THE EFFECT OF SOME PROCESSING METHODS ON THE FLATULENCE FACTOR IN SOME COWPEA VARIETIES *(Vigna unguiculata* (L) Walp)

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DECLARATION

I hereby declare that this thesis is a record of my own research work and has not been submitted for the award of a degree at any other university. All quotations are indicated and sources of information are specifically acknowledged by means of references.



Godwin Chiaka Njoku

Date: 27/4/98

CERTIFICATION

This thesis entitled "The effect of some processing methods on the flatulence factor in some cowpea varieties (*Vigna unguiculata* (L.) Walp) by Godwin Chiaka Njoku meets the regulations governing the award of the degree of Master of Science (M.Sc.) in Biochemistry of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my father, Late Anthony Osuji Njoku and Late Professor N. B. Mijindadi who were the brains behind my higher education.

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ABSTRACT

Oligosaccharide contents of 5 cowpea varieties were studied in raw and processed samples. Thin layer chromatography of 80% ethanol extracts of the defatted samples revealed the presence of verbascose, stachyose, raffinose, sucrose and in some cases, traces of glucose- Quantitative analysis using phenol-sulphuric acid assay method revealed that the average content of the oligosaccharides in whole raw cowpea were raffinose 1.19g/100g and stachyose 3.88g/100g with no varietal difference in the levels of raffinose. Stachyose levels showed difference due to variety.

The results showed a steady decrease in raffinose and stachyose contents in the order-dehulling, cold water soaking, cooking, processing into bean cake, alkali (potash) treatment and hot water soaking (blanching).

Dehulling resulted in a mean reduction of 30.25% for raffinose and 26.80% for stachyose while cold water soaking for 16hrs gave a mean reduction of 40.34% for raffinose and 35.29% for stachyose. Cooking for 40mins was sufficient to promote a mean reduction of 47.06% for raffinose and 36.85% for stachyose. Mean reduction of 47.90% for raffinose and 47.16% for stachyose were obtained when the cowpea was processed into bean cake. Alkali (potash) treatment reduced the mean raffinose level by 68.90% and 61.34% for stachyose.

Hot water soaking for 4 hrs brought about a mean decrease of over 80% for both raffinose and stachyose which when analysed gave an equivalent of about 82.2% reduction of flatulence activities.

Statistical analysis at ($P < 0.05$) also showed that processing time and treatment (processing method) were more important factors affecting the levels of the oligosaccharides than variety.

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CHAPTER ONE

1.0 INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp), a member of the plant family of leguminosae is cultivated throughout West Africa and is the most widely consumed legume seed in Nigeria and other West African countries (Faris, 1965; Onigbinde and Akinyele, 1983).

Cowpea is an important food for children as well as adults and is consumed in a variety of ways. In households, they are cooked alone; with yam, maize or rice; steamed as in moin-moin; fried as in bean cake (akara) and made into bean soup called "gbegiri" (Aykrod and Doughty, 1982; William, 1984). In West and East Africa, the tender green leaves are cooked like spinach, the green seeds and pods can also be cooked and eaten. Cowpeas can be used as fodder, ground cover or green manure.

Cowpea like other legumes is a good, cheap source of plant protein, vitamins, minerals and fibre in developing countries where the supply of food of animal origin is limited due to non-availability and high cost. The proximate composition of cowpea varieties range from 19.97 to 24.13 g/100g for protein, 60g/100g average for carbohydrate, total

dietary fibre 27g/100g, oil 0.87 - 3.53 g/100g and rich in water soluble vitamins and minerals (Ritchey *et. al.*, 1976; Phillip and McWatters, 1991; IAR Report, 1994).

Cowpea and other legumes have received increased scientific and public attention regarding their potential significance as a part of a healthy diet. The greatest interest is in its hypocholesterolemic effect (Trowell, 1973; Hellendoon, 1979; Anderson *et. al.*, 1990), hypoglycemic effect (Trowell, 1973; Jenkins *et. al.*, 1984), reduction in the risk of colonic cancer and its use as a weaning food (Hellendoon, 1979; Uebersax *et. al.*, 1991).

However, there are some qualitative problems which if solved will make cowpeas and their products enjoy wider acceptability and utilization. These problems include - deficiency in the sulphur containing amino acids especially methionine (Evans and Bandemer, 1967; FAO, 1970); the physical constraints - cooking time, convenience and expense in terms of cooking fuel and the hard-to-cook defect (Sefa-Dede *et. al.*, 1978; Keshun liu *et. al.*, 1992; IAR Report, 1994); the biological constraints (ie the presence of some anti-nutrients) - trypsin inhibitors, phytohaemagglutinins, tannins and phytates (de-Boland *et al.*, 1975; Liener, 1976; Elias *et al.*, 1979; Gomes *et al.*, 1979; Gupta and Wagle, 1980; Maga,

1982), poor digestibility of legume proteins (Nielson, 1991) and the flatulence factor (Calloway, 1966; Richard *et al.*, 1968; Sugimoto and Van Buren, 1970; Cristafaro *et. al.*, 1974; Phillip and Abbey, 1989).

Some of these physical and biological constraints do not pose much problems since cowpeas have to be processed by heat treatment before consumption. The actions of nearly all these antinutrients are eliminated by heat applied in proper cooking or by preliminary soaking, particularly when the soaking water is changed (Nutr. Foundation Inc. 1967ab., Ogun *et. al.*, 1989). Decortication of cowpea significantly reduces the tannin content (Desphande *et. al.*, 1982; Ikemefuna, 1989). Cowpea can be supplemented with methionine or with cereals which are high in sulphur - containing amino acids but low in lysine (Onayemi and Potter, 1976). This supplementation improves the protein quality.

Flatulence is a major constraint militating against the consumption of cowpeas and other legumes by humans. The phenomenon has been attributed mainly to alpha-galacto-oligosaccharides of the raffinose family which are relatively heat stable and are present in legume seeds (French, 1954; Cerning-Beroard and Fliatre, 1976; Onigbinde and Akinyele, 1983). The monogastric animals do not possess the enzyme alpha-galactosidase in the gastro-intestinal tract. This

enzyme is responsible for the hydrolysis of these oligosaccharides. Also the sucrase or invertase which is present cannot hydrolyse a substituted sucrose. These oligosaccharides escape breakdown and absorption in the stomach and small intestine and are made available for anaerobic fermentation by microorganisms present in the colon. This anaerobic fermentation results in the production of flatus and other attendant discomfort.

Human and animal trials confirmed that there was an increased flatus production after the consumption of legumes and their products (Calloway, 1966; Richard *et. al.*, 1968; Rachis *et. al.*, 1970ab; Murphy *et. al.*, 1972; Tomlin *et. al.*, 1991).

Different investigators have demonstrated both *in vivo* and *in vitro* that the addition of antibiotics or bacteriostatics such as vioform or mexaform to diets containing leguminous seeds inhibited flatus formation (Richard and Steggerda, 1966; Steggarda, 1968). The addition of such substances to foods for human consumption is obviously unacceptable. It has also been reported that certain phenolic compounds present in some legumes inhibit the activities of intestinal anaerobic bacteria (Arai *et. al.*, 1966; Rachis *et. al.*, 1970a).

Although several practices including soaking, cooking and dehulling (Ogun *et. al.*, 1989; Nnanna and Phillips, 1990; Somiari and Balogh, 1993); enzyme treatment (Sugimoto and Van Buren, 1970; Thananunkul *et. al.*, 1976); fermentation (Reddy and Salunkhe, 1980); germination (Chang *et. al.*, 1989; Trugo *et. al.*, 1990); high temperature extrusion (Borejszo and Khan, 1992) and ultrafiltration (Omosaiye *et. al.*, 1978) could reduce the oligosaccharide contents of legumes, the reduction obtained by several investigators have so far been inconsistent or insufficient to suggest a significant reduction in flatus activities.

For example cooking brought about an increase in the levels of oligosaccharides in four Indian pulses (Rao and Belevady, 1978). No increase in flatus activity was recorded when malted legumes were fed to experimental rats (Price *et. al.*, 1988). Savitri and Desikachar, (1985) reported an apparent increase in total oligasaccharides after cooking seven different legumes.

Different investigators have reported that these oligosaccharide levels vary in different varieties of legumes. For instance, studies on horse beans (*Vicia faba*) (Cerning *et. al.*, 1975), common beans (*Phaseolus vulgaris*) (Trugo *et. al.*, 1990) and cowpeas (*Vigna unguiculata*) (Onigbinde and Akinyele, 1983) have demonstrated considerable variation in the composition of these oligosaccharides in the varieties.

Also with the production of improved cowpea varieties through new plant breeding techniques such as genetic manipulation and agronomic practices such as application of fertilizers and pesticides, it is possible to alter the oligosaccharide levels of the legumes.

Hence, the purpose of this study is to screen some IAR cowpea varieties available in Nigeria for their raffinose and stachyose contents and further determine the effect of some processing methods on the oligosaccharide contents of these varieties.

CHAPTER TWO

2.0 LITERATURE REVIEW

The FAO survey (FAO, 1957) estimated that on a wide basis, some 10 to 15% of the people are under nourished and up to half suffer from hunger or malnutrition or both. These nutritional deficiencies are most serious with the developing countries. According to Schertz, (1971) about two-thirds of the world, the poor countries, consume only half of the worlds protein; and this is mostly cereal protein.

Undernourishment or malnutrition may arise from inadequate calorie intake, protein deficiencies and a shortage of vitamins and minerals. Of these deficiencies, protein deficiency has aroused the greatest concern. Protein hunger may largely be overcome by an increase in availability in animal or plant protein at price levels the affected population can afford or through supplementation (Onayemi and Potter, 1976). The world over, vegetable proteins account for approximately 70% of the protein in human diet and animal protein some 30%. In the developing countries even less of the total dietary protein comes from animal source because it is scarce, costly and not within the reach of the poor sector. Cereal proteins make up more than 70%, a sizable fraction of the total vegetable protein consumed since the diet is mainly

based on processed cereal grains and root tubers.

The protein content of cereals normally ranges from about 8 to 16 g/100g. In each of the cereals, protein quality is below the optimum need of man particularly for children, pregnant and lactating women (Nnanyelugo, 1982).

Food legumes, because of their high protein content, in general constitute the cheapest protein source and natural protein supplement to staple diets. Cowpea, in Africa, at least represent the legume of choice for many such population (FAO, 1969). Food legume protein varies from 20 to 40 g/100g (Aykroyd and Doughty, 1982). Methionine and to some extent cysteine, both sulphur - containing amino acids, are usually the limiting amino acids.

The cereals and legumes complement each other very satisfactorily, both in terms of protein content and quality. The supplementary effect of legume protein to cereal grain on a dry weight basis show that there is an increase in protein content of diets when they contain 10% beans. This increase which is from 7.7 - 8.6 g/100g for sorghum and 11.0-12 g/100g for whole wheat is significant, particularly for children who require relatively high protein intake than do adults. The product of protein quality and protein content relative to a reference protein (utilizable protein) was higher for diets made with cereal and beans (Bressani *et. al.*, 1974).

Apart from being deficient in sulphur - containing amino acids, grain legumes contain anti-nutritional factors. The most common of such factors are the trypsin inhibitors (Pusztai, 1967; Kakade *et. al.*, 1969). These anti-nutritional factors are important chiefly in raw grain legumes, since heat processing destroys them in most cases. Phytohaemagglutinin compounds are also present in most legumes, characterised by causing blood clotting (Liener, 1974). The goitrogenic factors and cyanogenic glycosides (Montgomery, 1980) are also known in grain legumes. As in the case of trypsin inhibitors and phytoheaemagglutinins, they are destroyed during processing.

The heat stable factors of nutritional importance include - toxic alkaloids of the bitter taste (most serious in *lupinus* species); lathyrogens (in *lathyrus sativus*); favism (in *Vicia faba*) and flatulence factor (Sharma, 1961; Calloway, 1966; Nagarajan, 1969; Donoso *et. al.*, 1969; Cristafaro *et. al.*, 1972; Cristafaro *et. al.*, 1974). Fortunately all these factors are not common to all species and therefore routine screening of all material may not be necessary.

2.1 Cowpea Utilization

In tropical Africa cowpeas are primarily used in the form of dry seed cooked as a pulse in a large variety of dishes (Aykrod and Doughty, 1982). Preference is for brown, white or

cream seeds with a small eye and wrinkled or rough seed coat (Williams, 1974). In households, in Nigeria, they are cooked alone, with yam, maize or rice; steamed as in moin-moin; fried as in bean cake (akara) or made into bean soup "gbegiri" (Aykrod and Doughty, 1982; Williams, 1984). In many areas of West and East Africa, the tender leaves are cooked like spinach or as a relish. Generally the grower also likes to obtain some dry-seed yields from the plucked crop. Green beans or cut green pods used as a vegetable are of secondary importance. Cowpeas are also grown for fodder, ground cover or green manure but to a much lesser extent than for pulse.

In Asia, the pulse uses of cowpeas are important primarily in the drier regions such as India where increasing amount of the crop are used as "dhal" (split decorticated grains) (Aykroyd and Doughty, 1982). In the more humid areas of south east Asia and southern China, the long bean is the predominant form and is used as cut green beans.

In south America, cowpeas are grown mainly as pulse with a wide range of seed colours. Sometimes, they are used as forage, hay and cover; and the seeds may replace soybean or other protein source used in animal feed.

In USA, dry seeds are grown mainly in California and Western Texas, they are used as green peas for canning of fresh peas (Rachie, 1985).

2.2 Chemical Composition

The chemical composition of cowpea is similar to that of most edible legumes. It ranges from 19.97 - 25.4g/100g for protein, 0.87-3.5 g/100g for fat, 60.8 - 66.4 g/100g for carbohydrates, 5.0 - 6.9 g/100g for crude fibre. The ash content ranges from 3.1 - 3.9 g/100g and the moisture 8.31 - 10.64 g/100g. The vitamin contents are 0.41 - 0.99 mg/100g thiamine, 0.29 - 0.76 mg/100g riboflavin, 2.15 - 3.23 mg/100g niacin . (Elias *et. al.*, 1964; Ritchey *et. al.*, 1976; IAR Report, 1994).

Thus most of its nutritional value is provided by proteins and carbohydrates (Bressani, 1985). The seed protein can be separated into 4 different types - albumin, globulins, prolamins and glutelins. The predominant and most characteristic type of protein found in legumes (peas and beans) is the globulins which constitute about 80 g/100g of the total proteins. The globulin type can be further fractionated into legumin and vicilin (Milner, 1974). Prolamins and glutelins constitute the bulk of the proteins of most cereals. Since prolamins contain only small amount of lysine, the poor quality of many cereals is often attributed to their prolamin content (Kakade, 1974).

The lysine content of cowpea is relatively high, making it an excellent improver of the protein quality of cereal

grains (Bressani *et. al.*, 1974). However, cowpea proteins as in the case of other legumes is deficient in sulphur - containing amino acids particularly methionine (Onayemi and Potter, 1976) Variability in protein content from 19.79 - 25.4 g/100g is influenced by genotype as well as by environmental factors (Bliss, 1975).

The total carbohydrate content in seeds of legumes (cowpea) varies from 50.66 to 68 g/100g, with starch contributing as much as 32 - 48 g/100g. Total soluble sugars content is from 13.75 to 19.75 g/100g, amylose content range from 20.88 - 48.72 g/100g while amylopectin varies from 11.42 to 36.58 g/100g. (Srinivasa, 1976; Ritchey *et. al.*, 1976; Arora and Das, 1976; Reddy *et. al.*, 1984). Sucrose content range from 0.36 - 1.4 g/100g, raffinose content range from 1.26 - 4.12 g/100g while stachyose content is from 1.21 - 4.98 g/100g (Onigbinde and Akinyele, 1983). Verbascose content is about 0.9 g/100g average, while xylose is 1.67 mg/g, fructose about 0.72 mg/g average. Galactose, glucose and maltose are in trace amounts (Akpapunam and Markakis, 1979; Phillip and Abbey, 1989).

2.3 Potential Health Benefits

Beans (cowpeas) have long been recognized as a valuable and cheap source of dietary fibre. The total dietary fibre of

selected legumes ranges from 10.12 - 34 g/100g (Anderson and Bridges, 1988). The potential health benefits have been attributed to this high content of dietary fibre when compared to other food sources (Trowell, 1973; Hellendoon, 1979; Hughes 1991).

Dietary fibre consist of the portion of plant cells that cannot be digested by enzymes in the human digestive tract. It is not one entity but a complex mixture of substances that despite being chemically different share the common physiological characteristic of resisting enzyme digestion. Most of the substances found in dietary fibre are carbohydrates (cellulose, hemicellulose and pectin) with the phenolic compound lignin being the only non-carbohydrate currently defined as a part of dietary fibre (Trowell *et. al.*, 1976). Dietary fibre can be classified as soluble and insoluble dietary fibres. Lignin, cellulose and some hemicellulose typically constitute the insoluble dietary portion, while pectin, some hemicellulose and other polysacchrides make up the soluble dietary fibre (Oslon *et. al.*, 1987).

Beans along with other legumes, oats and barley contain a balance of both soluble and insoluble dietary fibre, containing approximately 7g/100g soluble dietary fibre and 13g/100g insoluble dietary fibre (Hughes and Swanson, 1989).

The health benefits associated with the consumption of beans results from a combination of physiological effects (Schneeman, 1986; Hughes, 1991) These health benefits include,

(i) Hypocholesterolemic effect and the reduction of coronary heart diseases (Trowell, 1973; Anderson and Chen, 1983; Anderson *et. al.*, 1984 Cummings, 1984; Chen and Anderson, 1986; Fleming and Floch, 1986).

(ii) Hypoglycemic effect, improved glucose tolerance and management of diabetes (Thompson *et. al.*, 1984; Jenkins *et. al.*, 1984; Jenkins *et. al.*, 1986; Tappy *et. al.*, 1986; Anderson and Bryant, 1986; Wolever, 1990).

(iii) Reduction in the risk of colonic cancer (Hellendoon, 1979, Jenkins, *et. al.*, 1986; Schneeman, 1987).

2.4 Constraints Limiting Cowpea Utilization

2.4.1 Protein quality

One of the primary constraints militating against cowpea is the protein quality. Cowpea protein as in the case of other legume proteins is deficient in sulphur containing amino acids with a level of 1.2 g/100g and 1.8 g/100g protein for methionine and cysteine respectively. The primary deficiency of methionine and cysteine in many instances is further complicated by a secondary deficiency of tryptophan (Evans and Bandemer, 1967)

This makes cowpea protein inferior when compared to protein from animal source. However the protein quality can be improved by supplementation with methionine (Onayemi and Potter, 1976). Cowpea is very rich in lysine and the amino acid profiles compliment those of the cereal proteins to give a high protein quality (Bressani *et. al.*, 1974).

2.4.2 Physical constraints

The processing of cowpea for preparation of bean products like moin-moin, bean cake (akara), bean soup (gbegiri) poses some problems. The seed coat has to be removed before it can be ground into paste. This means steeping the cowpea seed in water to loosen the seed coat or devising other means for removing the coat. Observation have shown that removing cowpea seed coat can be tedious and time consuming especially for those who use cowpea to prepare dishes on a large scale. For instance, it takes as long as 90 mins soaking time to remove the seed coat of Sampea 6 (1696) (IAR Report, 1994). Therefore the ease with which the coat can be removed is an important factor.

Cowpeas require some cooking before they are eaten. This thermal process provides tenderization of the cotyledon which increases product palatability, digestibility and inactivates endogenous toxic factors that would markedly limit the final nutritional value (Sefa-Dede *et. al.*, 1978; Uebersax and

Ruengsakulrach, 1989). Investigation showed that cooking time which is long, varies for different varieties. While sampea 7 and sampea 4 cook under one hour, sampea 6 takes over one hour to cook. The cooking time is particularly important in these days of shortage and high cost of cooking fuel (IAR, report, 1994).

Another physical constraint associated with cowpea consumption is the storage induced hard-to-cook defect. This term indicates a resistance of seeds to softening during cooking. This phenomenon is normally associated with certain legumes stored under high temperature and high humidity. The hard-to-cook seeds require longer cooking time than soft seeds and decreases the nutritional quality (Tuan and Phillips, 1991).

2.4.3 Biological constraints

Like other legumes, cowpeas can synthesize a variety of undesirable chemical substances termed antinutrients that are known to exert deleterious effect when ingested by man and animals. These include:

(a) Trypsin Inhibitors

These are naturally occurring proteinase inhibitors capable of combining with trypsin and other serine proteinase to form an inactive complex. The trypsin inhibitor - trypsin

complex are almost certainly held by secondary rather than primary bonds (Laskowski and Laskowski, 1954). According to Richardson, (1977) they are proteins which have the peculiar property of forming reversible stoichiometric protein-protein complex with various proteolytic enzymes thus bringing about competitive inhibition of their catalytic functions.

These plant proteinase inhibitors are generally small proteins having molecular weight under 50,000 and more commonly under 20,000 (Vogel, *et. al.*, 1966). A number of inhibitors from several legumes have minimum molecular weights of below 10,000 and are often present as dimers or tetramers (Kakade and Simons, 1970). Nearly all plant inhibitors inhibit enzymes of animal or microbial origins having trypsin - like or chymotrypsin - like specificities.

Trypsin inhibitor is an important serine proteinase inhibitor of the digestive tract of animals. It soon became apparent however that many of the so-called trypsin inhibitors were also inhibitory to the related enzyme chymotrypsin (Vogel *et. al.*, 1966). In some cases the reactive site of inhibitor was the same for both enzymes but several of the other inhibitors were demonstrated to be "double-headed" or polyvalent, that is, containing different reactive site for the independent inhibition of the two proteolytic enzymes. Sometimes the trypsin and chymotrypsin inhibitors are strictly

specific for these two enzyme but other examples have subsequently been shown to inhibit a range of other serine proteinase (Elastase, thrombin, Plasmin and Kallikrein) (Bidlingmayer *et. al.* 1972; Richardson, 1977).

Recently a proteinase inhibitor of trypsin and chymotrypsin was isolated from potato tuber (*Solanum tuberosum* L.). The molecule consist of one polypeptide chain with molecular weight of 17 kilo Dalton. The inhibitor interact with trypsin or chymotrypsin in 1:1 molar ratio and the enzyme-inhibitor complex undergoes substrate - dependent dissociation. The inhibitor does not suppress the activity of subtilisin and pancreatic elastase. The formation of "triple" complex containing one trypsin molecule and one chymotrypsin molecule indicates that the inhibitor has two independent active centres for these enzymes (Revina *et. al.*, 1996). The physiological functions of trypsin inhibitors include:

(i) As regulatory agents in controlling endogenous proteinase (Vogel *et. al.*, 1966; Liener and Kakade, 1969; Ryan 1973).

(ii) As storage proteins (Ryan, 1973; Vogel *et. al.*, 1966; Liener and Kakade, 1969).

(iii) Plant protection (Vogel *et. al.*, 1966; Kafatos *et. al.*, 1969; Matsubara and Feber 1971; Fritz and Tschesche, 1971; Green and Ryan, 1972).

(iv) Endozoic dispersal of seeds (Marshall and Lauda, 1975)

(v) Therapeutic application (Richardson, 1977; Silverstone, 1985).

(vi) Colostrum Protection (Laskowski and Laskowski, 1954).

The exact nutritional significance of trypsin inhibitor is difficult to assess (Pusztai, 1967). If the diet of rats and chicks contain high levels of these compounds, the protein in the diet is poorly utilized and tissue growth and repairs are impaired. This is due to either the limited availability of amino acid methionine or to the inactivation of proteases (Rachis and Gumbman, 1981). The inactivation of proteases resulted in excessive enzyme secretion and pancreatic hypertrophy (Bender, 1987). Metabolic disturbance in cysteine and methionine utilization was also suggested (Pusztai, 1967).

The nutritional value of many leguminous seeds has been reported to improve during germination (Collins and Sanders, 1976) and in most cases following controlled heat treatment (Liener and Kakade, 1969; Ogun *et. al.*, 1989). Both processes inactivate and reduce the levels of these inhibitors.

The level of trypsin inhibitor in cowpea is intermediate with an activity ranging from 16.5 - 35.3 Tiu/mg sample (Elkowicz and Sosulski, 1982; Nnanna and Phillips, 1990). Ogun *et. al.*, (1989) observed a reduction from an average of

27.6 to 10.7 Tiu/mg sample when 4 cultivars of cowpea were soaked in hot water.

(b) **Phytohaemagglutinins**

Phytohaemagglutinins are proteins or glycoproteins which can bind to specific carbohydrate residue in cell membrane and capable of agglutinating red blood cells (Lis and Sharon, 1973; Goldstein and Hayes, 1978). The fact that these protein fractions were capable of agglutinating red cells gave them the name haemagglutinins. It was also established that relative haemgglutination activities of various seed extracts were quite different when tested with erythrocytes from different animals. This specificity toward specific types of blood cells led to coining the word lectin (Latin, *legere*-to choose).

The action of lectins is due to the binding to intestinal mucosal cells causing malfunction, disruption and lesion in the small intestine. These actions interfere with the absorption of nutrient from the guts (Liener, 1974; Jaffe, 1980). *In vitro* experiment with intestinal loops taken from rats fed this lectins revealed 50% decrease in the rate of absorption of glucose across the intestinal walls compared to controls (Liener, 1974). Ricin, the lectin of castorbean is extremely toxic, about 100 times more toxic than most of the other bean lectins (Jaffe, 1969). Concanavalin A, the lectin

from Jack bean when injected into animals caused the agglutination of red blood cells, followed by hemolysis and finally death (Edmundson *et. al.*, 1971). However, the level in cowpea was observed to be low (Elkowicz and Sosulki, 1982).

Since the toxicity of these compounds is dramatically reduced by cooking with moist heat, their presence in human diets is of little cause for concern. Toxicity is also reduced because many toxic compounds are destroyed or neutralized in a normal digestive tract and most are poorly absorbed. Poor absorption means that lectins reach the colon in a biological intact form and thus can have a beneficial effect. They appear to protect the human body against colon cancer either by causing hypersecretion of intestinal mucus or by exerting a direct toxic effect on tumor cells (Liener, 1980).

(c) **Tannins**

The term tannins referred originally to substances with the ability to tan leather (Haslam, 1979). It is now generally used to include any naturally occurring soluble compound of high molecular weight (500 - 3,000) and containing a large number of phenolic hydroxyl groups (one to two hundred molecular weight), to enable it to form an effective cross-link with proteins and other molecules (Bate-Smith and Swain, 1962; Kumar and Singh, 1984).

The hydrolysable and condensed tannins are two groups of the compound widely distributed in the plant kingdom which are differentiated by their structure and reactivity toward hydrolytic agents (Haslam, 1966). However, the principal forage tannins are usually of a condensed type (Mcleod, 1974). The presence of condensed tannins have been demonstrated in legumes and pasture species (Burns *et. al.*, 1967). In most legumes and cereals, the seed coat or outer portions contain fairly high amount of tannins (Rao and Prabhavathi, 1982).

Tannins react with protein to form protein-tannin complexes. (Haslam, 1981). Proteins which strongly associate with tannins are rich in the amino acid proline, relatively large and hydrophobic, posses conformationally open and flexible structures (Geneviena *et. al.*, 1994).

Polyphenols (tannins) have a harsh astringent taste and produce in the pallate a feeling of roughness, dryness and constriction. The primary reaction whereby astringency develops is via precipitation of proteins and mucopolysaccharides in the mucous secretion (Goldstern and Swain, 1963; Geneviena *et. al.*, 1994). The deleterious effect of dietary tannins on the feeding of the insects, larvae and animals on tannin rich plants is quantitatively defensive. Tannins repel predators by virtue of their strong astringent taste and because of their anti-nutritional characteristics

once ingested. Their role is elegantly summarized as plant chemical defence (Bate-Smith, 1973). Unpalatability due to astringent tannins lead to reduction in the voluntary food intake in ruminants (Mcleod, 1974). Tannins also diminish the permeability of the gut wall by reacting with the outer cellular layer of the gut, so reducing absorption. The reduced digestibility of tannin rich feeds can be explained on the basis of inhibiting digestive enzymes (Bressani and Elias, 1979; Griffith and Mosley, 1980)

In the body, they bind proteins, precipitate the proteins of the epithelium, penetrate through the superficial cell and cause liver damage (Mehansho *et. al.*, 1987). A 3-5% solution of tannin is toxic orally and retards growth. Tannin inhibits virtually every digestive enzyme and reduces the bioavailability of iron and vitamin B_{12} (Liener, 1980; Butler, 1989). Tannins are both mutagenic and carcinogenic (Stolz, 1982).

Cowpeas are fairly high in tannins (Laurena *et. al.*, 1987) Ogun *et. al.*, (1989) reported a range of 0.08-0.15 g/100g dry weight for four cultivars of cowpeas. Polyphenols (condensed tannins) are heat stable and are located mainly in the seed coat of cowpeas and other legumes. The level of tannins in cowpeas can be significantly reduced by decortication (Desphande *et. al.*, 1982) and a complete

elimination was observed when the seed coat of cowpeas was removed (Ogun *et. al.*, 1989).

(d) **Phytates**

Phytates represent a complex class of naturally occurring compounds that can significantly influence the functional and nutritional properties of food (Maga, 1982). The compound phytic acid can be commonly called myo-inositol hexaphosphoric acid or scientifically 1, 2, 3, 4, 5, 6 - hexakis (dihydrogen phosphate) myo-inositol (IUPAC-IUB, 1968). The term phytin implies a calcium - magnesium salt of phytic acid. The phytate ion complexes with di-and trivalent metallic ions (Zn^{++}, Ca^{++}, Mg^{++}, Fe^{++} and Fe^{+++}) and is the principal storage form of phosphorus in many kinds of seeds.

The ability of phytic acid to complex with metals is one of the main nutritional concerns associated with phytates. Phytic acid and their derivatives can bind essential dietary minerals, thus making them insoluble and biologically unavailable or only partially available for absorption. This problem becomes especially important when two or more cations are present, in that a synergistic binding effect can occur. This is particularly true for zinc and calcium and also zinc and copper. The greatest impact of phytates relative to human nutrition is its reduction in zinc bioavailability.

The physiological roles of phytates in seeds have been suggested to include, a phosphorus store (Hall and Hodges, 1966), an energy store (Biswas and Biswas, 1965) and an activator of dormancy. (Sobolev and Rodionova, 1966). Phytate serve as source of phosphate, cations and myo-inositol during germination due to the action of phytase (Mayer, 1956; Darbre and Noris, 1957; Williams, 1970). The dry matured beans and cowpeas contain approximately 1 g/100g dry weight (Makower, 1969; Ogun *et. al.*, 1989) and more than 90% of the phytic acid in beans is soluble in water. Proper processing or preliminary soaking, particularly when the soaking water is changed can eliminate the action of phytates considerably (Ogun *et. al.*, 1989).

2.5 The Flatulence Factor - Their Presence in Legumes

Flatulence is one of the constraints limiting the wider acceptability and utilization of legume seeds in humans. This phenomenon has been attributed mainly to the presence in legumes of alpha-galacto-oligosaccharides of the raffinose family (French, 1954; Gross, 1962). The alpha-galactoligosaccharides of the raffinose family, constitute of sugars related to raffinose by the fact of having one or more alpha-D-galactopyranosyl group in their structures. These D-galactosyl groups are found in nature joined to sugars such as D-glucose, to sucrose, to certain polysaccharides and to a few non-sugars such as glycerol and inositol (French, 1954).

These members include mainly raffinose, stachyose, verbascose and ajugose. The structural relationship shows that the group increase from lower to the higher series by the successive addition of one alpha-D-galactopyranosyl group to sucrose (Fig.2.1).

According to Hardinge *et. al.*, (1965) soybean has the highest levels of raffinose (1.9g/100g) and stachyose (5.2g/100g) while chickpea (*Cicer arietinum*) and Pigeon peas (*Cajanus cajan*) have the highest levels of verbascose (4.2g/100g) for 10 investigated legume seeds. About 0.48 g/100g of higher oligosaccharide which was confirmed to be ajugose is contained in some Nambyquarea variety of groundnuts (*Arachis hypogea*) (Tharanathan *et. al.*, 1976). The presence of ajugose has also been reported in horsebeans (*Vicia faba*), smooth peas (*Pisum sativum*), wrinkled peas (*Pisum sativum*) and lupines (*Lupinus* species) (Cerning-Beroard and Filiatre, 1976).

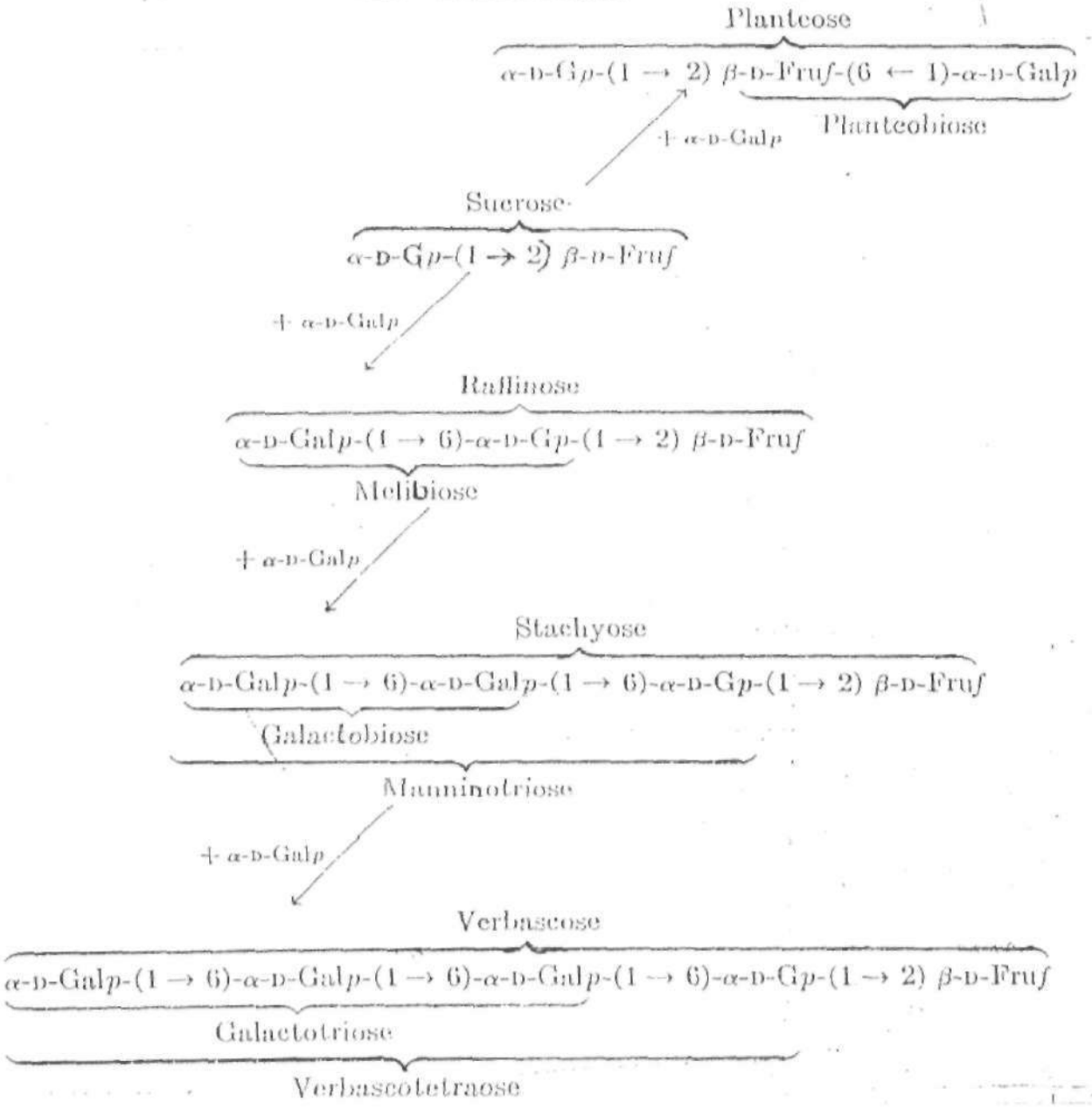


Fig. 2.1: Structural relationship of the rafinose oligosaccharides

Source - French, 1954

In their own investigation, Onigbinde and Akinyele, (1983) reported the levels of raffinose to range from 1.26 - 4.19 g/100g, and for stachyose to range from 1.21 - 4.98 g/100g for 20 varieties of cowpeas used for their study. Phillip and Abbey, (1989) reported an average value of 3.76 mg/g for verbascose in cowpeas.

2.6 Enzymology of Raffinose Oligosaccharides

The alpha galactopyranosidases (α - D - galactoside galacto hydrolases E.C. 3.2.1.22) (Suzuki *et. al.*, 1970) are a group of exotype carbohydrases which release α - D - galactose from melibiose, raffinose, stachyose, verbascose, *O*- and *P*- nitrophenyl α -D- galactopyranoside, methyl - α - D - galactopyranoside, galactinol, 4, *O* - α - D-galactopyranosyl - D - galactose, 6 - *O* - α -D-galactopyranosyl - *O*-β-D-galactopyranosyl -1- glycerol and glycoproteins. (Suzuki *et. al.*, 1970; Mulimani and Ramalingam, 1995).

Alpha-galactopyronosidases are found in yeast, bacteria, molds and higher plants. It has been extracted from *Mortierella vinacea* (Thananunkul *et. al.*, 1976), *Aspergillus saitoi* (Sugimoto and Van Buren, 1970) *Aspergillus oryzae* (Khare *et. al.*, 1994), *Gibberrelia fugikuroi* (Mulimani and Ramalingam, 1995).

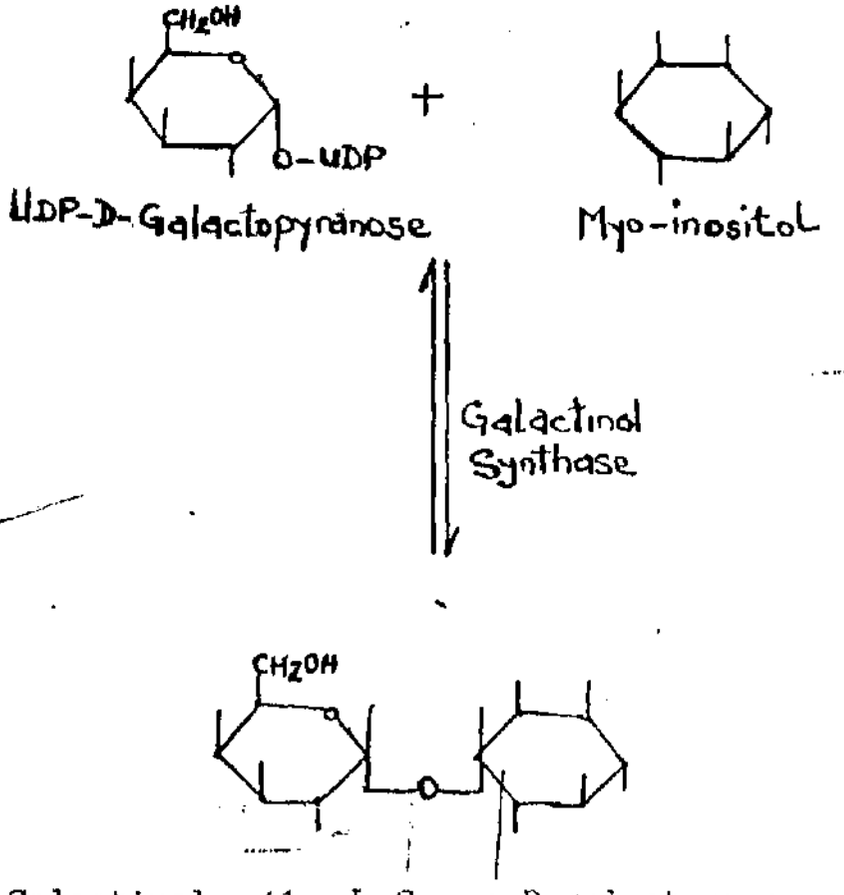
The crystalline preparation contains 10.8% neutral sugars and 2.7% D-glucosamine, indicating that it is a glycoprotein

in nature (Suzuki *et. al.*, 1970). The enzyme from *Mortierella vinacea* has the following properties - stable at neutral and alkaline pH, heat stable up to 55°C, Ag^{+} and Hg^{2+} were the most potent inhibitors, D-glucose and D-galactose are relatively potent inhibitors. The enzyme hydrolyses various glycosides and oligosaccharides containing α-galactosidic linkage at their non-reducing ends and exhibits some glycosyl transfer activities. The enzyme only cleaves α- (1⟶6)- and α- (1⟶4) linked D-galactosides. (Suzuki *et. al.*, 1970). Similar properties were also observed in the enzyme from other sources (Sugimoto and Van Buren, 1970; Thananunkul *et. al.*, 1976).

Hydrolysis by yeast invertase (E.C. 3.2.1.26) has been found to yield D-fructose and the complimentary aldose-D-glucose, melibiose, manninotriose and verbascotetraose. Planteose is an exception to the general behaviour of the raffinose oligosaccharides because it lacks an unsubstituted β-D-fructo-furanoside group (French, 1954). Invertases from the animal intestinal tract generally act as D-glucosidases rather than as D-fructosidases. Hence the Oligosaccharide containing a sucrose unit which the D-glucose is substituted by D-galactose resist the action of animal invertases. Hence human invertase does not hydrolyse the galactosides of raffinose family (French, 1954).

2.7 Synthesis of Raffinose Family of Oligosaccharides

Evidences from *in vivo* experiments have shown that sugars of the raffinose family are synthesized by galactosyl transfer from galactinol, (Sensor and Kandler, 1967). Galactinol (IL-*O*- α-D-galactopyranosy-(1⟶1)-D-myo-inositol) is synthesized from UDP-D-galactose and myo-inositol (Frydman and Neufeld, 1963).



Galactinol (1 L-*O*- α-D-galactopyranosyl-(1⟶1-D-myo - inositol)

Galactinol participate in a series of galactosyl transfer reaction, successively donating single galactosyl residue to sucrose, raffinose and higher homologue of raffinose family. At each step, free myo-inositol is produced as one of the products. The reaction is reversible and is catalysed by galactinol: galactosyl transferase (Loewus, 1971).

Transfer reaction

Sucrose + Galactinol ⟷ Raffinose + myo-inositol

Raffinose + Galactinol ⟷ Stachyose + myo-inositol

Stachyose + Galactinol ⟷ Verbascose + myo-inositol

Verbascose + Galactinol ⟶ Ajugose + myo-inositol

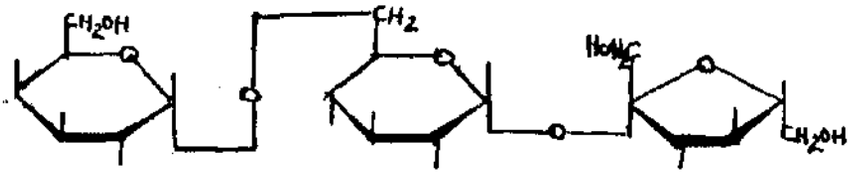
Galactinol (1 L-*O* - α-D-galactopyranosyl - (1⟶1) -D-myo-inositol) is the galactosyl donor for the sugars of raffinose family and myo-inositol is a co-factor (Sensor and Kandler, 1967; Tanner and Kandler, 1968). The galactinol is regenerated by transfer of a galactosyl residue from UDP-Galactose to free myo-inositol (Frydman and Neufeld, 1963). Myo-inositol represses galactinol hydrolysis by the transferase which has a little hydrolytic activity toward galactinol.

The biosynthesis of raffinose, the simplest member of the family can arise by galactosyl transfer from galactinol to sucrose (Pridham and Hassid, 1965). In the synthesis of stachyose in *Phaseolus vulgaris* seeds, the enzyme galactinol: raffinose - 6 - galactosyl transferase transferred galactose

from galactinol to raffinose (Tanner and Kandler, 1966). The biosynthesis of verbascose has also been shown to proceed via galactinol in extracts from *Vicia faba* seeds. Hence the synthesis of raffinose family of oligosaccharides proceed through galactosyl transfer from galactinol (Sensor and Kandler, 1967).

2.8 Occurrence and Properties of the Oligosaccharides

Raffinose: [*O*- α-D-Galactopyranosyl - (1⟶6)-*O*- α-D-glucopyranosyl - (1⟶2)-β-D-fructofuranoside]



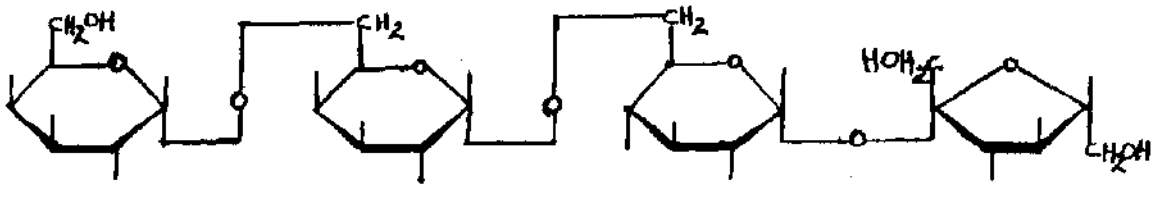
$C_{18}H_{32}O_{16}$ (M.W. 504)

Raffinose is the most abundant oligosaccharide of the plant world and occurs in sugar beat, cotton seed, manna and legume seeds.

It occurs as white needles or prisms. It crystallizes from water, from aqueous alcohols or acetic acid as the pentahydrate ($+5H_2O$) of melting point 78°C. The anhydrous form has a melting point of 118°-119°C. It has a specific

rotation [α]D^{20} of + 104° in water (hydrate) and + 123.3° in water (anhydrous). It has a slightly sweet taste, soluble in water, slightly soluble in methanol. It is insoluble in ethanol, diethylether. It does not reduce fehling solution, hence a non-reducing sugar. It is hydrolysed by dilute acids to give galactose, glucose and fructose; hydrolysed by emulsin to give sucrose and galactose and by invertase from yeast to give melibiose and fructose (French, 1954; Gross, 1962).

Stachyose: (*O*-α-D-Galactopyranosyl - (1⟶6) -*O* - α - D - galactopyranosyl - (1⟶6) -*O*- α-D-glucopyranosyl-(1⟶2)-β-D-frucofuranoside)

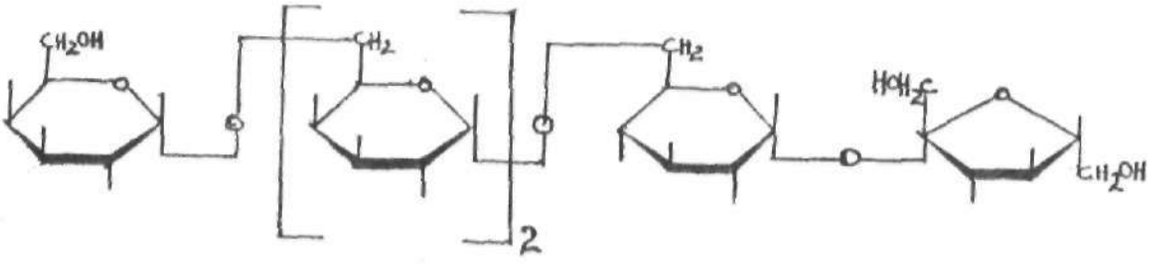


C_{24} H_{42} O_{21} (M.W. 666).

This is the first and best known sugar of this class, the classical tetrasaccharide. The best source is the juice from the rhizomes of *Stachys tuberifera* from which it was first extracted. It also occurs in ash manna and seeds of leguminosae. The plant *Stachys tuberifera*, has been used in the orient as vegetable (Japanese: Chorogi) and for a time it was widely grown in France and marketed as Japanese artichokes which contains 14g/100g of stachyose.

It occurs in the form of plates and a tetrahydrate (+ $4H_2O$). It loses water at 115°C in vacuum. The melting point of the anhydrous form is 167 - 170°C. It has a specific rotation at 15°C measured at the D line of Na of + 133.9° in water and + 148.4° (anhydrous in water). It is soluble in water, and insoluble in ethanol and diethyl ether. It has a sweet taste and does not reduce fehling solution, hence it is a non-reducing sugar. It is hydrolysed by dilute acid to give glucose (1 mole), galactose (2 moles) and fructose (1 mole) (French, 1954; Gross, 1962).

Verbascose [*O*- α-D-Galactopyranosyl-(1⟶6)-*O*- α-D-galactopyranosyl - (1⟶6) - *O*- α-D-galactopyranosyl-(1⟶6)-*O*- α-D-glucopynanosyl - (1⟶2) - β-D-fructofuranoside)



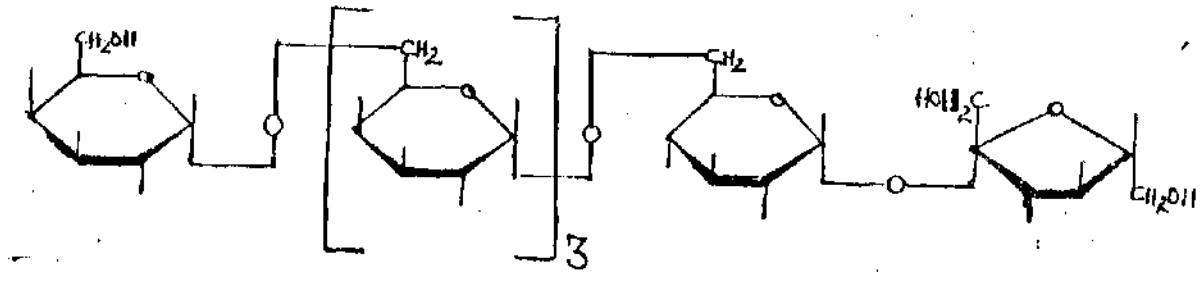
$C_{30}H_{52}O_{26}$ (M.W. 910).

It occurs in roots of mullein (*Verbascum thapsus*), cocoa beans and legumes. It occurs as needles and crystallizes as a pentahydrate (+$5H_2O$). It has the following physical properties - the melting point is 188° - 189°C; specific

rotation is + 148°C; It is soluble in water, insoluble in ethanol and diethylether; It has a sweet taste; Does not reduce fehling solution and hence a non-reducing sugar. When completely hydrolysed it yields glucose (1 mole), fructose (1 mole), galactose (3 moles) (French, 1956; Gross, 1962).

Ajugose: (*O*- α-D-Galactopyranosyl - (1⟶6)-*O*- α-D-galacto pyranosyl-(1⟶6)-*O*- α-D-galactopyranosyl - (1⟶6) - *O* - α - D- galactopyranosyl - (1⟶6) - *O*-α-D-glucopyranosyl - (1⟶2) - β- D - fructofuranoside).

It occurs in the root of *Ajuga nopponensis* Makino, *Verbascum thapsoforme* Schrad and some legume seeds like horse bean (*Vicia faba*), peas (*Pisium sativum*), Lupine (*Lupinus* spp) and some Nambyquarea varieties of groundnut (*Arachis hypogea*) (Cerning - Beroard and Filiatre, 1976; Tharanathan *et. al.*, 1976).



$C_{36}H_{62}O_{31}$ (Mol. Wt. = 990)

It forms a microcrystal in water-ethanol mixture. It has a melting point of 270°C and a specific rotation of + 190.8 in water. It is soluble in water, sparingly soluble in ethanol.

It tastes slightly sweet and does not reduce fehling solution, hence a non-reducing sugar. When completely hydrolysed it yields glucose (1 mole), fructose (1 mole) and galactose (4 moles) (French, 1956, Gross, 1962).

2.9 Evolution in the Leguminous Seeds

A study on the conversion processes in ripening seeds of *Phaseolus vulgaris* reveals that the synthesis of these sugars takes place in the seed (Loewus, 1971). During ripening, pools of myo-inositol and sucrose which accumulate during early stage of seed development disappear as galactinol is formed. Later galactinol decreases as galactinol: raffinose - 6 - galactosyl transferase activity rises and stachyose accumulates. Commercially frozen green peas when analysed contained no trace of oligosaccharide (Cristafaro *et. al.*, 1974), and the level of oligosaccharides of the raffinose family increase during maturity of the seeds while sucrose decreased (Shallenberger and Moyer, 1961; Keshun and Markakis; 1987) Tanusi, (1972) remarked that in the cotyledon, the oligasaccharides are gradually detected during growth in Soybeans in the sequences Sucrose —— raffinose —— stachyose —— Verbascose.

During germination in *Phaseolus vulgaris*, the same transferase which is already present in the seeds maintains its activity for about 48hrs, then slowly disappears but not before nearly all the stachyose has vanished. Raffinose and

sucrose levels increase in the first 24 hrs but raffinose decreased rapidly over the next 24 hrs while sucrose level fall slightly to a constant level (Loewus, 1971). Galactinol, which is already low in the seed, disappears during germination at precisely the same time as sharp drop in stachyose occurs. If the galactose produced as a result of breakdown of oligosaccharide were transferred back to myo-inositol, one might expect a rise in galactinol at this point.

A possible role of an α - galactosidase was suggested in this connection (Shadaksharaswamy and Ramachandra, 1968). During germination, the seeds undergo marked metabolic changes and the reserve carbohydrates including the oligosaccharides of the raffinose family are hydrolysed during this stage (Adjei - Twen *et. al.*, 1976). The disappearance of verbascose and stachyose during germination of black gram (*Phaseolus mango*) during the first 48 hrs was caused by enzymatic degradation as confirmed by increased alpha-galactosidase activity in germination seeds. (Reddy and Salunkhe, 1980).

Puzur *et. al.*, (1962) detected the presence of alpha-galactosidase among enzymes of germinating soybeans and showed that the galactose produced during enzymatic hydrolysis was utilized. Trugo *et. al.*, (1990) also reported a rapid decrease of oligosaccharides during the germination of different varieties of *Phaseolus vulgaris*, 64% for raffinose, 79% for stachyose after the 3rd day. This reduction of alpha-

galactosides was followed by an increase of about 40% of sucrose after the 3rd day of germination of winged beans (*Psophacarpus* spp) and this was a consequence of the seed metabolism which needs available sugars as energy source (King and Puwastien, 1987).

There is a superficial similarity between ripening (maturity) and germination as it relates to the oligosaccharide accumulation and utilization, but the two processes do not follow similar pathway of interconversion.

2.10 The Raffinose Family of Sugars in the Alimentary Canal

Disaccharide, lactose is metabolised by normal human subject since its β-glycosidic linkage is split by lactase (β-glucosidase E.C. 3.2.21). Sucrose is also metabolised since its α (1⟶2) linkage is hydrolysed by invertase (E.C.3.2.1.26). Similarly, the metabolism of the members of the raffinose family of sugars containing alpha-galactose units with unusual α (1⟶6) glycosidic bonds would require the presence of an alpha-galactosidase (α-D-galactoside galactohydrolase. E.C. 3.2.1.22) (Suzuki *et. al.*, 1970) which has the ability to split off the galactose unit of the alpha - galactosides. (Khare *et. al.*, 1994; Mulimani and Ramalingam, 1995).

However the monogastric animals do not possess this enzyme (alpha-galactosidase) in their gastro intestinal tract (Semenza and Auricchio, 1962). In particular, Gitzelmann and Auricchio, (1965) in the case of a galactosemic child, did not find any alpha-galactosidase activities in the homogenates of human small intestinal mucosa both for normal and galactosemic children. The invertase from the animal intestinal tract cannot hydrolyse the alpha - galactosides because the oligosaccharides contain a sucrose unit in which the D-glucose is substituted by D-galactosyl unit. Hence man is unable to utilize the galacto-sucrose series of sugars. The disaccharidases and oligosaccharidases of the human intestinal flora and mucosa has been extensively investigated and it was discovered that the colonic intestinal flora contain *Clostridium perfringens*, which have the ability to produce oligosaccharidase (Bornside and Cohn, 1965; Rachis, 1975).

Gitzelmann and Auricchio, (1965) showed that in a healthy child, oral raffinose and melibiose loads failed to produce changes in blood glucose, blood galactose or red cell galactose - phosphate levels. Only trace amount of either saccharides were excreted in the urine. Raffinose, stachyose and verbascose are able to pass intact only in small amount, less than 1% of the dose administered through the intestinal wall (Taeufel *et. al.*, 1965). The passage is less for oligosaccharides of increasing molecular weight i.e. decreased absorption with increased molecular weight.

2.11 Utilization by Intestinal Bacteria - Formation of Flatus

In view of the fact that only very small amount of the oligosaccharides of the raffinose family are absorbed into the blood stream and excreted in the urine, these sugars during digestion remain within the lumen and get into the distal part - the large intestine and colon. Here they are anaerobically metabolized by the normal microflora. Following this observation, a hypothesis regarding the responsibility of intestinal micro-flora in the utilization of oligosaccharide was made.

It was observed that during a study of *in vitro* ileal and colonic dejecta from humans, an organism from both intestinal segments was able to utilize stachyose with increased production of gas (Calloway *et. al.*, 1966). This possibility was supported by the observation by Steggerda *et. al.*, (1966) that when various fractions of soybean meals were consumed by human subjects, the low molecular weight carbohydrate fraction was especially potent in their gas producing ability as compared with the fat, protein and complex carbohydrate fractions. Rachis *et. al.*, (1970ab) also observed that gas producing factor in soybeans resides principally in the low-molecular weight carbohydrate fraction. Murphy *et. al.*, (1972) also demonstrated that the 80% ethanol fraction has a high activity in the production of CO_2 in human flatus. They also showed that this fraction contained mainly low molecular weight oligosaccharides - raffinose and

stachyose. The observation that an organism from the intestinal segments was able to utilize stachyose confirmed the suggestion that the intestinal flora may contain an alpha - galactosidase that can cleave galactose from the sucrose moiety.

In 1968, Steggerda, found that the type of flora specifically responsible for flatus with its high concentration of CO_2 and H_2 in man and animals following the ingestion of bean product was the gram-positive, spore forming, anaerobic, motile bacterial type known as *Clostridia* group. Richard *et. al.*, (1968) in their *in vivo* experiments with the different parts of the gastro intestinal tract of a dog observed that the organisms responsible are the same as or closely related to the gram positive *Clostridium perfringens* type. Pure cultures of *Clostridium perfringens* exposed to bean homogenates produced gas similar in volume and composition to that observed in the different segments of the gastrointestinal tracts of the anesthetized dog in their preliminary experiment. The *in vitro* and *in vivo* responses to bacterial inhibiting agents were similar, thus the relationship of the *in vitro* experiment to conditions existing in the intact intestine and colon was established. (Richard and Steggerda, 1966). It was also demonstrated that the growth of *Clostridia* was stimulated by a substrate contained in bean (Richard *et. al.*, 1968) and that H_2 and CO_2 were the major constituents in flatus gas (Rockland *et. al.*, 1969).

In, 1970, Kurtzman and Halbrook isolated a carbohydrate-rich fraction from beans and found that a certain portion of the flatulent beans must not be absorbed by the guts and must be metabolized by *Clostridium perfringens*. Rachis *et. al.*, (1967, 1970a) confirmed that *Clostridia* can use oligosaccharides of the raffinose family. These sugars added to cultures isolated from dog colons can develop important amounts of gas with percentage of CO_2 and H_2 as high as those given by monosaccharides, glucose in particular. They deduced from their experiment that the gas producing factor is closely associated with oligosaccharides of leguminous seeds. They also suggested that a breakdown of the oligosaccharide to monosacchandes occurs before the gas producing mechanism takes place. It was thus understandable that a longer delay must occur before the onset of gas production with such oligosaccharides as raffinose and stachyose.

The production of flatus by monogastric animals is due to colonic fermentation of carbohydrates which escape breakdown in the stomach and small intestine. The oligosaccharides which are common in legumes are the major producers of flatulence when these foods are consumed (Naivikul and Appolonia, 1978; Onigbinde and Akinyele, 1983; Phillip and Abbey, 1989; Trugo *et. al.*, 1990).

These saccharides are comprised respectively of one, two and three galactose units joined together and to a sucrose in α-D-(1-->6) linkage. Due to lack of alpha-galactosidase in monogastric gastro-intestinal tract and also the inability of the invertase to hydrolyse substituted sucrose, these oligosaccharides escape breakdown and absorption in the stomach and small intestine. Hence they are made available for anaerobic fermentation by the microorganism, *Clostridium perfringens*, present in the colon. This then results in the production of flatus and other attendant discomfort (Calloway, 1960; Richard *et. al.*, 1968; Rachis *et. al.*, 1970; Murphy *et. al.*, 1972; Tomlin *et. al.*, 1991).

Most members of the enteric group of bacteria are facultative anaerobes and perform a mixed acid fermentation which yields ethanol (small amount) along with other products.

$$2(C_6H_{12}O_6) + H_2O \longrightarrow C_2H_5OH + 2CH_3CHOHCOOH + CH_3COOH + CO_2 + 2H_2O$$

Data suggest that members of the genus *Clostridium* possess a glycolytic system similar to the Embden-Meyerhof - Parnas pathway (Elsden, 1952). Paege *et. al.*, (1956) in a number of reactions between glucose and *Clostridium perfringens* noted along with a large number of different enzymes systems, a glycolytic enzyme system that is similar to the Embden - Meyerhof - Parnas pathway.

This sugar fermentation has one characteristic biochemical feature, which is not encountered in any other bacterial fermentation. There is a special mode of cleavage of the intermediate pyruvic acid to yield formic acid (Fig. 2.2).

$$CH_3COCOOH + CoASH \longrightarrow CH_3\ COCoA + HCOOH$$

Formic acid is therefore, frequently a major fermentative end product. It does not always accumulate. However, the bacteria possess the enzyme system, formic hydrogenlyase which splits formic acid to $CO_2 + H_2$. In such organism, formic acid is largely replaced as a fermentative end product by equimolar quantities of H_2 and Co_2.

Formation of molecular hydrogen as an end product of sugar fermentation is also charactersitic of many sporeformers of the genera *Clostridium* and *Bacillus*. The biochemical mechanism responsible for its production is, however, different. In spore formers, hydrogen is formed as a direct product of pyruvic acid cleavage (Stainer *et. al.*, 1990).

$$CH_3COCOOH + CoASH \longrightarrow CH_3\ COCoA + H_2 + Co_2$$

Methanogens present in the colon of some individuals may reduce CO_2 in the presence of H_2 to produce methane

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O$$

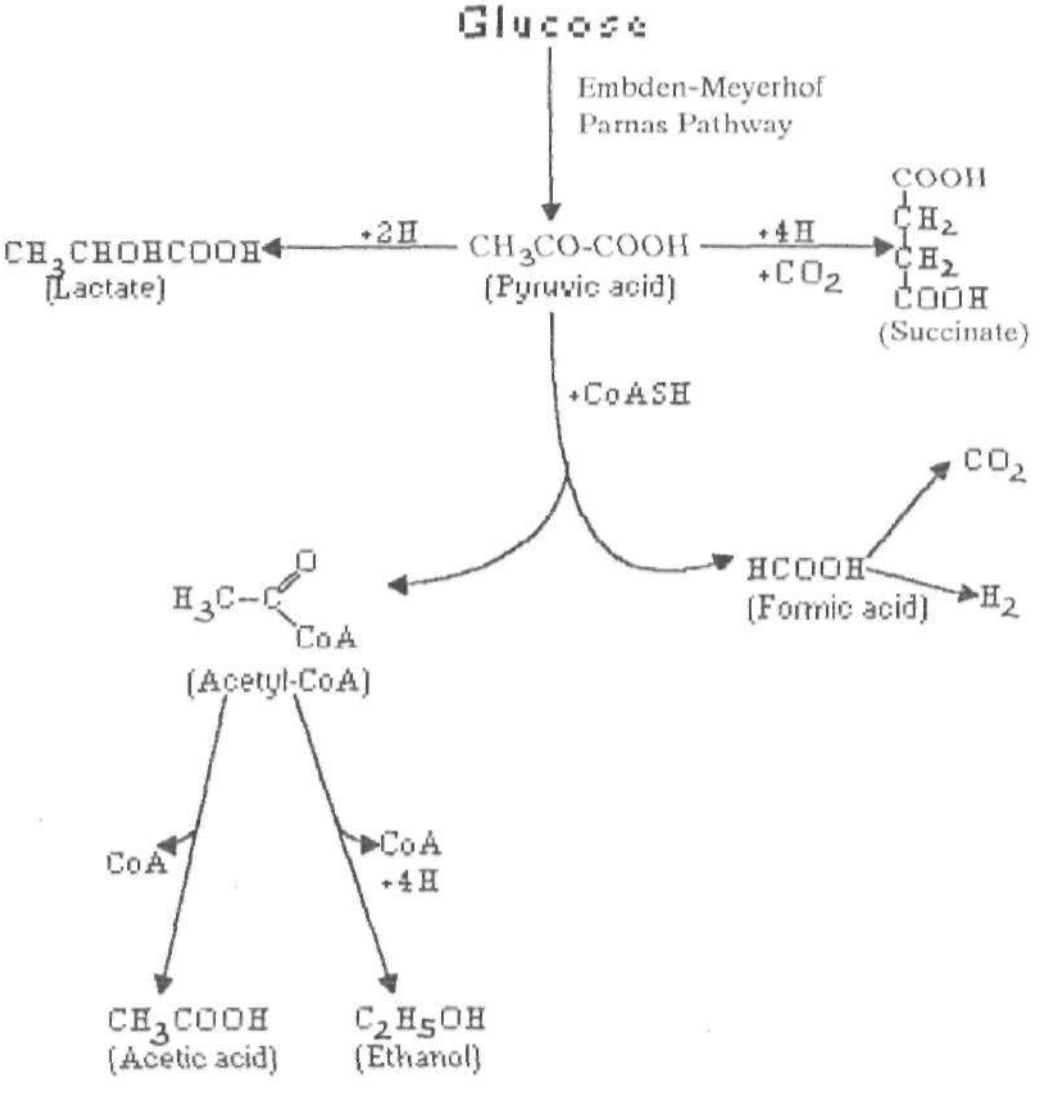


Fig. 2.2: Fermentative metabolism -- Mixed acid fermentation
Source — Stainer et al., (1990)

2.12 Composition of the Flatus

While monitoring flatus formation through breath hydrogen and methane, Calloway, (1966) observed that in human subjects, the time of which the sharp rise in hydrogen occurred suggested that major gas production took place when fermentable food residue reach the lower ileum where microorganisms are abundant.

In vitro experiment in which various substrates were incubated anaerobically with dejecta from well established - ileal and colonic stomata, showed that H_2 and CO_2 were always evolved and only these in quantity by ileal organisms, whereas colonic sample in some cases contain methane in addition to H_2 and CO_2. The composition of the flatus from ileal dejecta showed 62% CO_2, 36% H_2 and 2% N_2 using stachyose as substrate, and for colonic dejecta, 58% CO_2, 28% H_2, 12% CH_4, 2% N_2 using stachyose as substrate. (Calloway *et. al.*, 1966).

In their investigation, Richard, *et. al.*, (1968) observed that the flatus was composed of 40% CO_2, 53% H_2, 6% N_2 and 2% O_2 using different segments of the dogs gastro intestinal tract. The ingestion of raffinose and stachyose by rat in an individual life supporting system showed an increase in hydrogen concentration (Wagner *et. al.*, 1976). In different investigations, increase in flatus hydrogen produced with dietary oligosaccharide content was observed (Phillips *et al.*, 1988; Phillips and Abbey, 1989)

Rachis *et. al.*, (1970a), using anaerobic cultures isolated from dog colon biopsies showed that using raffinose as substrate, the gas produced was composed mainly of 35% CO_2, and 65% H_2; using stachyose as substrate gave a composition of 37% CO_2 and 63% H_2 of flatus produced. High concentration of CO_2 in flatus has been shown to correlate with the consumption of cooked dry beans (Murphy, 1963; Steggerda and Dimmick, 1966). Carbon dioxide was frequently found to constitute 60-70% of the total flatus volume during a period of maximum flatus egestion following a test bean meal (Murphy *et. al.*, 1972). A positive correlation between H_2 production and the content of raffinose, stachyose and verbascose was also established (Phillips and Abbey, 1989; Fleming, 1981). Tomlin *et. al.*, (1991) in their investigation using healthy human volunteers, observed that in most of the subjects, fermentation gases made the major contribution to the total daily flatus volume. Hydrogen and CO_2 were the main gases produced but 3 out of 10 subjects produced methane.

Following the time of maximum production of flatus and the composition of the flatus, it was concluded that the increase in gas production (flatus) is mainly as a result of bacterial fermentation of the oligosaccharides contained in legume foods (Calloway, 1966; Calloway *et. al.*, 1966; Rachis *et. al.*, 1970ab; Murphy *et. al.*, 1972).

2.13 Trials on Animals

In their experiment using rats, Hedin and Adachi, (1962) found that gas was formed in the intestine 2-8 hrs after feeding. They also established that red bean diet provoked an increase in the volume of gas in the percentage of CO_2 and H_2, the daily peak occurred approximately 4 hrs after feeding.

The relationship between the colon and various areas of the small intestine as gas producing areas following the introduction of different diets into surgically prepared intestinal segments of dog showed that the highest volume of gas of 31.9 ml/3hrs and percentage of CO_2 34.26 and H_2 33.16 was found in the colon. (Richards and Steggerda, 1966; Steggerda, 1968). They also showed that navybean homogenate increased the volume of gas when compared with methyl cellulose, and indicated that the bacterial action in duodenum, jejunum and ileum of dog may add significantly to the total intestinal gas volume.

Richard *et. al.*, (1968) fed diets containing beans directly into segments of the intestine of anesthetized female mongrel dogs and concluded that some constituents in the beans must interact with certain anaerobic bacteria inhabiting the ileum and colon. The flatus factor responsible can be related to carbohydrate oligosaccharide in the beans. Cristafaro *et. al.*, (1970) tested the effect on rats of crude oligosaccharide fraction from soybeans and observed that in each group of

diets, there was a marked increase in the volume of the intestinal gas, containing a high percentage of CO_2 when stachyose was present at 2 and 4% level. They also investigated the effect of verbascose in order to ascertain whether one or more galactose unit in the molecule could produce a greater volume of gas. The results showed that there was not much difference in the volume of intestinal gas, thus the presence of one or more α-galactose unit in the molecule had apparently no marked effect, contrary to what had been previously observed in which stachyose produced a highly significant increase in the volume of gas as compared with raffinose and lactose.

Steggerda, (1968) observed that a marked pH decrease (7.2-6.4) took place in the caecum of rats fed with a soybean flour diet (Stachyose, 2% of dry matter), this happening between the 4th and 8th hours after the meal. Cristafaro *et. al.*, (1970) also observed that the caecum pH decreased with increasing concentration of stachyose.

Rachis *et. al.*, (1970a) in an experiment with anaerobic cultures isolated from dog colon biopsies established that low molecular weight carbohydrates are implicated in the gas formation and suggested that glucose may be the form in which the anaerobic bacteria can most effectively use carbohydrates to produce CO_2 and H_2. Phillip and Abbey, (1989) using rats housed in glass life support chambers found a positive correlation between H_2 production and stachyose.

2.14 Trials on Humans

Trials on humans are more difficult than those on animals because they depend on the psychological and physical attitude of the subject. Their interpretation is therefore more complicated and can lead to imprecise conclusion (Cristafaro *et. al.*, 1974).

Blair *et. al.*, (1947) were able to show that the ingestion of soybeans considerably increased gas production in man. During a day's collection of rectal flatus, they found for one subject 200ml of gas on normal diet and up to 2,600ml on a diet with soybeans.

In 1961, Steggerda also made a striking observation on the average flatus production. While on the basal diet, gas volume was 16ml/hr with 10-12% CO_2, but on a bean diet, the average volume changed to 190ml/hr and with above 50% CO_2. In 1966, Steggerda, *et. al.*, stated for the first time that this fraction causing flatus, contained relatively large amount of raffinose and stachyose.

Calloway, (1966), while using human volunteers, 2 men and 4 women showed that about 3 or 4 hours after consumption of beans, a sharp rise in breath hydrogen content occurred. Peak levels were attained between 5th and 7th hours as the same time as subjects experienced feelings described as "bloating" and "gas". When some human subjects were fed textured soybean

flours, 5 out of 6 subjects complained of flatus when the amount ingested was 50gm or more of dry material.

In 1972, Murphy *et. al.*, while using human subjects for chemical and physical fraction of beans (*Phaseolus vulgaris*) and raffinose and stachyose added to a nonflatulent diets at levels higher than those in beans, observed that the fraction rich in low molecular weight carbohydrates were producing the highest amount of CO_2. In another investigation involving 10 healthy volunteers (five men and five women, aged 19-25 years), the volume and composition of flatus gas over a 24 hr period was obtained. Flatus gases were collected during consumption of a normal diet in which 200g baked bean was added by means of a flexible gas impermeable rubber tube. In most of the subjects, fermentation gases made the major contribution to the total daily flatus volume. Hydrogen and CO_2 were the main gases produced with some subjects producing methane in addition (Tomlin *et. al.*, 1991).

2.15 The Raffinose Family of Sugars in Processed Legume Foods and Their Elimination

The raffinose family of sugars is present in food raw materials such as matured legume seeds, known as staple food in many countries (Aykroyd and Doughty, 1982). In soybean, which is one of today's promising new source of protein, stachyose content is about 5.2g/100g average of the edible portion and raffinose content average 1.9 g/100g, chick pea

(*Cicer arietinum*) and pigeon pea (*Cajanus cajan*) contain 4.2 g/100g verbascose (Hardinge, *et. al.*, 1965).

In an investigation on 20 varieties of cowpeas, Onigbinde and Akinyele (1983), reported a range of 1.26 - 4.19 g/100g for raffinose and 1.21 - 4.98 g/100g for stachyose. Phillips and Abey, (1989) reported an average of 3.76 mg/g for verbascose.

Recent technological development has shown that soybeans and soyabean flour are quite suitable for the preparation of protein beverages (Lidner, 1988), Cowpea milk, like soymilk is an inexpensive, lactose free, nutritional alternative to cow milk for almost half the worlds population who are lactose intolerant, infants allergic to cow milk and all increasingly health conscious members of society (Lidner, 1988). One of the major reasons for avoiding legumes (Cowpeas) and their product is the flatus formation in relation with oligosaccharides of raffinose family (Wolf and Cowan, 1971).

2.15.1 Flatus inhibition

Richard and Steggerda, (1966) observed that the intestinal gas production in dogs was completely inhibited by bacteriostatic agents (Vioform and Mexaform), and antibiotic mixtures which effectively destroy anaerobic bacteria in the intestinal tracts. The agents also inhibit flatulence in humans (Steggerda, 1968). In an experiment, a mixture of

neomycin and sulphathalidine was added to terminal cultures from isolated segments of a dog after exposure to bean homogenate for 3 hrs and incubated for 37°C. Result shows that at a small dose of 50mg of the antibiotics, the gas production of micro organisms were inhibited completely in cultures taken from the small intestine only. When the antibiotic dose was doubled, inhibition was both in small intestine and colon segments. To record whether the gas producing activity of the anaerobic bacteria could be inhibited *in vivo* as well as *in vitro*, oral dose of vioform was given 2 days before exposure to bean meal and cultures were taken from the segments of the intestine after exposure to bean homogenate for 3hrs. The results were the same for both *in vivo* and *in vitro* experiments (Richard *et. al.*, 1968).

Rachis *et. al.*, (1970ab) showed both *in vivo* and *in vitro* that certain aqueous ethanol extracts of soybean meal with up to 80% carbohydrate contained both gas producing and gas inhibiting factor. This gas inhibiting factor is associated with certain phenolic acids, inhibiting the activity of the intestinal anaerobic bacteria. The phenolic acids present in decreasing amount in soybeans are syringic, furelic and chlorogenic acid (Arai *et. al.*, 1966). These substances were tested both *in vivo* and *in vitro* and it was observed that syringic acid had an effect greater than that of the other phenolic acids (Rachis *et. al.*, 1970ab).

The growth inhibition appears to be bacteriostatic since growth resumes after the inactivated anaerobic bacteria were transferred to fresh thioglycollate-glucose medium. The bacteria not only assume their original state but also produced gas just as effective as before the syringic acid treatment (Rachis *et. al.*, 1970a).

2.15.2 **Traditional methods of processing**

Processing of leguminous seeds, for instance, cowpea can be accompanied by a lowering of the oligosaccharides present, especially when the technological steps include treatment in the presence of water (Cristafaro *et. al.*, 1974; Ogun *et. al.*, 1989; Somiari and Balogh, 1993). It is known that the oligosaccharides of the raffinose family are well solubilized in water (French, 1954).

Different investigators had looked at the effect of processing methods like soaking (Reddy and Salunkhe, 1980; Ogun *et. al.*, 1989; Somiari and Balogh, 1993) and cooking Reddy and Salunkhe, 1980; Somiari and Balogh, 1993) on the oligosacchiarides and observed that there was reduction on the levels of the oligosaccharide.

During germination/sprouting, the seed under goes marked metabolic changes and the reserve carbohydrates including the oligasaccharides of the raffinose family are hydrolysed (Adjei-Twun *et. al.*, 1976). Germination/sprouting has been

very effective in the reduction of oligosaccharides in different legume seeds due to an increase in the alpha - galactosidase activity in the seed during germination. It is evident that the oligosaccharide residual level is a function of the duration of sprouting/germination process (East *et. al.*, 1972; Reddy and Salunkhe, 1980; Nnanna and Phillips, 1990; Trugo *et. al.*, 1990). Dehulling can reduce to minimal levels these oligosaccharides which cause flatulence (Ogun *et. al.* 1989).

Although these processes could reduce the oligosaccharide contents of the legumes, the reduction obtained by several investigators have so far been inconsistent or insufficient to suggest a significant reduction in flatus activity. Some investigators reported conflicting results, for example, a report from Rao and Belavady, (1978) showed a significant increase in oligosaccharides, (verbascose, stachyose, raffinose and sucrose) contents of 134, 114, 92 and 57 percent in red gram (*Cajanus cajan*), black gram (*Phaseolus mungo*), bengal gram (*Cicer arietinum*), and green gram (*Phaseolus aureus*) respectively after cooking at 15lbs of pressure for 15 mins. An increase in oligosaccharide content after decortication was also observed (Nnanna and Phillips, 1989).

2.15.3 Non traditional Methods of processing

Non traditional method of processing of dry beans such as autoclaving and thermal extrusion are needed for expanded utilization of dry edible beans (Gujska and Khan, 1991; Kawamura, 1968). Flatulence causing sugars and sucrose are reduced in extruded compared to non-extruded samples of starch rich fractions of pinto beans with higher reduction at higher process temperature. Sugar content decreased from 5.29 percent in non-extruded to 3.69 percent in samples extruded at 163°C. Raffinose and stachyose contents were reduced 47 to 60 percent by extrusion (Borejszo and Khan, 1992).

Enzymatic treatment could be applied either with a preparation rich in α-galactosidases or with microorganisms capable of utilizing the oligasaccharides of raffinose family by secreting this enzyme externally. A mold *Mortierella vinacea* which produces α-galactosidase mainly in the mycelium was used to reduce the content of raffinose in beet molasses (Suzuki *et. al.*, 1969). In 1970, Suzuki *et. al.*, obtained a crystalline alpha-galactosidase from the same organism. Sugimoto and Van Buren, (1970) with the enzyme preparation from *Aspergillus saitoi* found that at the neutral pH of soymilk, the addition of 1ml of enzyme solution to 50ml of soymilk, incubated at 50°C resulted in complete hydrolysis of the galactosides. In their experiment, Thananunku, *et. al.*, (1976) observed that the immobilized enzyme from disrupted *Mortierella vinacea* caused 48.8% reduction of oligosaccharides

after 12 hrs. The crude alpha-galactosidase preparation from *Aspergillus niger* was effective in reducing the level of stachyose and raffinose in all the samples of cowpeas treated. Treatment with this preparation for 2 hrs at 50°C resulted in a mean decrease of 82.3% for stachyose and 93.3% for raffinose (Somiari and Balogh, 1993). Entrapped cells of *Aspergillus oryzae* on agarose beads resulted in a total of 52% raffinose, 70% stachyose and 66.3% total oligosaccharide of soymilk hydrolysed in 8 hrs at 50°C (Khare *et. al.*, 1994).

The removal of oligosaccharide from soybean by ultrafiltration was investigated by Omosaiye *et. al.*, (1978). They showed that ultrafiltration can produce soy protein concentrates of 60g/100g protein, 35g/100g fat (dry basis) and very low oligosaccharide content with potentially good functional properties. This may be used directly to formulate infant foods, high protein beverage or may be dried and used as source of good quality protein and fat in food product. Cowpea if substituted for soybean, could produce equally good protein concentrate though not as high in protein like soyprotein concentrate but almost free of fat and oligosaccharides.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Collection of Samples

The cowpea cultivars 176B (sampea 4), 355 (sampea 5), 1696 (sampea 6), IAR 48 (sampea 7) and a local cultivar - Cananado was supplied by the Plant Science department of the Institute for Agricultural Research, ABU, Samaru - Zaria.

3.2 METHODS

3.2.1 Processing of Cowpea

Cold Water Soaking. The method of Somiari and Balogh, (1993) was used. Whole cowpeas were soaked in tap water (1g: 10ml) for 4, 8, 12 and 16 hours at ambient temperature (28°C). The water was drained off, the beans rinsed 3 times with tap water and mashed in a porcelain mortar. The mashed beans were dried for 24 hours at 60°C in an oven and milled in a Wiley mill (Author H. Thomas Co. Philadelphia Pa.) with a 60 mesh screen into flour, defatted and analysed for oligosaccharide contents.

Blanching. A modified version of the procedure of Ogun *et. al.*, (1989) was used. Whole cowpeas were soaked in hot water (1g: 10ml) for 4, 8, 12 and 16 hours at 80°C in glass beakers placed in a hot water bath. After soaking, the water

was drained off, the beans rinsed 3 times with tap water and mashed in a porcelain mortar. The mashed beans were dried in an oven for 24 hours at 60°C, milled in a Wiley mill (60 mesh) into flour. The flours were defatted and analysed for oligosaccharide contents.

Cooking in tap water. The procedure of Somiari and Balogh, (1993) was used. Whole cowpeas were cooked in tap water (1g. 10ml) for 10, 20, 30, 40 mins on a hot plate. The cooking water was drained off, the beans rinsed in water 3 times, mashed and dried in the oven for 24 hours at 60°C. The mashed bean was milled into flour using a Wiley mill (60 mesh), defatted and analysed for oligosaccharide contents.

Alkali (potash) treatment. The whole cowpeas were cooked in tap water (1g: 10ml) containing potash (1g of potash: 250g cowpea). The cowpeas were boiled until properly cooked, the cooking water drained off and the beans rinsed 3 time in water. The beans was mashed and dried for 24 hours at 60°C. The mashed beans was milled in a Wiley mill (60 mesh) into flour, defatted and analysed for oligosaccharide contents.

Dehusking or Decortication. The method of Ogun *et. al.*, (1989) was used. The whole cowpea grains were soaked in tap water, the husks take up water than the rest of the grains and become easily separable. After soaking, the husks were rubbed off the grains while they were still moist. The cowpeas were

washed after peeling and mashed in a porcelain mortar. The mashed beans were dried for 24 hours at 60°C, milled in a Wiley mill (60 mesh) into flour, defatted and analysed for oligosaccharide contents.

Processing into bean cake (akara). The dry cowpea seeds were first soaked in water to loosen the seed coats, which are then removed by manual rubbing or stirring in a mortar. The hydrated cotyledon are then ground or pounded into a thick paste, diluted with additional water, whipped into a foam, mixed with seasonings and fried in deep vegetable oil (groundnut oil) until the balls became brown. The brown balls are then mashed, dried in a oven at 60°C for 24 hours. The dried mash was milled into flour and defatted for about 24 hrs. The defatted sample was then analysed for oligosaccharide contents.

Raw unprocessed cowpea flour. The whole raw cowpeas were rinsed in water, dried for 24 hrs at 60°C. The cowpeas were then mashed, milled into flour, deffated and analysed for Oligosaccharide contents.

3.2.2 Defattening of flour

About 20g of the dried milled flour was wrapped in a whatman filter paper, fastened tightly with wire to prevent leakage of flour and placed in an extraction thimble. (30 x 100cm). the thimble was placed in a soxhlet extractor

containing petroleum spirit (bp. 40 to 60°C) and the oil extracted for 6 hrs (the bean cake flour was extracted for 24 hrs). The wrapped flour was then removed and dried to a constant weight in an oven at 60°C to remove residual petroleum spirit (Hymowitz *et. al.*, 1972).

3.2.3 Extraction of oligosaccharides

Five grams of samples were heated (60°C) with 50ml of 80% ethanol for 1 hour on a hot plate with magnetic stirring. After centrifugation (5000 G), residues were extracted twice using 20ml of ethanol and centrifuged each time at 5000 G for 10 mins. Extracts (supernatants) were combined. Five ml of 10% lead acetate was used to precipitate non-carbohydrate compounds (proteins) from the extract and centrifuged for 10mins at 5000G. The excess lead was removed from the extract by adding 0.2g sodium carbonate. After centrifuging at 5000G for 10mins, the volume was concentrated to 35 ml. (This is a slight modification of the method used by Borejszo and Khan, (1992).

3.2.4 Separation of oligosaccharides by thin layer chromatography. (Qualitative analysis) (De Stafanis and Ponte, 1968).

Preparation of chromoplates. Forty grams of kieselgel 60G (Silica gel), 5g of anhydrous Calcium sulphate and 95ml of 0.02M sodium acetate solution were manually shaken in a glass stoppered flask for 1 min. The slurry was transferred to a

spreader and a layer of 0.5mm thick was deposited on clean glass plates. The plates were allowed to dry overnight at room temperature, then were used without further preparation, except for scoring into lanes 3cm in width. However, if the plates are not used overnight and stored, the plates are activated by heating in an oven for 30 mins at 110°C before use. (Stahl and Kaltenbach, 1965).

Procedure. Thirty microlitres of sugar extract were applied to the plates using a micro pipette (Cole-Parmar, Chicago) Applications were made in small increments (less than 1uL) under a stream of warm air from a heat gun to facilitate rapid drying and thus minimize spot diffusion.

Ascending chromatography was conducted in closed glass tank lined with Whatman No. 4 paper saturated with the developing solvent. (The solvent system was chloroform-acetic acid - water (3.0, 3.5; 0.5 v/v). The solvent front was run to a distance of 15cm from the origin. This is a modification of the De Stafanis and Ponte, (1968) procedure to facilitate the proper separation of the sugars. After a run, the plates were dried with the aid of an oven and was spread with the detecting reagent.

Detection of spots. The spotting agent containing diphenylamine was used. The reagent was made up by dissolving 1g diphenylamine and 1ml aniline in 100ml of acetone; Prior to

spraying, 10ml of the acetone solution was mixed with 1ml 85% phosphoric acid. The sprayed plates were heated for 10mins at 130°C. The sugars appeared as dark grey or brown spots (Stahl and Kaltenbach, 1965).

3.2.5 **Quantitative analysis of sugars using phenol-sulphuric acid method.** (Dubois, *et. al.*, 1956)

For quantitation, the kieselgel area (2 x 2cm) corresponding to each oligosaccharide (Raffinose and Stachyose) spot was scraped from unsprayed duplicate plates and eluted with 3ml of distilled water for 2 hrs and filtered to give the sugar solution.

Procedure. One ml of sugar solutions was pipetted into a series of test tubes of internal diameter between 16 and 20mm and 1ml of 5% phenol was added to each tube. Then 5ml of conc. H_2SO_4 was added rapidly, the stream of acid being directed against the surface rather than the side of the tubes in order to obtain good mixing. The tubes were allowed to stand for 10mins, then they were shaken and placed for 20 mins in a water bath at 28°C before readings were taken. The absorbance of the characteristic yellow orange colour was measured at 490nm. Blanks were prepared by substituting 1ml of the eluent of (2 x 2 cm) area of the gel that does not contain sugar for that containing sugars.

Preparation of standard curve for the determination of the sugars. A standard solution of 100ug/ml of raffinose, and 100ug/ml of stachyose were used for the preparation of the standard curves.

The standard solutions were serially diluted in a series of test tubes to give concentrations of 6.25, 12.50, 25.00, 50.0 and 75.0 ug/ml. The colour was developed using phenol-sulphuric acid assay method. In the blank 1ml of sugar was substituted with 1ml of distilled water. The absorbance at 490nm was read off using a spectrophotometer. The data obtained were used to plot the separate standard curves for the determination of raffinose and stachyose.

Calculation of concentration using the standard curve. Thirty microlitres of the sugar extracts were spotted, area (2x2cm) corresponding to each oligosaccharide spot was scraped from the unsprayed duplicate plate and eluted with 3ml of distilled water. One millilitre of the filtrate was used for the analysis which is equivalent to the oligosaccharide contained in 10µl of the extract.

If the absorbance of the oligosaccharide in 10μL of extract gave a concentration say Y μg from the standard curve.

0.01ml of extract gave a concentration Y μg

..35ml of extract will give a concentration of

$$\frac{Y}{.01} \times \frac{35}{1} \mu g = 3500Y \mu g$$

5g of the sample contains 3500Y μg

..100g of sample will contain $\frac{3500}{5} \times \frac{100\%}{1}$

$$= 70,000Y \mu g\%$$

$$= 0.07 Yg/100g$$

3.2.6 Statistical analysis

Mean and standard deviations were calculated for the oligosaccharide data. All results were evaluated using analysis of variance and Duncan's multiple range test procedure of the statistical analysis system at ($P<0.05$) (SAS, 1985).

CHAPTER FOUR

4.0 RESULTS

4.1 Separation of the Oligosaccharides by Thin Layer Chromatography

Thin layer chromatography revealed the presence of sucrose, raffinose, stachyose and a spot below the stachyose on the plate which was suspected to be a higher molecular weight alpha-galactoside (verbascose) in the ethanol extract of all the varieties of cowpeas investigated. Only very small traces of glucose were present except for the extract from bean cake.

It was observed that after using the spotting reagent (diphenylamine- aniline) the glucose spot from the bean cake extract was deeper in colour than those from the other extracts.

The system employed produced spots of desirable geometry, compact and circular for all the sugars with no tailing and no distorted shapes. It was observed that the ascending chromatography to a distance of 15cm from the origin resulted in a better and discrete separation than 10cm as recommended by De Stafanis and Ponte, (1968).

The sugars were identified using standards except for the higher molecular weight alpha-galactoside for which no standard was available.

4.2 The Composition of Raffinose and Stachyose in the raw Samples of the Cowpeas

The raffinose and stachyose contents of the raw cowpeas are presented in Table 4.1. The raffinose level ranged from 1.12 g/100g in sampea 7 to 1.33 g/100g is sampea 4. The stachyose level also ranged from 3.50 g/100g in cananado to 4.20 g/100g in sampea 4. The total oligosaccharides (raffinose + stachyose) ranged from 4.64 g/100g in cananado to 5.53 g/100 in sampea 4. The mean value of raffinose was 1.19g/100g and stachyose gave a mean value of 3.88g/100g for the samples investigated.

4.3 Cold Water Soaking at Ambient Temperature

Soaking affected the level of the oligosaccharides in the samples to various extents. The effect of soaking on the mean raffinose and stachyose levels for different time intervals is shown in Fig. 4.1. The raffinose level was lowered from a mean of 1.19 g/100g in the raw samples to 0.95g/100g in 4 hrs, 0.87 g/100g in 8 hrs, 0.82 g/100g in 12 hrs and 0.71 g/100g in 16hrs soaked samples. The fig. also showed that the mean stachyose level changed from 3.88g/100g in the raw sample to 2.51 g/100g in 16hrs soaked sample.

The effect of soaking on the raffinose levels on the 5 varieties is shown in fig. 4.2. The raffinose level of sampea 4 was reduced from 1.33 g/100g in the raw sample to 0.80g/100g in 16 hrs; sampea 5 from 1.19 g/100g in the raw to 0.66g/100g

Table 4.1 Oligosaccharide contents of 5 unprocessed cowpea varieties

	Alpha-Galactosides g/100g	
Variety	Raffinose	Stachyose
Sampea 4	1.33±0.20[a]	4.2±0.10[a]
Sampea 5	1.19±0.01[a]	3.92±0.25[abc]
Sampea 6	1.19±0.03[a]	3.64±0.20[bc]
Sampea 7	1.12±0.03[a]	4.13±0.18[ab]
Cananado	1.14±0.03[a]	3.50±0.20[c]
Mean	1.19	3.88
S.D	0.08	0.30

Each value is the mean of two determinations. Sample size (n)=5

Means with the same letter in columns are not significantly different (P> 0.05)

fig 4.1

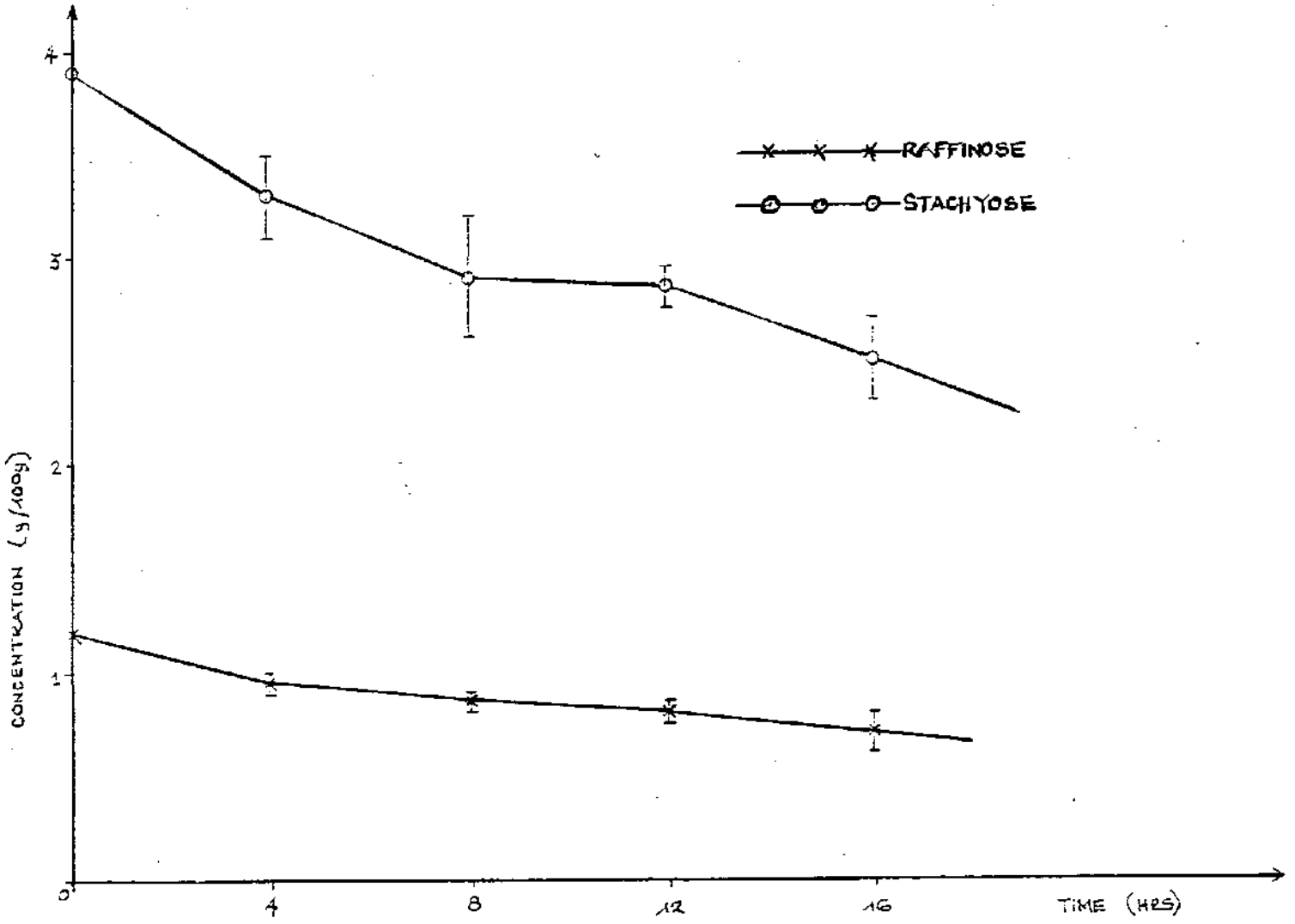


FIG. 4.1 : EFFECT OF COLD WATER SOAKING ON THE MEAN RAFFINOSE AND STACHYOSE CONTENTS OF FIVE (5) COWPEA VARIETIES.

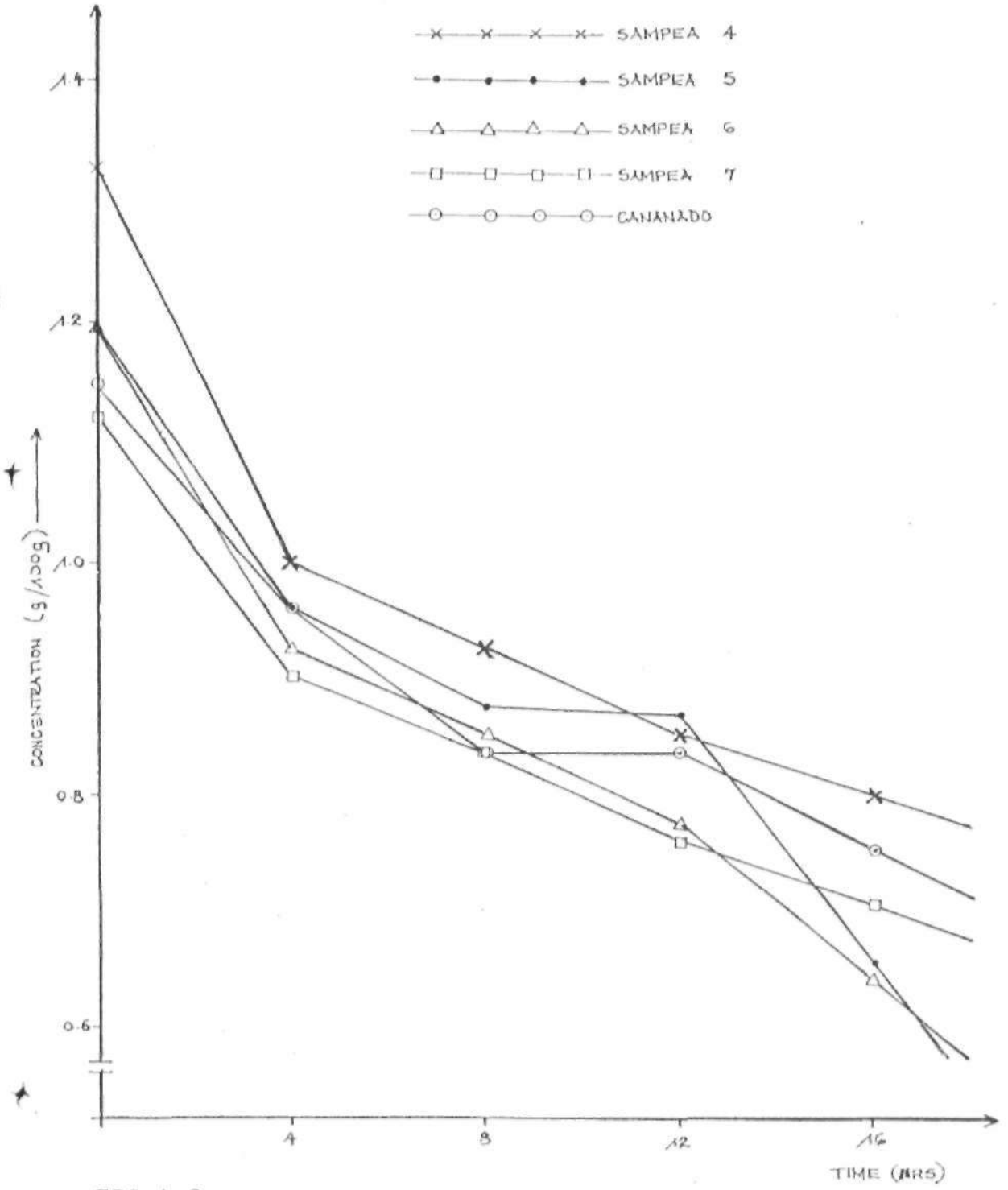


FIG. 4.2: EFFECT OF COLD WATER SOAKING ON THE RAFFINOSE LEVELS OF FIVE (5) COWPEA VARIETIES.

in 16 hrs; sampea 6 from 1.19g/100g in the raw sample to 0.64 g/100g in 16hrs; sampea 7 from 1.12 g/100g in the raw sample to 0.70 g/100g in 16hrs and cananado from 1.14 g/100g in the raw sample to 0.75 g/100g in 16 hrs.

Fig. 4.3 illustrates the effect of soaking on the stachyose levels of the varieties investigated. The stachyose level of sampea 4 was reduced from 4.20 g/100g in the raw sample to 2.77 g/100g in 16 hrs; sampea 5 from 3.92 g/100g in the raw to 2.60 g/100g in 16hrs; sampea 6 from 3.64 g/100g in the raw sample to 2.5 g/100g in 16hrs; sampea 7 from 4.13 g/100g in the raw sample to 2.45g/100g in 16 hrs and for cananado from 3.50 g/100g in the raw sample to 2.25 g/100g in 16hrs. The levels of raffinose and stachyose for other time intervals are shown in Figs. 4.2 and 4.3 respectively.

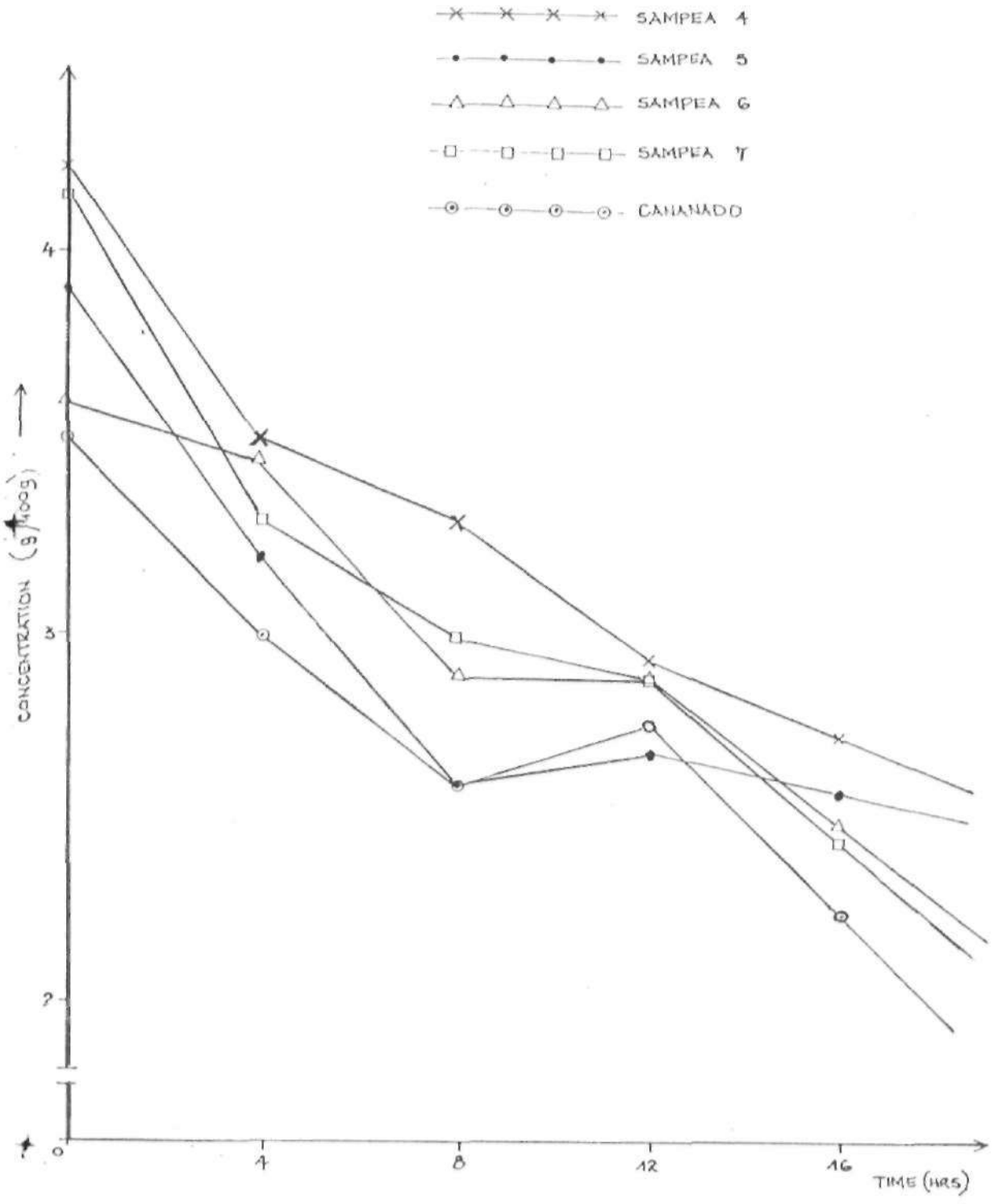


FIG.4.3: EFFECT OF COLD WATER SOAKING ON THE STACHYOSE CONTENTS OF FIVE (5) COWPEA VARIETIES.

4.4 Blanching (Hot Water Soaking at 80°C)

Fig. 4.4 shows the mean raffinose and stachyose contents of the 5 cowpea varieties. The mean raffinose level reduced from 1.19 g/100g in the raw samples to 0.13 g/100g in 16 hrs. The fig also shows that the mean stachyose level also reduced from 3.88 g/100g to 0.56 g/100g in 16 hrs of soaking.

Table 4.2 shows the levels of raffinose in each of the varieties after blanching, while table 4.3 illustrate that of stachyose. Sampea 4 showed a reduction from 1.33g/100g and 4.20 g/100g in the raw sample to 0.14g/100g and 0.56 g/100g for raffinose and stachyose in 16 hrs respectively. Sampea 5 showed a reduction from 1.19g/100g and 3.92g/100g in the raw sample to 0.14g/100g and 0.56g/100g in 16 hrs for raffinose and stachyose respectively. Sampea 6 showed a reduction from 1.19g/100g and 3.64g/100g in the raw sample to 0.12g/100g and 0.52g/100g in 16hrs for raffinose and stachyose respectively. Sampea 7 showed a reduction from 1.12g/100g and 4.13 g/100g in the raw sample to 0.10g/100g and 0.60g/100g in 16 hrs for raffinose and stachyose respectively. Cananado also showed a reduction from 1.14g/100g and 3.5g/100g in the raw sample to 0.14g/100g and 0.56 g/100g in 16hrs for raffinose and stachyose respectively. The levels of the sugars at different soaking times are also shown on the tables.

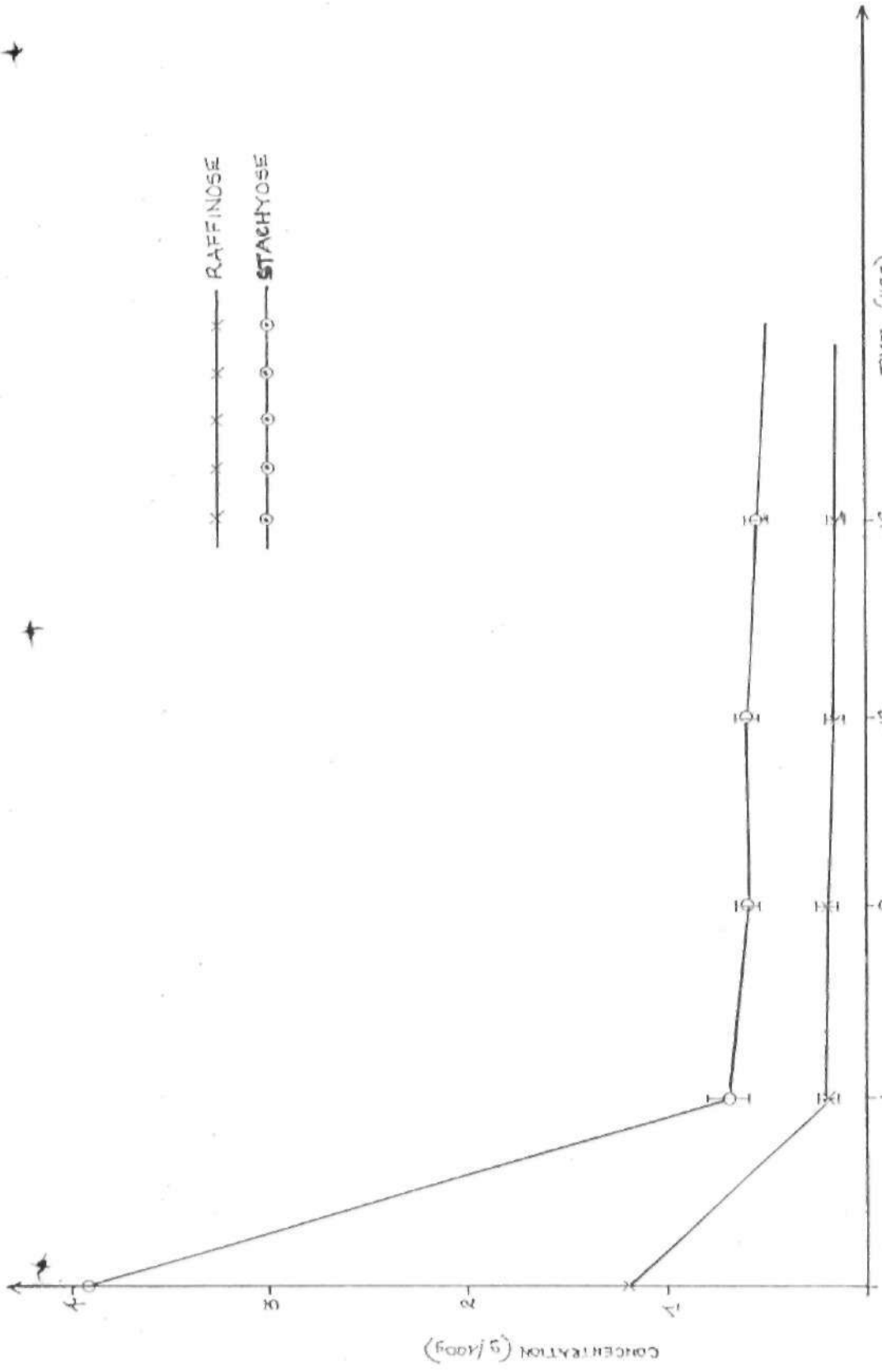


FIG. 4.4: EFFECT OF HOT WATER SOAKING ON THE MEAN LEVELS OF RAFFINOSE AND STACHYOSE OF FIVE (5) COWPEA VARIETIES.

Table 4.2 The effect of blanching (80°C) on the raffinose* level (g/100g) of the 5 cowpea varieties

Soaking time (hrs)	Sampea 4	Sampea 5	Sampea 6	Sampea 7	Cananando
0	1.33	1.19	1.19	1.12	1.14
4	0.21	0.21	0.18	0.22	0.22
8	0.11	0.18	0.18	0.21	0.21
12	0.11	0.14	0.16	0.14	0.16
16	0.14	0.14	0.12	0.10	0.14

*Each value is the mean of two determinations. n=5

Table 4.3 The effect of blanching (80°C) on the stachyose* level (g/100g) of the 5 cowpea varieties

Soaking time (hrs	Sampea 4	Sampea 5	Sampea 6	Sampea 7	Cananando
0	4.20	3.92	3.64	4.13	3.50
4	0.56	0.74	0.63	0.84	0.63
8	0.56	0.56	0.63	0.63	0.56
12	0.56	0.56	0.60	0.63	0.56
16	0.56	0.56	0.52	0.60	0.56

*Each value is the mean of two determination. n=5

4.5 Cooking in Tap Water

Cooking brought about a reduction in the mean levels of raffinose and stachyose in all the 5 varieties as shown in fig. 4.5. The mean level of raffinose was lowered from 1.19 g/100g in the raw sample to 0.63g/100g in 40mins and from 3.88g/100g in the raw sample to 2.45g/100g for stachyose in 40 mins of cooking.

Fig. 4.6 illustrates the effect of cooking on the raffinose contents of all the varieties. The raffinose level for sampea 4 was reduced from 1.33g/100g in the raw sample to 0.73 g/100g in 40mins of cooking; the level in sampea 5 reduced from 1.19g/100g in raw sample to 0.66 g/100g in 40 mins. There was also a decrease in raffinose level in sampea 6 from 1.19g/100g in the raw sample to 0.62g/100g in 40mins of cooking. Sampea 7, had a reduction of raffinose level from 1.14g/100g in the raw sample to 0.62 g/100g in 40 mins. The same trend of reduction was observed in cananado from 1.14g/100g in the raw sample to 0.54 g/100g in 40mins of cooking.

Stachyose also showed reduction after cooking. Sampea 4 was reduced from 4.20 g/100g in the raw sample to 2.59g/100g in 40mins of cooking; sampea 5 from 3.92 g/100g to 2.38g/100g in 40 mins; sampea 6 from 3.64g/100g to 2.38g/100g; sampea 7 from 4.13g/100g to 2.53 g/100g and cananado from 3.5g/100g to 2.35g/100g all for stachyose in 40 mins of cooking (Fig. 4.7).

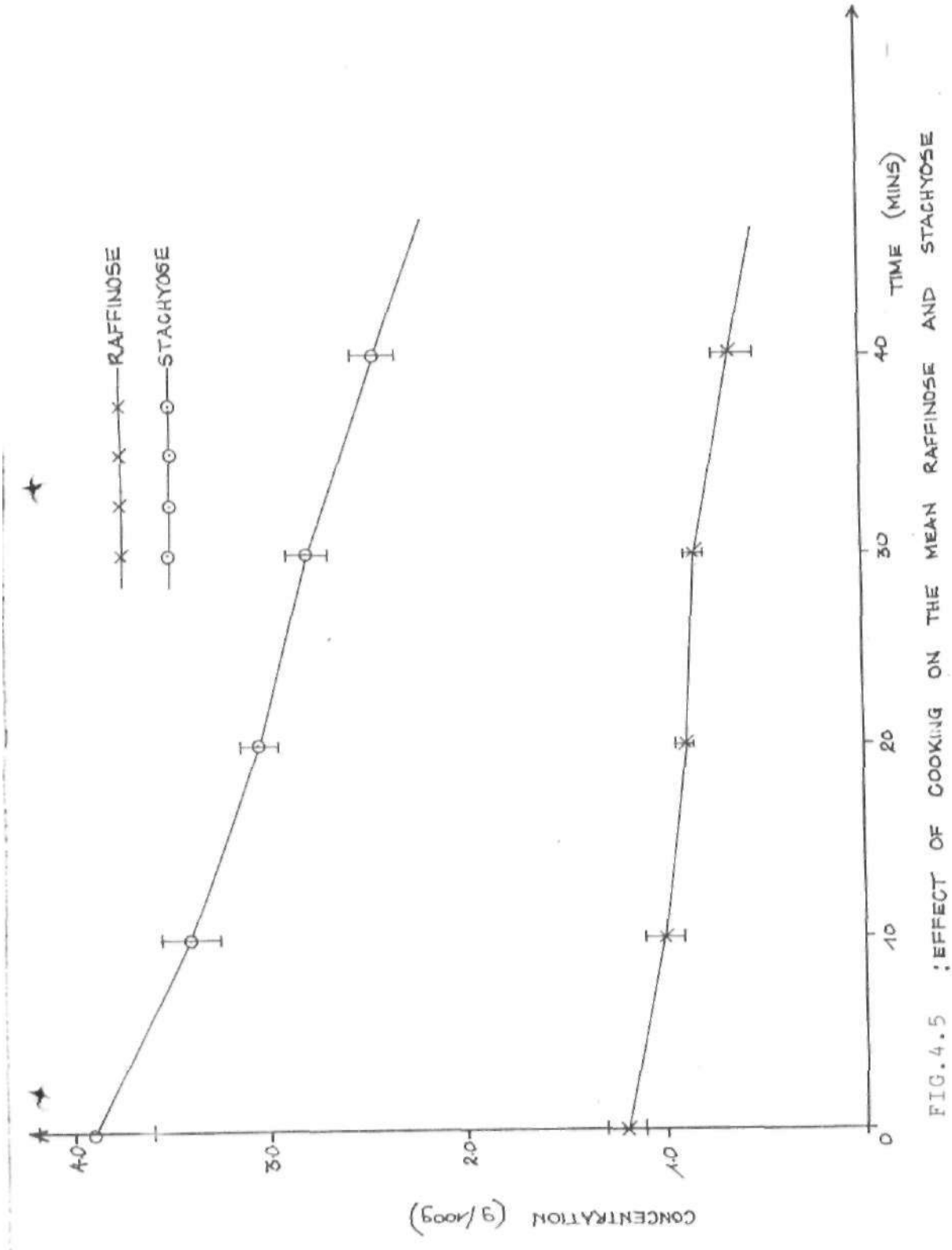


FIG. 4.5 : EFFECT OF COOKING ON THE MEAN RAFFINOSE AND STACHYOSE CONTENTS OF FIVE (5) VARIETIES OF COWPEAS.

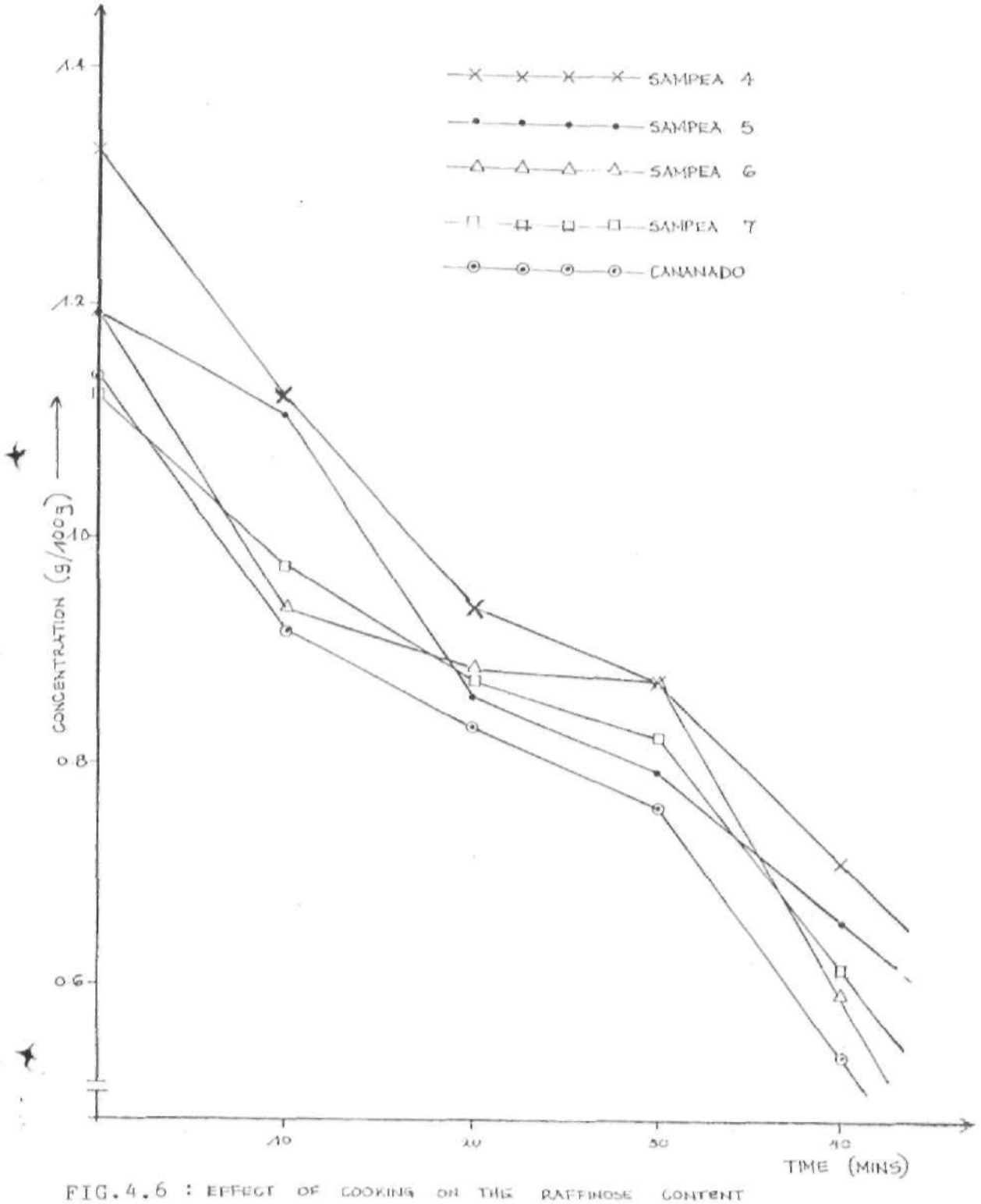


FIG. 4.6 : EFFECT OF COOKING ON THE RAFFINOSE CONTENT OF FIVE (5) COWPEA VARIETIES.

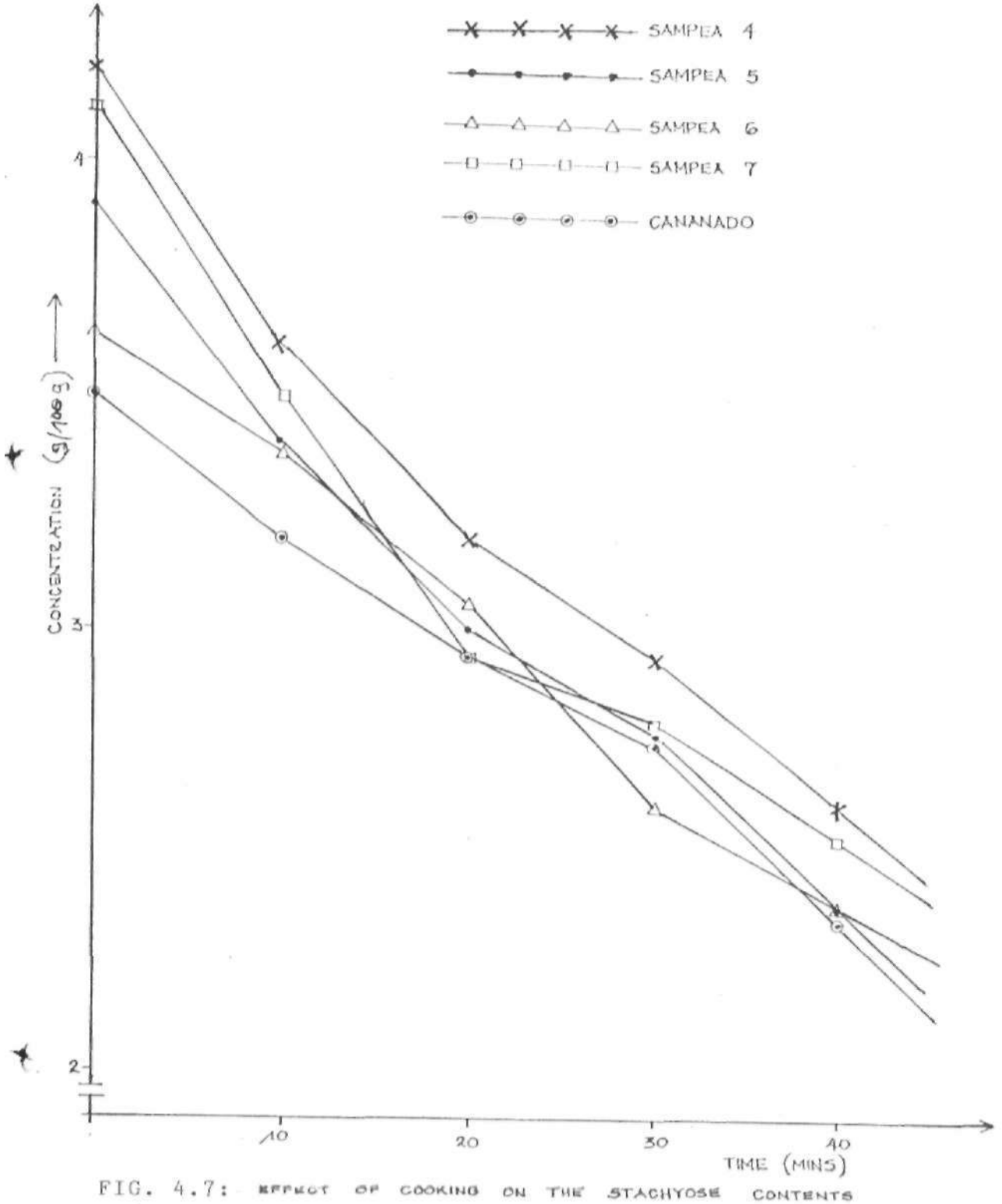


FIG. 4.7: EFFECT OF COOKING ON THE STACHYOSE CONTENTS OF FIVE (5) COWPEA VARIETIES.

4.6 Alkali (Potash) Treatment

This treatment affected the oligosaccharide levels significantly ($P<0.05$) Table 4.4 shows that cooking with potash decreases the raffinose content from a mean value of 1.19g/100g in the raw samples to 0.38g/100g, and the mean stachyose level also decreased from 3.88g/100g in the raw samples to 1.5g/100g after cooking. The raffinose levels ranged from 0.30g/100g in sampea 7 to 0.44 g/100g in sampea 5 and cananado, while the stachyose level ranged from 1.19 g/100g in sampea 5 to 1.75g/100g in sampea 6 after cooking.

4.7 Processing Into Bean Cake (Akara)

Table 4.4 shows the raffinose and stachyose levels of the cowpea varieties when processed into bean cake. The raffinose levels ranged from 0.54 g/100g in cananado to 0.67 in sampea 7 and sampea 4. The stachyose levels ranged from 1.86 g/100g in cananado to 2.3g/100g in sampea 4. The mean levels of all the 5 varieties of cowpeas was 0.62g/100g and 2.05g/100g for raffinose and stachyose respectively after processing into bean cake.

4.8 Dehulling

Table 4.4 also shows that the mean raffinose and stachyose levels were 0.83g/100g and 2.80g/100g respectively after dehulling. The raffinose level ranged from 0.67g/100g in sampea 5 to 1.09 g/100g in sampea 4; stachyose level ranged from 2.66g/100g in cananado to 3.01g/100g in sampea 7 after dehulling.

Table 4.4 Oligosaccharide contents of alkali (potash) treated, bean cake (akara) and dehulled cowpea varieties

Variety	Raffinose (g/100g)			Stachyose (g/100g)			Raffinose + Stachyose (g/100g)		
	Potash	Akara	Dehulled	Potash	Akara	Dehulled	Potash	Akara	Dehulled
Sampea 4	0.35	0.67	1.09	1.65	2.30	2.84	2.00	2.97	3.93
Sampea 5	0.44	0.59	0.67	1.19	2.10	2.68	1.63	2.69	3.35
Sampea 6	0.35	0.63	0.74	1.75	1.95	2.80	2.10	2.58	3.54
Sampea 7	0.30	0.67	0.87	1.37	2.03	3.01	1.67	2.70	3.88
Cananado	0.44	0.54	0.76	1.56	1.86	2.66	2.00	2.40	3.42
Mean	0.38	0.62	0.83	1.50	2.05	2.80	1.88	2.67	3.62
S.D.	0.06	0.05	0.15	0.20	0.15	0.13	0.19	0.19	0.24

CHAPTER FIVE

5.0 DISCUSSION

The deepness of glucose spot from the ethanol extract from bean cake (akara) may be due to the activation of the enzyme alpha - galactosidase and invertase in the cowpea paste. The alpha-galactosidase hydrolyses the alpha-galactosides to galactose, sucrose and lower oligosaccharides. The invertase further breaks down the sucrose to glucose and fructose. This hydrolysis occurs during the process of soaking and vigorous stirring of the paste with addition of water to give a foamy constituency before frying to produce akara.

The 80% ethanol extracts of defatted cowpea meals contained sucrose, raffinose, stachyose and a higher molecular weight sugar suspected to be verbascose. There were also traces of glucose. Table 4.1 shows that the concentration of stachyose is higher than raffinose in all the cowpea varieties. Analysis showed that there was no varietal difference in the levels of raffinose in all the investigated samples. For the stachyose level, there were slight varietal differences but not in all the samples-sampea 4, sampea 5 and sampea 7 did not differ in their stachyose contents. sampea 5, sampea 6, and sampea 7 have the same levels of stachyose. sampea 5, sampea 6 and cananado did not differ in their

stachyose levels. Sampea 4 and cananado showed a varietal difference ($P<0.05$) in their stachyose contents.

The raffinose and stachyose contents of cowpeas investigated in this study were comparable to those in the literature. In their study, Onigbinde and Akinyele, (1983) obtained a range of 1.21g/100g to 4.84g/100g for stachyose and 1.26 - 4.19g/100g for raffinose content in 20 varieties of cowpeas investigated. In a review, Reddy *et al.*, (1984) gave stachyose content of 2.0 to 3.6 g/100g and raffinose content 0.40 - 1.2 g/100g for cowpeas. The values obtained in this study quite agree with the values obtained by Somiari and Balogh (1993) for stachyose (3.3 to 4.8g/100g) but lower than the values for raffinose (2.2 - 2.8 g/100g). Result obtained by Ogun *et. al.*, (1985), 0.9-2.6 g/100g for raffinose and 2.6 - 3.5 g/100g for stachyose quite agree with results of this study. However values obtained by Phillips and Abbey, (1989) of 0.47 g/100g mean for raffinose and 2.24 g/100g mean for stachyose were lower than results obtained in this study.

Existing difference in extraction conditions, and methods for sugar determination may be responsible for the observed differences. Unfortunately, we were unable to locate a source of authentic verbascose, making it impossible to analize the varieties for that sugar. Since flatulence in legumes is mainly ascribed to both raffinose and stachyose (Steggarda *et. al.*, 1966; Murphy *et. al.*, 1972), the sum of both oligosaccharides in the whole raw cowpeas and processed

cowpeas has been used to estimate the percentage of flatulence activities of investigated cowpeas.

When cowpeas are soaked in cold water, they swell and the amount of water absorbed per unit weight increases with soaking time (Sefa-Dedeh *et. al.* 1978). In cowpeas, the protein and starch matrix of the cotyledon as well as the anatomical characteristics such as seed coat thickness and size of micropyle may contribute significantly to the total amount of water absorbed. Soaking at a bean to water ratio of 1:10 (Shun ku *et. al.*, 1976) affected the mean level of oligosaccharides in the samples to various extents (Fig. 4.1).

There was reduction in the levels of both raffinose and stachyose as soaking time increased resulting in the highest reduction in 16hrs. The rate of reduction was highest within the first 4hrs for both stachyose and raffinose (Fig. 4.2 and 4.3).

The mechanism by which the oligosaccharides are eliminated is not quite clear. Although leaching has been suggested (Price *et. al.*, 1988), results from Somiari and Balogh, (1993) and that of Silva and Braga, (1982) suggest that leaching cannot be the only factor. When the seeds are soaked, they undergo metabolic changes, the dormant enzyme - the alpha - galactosidases and the invertase are activated and this leads to the breakdown of the oligosaccharides. This hydrolysis increases with time until maybe the seed becomes

saturated. (Adjei-Twun *et. al.*, 1976). These two factors - leaching and enzyme action may be responsible for the reduction in levels of the oligosaccharides.

The mean concentration for raffinose and stachyose are 0.71g/100g and 2.51g/100g respectively giving a mean percentage reduction of about 40.34% and 35.29% for raffinose and stachyose respectively (Tables 4.5 and 4.6) in 16hrs of soaking. Using the sum of raffinose and stachyose, the best percentage reduction of flatulence activities was 36.50% and this was in 16hrs of soaking. Ogun *et. al.*, (1989) reported a 12.90% reduction in the mean stachyose content and 14.29% for raffinose for 4 cultivars in 12 hrs. This values are lower than values obtained in this study which gave 26.80% and 31.09% reduction for stachyose and raffinose in 12hrs of soaking (Table 4.6). Somairi and Balogh, (1993) also reported a mean reduction of 28% and 26.2% for raffinose and stachyose respectively in 16hrs of soaking for 3 cowpea cultivars. These values are however lower than the values of 35.29% and 40.34% mean reduction for stachyose and raffinose obtained respectively in 16 hrs of soaking in this study. Fig. 4.2 shows that the highest rate of reduction for raffinose was within the first 4hrs for all the cowpea varieties. Although there was no substantial difference between the 8th and 12th hour for sampea 5 and cananado, the levels in 16hrs were lower than the levels in 4hrs. Except for these two varieties. there was a reduction in the raffinose level with time for all

Table 4.5: Mean concentration of α-galactosides (g/100g)

	Raffinose	Stachyose	Raffinose + Stachyose
Raw	1.19	3.88	5.07
Cold water soaking (hrs)			
4	0.95	3.29	4.24
8	0.87	2.90	3.77
12	0.82	2.84	3.66
16	0.71	2.51	3.22
Cooking in tap water (mins)			
10	1.01	3.42	4.43
20	0.88	3.03	3.91
30	0.83	2.77	3.60
40	0.63	2.45	3.08
Bean cake (akara)	0.62	2.05	2.67
Alkali (potash) treatment	0.37	1.5	1.87
Dehulling	0.83	2.80	3.62
Blanching (hot water soaking) (Hrs)			
4	0.21	0.68	0.89
8	0.18	0.60	0.78
12	0.14	0.60	0.74
16	0.13	0.56	0.69

Table 4.6: Mean percentage reduction of the oligosaccharides in the 5 cowpea varieties

Processing method cold water soaking (hrs)	Raffinose	Stachyose	Raffinose + Stachyose
0	0	0	0
4	20.17	15.20	16.37
8	27.00	25.25	25.64
12	31.09	26.80	27.81
16	40.34	35.29	36.50
Blanching (hot water soaking) (hrs)			
0	0	0	0
4	82.35	82.47	82.45
8	84.87	84.53	84.62
12	88.24	84.53	85.40
16	89.08	85.57	86.40
Cooking in tap water (mins)			
0	0	0	0
10	15.12	11.86	12.62
20	26.05	21.90	22.88
30	30.25	28.60	28.99
40	47.06	36.85	39.25
Alkali (potash) treatment	68.90	61.34	63.11
Bean cake	47.90	47.16	47.33
Dehulling	30.25	26.80	27.61

other varieties. Fig. 4.3 shows that although there was a reduction in the level of stachyose with time continuously for all time intervals except for sampea 5 and cananado, the highest reduction was in 16hrs for all the varieties. It was observed that except for sampea 6, the highest rate of reduction was within the first 4hrs. For sampea 5 and cananado, although there was an increase between the 8th and 12th hour, the final levels in 16hrs was much lower than that in 4hrs. Using the sum of raffinose and stachyose to estimate the percentage reduction of flatulence-activities, it was observed that cananado has the highest reduction of about 40.83% in 16hrs.

Statistical analysis ($P<0.05$) shows that although varietal difference affected the mean levels of raffinose and stachyose during soaking, soaking time is a more important factor affecting the mean levels of the oligosaccharides. (appendices 3a and b).

Considering the level of reduction obtained after soaking in this study, it is unlikely that this technique alone would bring about a remarkable reduction in the flatulence - inducing activity of cowpeas. A remarkable pit fall in this technique is the presence of off-odour in the cowpeas soaked for 12hrs and above. This may affect the acceptability of soaked cowpeas and their products.

Fig. 4.4 shows that there was a tremendous reduction in the mean levels of both raffinose and stachyose within the first 4hrs of hot water soaking. The figure shows no significant decrease in the oligosaccharides after 4hrs of blanching. This technique, produced the highest mean percentage reduction of the oligasaccharides of over 80% in 4hrs (Table 4.6).

Ogun *et. al.*, (1989) obtained a reduction of about 21% for raffinose and 16% for stachyose. The technique and duration of time used by these investigators were different from that employed in this study. This technique may not be economically viable and easily applicable in households but it could be applied in industries to produce an oligosaccharide-free cowpea that can be used to produce high protein concentrates free from the flatulence activities. An additional advantage is that, the product is intact, no disruption of the Cotyledon, fairly cooked and the off-odour observed in cold water soaking is totally eliminated.

Analysis shows that at ($P<0.05$), variety has no effect on the mean levels of raffinose. The only important factor is the time of soaking which affected significantly the mean levels of raffinose. For the mean stachyose levels, both the variety and time affected the levels but the time factor is the more important. (appendices 4a and b). Hot water

soaking time has a negative correlation with the levels of raffinose ($r = -0.75$) and stachyose ($r = -0.73$).

Cooking cowpeas provides tenderization of cotyledon which increases product palatability and inactivates endogenous toxic factors that would markedly limit the final nutritional value. Cooking also brought about a reduction in the levels of raffinose and stachyose. Even though the level of reduction was not proportional to the duration of cooking, the longest cooking time resulted in the greatest reduction in the mean levels of oligosaccharides in all the varieties (Fig. 4.5). The first 10mins resulted in the highest rate of reduction for both raffinose and stachyose. The average percentage reduction for 40mins was 47.06% and 36.85% for raffinose and stachyose respectively. As shown in fig. 4.6 the rate of reduction was highest in the first 10mins except for sampea 5. It also shows that the rate of reduction between 20 and 30mins was the lowest especially for sampea 6. The level of stachyose on cooking also decreased with time (fig. 4.7) for all varieties.

The mechanism by which the oligosaccharides were eliminated in this case may be due to leaching since the oligosaccharides are relatively heat stable. The highest mean percentage reduction of flatulence activities as computed by the sum of raffinose and stachyose was 39.25% in 40mins of cooking.

Cooking beyond 40mins disrupts the cotyledon of some varieties especially sampea 7. Cooking time differs from variety to variety, hence the cooking was limited to 40mins.

Somiari and Balogh, (1993) reported a mean reduction of 28.6% and 44% in stachyose and raffinose levels respectively in 50mins of cooking 3 varieties of cowpeas. The results from this study is higher than that reported by Somiari and Balogh, (1993) even though cooking was limited to 40mins (Table 4.6). Onigbinde and Akinyele, (1983) reported a mean decrease of about 46% and 50% in stachyose and raffinose contents of 20 cowpea cultivars respectively after cooking. This result agrees with the results obtained in this study.

In a marked contrast, Rao and Belavady, (1978) reported a significant increase in oligosaccharides (Verbascose, stachyose, raffinose, and sucrose) contents of 134, 114, 92, and 57% in red gram, black gram, bengal gram and green gram respectively after cooking at 15lb of pressure for 15mins. This marked difference may be due to the technique they employed. In their technique, the cooking water was not decanted rather the oligosaccharides were extracted from the pulses along with cooking water. This increase also may not be explained by the heating effect since the oligosaccharides are stable to heat. Using the same technique of Rao and Belavady, (1978) but with higher temperature (116°C) for 40mins, Reddy and Salunkhe, (1980) reported a corresponding

decrease in verbascose and stachyose contents to 73% and 81% of the original. Cooking for 40mins reduced the oligosaccharides to 74.5% of the original.

Analysis showed that there was a change in raffinose and stachyose levels due to variety but changes in the oligosaccharides levels due to cooking time was the more important factor affecting the mean levels of the oligosaccharides ($P< 0.05$) (appendices 5a and b).

Alkali treatment using potash (sodium sesquicarbonate and sodium bicarbonate) decreases cooking time and alters the texture of the cowpea seed, (Onwuka, 1983). At a ratio of 1:250 (w/w) of potash to cowpea, the product does not have a bitter taste and is accepted organoleptically. In addition to the reduction in cooking time, alkali treatment reduces the levels of oligosaccharides in the cowpeas (Table 4.4). This technique resulted in marked mean percentage reduction of 68.90% and 61.34% for raffinose and stachyose respectively, hence a 63.11% mean reduction in flatulence activities of the cowpeas (Table 4.6).

Inspite of the marked reduction of flatulence activity and attendant discomfort resulting from consumption of cowpea treated with potash, this treatment causes severe losses of thiamine and riboflavin and a possible formation of lysinoalamine. Hence the traditional Nigerian use of potash

is not advocated (Edijala, 1980). This marked reduction in the levels of the oligosaccharide may be due to increased leaching resulting from the alteration of the texture of the seed coat and cotyledon.

Processing into bean cake (akara) is a popular processing method in Nigeria. This resulted in reduction of mean raffinose and stachyoses levels to 0.62g/100g and 2.05g/100g respectively (Table 4.5). This accounted for a mean reduction of about 47.90% for raffinose and 47.16% for stachyose. This gives a 47.33% mean percentage reduction of flatulence activities (Table 4.6). This processing method is a combination of soaking, dehulling, whipping to produce a foamy paste and deep frying in vegetable oil. The effect of these processing methods may not be additive.

Apart from completely eliminating the tannin contents of cowpeas (Ogun *et al.*, 1989), dehulling also reduces the levels of oligosaccharides to various extents since the cowpeas have to be soaked to facilitate decortication. After decortication, mean raffinose and stachyose levels of 0.83g/100g and 2.80g/100g were obtained respectively (Table 4.5), resulting in a mean percentage reduction of 30.25 and 26.80% for raffinose and stachyose respectively. This resulted in 27.61% mean reduction of flatulence activities after dehulling (Table 4.6). Comparison of the effect of treatment (Cooking in potash, processing into bean cake and

dehulling) and variety on the mean levels of raffinose and stachyose showed that although variety had some effect on the raffinose and stachyose levels, processing method (treatment) was the more important factor influencing the levels of the oligosaccharides ($P < 0.05$) (appendices 6a and b).

An excellent comparison of the mean levels of raffinose and stachyose for the raw and processed cowpeas varieties is presented in fig. 4.8. At a glance, it is observed that hot water soaking has the highest mean percentage raffinose and stachyose and hence the best mean reduction of flatulence activities of over 80%.

Although this study does not report on the total amount of the possible oligosaccharides present in cowpeas, it is of physiological interest, in relation to cowpea digestion, that cooking to softness could lead to a reduction in levels of stachyose and raffinose which are the major oligosaccharides associated with the flatulence activity in cowpeas. A reduction in the levels of these sugars when raw cowpeas were dehulled, suggested that the level of the oligosaccharides could be further reduced if the cowpeas were dehulled before cooking, although the reduction may not be additive.

MEAN PERCENTAGE OLIGOSACCHARIDES IN RAW AND PROCESSED COWPEA VARIETIES.

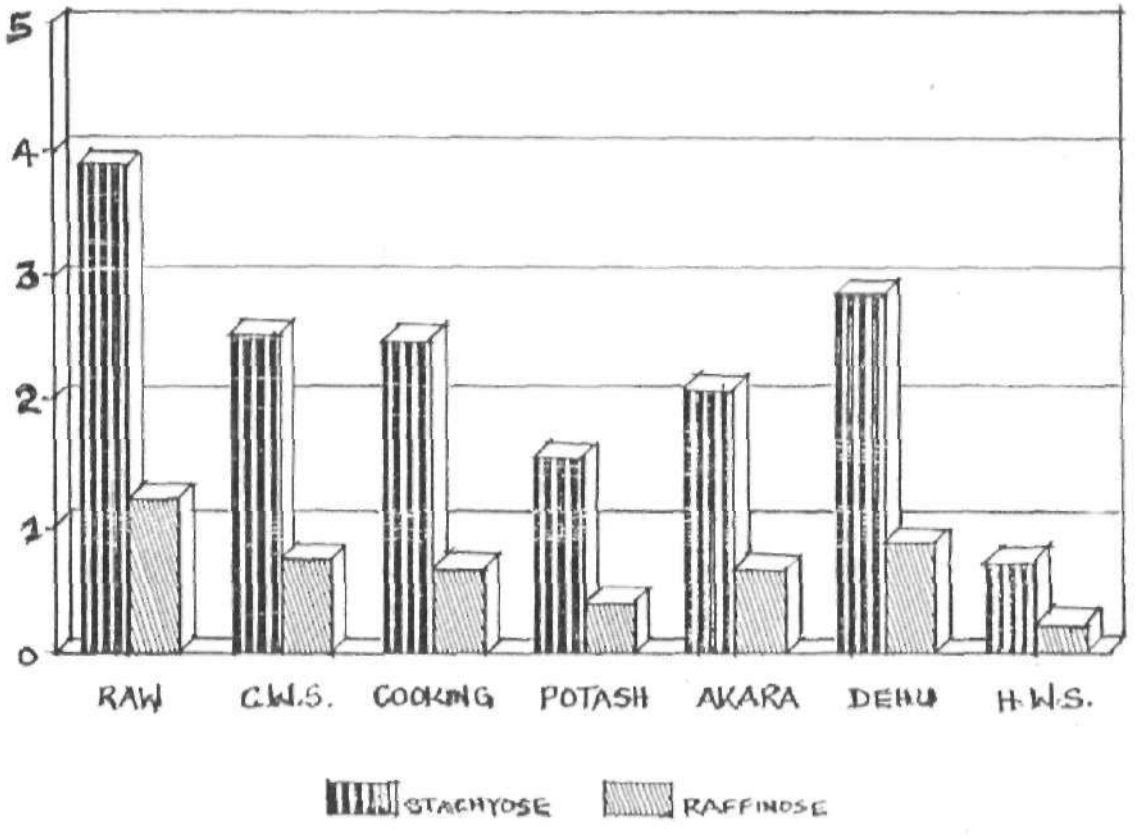


FIG. 4.8 : MEAN LEVELS OF RAFFINOSE AND STACHYOSE BEFORE AND AFTER COLD WATER SOAKING (C.W.S. 16hrs), COOKING (90mins), COOKING WITH POTASH, BEAN CAKE (AKARA), DEHULLING, AND HOT WATER SOAKING (H.W.S. 4hrs).

Based on the observation of Calloway and Murphy, (1968), a 5g portion of raffinose caused an elevation in flatus volume in man equivalent to that produced by 100g of beans on the average. Hellendoon (1969) showed that 100g of beans caused an elevation of about 300ml in flatus. The mean values of raffinose (0.63g/100g) and stachyose (2.45g/100g) in 40mins of cooking obtained in this study could cause an average total elevation of 184.8ml flatus per 100g of the cowpea. This represents about 38.4% reduction in the volume of flatus that could be attributed to the level of the two galactosides in the cowpea before cooking. The amount of stachyose (0.68g/100g) and raffinose (0.21g/100g) obtained for hot water soaking for 4hrs cause an average total elevation of about 53.4ml flatus per 100g of cowpea. This represents about 82.2% reduction. This technique is highly advocated because, the cotyledon remain infact, fairly cooked, and the off odour associated with cold water soaking is completely eliminated.

Although, the percentage reduction cannot be taken as absolute because cowpeas also contain verbascose which is potentially flatulent, this percentage reduction is not far from the absolute figure because stachyose and raffinose are the two major galactosides implicated in flatus production in cowpeas.

CHAPTER SIX

6.0 CONCLUSION AND SUGGESTION FOR FURTHER STUDIES

6.1 Conclusion

Cananado had the lowest total oligosaccharide of 4.64g/100g as compared to Sampea 4 with the highest of 5.53g/100g and may have the ability to produce less flatus.

In all the investigated varieties there was no varietal difference in the levels of raffinose. Slight difference was observed in the stachyose levels ($P< 0.05$).

The flatulence - causing sugars were found to be reduced by all processing methods contrary to results obtained by some investigators. (Rao and Belevady, 1978; Savitri and Desikakar, 1985). The level of reductions were significantly influenced by processing time and processing method (treatment) rather than by variety. Total oligosaccharides were drastically and effectively reduced by blanching which resulted in about 82.2% reduction in flatulence activities for 4hrs soaking. This technique compares favourably with enzyme treatment and has the added advantage of good product quality.

This study as well as that of others (Murphy *et al.*, 1972; Wagner *et al.*, 1976; Nnanna and Phillips, 1988) has shown a drastic reduction in flatulence potential by processes which reduce the levels of oligosaccharides.

Thus, even if other seed constituents may contribute to the qualitative problems of cowpeas which militate against their complete acceptability and utilization, removal of oligosaccharides by processes which are economically and technological feasible and which preserve functional and nutritional quality can reduce the problem. This will help to increase the spectrum of protein source.

6.2 Suggestion

(1) More IAR and local varieties of cowpeas should be screened for their raffinose oligosaccharides with a view to recommending the varieties with less flatulence activities.

(2) Beside research to improve nutritional quality, agronomic practices, resistance to pests, effort should be made by breeders and researchers toward reducing the raffinose oligosaccharide levels in raw cowpea by using advanced techniques like genetic engineering.

(3) In this era of high cost and shortage of cooking fuel, further research should be carried out to determine the level of reduction of these oligosaccharides between zero and 4 hours time intervals of blanching at 80°C. This would be more economical in terms of energy and time if blanching at a time less than 4 hrs could sufficiently reduce the levels of these oligosaccharides.

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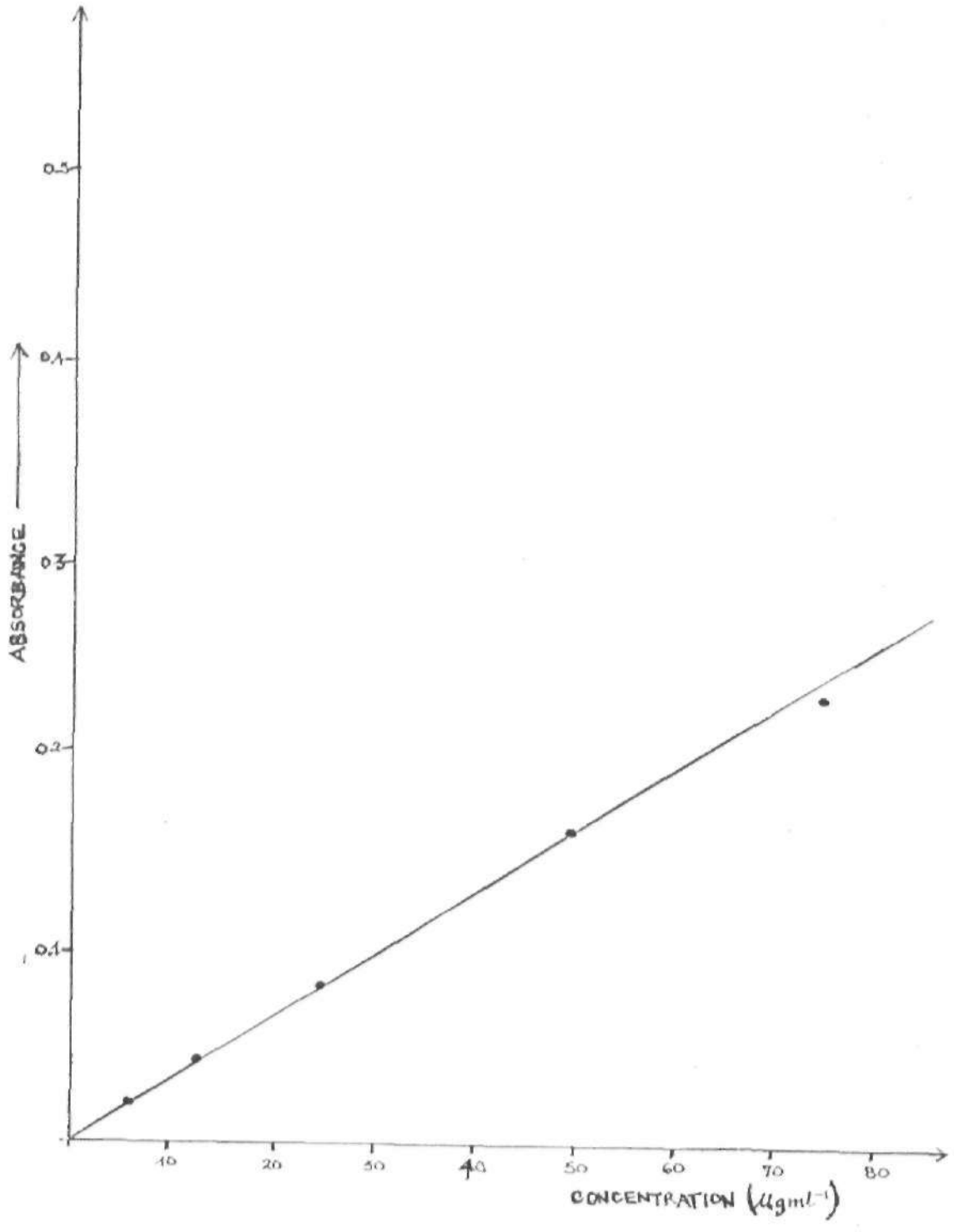
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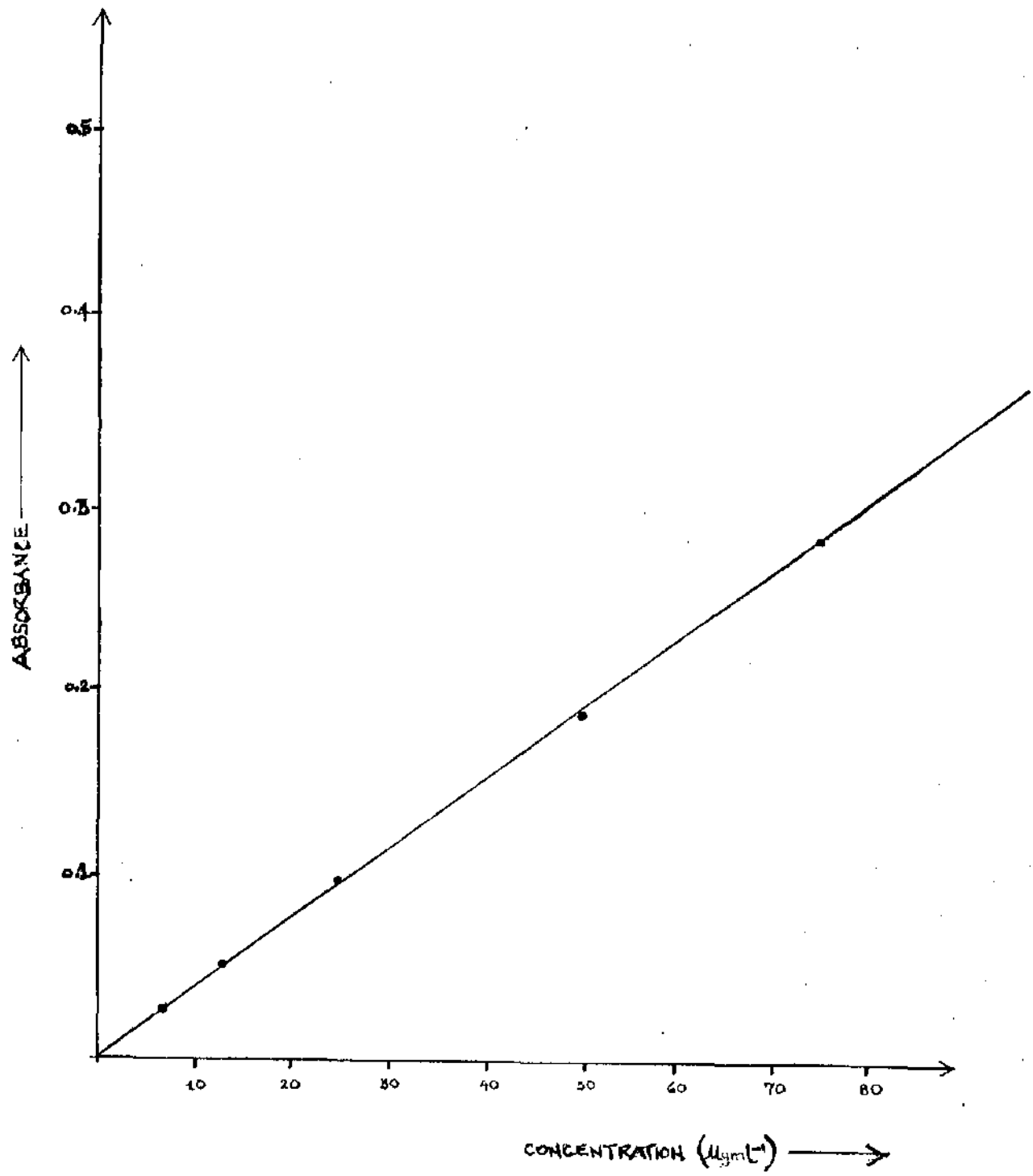
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APPENDIX 1: STANDARD CURVE FOR RAFFINOSE DETERMINATION



APPENDIX 2: STANDARD CURVE FOR STACHYOSE DETERMINATION



Appendix 3a: Effect of cold water soaking time on the mean raffinose and stachyose levels of 5 cowpea varieties

Soaking time (hrs)	α - Galactosides (g/100g)	
	raffinose	Stachyose
0	1.1940[a]	3.8780[a]
4	0.9500[b]	3.2900[b]
8	0.8700[c]	2.8850[c]
12	0.8200[c]	2.8240[c]
16	0.7100[d]	2.5140[d]

Means with the same letters in Columns are not significantly different ($P > 0.05$). $n=5$.

Appendix 3b: Effect of variety on the mean raffinose and stachyose contents during cold water soaking of 5 cowpea varieties

Variety	α - Galactosides (g/100g)	
	Raffinose	Stachyose
Sampea 4	0.9840[a]	3.3420[a]
Sampea 5	0.9100[b]	2.9840[bc]
Sampea 6	0.8800[b]	3.0890[b]
Sampea 7	0.8640[b]	3.1520[c]
Cananado	0.9060[b]	2.8240[c]

Means with the same letter in Columns are not significantly different (P> 0.05). n=5.

Appendix 4a:The effect of blanching time on the mean raffinose and stachyose levels of 5 cowpea varieties.

Soaking time (hrs)	α - Galactosides (g/100g)	
	raffinose	Stachyose
0	1.1940^{a}	3.8780^{a}
4	0.2080^{b}	0.6800^{b}
8	0.1789^{bc}	0.5880^{c}
12	0.1420^{cd}	0.5820^{c}
16	0.1280^{d}	0.5600^{c}

Means with the same letters in Columns are not significantly different (P> 0.05). n=5.

Appendix 4b: The effect of variety on the mean raffinose and stachyose contents during blanching of 5 cowpea varieties.

Variety	α - Galactosides (g/100g)	
	Raffinose	Stachyose
Sampea 4	0.3800^{a}	1.2820^{ab}
Sampea 5	0.3720^{a}	1.2040^{bc}
Sampea 6	0.3660^{a}	1.1400^{c}
Sampea 7	0.3580^{a}	1.3300^{a}
Cananado	0.3740^{a}	1.1260^{c}

Means with the same letter in Columns are not significantly different ($P > 0.05$). n=5.

Appendix 5a:The effect of cooking time on the mean raffinose and stachyose levels of 5 cowpea varieties.

Soaking time (mins)	α - Galactosides (g/100g)	
	raffinose	Stachyose
0	1.1940[a]	3.8780[a]
10	1.0020[b]	3.4180[b]
20	0.8820[c]	3.0320[c]
30	0.8320[c]	2.7720[d]
40	0.6300[d]	2.4460[e]

Means with the same letters in Columns are not significantly different (P> 0.05). n=5.

Appendix 5b: The effect of variety on the mean raffinose and stachyose contents during cooking of 5 cowpea varieties.

Variety	α - Galactosides (g100g)	
	Raffinose	Stachyose
Sampea 4	0.9980^{a}	3.3100^{a}
Sampea 5	0.9140^{b}	3.0940^{bc}
Sampea 6	0.9000^{b}	3.0100^{bc}
Sampea 7	0.8860^{b}	3.1840^{ab}
Cananado	0.8420^{b}	2.9480^{c}

Means with the same letter in Columns are not significantly different (P> 0.05). n=5.

Appendix 6a: The effect of treatment (Raw, alkali treatment, bean cake and dehulling) on the mean raffinose and stachyose levels of the cowpea varieties.

Treatment	α - Galactosides (g/100g)	
	Raffinose	Stachyose
Raw	1.194[a]	3.8780[a]
Akara	0.6200[c]	2.0480[c]
Potash	0.37600[d]	1.5040[d]
Dehulling	0.8260[b]	2.7980[b]

Means with the same letter in Columns are not significantly different ($P > 0.05$). n=5.

Appendix 6b: The effect of variety on the mean raffinose and stachyose content during different treatments of 5 cowpea varieties.

Variety	α - Galactosides (g/100g)	
	Raffinose	Stachyose
Sampea 4	0.8600[a]	2.7475[a]
Sampea 5	0.7225[b]	2.4725[bc]
Sampea 6	0.7275[b]	2.5350[bc]
Sampea 7	0.7400[b]	2.6350[ab]
Cananado	0.7200[b]	2.3950[c]

Means with the same letter in Columns are not significantly different (P> 0.05). n=5.