOUS SUBSETS

TRYPSIN INHIBITOR

	Sample	N	Subset for alpha = .05		
			a	b	c
Duncan[a]	WCCL	3	.1700		
	CASS	3	.1700		
	WYAM	3	.1700		
	WCCM	3		.2600	
	RCCL	3		.2600	
	SPOTA	3		.2600	
	BYAM	3			.3500
	RCCM	3			.3500
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

TANNIN CONTENT

	Sample	N	Subset for alpha = .05			
			a	b	c	d
Duncan[a]	RCCL	3	9.0000E-02			
	RCCM	3	1.0000E-01	1.0000E-01		
	BYAM	3	.10000	.10000		
	WCCL	3		.11000	.11000	
	SPOTA	3		.11000	.11000	
	WCCM	3			.12000	.12000
	WYAM	3			.12000	.12000
	CASS	3				.13000
	Sig.		.240	.254	.254	.240

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

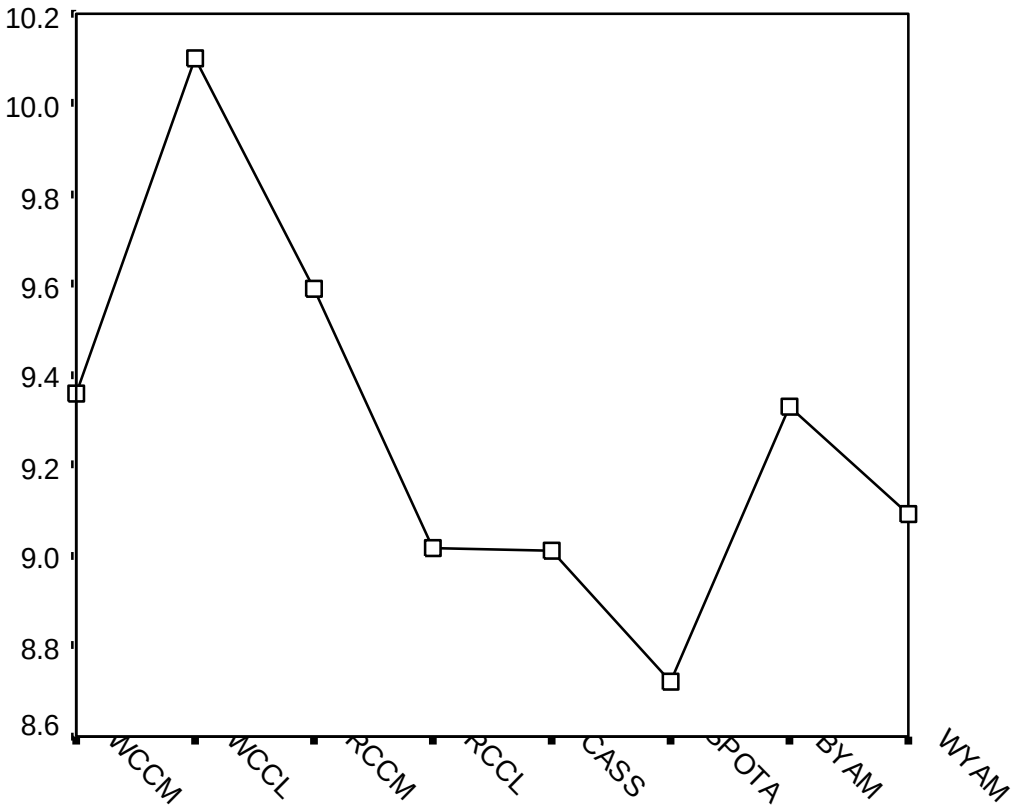
CYANIDE CONTENT

	Sample	N	Subset for alpha = .05							
			a	b	c	d	e	f	g	h
Duncan[a]	RCCL	3	7.3100							
	RCCM	3		7.4500						
	WYAM	3			7.9300					
	BYAM	3				8.2800				
	SPOTA	3					8.5100			
	WCCL	3						8.9200		
	WCCM	3							9.8400	
	CAS	3								21.0300
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000.

MEAN PLOTS



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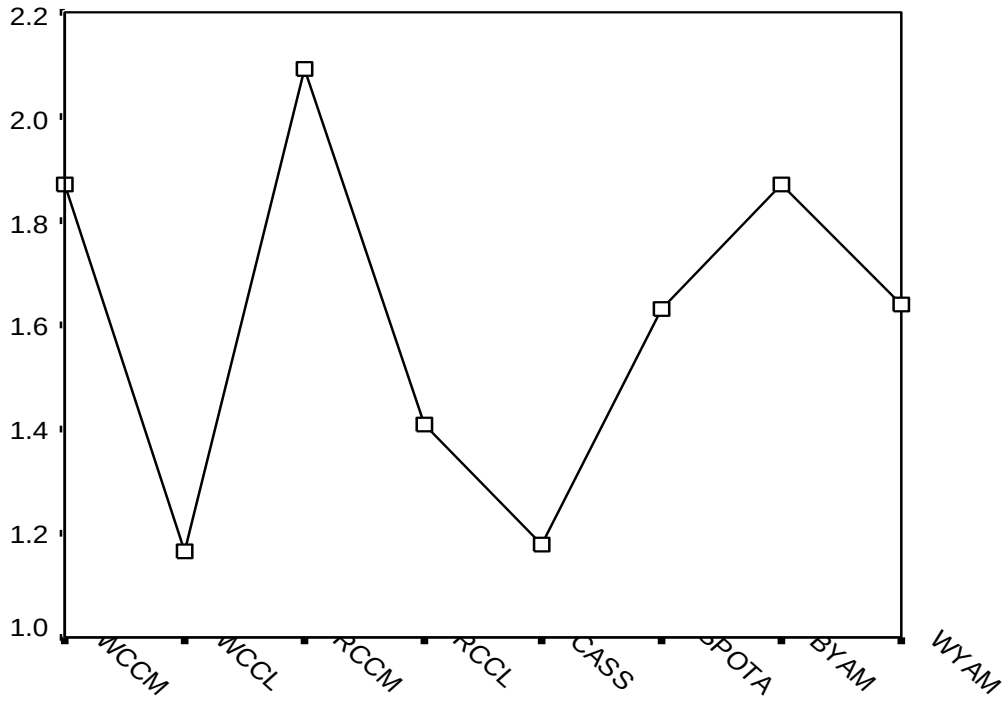

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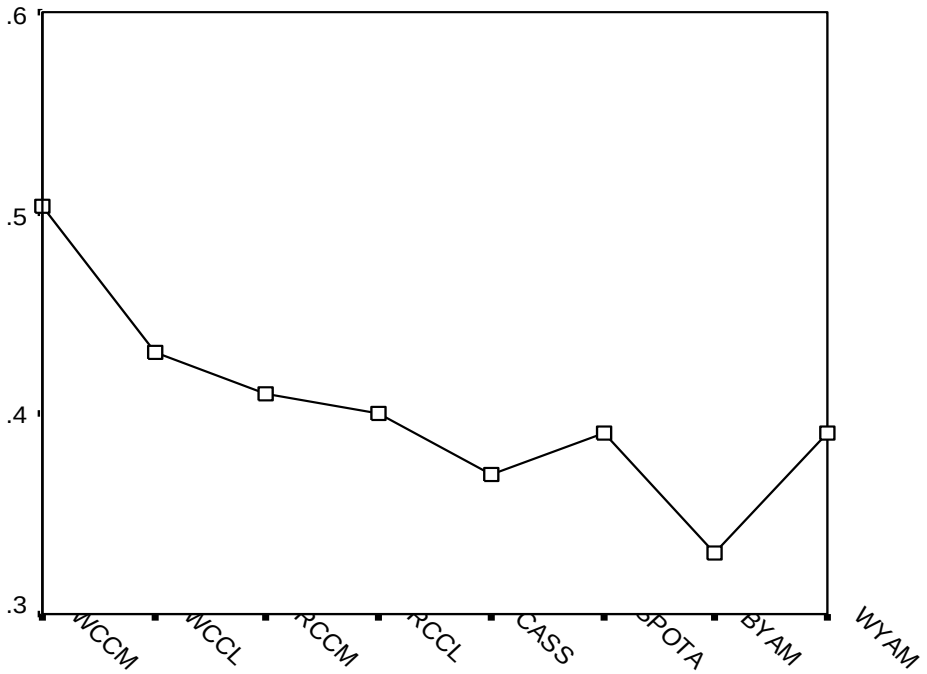

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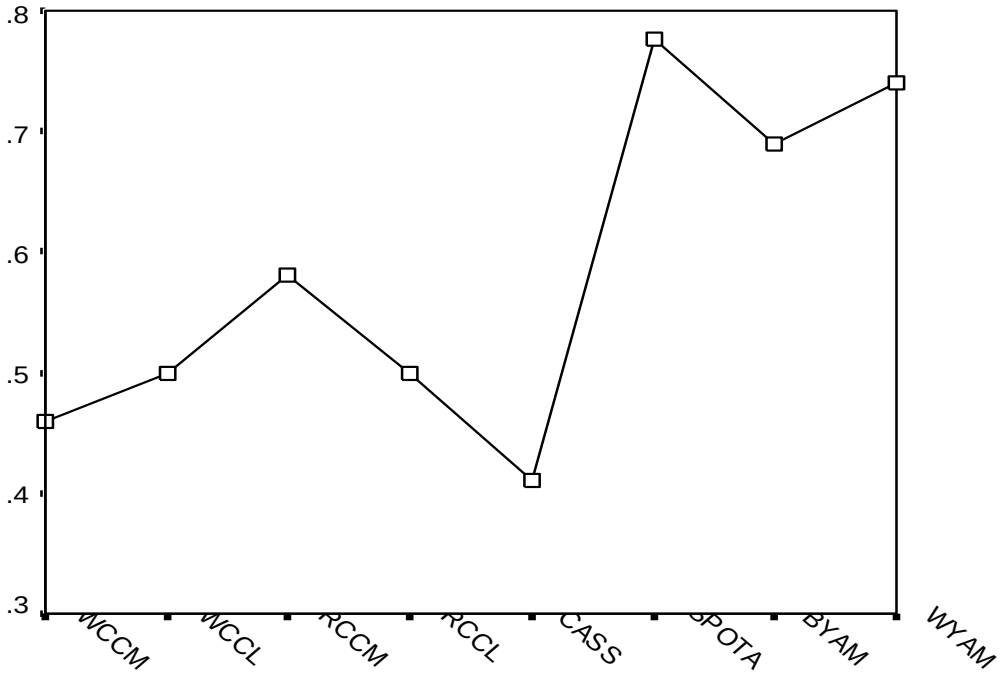

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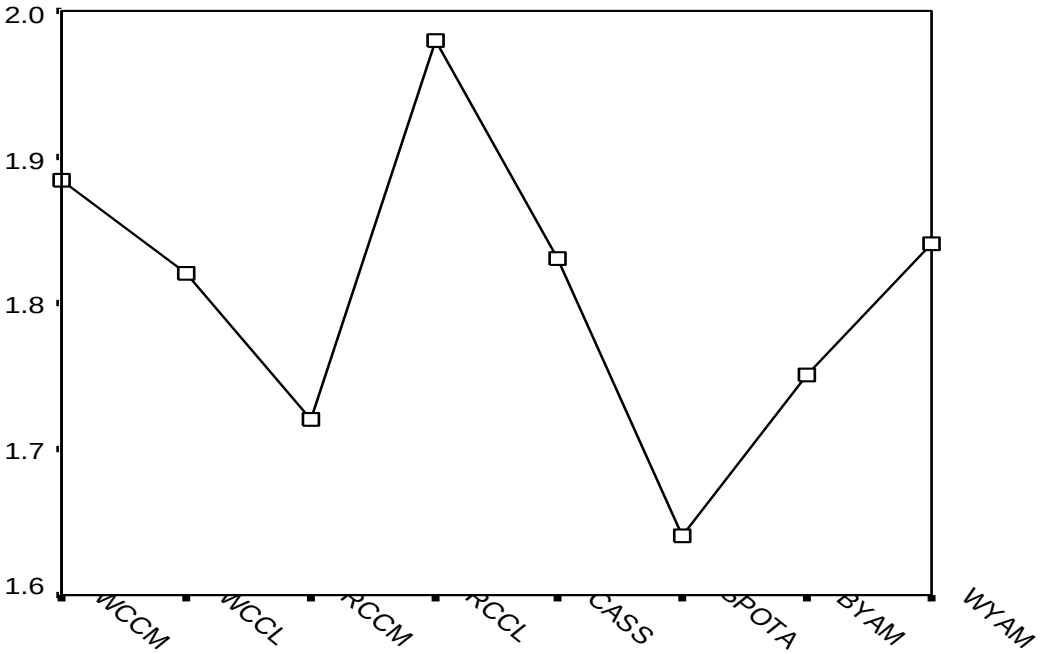

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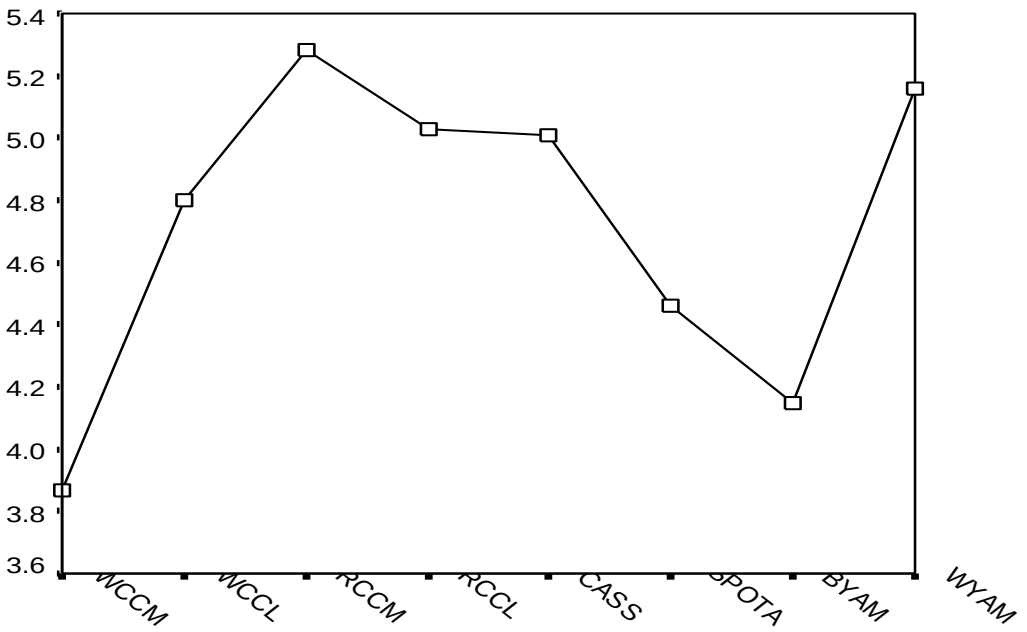

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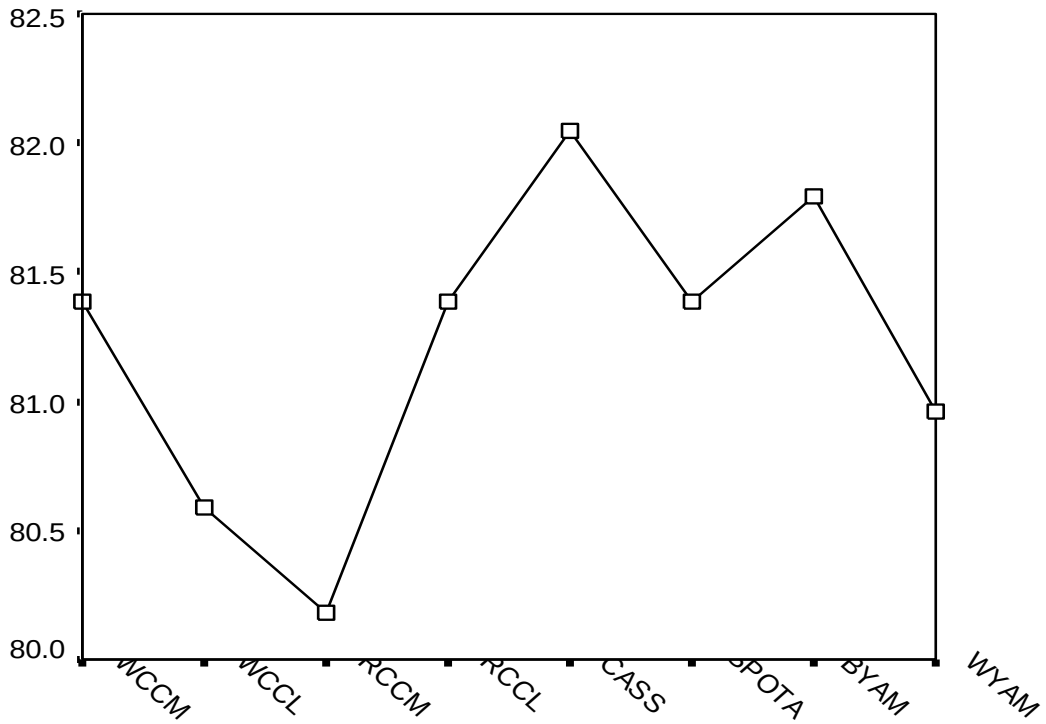

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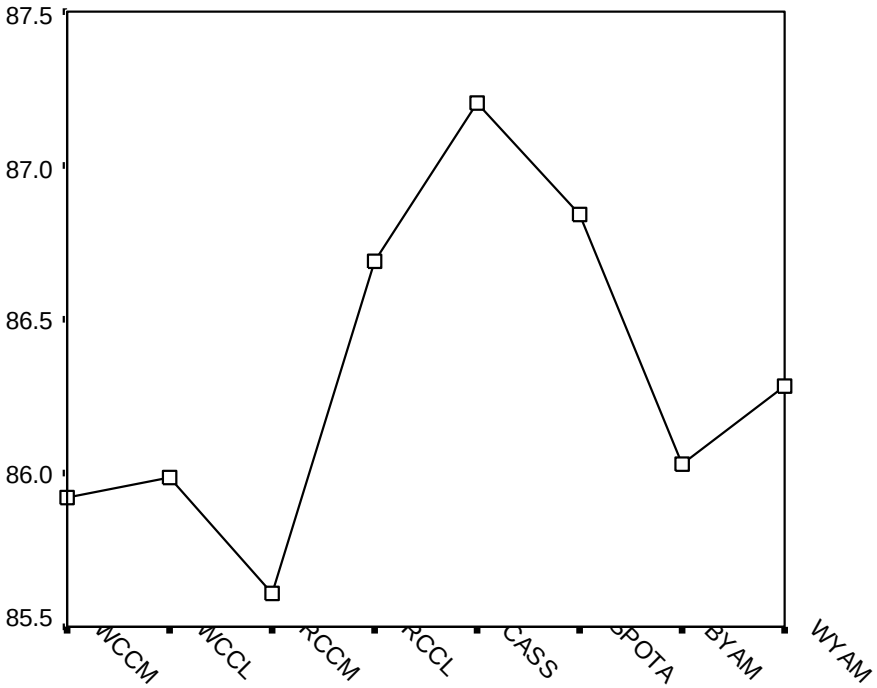

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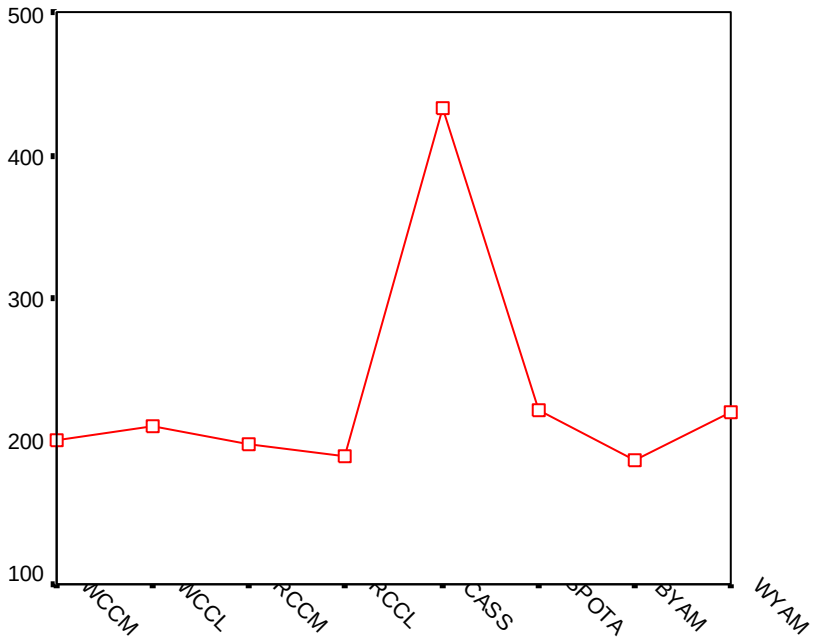

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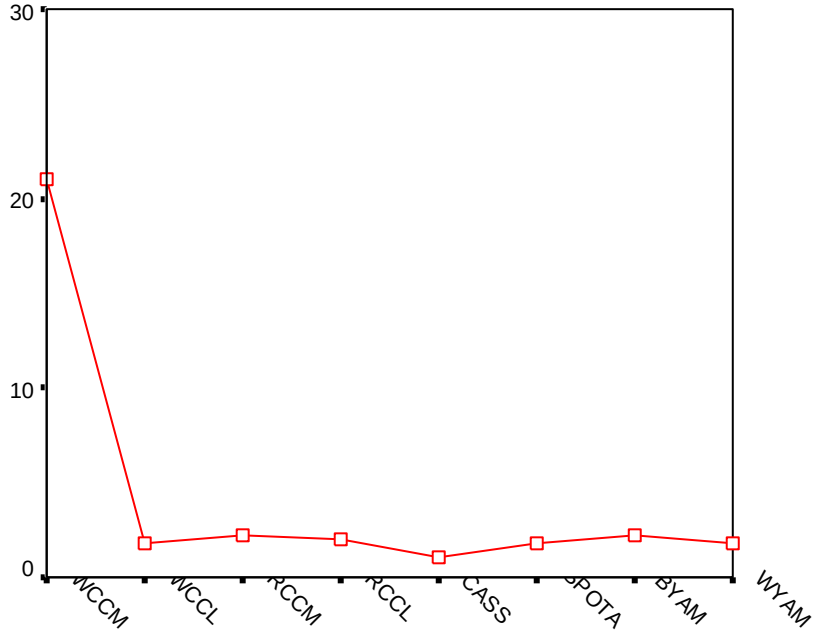

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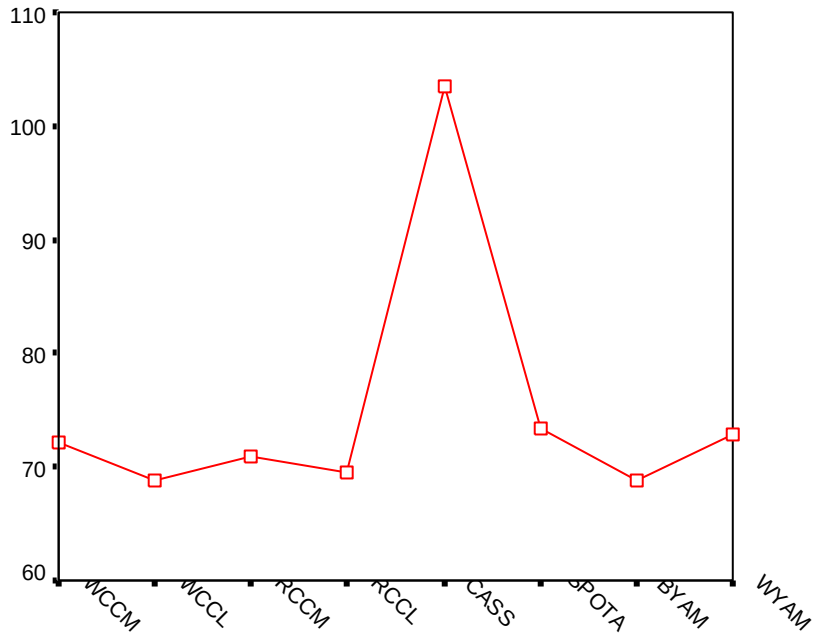

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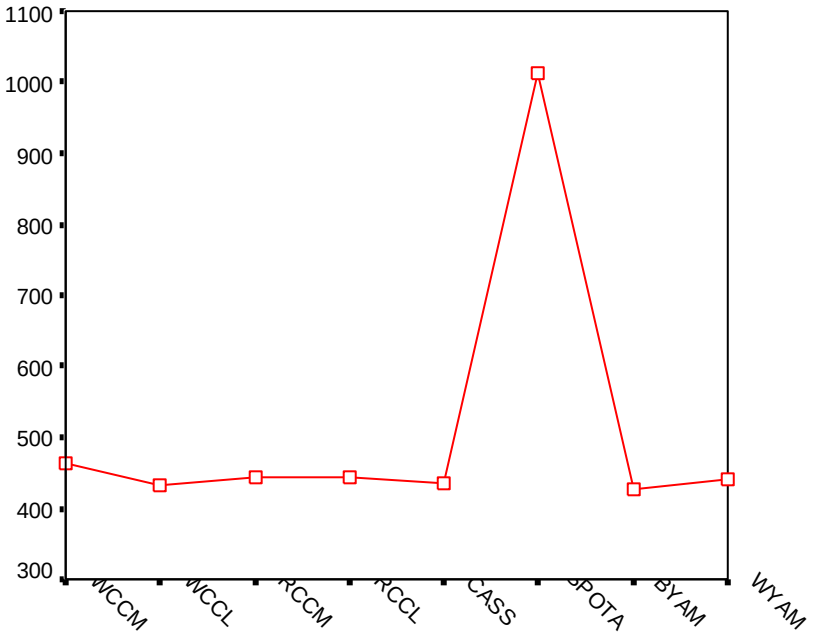
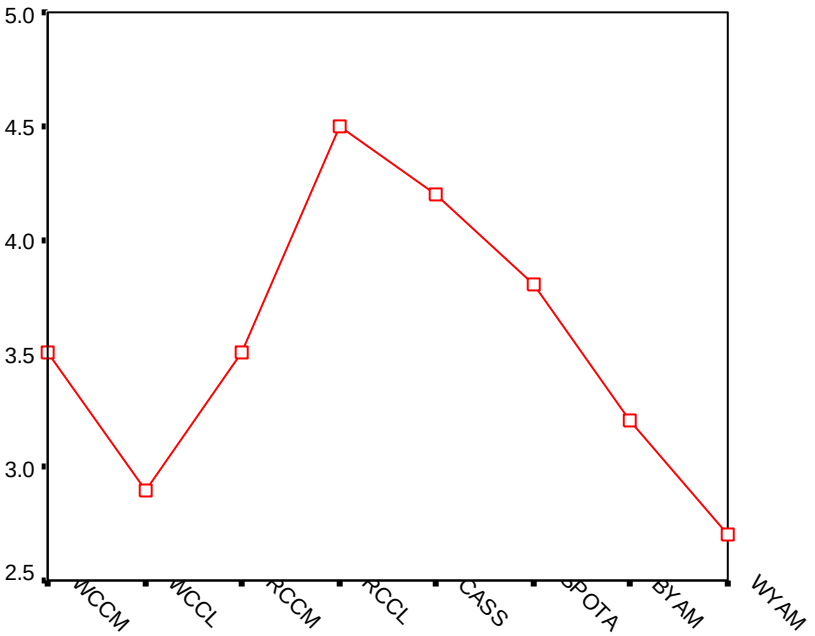


Sample



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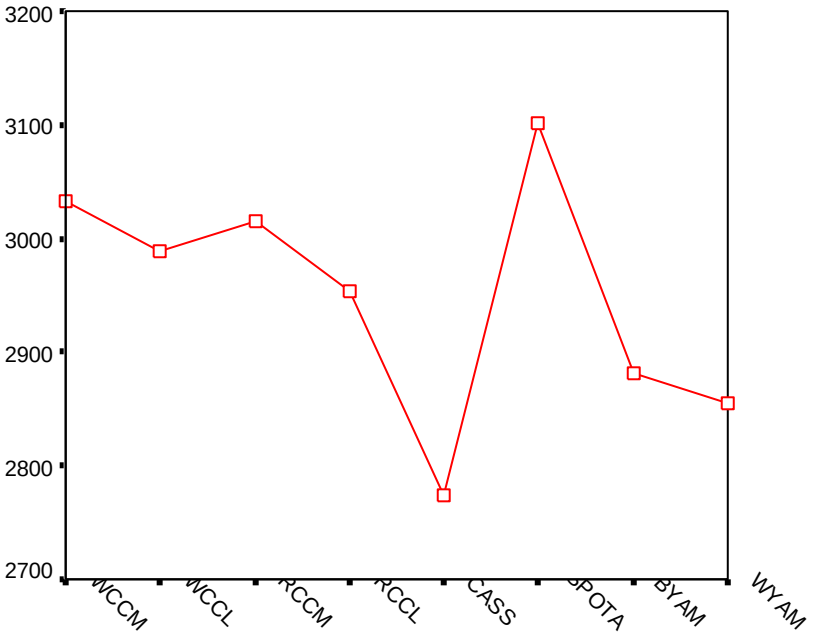

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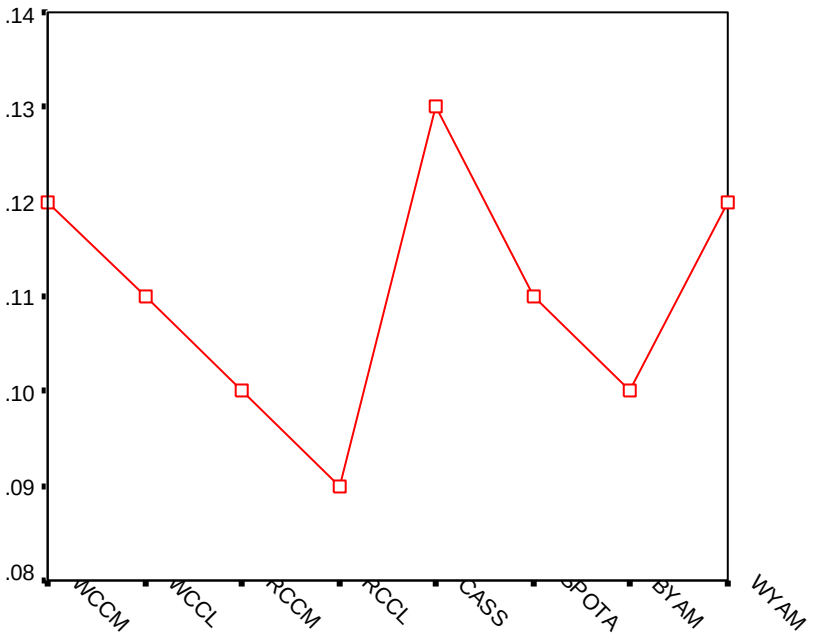

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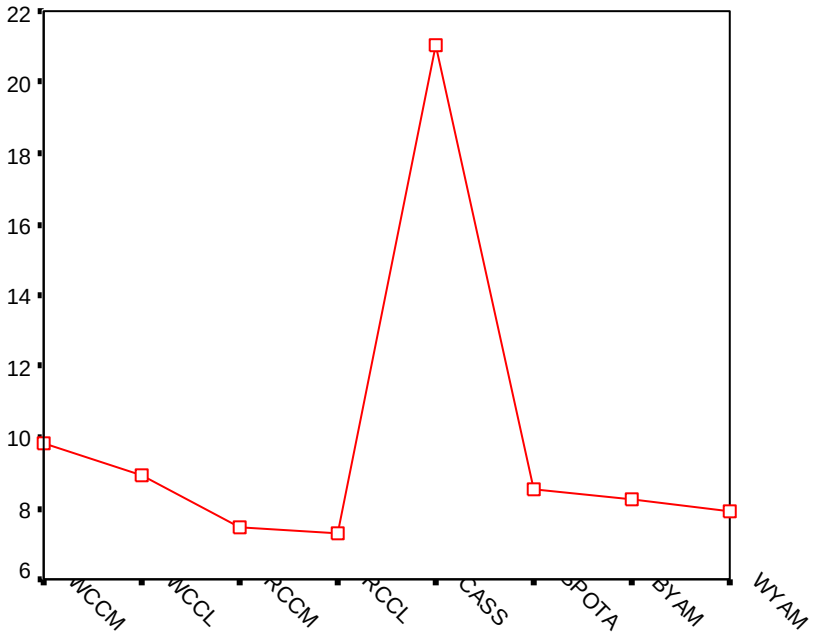

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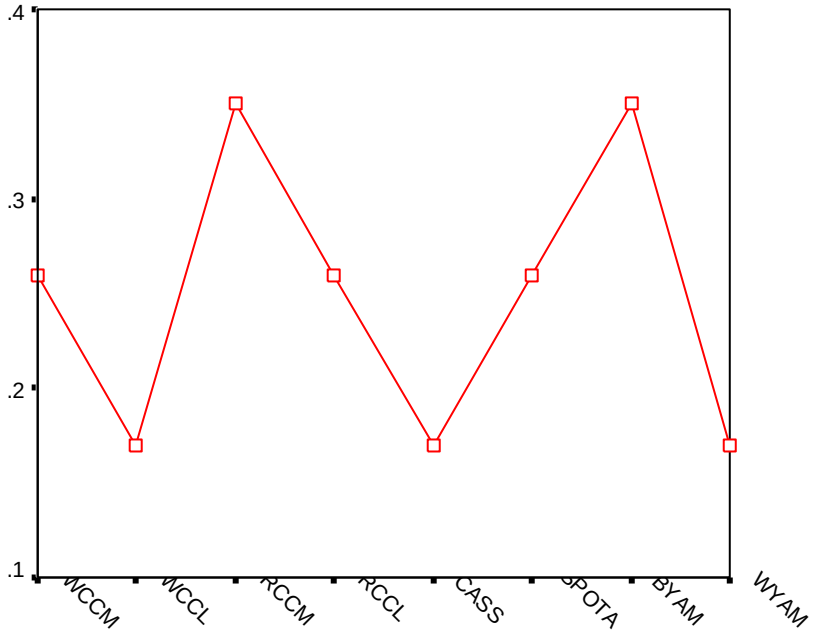

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