A STUDY OF PROCESSING AND STORAGE EFFECTS ON THE MAJOR CONSTITUENTS OF CHICKPEAS (*Cicer arietinum* L.)

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TO

THE WHOLE OF MY FAMILY

(MAY THEY REMAIN UNITED)

... "Prove thy servants I beseech thee ten days; and let them give us pulse to eat and water to drink then let our countenances be looked upon before thee and the countenances of the children that eat of the portion of the King's meat... At the end of 10 days their countenances appeared fairer and fatter in flesh than all the children which did eat the portion of the King's meat. Thus Melzar took away the portion of their meat and the wine that they should drink and gave them pulse."

Book of Daniel.

ABSTRACT

Nutritional changes in chickpeas (Cicer arietinum L.) due to various food processes and storage have been studied.

Carbohydrates, lipids and proteins were all quantified. Scanning electronmicrographs of the samples were also taken. Analyses were conducted directly after processing and after one-year storage. Results were compared to raw seed and one-year stored raw seed controls.

A batch of chickpeas was either presoaked in an aqueous salt medium or in distilled water. The medium solution effectively reduced the cooking time needed for the seeds while water-soaked seeds were used as further controls to observe any adverse effects due directly to the medium.

1. Raw seeds were decorticated and their nutrient composition determined. There was a significant loss of available carbohydrates and protein but lipids were not affected by decortication. Storage brought about further losses in available carbohydrates and protein and significant losses in lipids.

2. Samples of medium-soaked seeds were dehydrated to a moisture content of approximately 3%, resulting in available carbohydrate loss. Proteins were not significantly affected. Storage resulted in further losses in available carbohydrates. No significant losses in protein occurred but its nutritional value was reduced. There was a loss in lipids.

3. Samples of medium-soaked seeds were either immediately frozen at -18°C or precooked and then frozen at -18°C. Losses in available carbohydrates but increases in protein and lipid were noted. Further losses occurred in available carbohydrates on storage but proteins and lipids were well retained.

Precooked frozen seeds also showed losses in available carbohydrates but no apparent change was observed in protein and lipid. Further losses occurred in available carbohydrate on storage. Protein content, its nutritional value, and total lipid were significantly reduced.

4. Samples of medium-soaked seeds were precooked and canned. These cans were stored at either 22°C or 30°C. Analysis immediately after canning revealed that available carbohydrate contents were reduced, protein was increased while lipid remained unchanged. Storage at 22°C led to further losses in available carbohydrates, lowering of nutritional value of protein and highly significant loss in lipid. Storage of cans at 30°C did not cause any additional losses.

Despite the losses in certain samples mentioned there are still certain advantages offered by all the above-mentioned processing and storage conditions which will be further discussed.

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INTRODUCTION

Cicer arietinum L (chickpeas), also referred to as Bengal gram and Chana in India, Garbanzo beans in North and South America, and chickpeas in England is a member of the Leguminoseae.

Chickpeas were sub-divided into four races: orientale, asiaticum, mediterraneum, and eurasiaticum (MAESON, 1972). Although this classification may have taxonomic merit, a less precise classification, but more practical for food scientists, is to divide the species into two broad groups (AUCKLAND, 1976). These are:

a) Those producing large seeds (more than 26g per 100 seeds) which are approximately rounded and pale cream in colour. The plants are relatively tall with white flowers and no anthocyanin pigmentation. These are found in the Mediterranean area, Near East, Central and South America, and cultivated on a small scale in the Indian Subcontinent where they are referred to as the 'Kabuli' (possibly place of origin) type. They contribute 10→15% of the world's production.

b) Those of smaller irregular-shaped seeds of various colours. The plants are short with smaller leaflets, sometimes prostrate. The colour of the flowers and stems are due mostly to anthocyanin pigments. Characteristic of East Asia, Ethiopia, parts of Iran and Afghanistan and the Indian subcontinent where they are known as 'Desi' (indigenous) types.

Chickpea is the World's third pulse crop, fifth legume crop, and fifteenth seed crop (FAO, 1972). Total world area under the crop is estimated as 10.54 million hectares and it is grown in

31 countries. India has nearly 74% of the world acreage and total production. Other countries where the crop is important are (in descending order) Pakistan, Ethiopia, Mexico, Burma, Spain, Morocco, Turkey, Iran, and Tanzania. Most of the crop is consumed locally and the export trade is minimal.

These production figures are being offset partly by the 'Green Revolution'. The latter refers to the recent rapid rise in yields and production of wheat, rice, and maize in a number of developing countries. These increases have resulted from development of high-yielding crop varieties that have showed spectacular improvements of growth. Unfortunately, the pulse production is on the decline as a consequence. This is undesirable nutritionally due to drop in protein output; protein in pulses are higher than in cereals.

Legumes have direct beneficial effects in agriculture as well. The latter are symbionts of bacteria that form root nodules. These take free nitrogen from air and 'fix' it in plant root tissue. This increases directly the level of soil nitrogen and this in turn causes increase in yields of cereals that follow legumes in plant rotation. This rotation procedure also decreases weed, disease and insect problems.

Chickpeas are important sources of protein and calories in India, Africa, Mexico, Central and South America. It is often regarded as the 'Poor man's Food'. It is worth noting that a crop comparatively inexpensive to grow with a relatively high protein content and which helps to support whole populations of low-income groups in underdeveloped countries is so ignored in the Western nations. The role they play in the diets of many developed countries appears to be limited by their present low yields, their consequent cost, and certain defects in their nutritional and food use quality. The Protein Advisory group (PAG) of the United Nations recommends urgent research attention to these legumes (MILNER, 1972).

In different parts of the World chickpeas may be eaten raw as immature green seeds or as cooked or milled dried pulses. The seeds may be parched, or roasted over open fires in metal pans or on hot sand. In India probably more than 75% of the chickpea production is milled to produce 'dhal' (Indian soup-like dish). In several Middle Eastern countries milled chickpeas are mixed with wheat and other cereal flours to make a variety of fermented breads and sweet breads, in addition to being used together with meat, vegetables, and spices in many delicious dishes.

Although the West have provisions to fulfil the minima of nutritional needs they have discovered that this does not have optimum conditions for health and longevity. More and more advisory committees have published guidelines for modification in their food pattern in order to avoid diseases of affluence such as atherosclerosis, diabetes, and obesity. People are becoming more aware of their nutritional needs for vegetable proteins (COWEN, 1979).

The marked increase in the trend to vegetarianism (HARRIS, 1975) among certain segments of the world's population may be an indication of things to come. These changes may have dramatic health repercussions and may necessitate changes in food processing techniques. With increased emphasis on energy conservation it is very likely that a major change will be observed in the nature of dietary habits. This stems partly from the much greater cost in terms of energy and water to produce animal foods in comparison to plant foods used directly by man.

Efforts in trying to increase the popularity of the chick-pea may be short-lived because of the improper storage conditions that cause 'hard-shell' (toughening of seed coat) in the grain, so decreasing cooking quality. In a recent meeting in El Salvador, farmers complained about poor storage stability of the chickpea in their country (PAG, 1972).

It is well accepted that legume foods become hard and difficult to cook when stored for long periods of time. In the past farmers did not attempt to increase yield because as the stored grain became hard its value decreased and losses increased. If yield is to increase, the problem of storage will become important. Therefore, studies of this problem should be undertaken to help increase chickpea production and availability.

The problem of hardened seeds can be controlled by using appropriate storage conditions or by processing. Processed chickpea can also serve as a vehicle for other nutrients.

When compared to cereal grains, there are very few processing techniques applied to beans to increase their uses. Research should be carried out to diversify processing forms and uses. In addition, if legume foods are processed the frequency and amount eaten daily may increase.

The acceptability of a food after processing is also an essential factor that must be considered (BOMBAL, 1974). This includes properties in the food of good texture and consistency of cooked product, colour, taste and flavour, cooking time, storage capacity, and cost.

Cooking time is important because of the excess amount of time and fuel used. Legumes take a considerably longer time for cooking than any other vegetable product. Cooking time outrates all other preferences in the case of legumes. It would not be rash to state that one of the drawbacks in popularising the soybean was the long cooking time it took to prepare (PUSHPAMMA & SUDERSHAN, 1972).

In the USA, where peas (Pisum sativum) and beans (Phaseolus vulgaris) are used, the per capita consumption is reported to be on the decline (ROCKLAND and METZLER, 1967). This has

been attributed to the prolonged preparation time involved.

The food processing method used should take into account the need for the preparation of stable, pre-cooked products. Some of the home processes can be adapted to industrial scale where conditions may be set up to preserve and improve nutritive value. The possibility for the increased use of legume foods deserves further research.

Processes have been used for chickpeas which reduce their cooking time also preserving texture, consistency, colour, taste, and helping towards the acceptance of the product to the consumer (ROCKLAND et al, 1973)

The purpose of this present study was to investigate some of these processes fully and to determine the effects these have on the major constituents of chickpeas. In this context the major constituents studied were carbohydrates, proteins, and lipids.

Extensive nutritional studies have so far been carried out on chickpeas. However, very few detailed or in-depth biochemical investigations on this important pulse have been carried out to date (KRISHNAMURTI, 1975).

The present study will furthermore attempt to remedy this.

CHAPTER 1

SIGNIFICANCE & USE OF FOOD PROCESSING METHODS

a) Introduction

Ever since the [illegible] the place held by processed foods in the diet has been increasing steadily. Our meals rely more on foods which have been subjected to some sort of industrial operation or commercial long-range storage. The impact of industrial processing is increasing not only in width but also in depth (BERK, 1970).

Food processing has five main objectives; improvement of nutritional value, improvement of sensory acceptability, cost reduction of food, improvement of convenience for the consumer, increase shelf life of product. The primary factor, however, is the one for preservation. With the high concentration of urban population, an adequate amount of food cannot be supplied in the great variety encountered at present without preservation techniques.

There is a strong belief that if foods are cooked fresh no nutrients are lost. If the same food passes through a food processor these are destroyed. There are controversial views on this matter. For a better understanding all the reasons for food processing will need to be considered in detail.

The term 'processing' covers enormous fields of differing treatments. Losses resulting could be intentional as encountered when cereals are being milled to remove unwanted bran, when vegetables are trimmed, when fish is eviscerated, or foodstuff extracted from raw material. Inevitable losses occur when food is cooked. The amount is dependent on the

susceptibility of the nutrient to the type of cooking employed and the care used to minimise the loss. The accidental losses are due to inadequate control of processing and storage. If processing is not carried out under standardised conditions it may contribute to the variability in nutrients reported in food.

When considering these points certain principles need to be borne in mind.

(i) Some losses are inevitable. Processing is carried out for a variety of purposes including preservation, improvement of palatability, texture, improving eating properties, creation of new products, removal of inedible parts, destruction of toxins. These involve application of heat and water both of which provide a means for nutrient loss.

(ii) Manufacturing processing sometimes involve partial or complete cooking so factory losses simply replace those that would inevitably take place at home.

(iii) One must also account for the fact that the term 'fresh' is not always explicit. The nutrients of a food are dependent on the initial nutrient content and the losses that may be incurred between harvesting and processing. Part of these losses may have occurred on transport, and by instantly processing food before transporting it, huge losses in nutrients may be avoided.

(iv) Advantage of enrichment with supplements may be gained or simply the availability of a nutrient may be increased by destroying a toxic substance present.

(v) Finally, there is not always a choice between fresh and processed food as crops may be seasonal. It is often a choice between processed food and none at all.

Interest in the nutrient content of foods has significantly increased recently, partly due to the requirements of the U.S. Federal Department of Agriculture (FDA) (NESHEIM, 1974).

b) Processing Methods

Foods are subjected to vast numbers of processes. More emphasis was placed on the following methods of processing; dehydration, freezing, canning, cooking and blanching.

Each method has its own advantageous or disadvantageous effect on any particular food. The greatest loss is generally due to extraction into water during blanching and by heat damage. Conditions for maximum nutrient retention are, however, not always the same as those conferring maximum palatability.

Pasteurisation and sterilisation are the more common methods used. They lengthen shelf-life of a product but may spoil texture, colour, flavour and nutrient value.

Blanching is sometimes an essential preliminary before freezing, drying or canning. Here, foods are subjected to increased temperatures for a few minutes. This serves to inactivate enzymes, decrease volume of food prior to canning, expel gases which may increase the pressure in cans, maintain colourand clean product.

Attention is mainly focussed, in this study, around the processes of dehydrating, freezing, canning, and cooking.

1. Drying:

Most of the modern processes of dehydration consist of the removal of moisture from the food by careful application of heat, usually in the presence of a controlled flow of air, or under conditions of reduced pressure. Improvements in these techniques have greatly improved nutritive value and acceptability of the products. This has been mainly due to a better understanding of the time and temperature tolerances of particular foodstuffs and vast improvements in methods of heat transfer.

When devising a drying process applicable to any food produce, certain factors need to be kept in mind.

(i) Drastic changes in the physical properties of the cell must be avoided. When a dried vegetable is put into water the walls absorb water and soften. Owing to their natural elasticity they tend to return to their original shape.

The elasticity of the cell wall is reduced by heat treatment whether during drying or storage at elevated temperatures.

(ii) Undue heating during the early stages of drying must be avoided as over-cooking would result, leading to disintegration on reconstitution.

(iii) The drying temperature must not be so low as to allow the growth of bacteria.

(iv) The high costs of lowering the moisture content to too low a level, must also be taken into consideration.

Protein constituents are affected by heating but quality is maintained if the temperature of the food is not increased over 100 ^{o}C. After drying

subsequent loss of protein quality may be minimised if there is cool storage and the moisture is kept below 5%.

A dried food containing 3-6% of water and in equilibrium with 10-30% relative humidity is unstable and hygroscopic. During storage,losses of flavour may occur together with a slow increase in toughness and decrease in rehydration. Brown pigments may also be produced. These changes are decreased by storage at a lower water content and at low temperature. Problem is that rancidity is increased at low water content.

In a general way it may be said the various deteriorations that occur during drying continue during storage at a rate controlled by the moisture content and temperature.

In the storage of dried foods the small changes of water content may influence considerably the admissible storage time. Changes of this kind will be very limited when the 'free water' (free for evaporation) content is sufficiently low or has been removed completely. This has led to industrial processes in which a part of the 'free water' has been transformed into 'bound water' (bound against dehydration) by addition of water-binding substances (e.g. sugars and glycerol) which act in some way as dehydrating agents.

It was postulated, that as long as 'bound water' remained, no irreversible reactions would occur (KUPRIANOFF, 1976). Optimum water content must not be identified with bound water content.

2. Freezing Methods:

Freezing is the best method of preservation from an organoleptic and nutritional point of view. The temperature of storage, however, must be a compromise between optimum and practical and is usually $-18^{o}C$ (BENDER, 1978). Losses will also depend on the rate of thawing of food prior to cooking.

Since a primary purpose of food processing is preservation for later consumption, the effects of length and conditions of storage are important considerations in the nutrient content of foods as they are consumed.

In the case of quick-frozen foods, the storage damage is much more severe than loss of nutritional value during processing itself. KRAMER (1974) has extensively reviewed the influence of storage temperatures on nutrient retention. Storage at $-5^{o}C$ will usually maintain sensory quality of most frozen foods. Recent work has shown that for most produce, storage at $-18^{o}C$ provided good protection against nutrient lossfor a year (KRAMER et al, 1976).

When food is frozen slowly the moisture in it tends to form large ice crystals, which lacerate the delicate tissues (plant or animal), so that when the food is thawed again, intracellular nutrients can drain away with the moisture. Quick-freezing of chickpeas does not allow the build up of large ice crystals and consequently cell damage does not result. When the food is thawed it resumes its original condition without significant loss of nutrients.

The most critical stage, the zone of crystallization must be passed through as quickly as possible.

3. Canning:

Canned foods have become a major factor in the food industry, constituting a sizeable and important portion of the diet. They are stable, not requiring refrigeration, and are easily transported. This makes it possible for the consuming public to have a varied and nutritious diet at all seasons of the year and in all geographical locations. There is a tremendous potential for a growth of the industry in all parts of the world.

Food canning is the procedure for preserving food by the combination of sealing in an hermetic container and heating

to destroy spoilage and pathogenic microorganisms and to inactivate enzymes.

Canned products usually are regarded to have an indefinite shelf-life in terms of the microbiological point of view. It is frequently assumed that the nutrient content of food is also relatively stable following canning. The latter statement is not strictly true since there is a decline in organoleptic and nutritional properties during storage due to various chemical reactions.

Reasons for this could be due to the leaching of nutrients into the liquor, slow chemical destruction during storage, or uptake of metals from the can itself. These may be reduced or delayed by low temperature storage.

4. Extra treatments in Processing;

A large number of studies have been carried out on food treatments which involve pre-soaking and pre-cooking processes.

Wheat, rye, and other cereal grains have been hydrated in aqueous media by ROCKLAND (1970) prior to drying treatments.

Quick-cooking rice has been prepared by soaking in an aqueous medium containing an edible oil and a surface active agent. The rice is steamed to gelatinise it, after which it is air dried before being expanded (TAKEDA CHEM. INC., 1970). Related conditions for rice have also been used by SLOWINSKI et al (1970).

Quick-cooking soybean products (ROCKLAND, 1972), lima beans small white beans, and peas (ROCKLAND & METZLER, 1967) have been prepared, combining the pre-soaking (in an aqueous medium) with pre cooking for a standardised time period before packing.

The beneficial effects of water soaking and chemical treatments were possibly due to leaching of toxic metabolites from the seeds, antifungal and anti-catabolic effects of the treatments (BASU et al, 1974; BASU & DAS-GUPTA, 1974).

More recent studies on various grain seeds show that hydration followed by drying back, significantly prolonged the viability of seeds under ambient ageing conditions (BASU et al, 1975). They also suggest that the beneficial effects cannot be interpreted solely on the basis of leaching out of toxic substances from the seeds. Several combinations of pre-soaking aqueous media have also been used by SIDDAPPA (1959) prior to canning.

ROCKLAND et al (1975) have stated that additives to the aqueous pre-soaking medium not only act as alkaline agents, but also act as protein dissociating, solubilising or tenderising agents.

c) Processing Implications to Chickpeas

Food grains and pulses, undergo substantial qualitative and quantitative losses as a result of rodent and insect infestation and by the infection of fungi during post-harvest handling, storage, and distribution. Such losses are particularly heavy in India, where more than 70% of the food grain produced is stored and consumed in villages under very inadequate conditions for controlling infestation and infection.

Chickpeas stored in overground structures are attacked by the pulse beetle (Bruchus chinensis) which causes heavy destruction of the seeds by creating favourable conditions for microbiological infection. The pulse beetle passes through 8 overlapping generations in one year. The progeny of 50 eggs can reduce the weight of seeds by about 49% in just 6 months. Seeds infested by these pests are contaminated with metabolites, such as uric acid, and show increased acidity becoming unfit for human consumption in a short time (RAO, 1960). The milling yields of the legume are also decreased as a result. These

beetles can be killed in all stages by heating the peas for varying periods at 45-55°C. It is also possible to disinfect all seeds by solar heating with the diurnal temperature varying from 37-54°C. There is no impairment to germination under these conditions (MOOKHERJEE et al 1968).

Heat treatments are also required to prepare chickpea seeds for food use due to the content of mildly toxic and anti-nutritional factors.

Whole chickpea seeds are normally soaked in fresh water for up to 24 hours to allow them to rehydrate, during which time the first stages of germination have been initiated, and then are generally cooked in fresh boiling water until tender. The cooking time range is dependent on variety, origin, age, moisture content of seed, and hardness of water used for soaking (ROCKLAND & NISHI, 1979).

Cooking removes or inactivates heat labile anti-nutrients and permits more efficient digestion and assimilation of protein and starch which are the main nutrients of legume seeds.

In industrial countries, canning has become one of the most effective methods for processing and preserving legume seeds in a convenient form.

In the Far East, problems of slow rehydration and cooking have been circumvented by milling the dry seeds to remove seed coats which retard water imbibition. In the absence of seed coats, split seeds or 'dhal' rehydrate and cook more quickly in boiling water.

The convenience and utility of the 'dhals' are offset, in part, by milling losses which range from 15-25% of the dry seed weight. These losses include both mechanical losses during milling as well as loss of seed coats which constitute generally 11% of the original seed weight.

It is estimated that an 8% increase in food legume yields by improved processing technology would increase available supply of milled pulses by 800,000 tonnes, containing 240,000 tonnes of crude protein, having a value of $290 million (KURIEN, 1977). Seed coats also contain a high percentage of fibre hence 'dhals' and flourspreparedfrom milled seeds contain very little fibrous material.

There are numerous investigations in progress to determine the importance of natural fibre in human health and nutrition (COLMEY, 1978). If these studies corroborate current findings and projections concerning the importance of natural fibre in human diets, traditional Eastern practices of decorticating legume seeds to increase hydration and cooking may be counter productive (ROCKLAND & NISHI, 1979).

A new processing technology has evolved during a twenty year period of basic and applied research on physical, chemical and biological properties of legumes. This involved the introduction of Quick-cooking characteristics to legume seeds. It may have some unique application in extending utilisation of tropical grains and legumes for human food (ROCKLAND, 1978).

The main principle of this technique requires the loosening of the seed coats by either a brief blanching in steam or boiling water. This is followed by soaking the whole seeds for 1 - 24 hours, depending on conditions, in a dilute solution of food-grade salts. The seeds are then drained to remove excess solution. Pre-cooking is sometimes carried out. The product is then ready to be processed.

The processed whole legume requires only 10-15% of the time required to cook analogous, standard water-soaked seeds. This reduction in absolute cooking time is dependent on the characteristics of the seeds themselves e.g. hard shells. The processed, cooked products have been said to have an excellent

integral whole bean appearance, an increased natural flavour and a smooth creamy texture (ROCKLAND et al, 1975). These may be used as boiled beans, winter vegetable, ingredients in soups and salads, or combined with other ingredients for the preparation of traditional casserole - type foods.

The use of quick-cooking legumes can effect a significant decrease in energy requirements, especially in the fuel-poor, less developed countires which utilise grain legumes as primary protein calories.

Some of the above principles and guidelines concerning the drying, freezing, canning and other treatments to food have been incorporated into this study on chickpeas.

d) Scheme of Research

In legumes the carbohydrates, which make up most of the pulse grains have been more or less neglected. It may be argued that legumes are primarily a protein crop and that carbohydrates will come from cereals. In cereals, however, emphasis is once again being placed almost entirely on protein and amino acids (SHELDRAKE, 1972).
This is a natural consequence of thinking that the major nutritional problem is not one of calorie supply but primarily of protein as a limiting factor. In the under-developed countries this is not always true and a change may have to be made to this basic assumption (SHELDRAKE, 1972).

The success achieved in the treatment of kwashiokor (CHAUDHURI, 1960) by using chickpeas has lately stimulated an interest in its therapeutic effectiveness in diabetes (KHANRA et al, 1963) and heart disease (MATHUR et al, 1961). The relationship to heart disease has been attributed largely to the lipid compositon of chickpea.

Therefore, in the present study not only will carbohydrates and proteins be investigated but also the lipid composition of this

important Asian pulse.

(var. - RABAT)

Chickpeas of the 'Kubuli' type grown in Turkey were purchased from [illegible] market. These seeds are generally solar dried after harvesting prior to packing into plastic bags for the export market. The seeds were sorted and separated from stones and other contaminants before putting them aside to form the main 'reservoir of raw seeds' of experimentation.

An outline of the procedures that have been adopted is shown in Fig 1. From the main reservoir of raw seeds a batch was either presoaked in distilled water ('X' in Fig 1) or in an aqueous salt medium ('Y' in Fig 1). The final processes consisted of refrigeration, freezing, drying, and canning. Samples from 'X' and 'Y' were put through the final processes either directly or after precooking (Fig 1).

The canned samples were stored either at 22°C or at 30°C for one year.

From the raw seeds a third batch was taken ('Z' in Fig 1) in which the seeds were directly decorticated to simulate the milling process.

Samples were analysed immediately after processing and also after one year's storage.

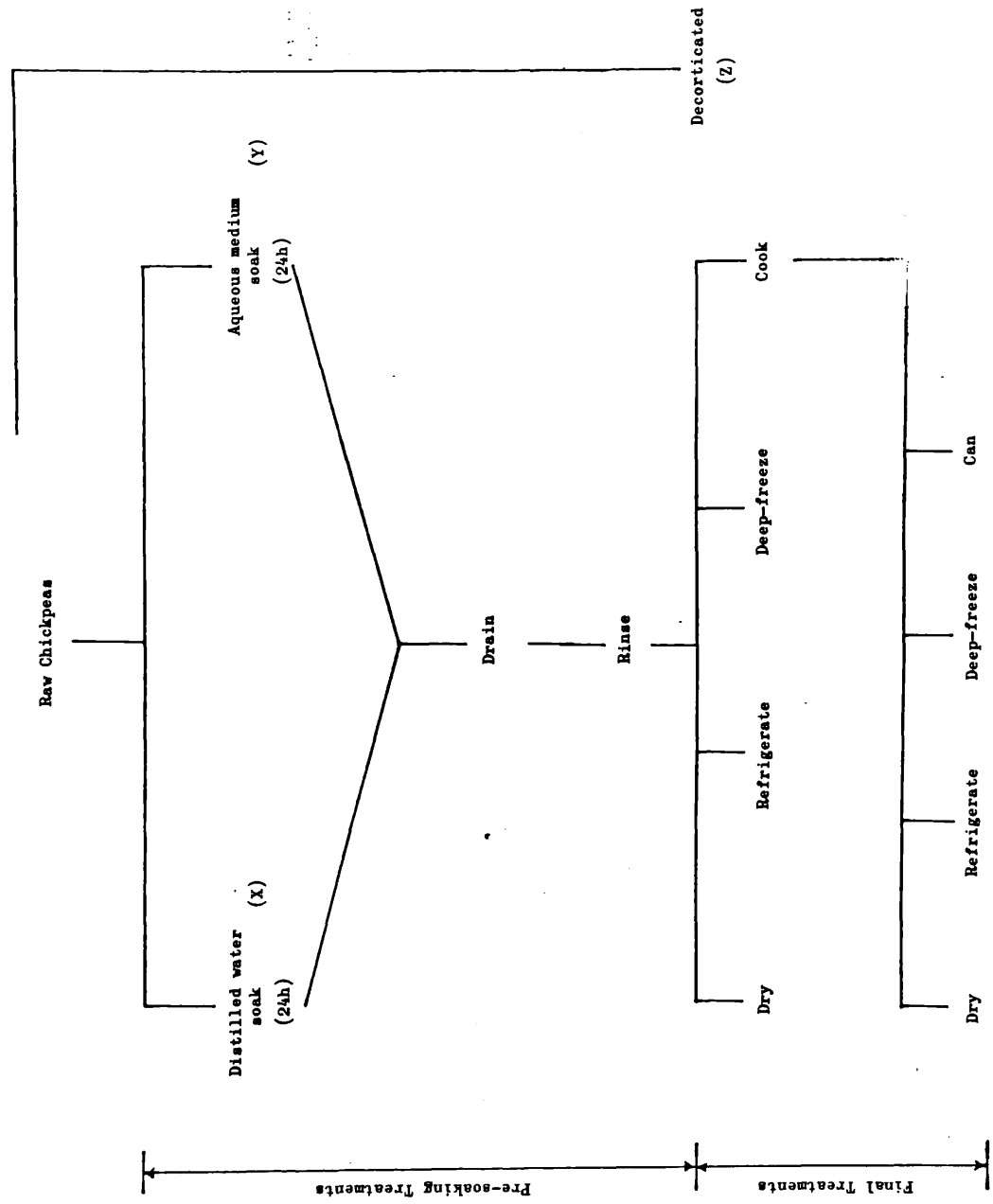


Fig 1. SEQUENCE OF PROCESSING OPERATIONS AS MODIFIED FROM ROCKLAND (1978)

e) Presoaking & Cooking of Chickpeas

The procedure for the hydration of chickpeas was as follows:

Two samples of 4kg each were used. One was soaked for 24 h in distilled water at ambient temperature and atmospheric pressure. The second was treated by soaking in a mixture of 2% sodium chloride (NaCl), 0.375% sodium bicarbonate ($NaHCO_3$), and 0.125% sodium carbonate (Na_2CO_3) solution (w/v) for 24 h under the same conditions as above (ROCKLAND et al, 1975). The latter will be referred to as medium soaked seeds. The seeds were then removed and drained in both cases.

The medium soaked seeds were rinsed in distilled water to remove excess salts. The washing was kept to a minimum to avoid removing more than just the agents adhering to the surface. Otherwise, the seeds would have lost their quick-cooking properties.

Samples (2kg) from each of the treatments were removed and cooked separately in hot water for 13 min at a temperature of $90^{o}C$. These were then drained and ready for final processing.

f) Dehydration (drying) of Chickpeas

The drying procedure for chickpeas was determined as follows:

A sample of raw seeds was given an initial soaking for 24 h. Drying of the hydrated seeds was carried out in a cross-flow dryer at 50°C with an air velocity of 170 – 200 m min^{-1} and approximately 6kg m^{-2} tray loading.

Samples were removed every hour to determine moisture content using a 'Marconi' moisture meter. The results are shown in appendix 1 (p 226). An 18 h time period gives a moisture level of 3% in the seeds.

Two hundred and fifty grammes each of uncooked water and medium soaked seeds were dried at 50°C for 18 h. These were then stored in separate plastic screw-capped bottles at a temperature of 10°C.

g) Refrigeration & Freezing of Chickpeas

After soaking and cooking of the seeds, 4 treatments (2kg each) are produced. These include water-and medium - soaked seeds which were either cooked or uncooked.

Duplicates of 250g samples of each treatment were transferred into 8 separate plastic screw-capped containers.

One set was placed under refrigeration at 4°C. The other set was placed on the lowest shelf of the deep-freeze pre-set at -18°C. The temperature drop to -18°C was accomplished as rapidly as possible in order to avoid the formation of ice crystals within the seeds.

h) Canning of Chickpeas

A general outline for the canning process is shown in Fig 2.

Equal weights of samples from both water and medium-soaked cooked seeds were used.

A 1 lacquered (sulphur resistant finish) cans were filled with the two treated seed samples. To each can containing 170g chickpeas, 135g brine solution was added giving a total fill of 305g. The brine solution consisted of 2% NaCl and 0.1% Citric acid (w/v). A total of 16 cans were filled.

The 'exhausting' step was not needed as the can closing machine was fitted with a 'steam - flow close' device to remove air from the head space of the can immediately prior to closing.

The cans were then cooled rapidly in cold water to prevent the growth of any thermophile bacteria.

All the cans were labelled appropriately. Four cans each of medium - and water - treated seeds were stored at 22°C while the others were stored at 30°C.

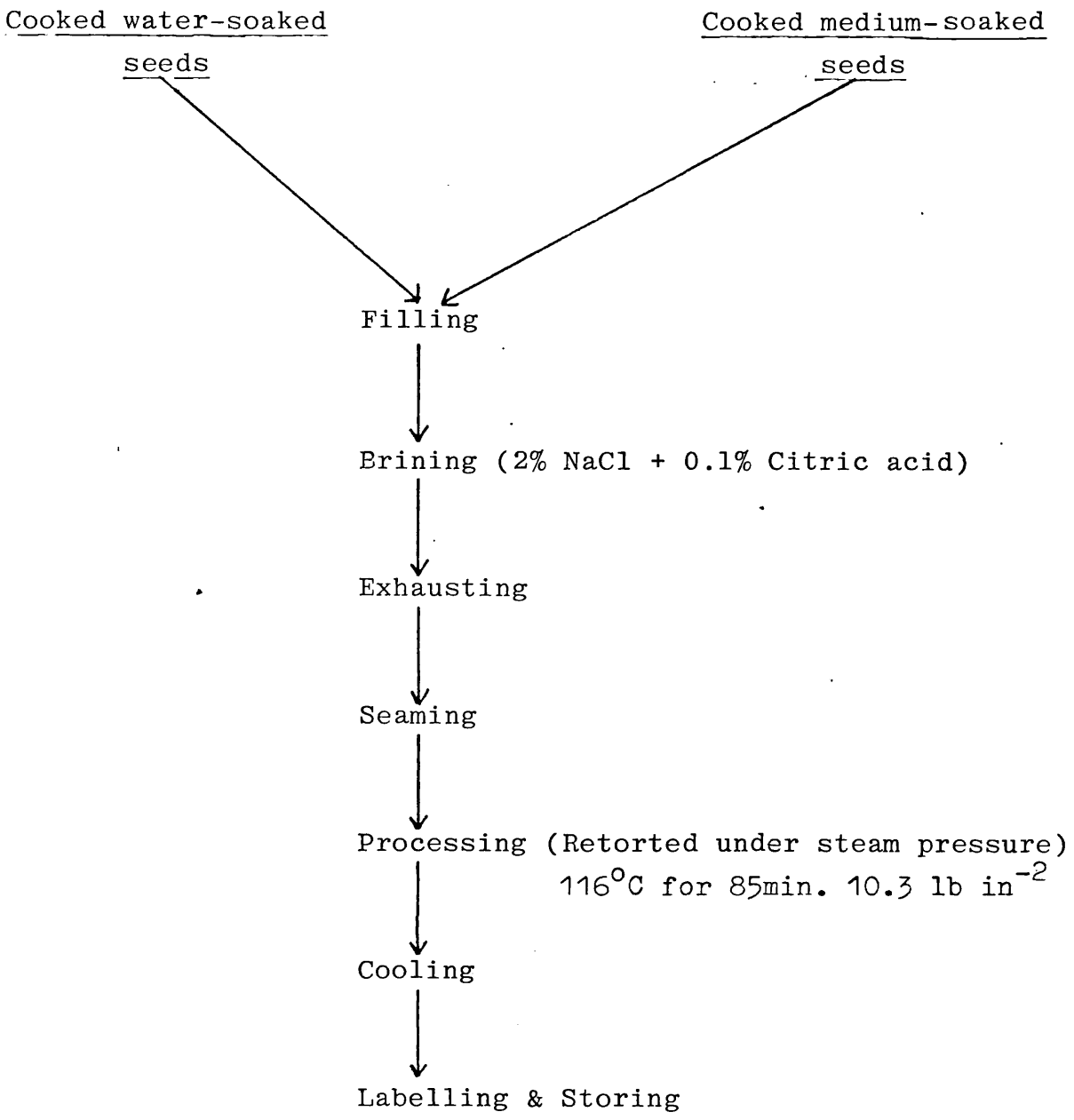


Fig 2.

Flow diagram for the Canning of chickpeas

(Supplied from Metal Box Ltd)

C H A P T E R II

PRELIMINARY INVESTIGATIONS OF RAW & PROCESSED CHICKPEAS

Introduction

Preliminary data of the raw seeds and those under various processing conditions were obtained especially as regards their viability and their dimensions. The cooking times, moisture contents, and appearance of the seeds were also established.

The different samples used are given below and represent those in Fig 1 (p 35). The abbreviations given will denote the stated samples throughout the rest of the study. A suffix '-S' will be added to the abbreviations of some samples to denote one year storage of the respective sample. A suffix '22' or '30' is added to denote the storage temperatures for the canned seeds.

1 - Raw seeds (control)
2 - Decorticated (milled) seeds — De
3 - Medium hydrated dried seeds — D
4 - Frozen water hydrated uncooked seeds — WF
4 - Frozen medium hydrated uncooked seeds — MF
4 - Frozen water hydrated cooked seeds — WPF
4 - Frozen medium hydrated cooked seeds — MPF
5 - Water hydrated canned seeds — WC
5 - Medium hydrated canned seeds — MC

Tests were not carried out on refrigerated seeds as they were found to be contaminated by bacteria and fungi. The water soaked, dried processed samples showed a wrinkled appearance

on reconstitution in cooking water. This factor reduced the attractiveness of the (otherwise whole-bean appearance) chickpeas seeds. These processed samples were therefore discarded.

Determining the viability of raw Chickpeas

Seeds for growing must be able to germinate almost 100% and produce rigorous seedlings in the fields whereas seeds for processing may only be required to be [illegible] and free from contaminants.

In practice, the latter requirement bears a close relationship with the maintenance of a reasonably high germinative capacity, although the seeds may not be capable of successful seedling establishment (DUFFUS & SLAUGHTER, 1980).

This idea has been supported by studies which have shown that the amount of material leached from seeds is inversely proportional to the viability of the seeds (ROBERTS, 1972).

Procedure:

Unbroken seeds were washed and soaked in five times their volume of water for 5h at room temperature in a dark cupboard. By the end of the soaking time seeds were soft & some broken testas were observed. Cellulose sponge sheets, 3cm thick (FORDHAM et al, 1975), boiled in water for 10 min were placed in sterilised plastic trays (100cm x 90cm) and allowed to cool to room temperature. The soaked seeds were washed thoroughly with water, placed in a layer over the sponge and water was poured to half the height of the sponge (1.5cm) to provide moisture during germination. The trays were then covered with aluminium foil and held at 25°C in a dark cupboard for 48 h.

The advantage of using this method was that the moisture content could be regulated, whereas with other growing media, e.g. Cheesecloth (CHEN, 1970) or paper towels (COULTER, 1972), the seeds needed to be washed every 4h to prevent the growth of moulds.

Determining moisture content in chickpeas

Determination of moisture is one of the most important analytical measurements in the processing and testing of food index of stability and quality and also a measure of yields and quantity of food solids.

Relative humidity of the food, nutrients provided by the food, the types of microbes present, length and temperature of storage are the main factors that control microbial growth.

Moisture content must be known accurately in the evaluation of processing losses, estimated yields and in determining whether or not the product meets the requirements of the pertinent standard of marketing restriction.

Procedure:

A known weight of seeds (20g) were accurately weighed and put into a tared moisture dish and left in a drying oven at $70^{o}C \pm 3^{o}C$ for 8h. The dish was then removed from the oven and cooled to room temperature in a dessicator and weighed again. The procedure was repeated until a constant weight was obtained. (AOCS, 1956).

Calculation

The moisture content of the seeds was calculated by using the following formula.

$$\text{Moisture \% of seeds} = \frac{\text{Loss in weight of seeds} \times 100}{\text{Original weight of seeds}}$$

Determining the weight of a hundred chickpeas

The samples of chickpea were examined and any foreign matter was removed before analysis. The seeds were then weighed (app. 80g) and the number of individual seeds within this portion were counted.

The weight of one hundred seeds was calculated using the formula:

$$\text{Weight of 100 seeds (g)} = \frac{\text{Weight of seeds(g)}}{\text{Number of seeds}} \times 100$$

Determining the cooking time needed for raw and processed chickpeas

Samples of chickpeas (30g) were placed in separate metal cooking pans containing boiling water and heated on a gas stove on maximum flame.

The time taken to bring the seeds to an edible state was noted. This referred to the stage when all the seeds in a sample were soft enough for a metal needle to be pierced through them with ease.

The dried and decorticated chickpeas were hydrated in water for 6h prior to cooking.

Results & discussion

Appearance and cooking times of raw and processed chickpeas

Considerable expansion of size in canned and frozen chickpeas was observed. This was due to the high amount of water absorption during the presoaking treatments. A pictorial representation of raw and processed chickpeas are given in Fig 3.

The original raw seed was cream to light brown in colour.

The frozen uncooked seeds showed a more yellow tint, the water-soaked ones being slightly paler than the medium-soaked ones. The medium may have aided in preserving more of the original colour of the raw seeds.

On cooking, however, a slightly paler seed was obtained in both medium-and water treated frozen samples. The seed coat in the cooked samples was softer in texture when compared to the uncooked ones. This was probably due to the weakening of the cell walls in the cooked samples due to heating.

The canned chickpeas all showed a light brown to brown colouration but were darker than the original raw seeds. The seeds were softer than the cooked frozen samples and were also of a more creamy texture. Splitting of the seeds between the cotyledons had occurred in some cases but in general most of the seeds were intact and whole in appearance. The brine solution in the cans was slightly coloured.

From results in table 1, in general, it can be said that soaking of chickpeas bring a reduction in their cooking times.

Decorticated seeds show a significant reduction in cooking time when compared to raw seeds. This is due to two main reasons.

Fig 3.

Raw and processed Chickpea seeds used in this study.

A = Raw

B = Medium-soaked dried

C = Water-soaked frozen

D = Medium-soaked frozen

E = Water-soaked precooked frozen

F = Medium-soaked precooked frozen

G = Water-soaked canned

H = Medium-soaked canned

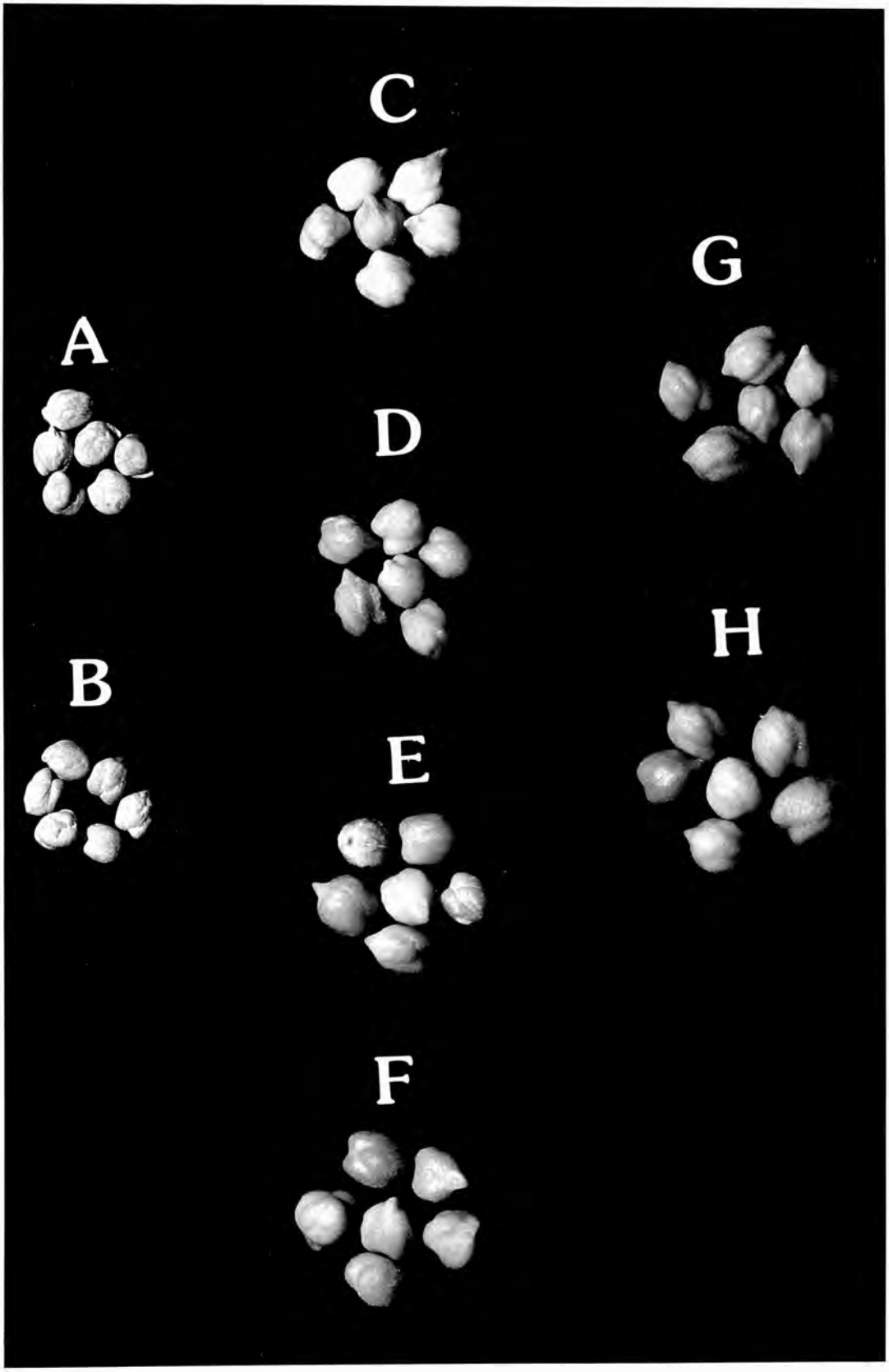

A
B
C
D
E
F
G
H

TABLE 1 Cooking times for raw and processed chickpeas. Results are expressed in min ± standard error (nearest 0.5 min).

SAMPLE	COOKING TIME (MIN)
Raw seed	190 ± 20.0
De*	120 ± 15.0
D*	15 ± 2.5
WF	135 ± 10.0
MF	11 ± 1.0
WPF	30 ± 10.0
MPF	4 ± 1.5
WC	30 ± 15.0
MC	4 ± 1.5

* Suspended in cooking water for 6 hours prior to cooking.

The hydration step has aided in softening the cell walls of the seeds hence reducing the cooking time needed to fully tenderise the skin.

The testa prevents rapid entry of water into the cotyledons hence most of the water enters through the micropyle which itself allows a limited rate of entry. In milling, the testa is removed. As a consequence, moisture can penetrate more easily and rapidly.

The effect of the medium solution on the cooking time of dehydrated seeds is quite pronounced. They show an average reduction of 105 min cooking time when compared to decorticated seeds. This indicates that the medium itself not only allows a means of water entry into the seeds but also must affect it by other means. Sodium chloride is known to act as a tenderising agent (ROCKLAND, 1972). This compound in the medium solution probably has the principal effect of softening the testa and proteinaceous components of the cotyledons. Sodium chloride is aided by carbonate and bicarbonate of sodium which also have protein dissociating, solubilising, and tenderising properties. These components in the medium contribute largely to the goal of attaining a product that appears to be quick-cooking only needing about 15 min cooking time.

This quick-cooking characteristic is further demonstrated when comparing water-and medium-soaked frozen chickpeas where the medium-soaked seeds showed an average reduction of 124 min, cooking time.

Pre-cooking for 13 min at 90°C (p 36) has further decreased the final cooking time needed; 30 min for water-soaked and 4 min needed for medium-soaked seeds.

Water-soaked canned seeds show similar cooking times to water soaked pre-cooked frozen chickpeas. The same applies to medium-soaked canned seeds and medium-soaked frozen chickpeas.

These overall results indicate that soaking chickpeas in medium containing certain sodium salts in the specific quantities indicated helps in reducing the overall time needed to cook them. This is especially important in food deficient areas which also suffer from deficiencies in fuels. Consequently, this medium treatment not only offers a means of saving cooking time but also of saving significant quantities of fuel which would have otherwise been used. This property is maintained even after 1 yr. storage of processed chickpeas.

Viability, moisture content & weight of chickpeas

The raw seeds showed approximately 95% germination. This indicates that most of the seeds were still viable prior to processing. It could be assumed, from the observations of DUFFUS and SLAUGHTER (1980), that the seeds were still also chemically sound.

The raw seeds showed an average moisture content of approximately 9% while 0.7% was lost on milling.

The dried seeds were dehydrated down to an average moisture content of 3.2%

Canned samples were observed to have the highest level of moisture showing an increase of nearly 60% when compared to the original raw seeds. This may be due to the fact that they are maintained in a liquid 'environment'. The latter also being the reason why they show an approximately 10% greater moisture content as compared to frozen seeds; the frozen seeds were drained before freezing them.

The raw seeds have a weight of 36.9g per 100 seeds which brings them into the 'Kabuli' types (Table 3).

TABLE 2 Moisture contents of raw and processed chick-peas.
Results are expressed as a mean % ± standard error.

SAMPLE	% MOISTURE
Raw seed	9.0 ± 0.4
De	8.3 ± 0.4
D	3.2 ± 0.7
WF	58.0 ± 0.5
MF	57.4 ± 0.7
WPF	59.2 ± 0.4
MPF	58.1 ± 0.4
WC	68.0 ± 0.5
MC	68.8 ± 0.2

TABLE 3 The weight of 100 chickpea seeds. (Raw and Processed). The results are expressed in g. ± standard error.

SAMPLE	WEIGHT OF 100 SEEDS(G)
Raw seed	36.9 ± 0.1
De	34.0 ± 0.2
D	34.6 ± 0.1
WF	76.6 ± 0.3
MF	74.3 ± 0.4
WPF	76.9 ± 0.2
MPF	70.4 ± 0.2
WC	95.6 ± 0.3
MC	100.3 ± 0.1

It is observed that canned chickpeas have higher weights than those of frozen seeds. This was probably due to the greater absorption of water on being suspended in a liquid environment within the cans. The values for medium-soaked canned seeds are higher than those of water-soaked canned ones. The salts in the medium not only penetrate the surface layers of the seeds but are distributed throughout the cotyledons of the seeds. The presence of these salts would account for the higher weight of medium-soaked canned seeds than water-soaked ones.

CHAPTER III

EFFECT OF PROCESSING & STORAGE ON CARBOHYDRATES

Introduction

McCANCE & LAWRENCE (1929) have suggested that carbohydrates in foods may be considered to fall into two broad categories: (i) the 'available carbohydrates' are those digested and absorbed by man, (ii) the 'unavailable carbohydrates' are not digested by endogenous secretions of the human digestive tract.

The available carbohydrates in this study include free sugars, dextrins, and starches. The unavailable carbohydrates consist of pectins, hemicelluloses & celluloses (SOUTHGATE, 1976) which are collectively termed dietary fibre(TROWELL, 1972).

Identification and quantification of free sugars in chickpeas has been carried out by a number of workers including SHALLENBERGER & MAYER, 1961; NIGAM & GIRI, 1961; SCHWEIZER et al 1978. Oligosaccharides of the raffinose family (e.g. raffinose, stachyose, and verbascose) containing α (1-6) galactose linkages indigestible to mammalian enzymes, are known to be important constituents in legumes (AMAN, 1979). LINEBACK & DE (1975) found that chickpea flour contained approximately 8% free sugars in which sucrose and stachyose predominated.

As free sugars are soluble components, they would be affected by the soaking and cooking treatments and some may leach out of the food into the soaking and cooking water respectively.

The more complex carbohydrates are liable to hydrolysis to simpler forms e.g. to reducing sugars. SALEM (1975) found that

during the baking of broad beans (120°C for 150 minutes) starch content decreased, being converted first into dextrins then into reducing sugars. This break-down of starch may, however, increase its digestibility.

The effects of heat on mono- and oligo- saccharides may be varied. These effects include caramelisation, pyrolysis, and interaction with other food components.

Caramelisation occurs when heating leads to loss of water from the sugar molecule and gives anhydro sugars. This is followed by further unknown reactions to give brown-coloured degradation and polymerisation products (GREENWOOD & MUNRO, 1979). Caramel, which is used as a colouring material with a characteristic smell and of uncertain composition, is produced by such reactions.

Following polymerisation, depolymerisation, and dehydration reactions which are involved in caramelisation, secondary thermal reactions take place. They cause carbon-carbon bond cleavage and similarly many pyrolysis products of low molecular weight are produced from both mono- and poly-saccharides (HOUMINER, 1973).

Both caramelisation and pyrolysis imply severe heating of sugar molecules. The most important reaction of carbohydrates with other food components is the so-called non-enzymic browning or Maillard reactions (HURRELL, 1971; FINOT, 1973; REYNOLDS, 1965) which occurs between reducing sugars and nitrogenous compounds, in particular certain amino acids and proteins. Although Maillard reactions are necessary in certain foods for the development of flavours and odours, they can cause a severe reduction in the the nutritive value of the protein component. In model systems, glucose has been found to be more reactive then fructose in the above reactions (LEWIS & LEA, 1959). Sucrose can also be an indirect precursor to this as a result of the splitting of its glycosidic bonds to yield glucose and fructose.

General browning effects obtained during processing have been extensively reviewed by MEYER (1969), BENDER (1978), PRIESTLEY (1979).

The effect of temperature on Maillard reactions must be considered in relation to other variables such as acidity and water activity so that the browning rate increases. The latter also increases with increasing pH (WILLIAMS, 1976).

Maillard reactions are complex and as yet not fully understood, although they do appear to follow common path ways.

A variety of groups such as aldehydes, ketones, and reducing sugars combine with amino groups in aldol condensation to form first a Schiff's base and then an N-substituted glycosylamine. These compounds undergo Amadori rearrangement, at which stage the compounds formed are still colourless and the reactions are reversible. The third stage is a Strecker degradation with loss of a molecule of carbon dioxide, followed by a condensation of the aldehydes so formed, or condensation with sugar fragments and various dehydration products in the heated food to form brown pigments. The first relatively stable compound formed in the Maillard reaction appears to be a 1-deoxy-2-Ketone (lysine-fructose) which is not hydrolysed by digestive enzymes.

The literature reveals considerable variations in the stability of nutrients in frozen foods (BENDER, 1978). This may be partly due to different processing conditions, but also because of the varying extent of inactivation of oxidising enzymes by any pretreatment. This may include the cooking times and conditions used prior to freezing. Even at -30°C part of the water is still unfrozen.

A factor worthy of consideration is that, while pre-soaking and

pre-cooking treatments remove some soluble carbohydrates, they may aid in preserving the remainder by destroying oxidising enzymes. Moreover, these losses may have taken place in the normal event of home cooking practices. MAPSON (1956) has shown that there is little difference between the vitamin C contents of fresh, freeze-dried and frozen garden peas after cooking. This is largely because fresh peas need 10 minutes boiling, compared with 3.5 minutes for frozen peas and 2 minutes for freeze-dried peas. One stage (e.g. freezing), may cause a great loss of nutrients, but another (e.g. fresh seeds), causes an even greater loss as the cooking time may now be much longer (BENDER, 1978). It would be interesting to find out to what degree the freezing process affects the retention of carbohydrates in chickpeas.

For many canned products stored at a temperature of approximately 21°C (70°F) the foods remain acceptable until corrosion of the can, or some accident occurs, resulting in leakage permitting microbial contamination. If the foods are stored at 38°C (100°F) they may become unacceptable within 3 months to a year due to their chemical degradation (JACKSON & SHINN, 1979).

Studies by MONROE et al (1949) on temperatures prevailing in 79 warehouses (stacking cans) situated throughout the United States, indicated that even in the hottest areas of the South, the average yearly temperatures did not exceed 27°C (80°F).

The present study has taken into account the above facts and canned chickpeas have therefore been stored at 22°C and 30°C. This may be of prime importance in areas with a warm climate where canned products are commonly used.

In the calculation of nutrient retention MURPHY et al (1975) have drawn attention to the different methods of calculating nutrient losses which may produce quite different results.

They define apparent retention as:

$$\frac{\text{nutrient content } g^{-1} \quad \text{cooked food}}{\text{nutrient content } g^{-1} \quad \text{raw food}} \text{ (dry weight) x 100}$$

while true retention was given as:

$$\frac{\text{nutrient content } g^{-1} \quad \text{cooked food x g} \quad \text{food after cooking}}{\text{nutrient content } g^{-1} \quad \text{raw food x g} \quad \text{food before cooking}} \times 100$$

This is because losses and gains in moisture and solids due to processing may have to be taken into account. However, a simpler method for expressing the results would be on a per seed basis, hence eliminating any increase or decrease in moisture during the various processes.

MATERIALS AND METHODS

a) Estimation of total available carbohydrates (Clegg anthrone method, OSBORNE & VOOGT, 1978)

This method may be used to estimate the amount of starches and dextrins together with soluble sugars (total available carbohydrates). The samples are digested with perchloric acid and the digested carbohydrates are determined colorimetrically by the anthrone method. The anthrone-sugar complex forms a green colour which has a maximum absorption at 630nm.

One modification was made, however. KALEQUE et al (1976) have shown that maximum extraction of starch from chickpeas, was obtained by stirring and macerating for 50 minutes in the extracting solution, hence this was included in the above method.

Reagents:

Perchloric acid 52%: 270 ml of perchloric acid (Sp gr 1.70) was added to 100ml water and was kept cold before use.

Sulphuric acid: 760ml of sulphuric acid (Sp. gr. 1.84) was added to 330 ml of water and kept cold before use.

Anthrone reagent: sufficient 0.1% anthrone was made up in the above sulphuric acid for each day's requirements. This solution had to be made fresh, daily.

Glucose standard solution: 100 mg glucose was dissolved in 1 litre of distilled water.

Extraction:

Two grams of chickpea seeds were macerated in a mortar with 10 ml water and then transferred into a 100 ml measuring cylinder. Thirteen ml of 52% perchloric acid was added ., and the mixture was stirred frequently with a glass rod for 50 minutes and then diluted to 100 ml. The mixture was passed through Whatman No.542 filter paper and made up to 250 ml.

Sample and standard preparation:

Ten ml of the above filtrate was diluted to 100 ml with water and 1 ml of this solution was placed in a test tube.

One ml of diluted glucose standard solution (1ml = 0.1 mg glucose) and 1 ml of sample were placed in separate test tubes. Five ml of freshly prepared anthrone 0.1% reagent were added rapidly to each test tube which was stoppered and the contents mixed thoroughly. The tubes were then placed in a boiling water bath for exactly 12 minutes and cooled quickly to room temperature. A green colour was formed with the anthrone reagent which was stable for at least 2 hours. The coloured solutions were transferred to glass cuvettes and the absorbance of the sample and standard were read (using a Unicam SP-800 spectrophotometer) against a blank solution containing 1 ml distilled water plus 5 ml anthrone reagent.

Calculations:

Total available carbohydrate was calculated as a percentage using the formula:

$$\text{Total available carbohydrate as \%} = \frac{25 \times b}{a \times w}$$

where, w = weight (g) of sample

a = absorbance of diluted glucose standard

b = absorbance of diluted sample.

b) Qualitative and quantitative estimation of neutral (soluble) sugars by gas-liquid chromatography (GLC)

In the extraction of carbohydrates two points have to be observed; the procedure should be completely exhaustive and prevent any artifacts being produced.

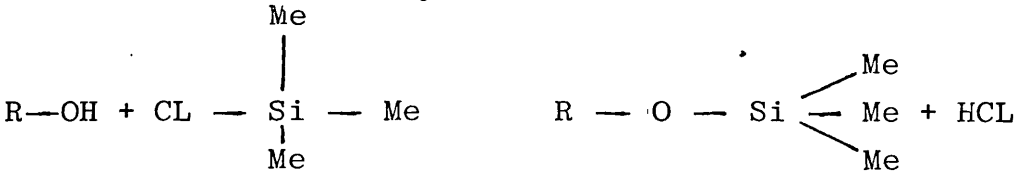
The solubility of the free sugars in aqueous solutions makes these the obvious choice as extracting media. However, there are several disadvantages in that the aqueous reagents extract many substances that interfere with the subsequent measurement of sugars. Most analytical approaches to the extraction of free sugars have therefore tried to combine the twin requirements of complete extraction of sugars and minimal extraction of interfering substances such as proteins (SOUTHGATE, 1976). Since the free sugars are generally soluble to a significant extent in aqueous alcoholic solutions, these reagents have formed the basis for most extraction procedures.

Several different techniques with aqueous alcohol have been employed and ethanol, methanol and iso-propanol have been used quite extensively (AOAC, 1975; FRIEDMANN et al, 1967: SOUTHGATE, 1969). A general precaution which has to be adhered to is that the extracting medium must be neutral and remain neutral during the extraction. Significant amounts of organic acids may be present in the sample and these will bring about a partial hydrolysis of polysaccharides. To prevent this happening, solid calcium carbonate is often added to the extraction medium (SOUTHGATE, 1976).

Separation of sugar derivatives by GLC is now well established (SWEELEY & WALKER 1964; CLAMP et al, 1971; HOLLINGAN, 1971).

Sugars are crystalline polyhydroxy compounds which are strongly hydrogen bonded, hence they have high melting points (200 - 300°C) (BIRCH, 1973). They can be converted into volatile derivatives such as acetates, methyl ethers, and trimethylsilyl ethers (TMS ethers). The first two can be used for monosaccharides, but at temperatures needed for higher MW. sugars (di-and tri - saccharides) thermal degradation effects occur; anhydro-derivatives may be formed or sugar ring inversions may occur. The TMS ethers (Fig 4) have the advantage of combined stability and volatility which allows the separation of di- and tri-saccharides.

Fig 4



(sugar) (trimethylsilylchloride) (volatile derivative)

Simplified scheme of the formation of trimethylsilyl derivative of sugars

Pyridine is universally accepted as the preferred solvent (BIRCH, 1973). The formation of TMS derivatives, in pyridine occurs very rapidly at ambient temperatures (SWEELEY, 1963).

Extraction:

Ten grams of chickpea seeds were ground in a mortar and the lipid which could otherwise cause interference in subsequent measurements had to be removed. This was done by extracting with 150 ml petroleum ether (40 - 60°C) and the ethereal layer discarded.

Free sugars were extracted by the methods used by DELENTE & LADENBURG (1972) with slight modifications.

Five grams of defatted and dried chickpeas were homogenised with a mixture of methanol: chloroform (1:1, v/v): water (1:2, v/v). To this was added 0.25g solid anhydrous calcium carbonate. After standing overnight at 4°C, the homogenate was agitated for 15 minutes with a magnetic stirrer. Approximately 5 ml of the suspension was centrifuged for 5 minutes at 20,000 rpm. To purify the sample, 1 ml of the supernatant was added slowly with agitation to 2 ml of a 1.8% barium hydroxide solution, followed by 2 ml of a 2% zinc sulphate solution. After standing for 5 min the samples was centrifuged for a further 5 min at 20,000 rpm to give a clear extract.

Silylation:

Silylation was carried out as follows: 1 ml of the extract was lyophilised and 1 ml of Tri-Sil 'Z' reagent (PIERCE) was added to it. This was heated at 60°C for 20 minutes. The sample was then ready for GLC analysis.

Gas-liquid chromatography:

GLC was carried out with a PYE series 104 chromatograph fitted with a flame ionisation detector and glass columns (180 x 0.4 mm and 180 x 0.2 mm). Graph shown in Fig 5.

The following packing materials and programmes were used:
(i) 3% OV-1 on Chromosorb W(HP)80/100 mesh. Temperature was programmed from 150 °C to 350°C, 16°C min^{-1} (DELENTE & LADENBURG, 1972).
(ii) 3% OV-17 on Gaschrom Q, 80/100 mesh. Temperature programmed from 150°C to 320°C, 8°C min^{-1}, (AMAN, 1979).
Number (ii) showed excellent results, hence this was used throughout the analyses.

Peak identification:

All the different sugar peaks were identified by both comparing.

Fig 5.

GLC analysis of free sugars.

Peaks were identified as follows:

1 fructose

2 galactose

3,5 glucose

4 sorbitol

6 unknown

7 sucrose

8 maltose

9 isomaltose

10 cellobiose

11 mellibiose

12 raffinose

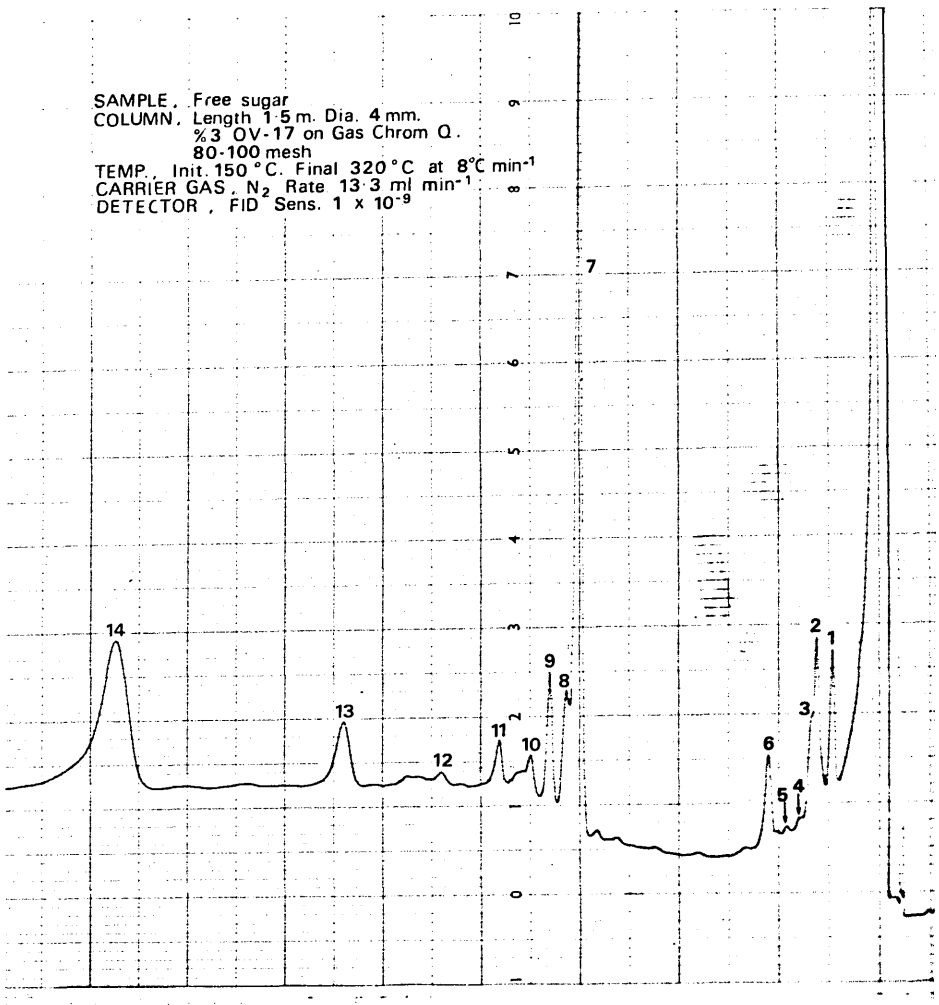
13 manninotriose

14 stachyose

Raw Seed

1 yr.-storage.

Raw Seed


SAMPLE , Free sugar
COLUMN , Length 1·5 m. Dia. 4 mm.
%3 OV-17 on Gas Chrom Q.
80-100 mesh
TEMP., Init. 150 °C. Final 320 °C at 8°C min-1
CARRIER GAS , N2 Rate 13·3 ml min-1
DETECTOR , FID Sens. 1 x 10-9
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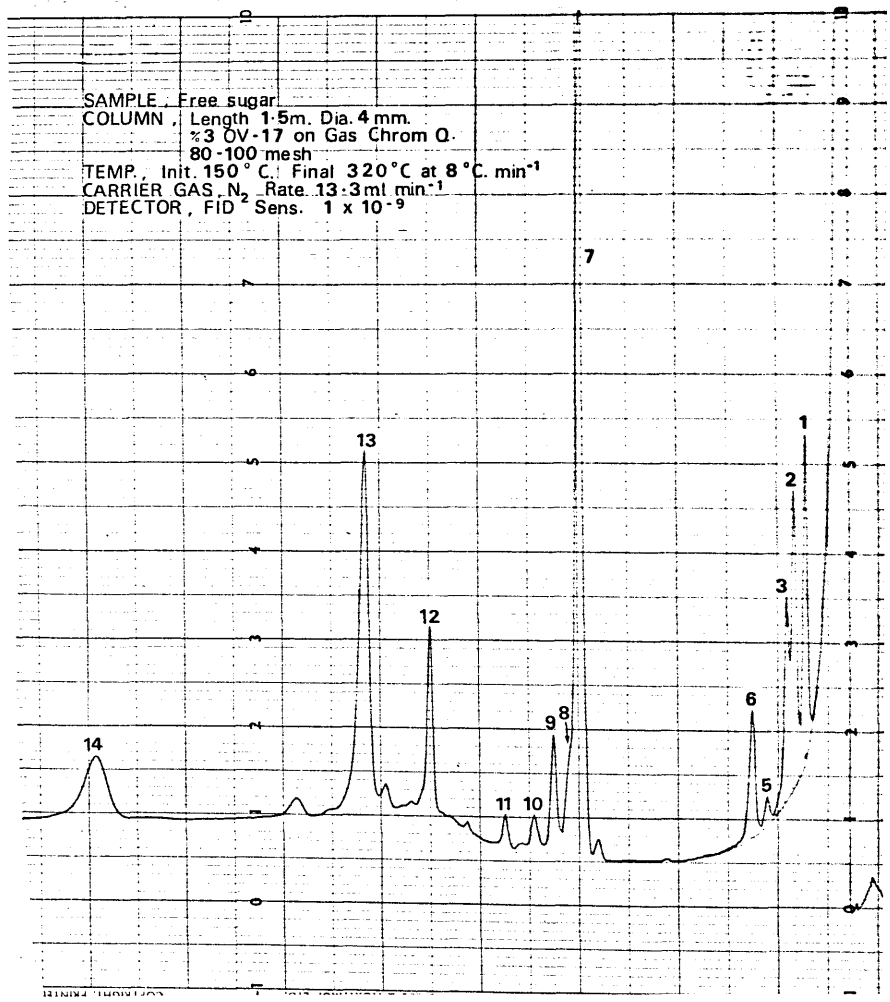
SAMPLE , Free sugar
COLUMN , Length 1·5m. Dia. 4 mm.
%3 OV-17 on Gas Chrom Q.
80-100 mesh
TEMP., Init. 150 ° C. Final 320 °C at 8 °C. min-1
CARRIER GAS , N2 Rate 13·3 ml min-1
DETECTOR , FID Sens. 1 x 10-9
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retention times with those of pure sugar standards, and by co-chromatography, where peak coincidence and height increase were observed.

Quantification:

All experiments were repeated four times. The results wherever possible are expressed as $\bar{x} \pm$ sd, where $\bar{x}$ is the mean of 4 replicates and sd is the standard deviation. In this case sd is the same as standard error of the mean ($\frac{2sd}{\sqrt{n}}$, n is number of samples) with 95% confidence limit.

All % retention values given in the tables represent increases or decreases as compared to the unprocessed raw seeds. An abbreviation, % retn, has somtimes been used to represent the same.

Results and discussion

Identification, quantification and the effects of storage in raw seeds:

The total available carhohydrates are comprised of free sugars, higher oligosaccharides and starch. In this study, the higher oligosaccharides in this group represent those containing more than five monosaccharide units. Investigation showed that the recovery of total carbohydrates ranged between 95 and 103% with the extraction procedures described.

The quantity of total available carhohydrates found in raw chickpeas was 208 mg $seed^{-1}$. This represented 186.5 mg $seed^{-1}$ of starch and higher oligosaccharides and 21.5 mg $seed^{-1}$ free sugars (Table 4).

The free sugars contribute to the flavour of the raw material and can affect cooking properties both by acting as flavour precursors and by causing browning due to the Maillard reaction. A knowledge of the types and amounts of individual sugars is therefore preferable for estimating changes occuring due to processing of the seeds.

Eleven free sugars were identified using GLC (Table 5). These were fructose, galactose, glucose, sorbitol, sucrose, maltose, cellobiose, mellibiose, raffinose, manninotroise, and stachyose. Diagramatic representations of the oligosaccharides are shown in Fig 6.

Fig 6

sucrose	glucose β (1 – 2) fructose
maltose	glucose α (1 – 4) glucose
cellobiose	glucose β (1 – 4) glucose
mellibiose	galactose α (1 – 6) glucose
raffinose	galactose α (1 – 6) glucose β(1-2)fructose
manninotriose	galactose α (1 - 6) galactose α(1-6)glucose
stachyose	galactose α (1 - 6) galactose α(1-6)-glucose β(1-2)fructose

TABLE 4 The effect of 1 year storage on total available carbohydrates in chickpeas.

	RAW SEED mg seed^{-1}	Raw - S	
		mg seed^{-1}	% Retention
FREE SUGARS	21.5 ± 0.5	34.7 ± 0.7	161.4
STARCH + HIGHER OLIGOSACCHARIDES	186.5 ± 0.6	141.0 ± 1.8	75.6
TOTAL AVAILABLE CARBOHYDRATES	208.0 ± 0.8	175.7 ± 0.4	84.5

Table 5 The effect of 1yr. storage on free sugars in raw and untreated chickpeas

	RAW SEED	Raw-S	
	mg seed^{-1}	mg seed^{-1}	% Retention
Fructose	1.2	1.8	150.0
Galactose	1.4	2.1	150.0
Glucose	1.0	1.6	160.0
Sorbitol	T	0.2	-
Unknown	0.7	1.4	200.0
Sucrose	7.1	10.5	147.9
Maltose	1.3	3.7	284.6
Cellobiose	0.2	0.8	400.0
Mellibiose	0.2	0.7	350.0
Raffinose	1.3	0.3	23.1
Manninotriose	4.7	2.2	46.8
Stachyose	2.4	9.4	391.7
Total free sugars	21.5±0.5	34.7±0.7	161.4

LINEBACK and DE (1975) working on chickpeas did not mention the presence of cellobiose, mellibiose and manninotriose in their free sugar fraction isolates. They did, however, report on two sugars which they failed to identify, but stated that acid hydrolysis of these yielded fructose, glucose and galactose. This would suggest that these sugars may have belonged to the raffinose family of oligosaccharides. The first unknown peak was eluted between that of raffinose and of stachyose. On comparing with the GLC peaks (Fig 5) there is a strong possibility that the unknown peaks were mellibiose and manninotriose repectively.

AMAN (1979) reported a relatively large amount of manninotriose in chickpea but the presence of cellobiose and mellibiose were not noted.

The hydrolysis or degraded products of oligosaccharides of the raffinose family would certainly contain at least small amounts of cellobiose and mellibiose in addition to other sugars. This can be deduced from the diagrammatic representation of these sugars in Fig 6.

The most abundant sugars were found to be sucrose, manninotriose and stachyose. This is in full agreement with the results of AMAN (1979) and also of LINEBACK and DE (1975); assuming for the latter that the second unknown sugar was manninotriose. NIGAM and GIRI (1961) have reported sucrose, raffinose and stachyose to be the most abundant sugars in chickpea.

Retention values show that, after storing raw seeds for 1 year at $10^{o}C$, there was a decrease in total carbohydrates from 208.8 to 175.7mg $seed^{-1}$ with a corresponding decrease in starch and higher oligosaccharides. Generally there were increases in most sugars and decreases in raffinose and in manninotriose (Tables 4 and 5).

It is apparent that the increase in monosaccharides and disaccharide is due to the hydrolysis of starch and of higher oligosaccharides. The loss in total carbohydrates suggests that there has been a gradual hydrolysis of higher oligosaccharides and starch (as indicated by the increase in maltose) to disaccharides and then to monosaccharides. These will then have been either used during the slow respiration of the seeds or will have also entered in the Maillard reaction by combining with amino acid or protein molecules.

General effects of processing:

When compared to raw seeds it is observed that there is a decrease in total available carbohydrates in chickpeas immediately after drying, decorticating, freezing and canning, followed by a further loss after one year of storage. This trend of loss is also similar in starch and higher oligosaccharides.

In general, the total free sugars have shown decreases in the first instance, due to processing but increases after one year of storage. In some cases these increases have led to sugar contents greater than that in the original raw seed.

From Figs 7, 8, and 9 it is observed that the losses due directly to processing range from 30% to 94% for monosaccharides, 10% to 54% for disaccharides 0% to 35% for tri- and tetra-saccharides, and 0% to 8% for starch and higher oligosaccharides. It appears that the reducing sugars have been most susceptible to damage or loss by processing.

After one year of storage of all processed samples, appreciable increases have been noted in fructose among the monosaccharides, in all the disaccharides (sucrose, maltose, cellobiose, and mellibiose), and in stachyose whereas the amounts of raffinose and manninotriose have decreased considerably.

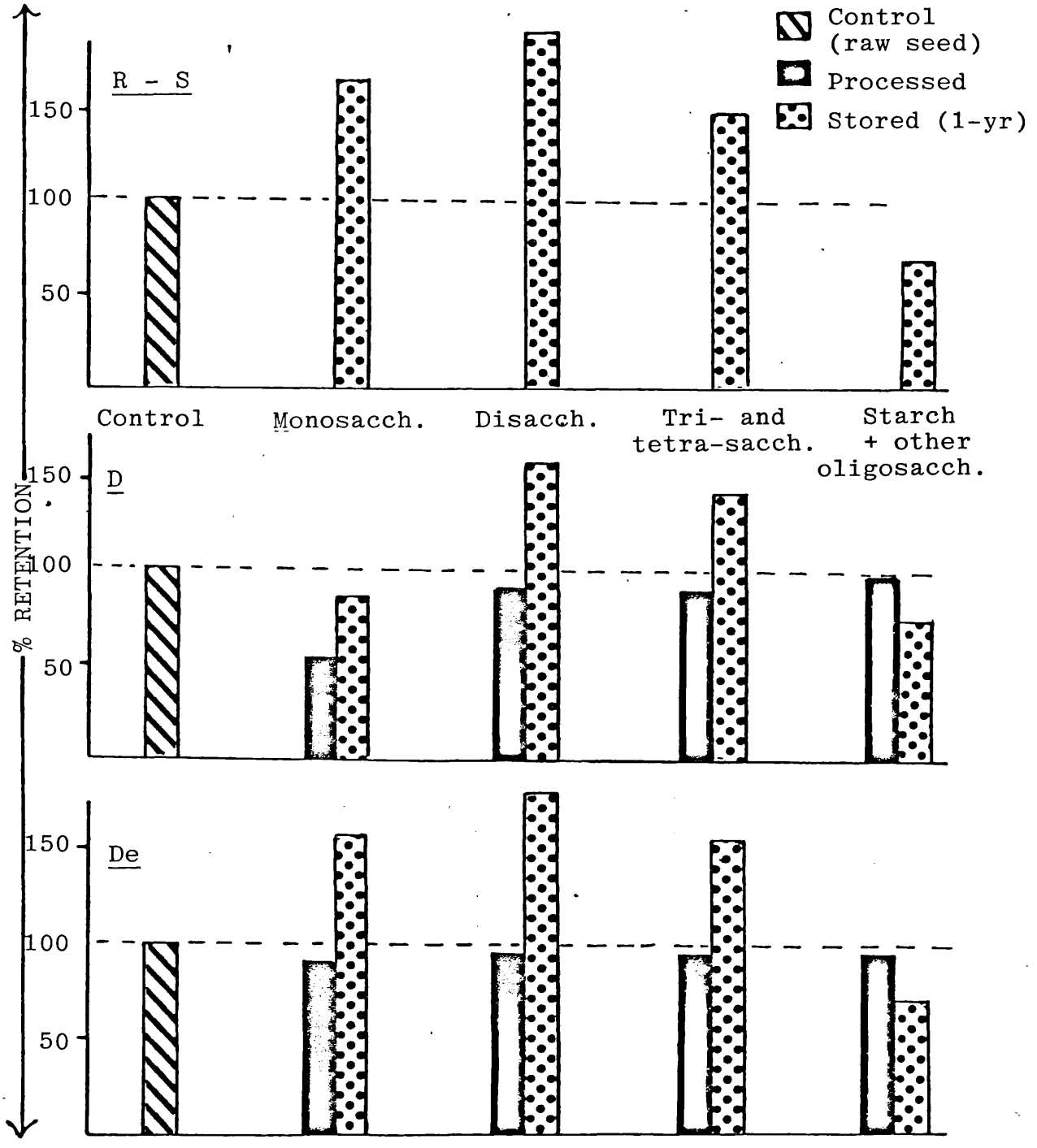


Fig 7 The effect of drying, decorticating and the subsequent storage of 1-year on % retention of carbohydrates in chickpeas.

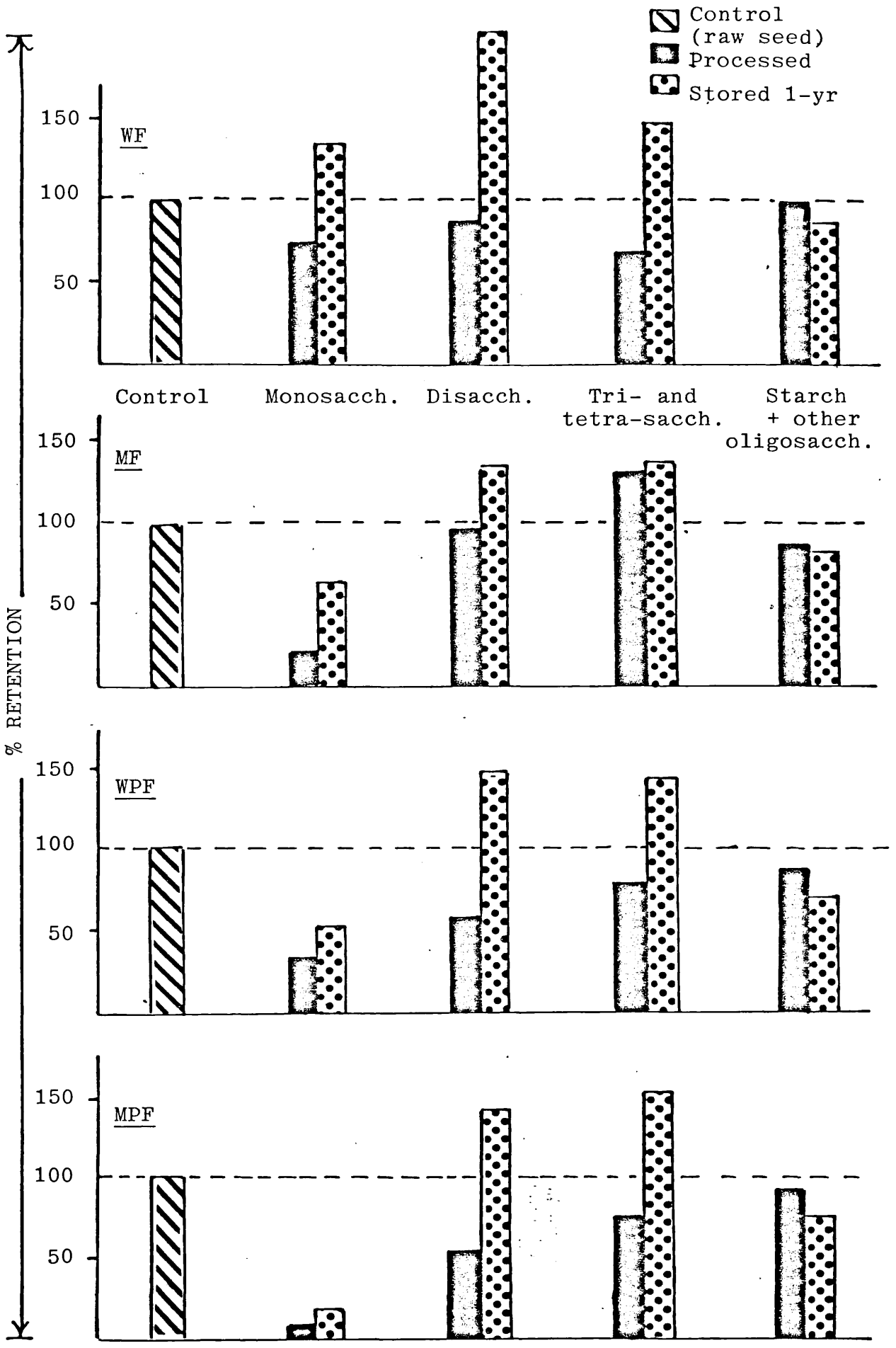


Fig 8 The effect of freezing and frozen 1-year storage on % retention of carbohydrates in chickpeas.

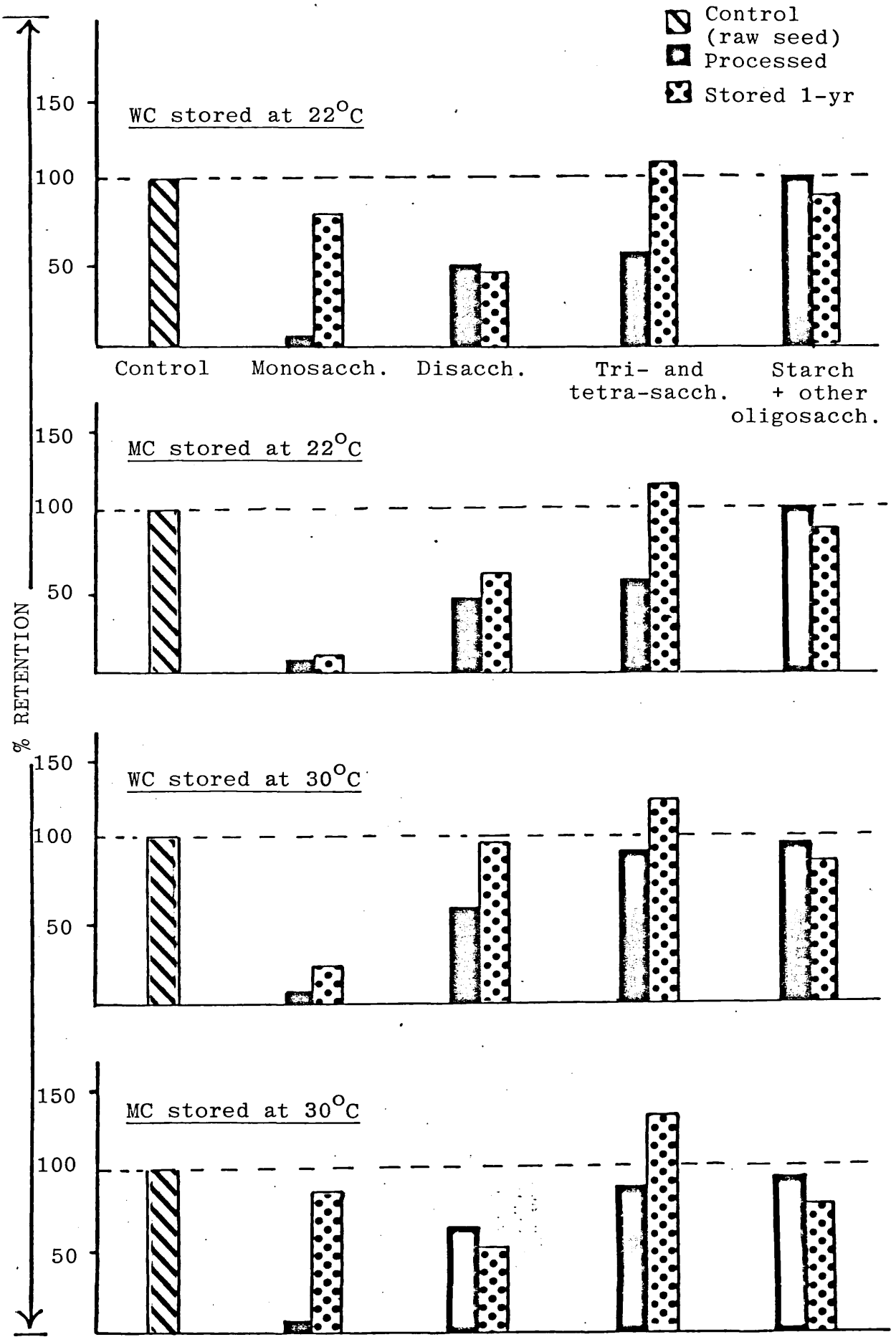


Fig 9 The effect of canning and canned 1-year storage at 22°C and 30°C on % retention of carbohydrates in chickpeas.

The increase in stachyose may be accounted for partly by the hydrolysis of larger molecules belonging to the raffinose family of oligosaccharides. It is also possible that certain oligosaccharides are present in the bound form to other macromolecules. Processing the seeds could have led to the breaking of these bonds hence leading to the release of molecules of lower molecular weight.

The increase in cellobiose, a degradative product of cellulose, implies that the cell wall may have also been weakened by the release of smaller fractions from it. Hydrolysis of starch is indicated by the build up of maltose.

The hydrolysis of the raffinose family trisaccharides has led to the accumulation of sucrose and mellibiose but it would be expected that these on further hydrolysis, would produce large amounts of galactose and glucose with lesser amounts of fructose. The results do not agree with this expectation hence a further explanation is needed for this observation. WILLIAMS (1976) stated that fructose browns more slowly then glucose and measurements of amino acid losses during the reaction indicate that it proceeds by routes different from the browning of aldose type sugars. LEWIS and LEA (1959) mention that this rate is ten times as slow as that of glucose. From this it can be assumed that during a year's storage the build up of fructose has occurred due to its slower reaction rate in the Maillard reaction as compared to glucose and galactose.

Effects of decorticating and storage:

The average content of total available carbohydrates in decorticated chickpea was found to be 200mg seed^{-1} comprised of 179.9 mg seed^{-1} starch and higher oligosaccharides and 20.1mg seed^{-1} free sugars. (Tables 6 and 7). This indicates a 3.8% loss in total available carbohydrates; 6.5% decrease in free sugars and 3.5% decrease in starch and higher oligosaccharides.

TABLE 6 The effect of drying, decorticating and the subsequent storage for 1 year on total available carbohydrates in chickpeas.

	RAW SEED	D		D - S		De		De - S	
	mg seed^{-1}	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention
FREE SUGARS	$\pm^{21.5}_{0.5}$	$\pm^{17.4}_{0.3}$	80.9	$\pm^{30.5}_{0.8}$	141.9	$\pm^{20.1}_{0.4}$	93.5	$\pm^{35.3}_{0.7}$	164.2
STARCH + HIGHER OLIGOSACCHARIDES	$\pm^{186.5}_{0.6}$	$\pm^{175.6}_{3.0}$	94.2	$\pm^{142.1}_{2.5}$	76.2	$\pm^{179.9}_{3.5}$	96.5	$\pm^{140.9}_{3.5}$	75.6
TOTAL AVAILABLE CARBOHYDRATES	$\pm^{208.0}_{0.8}$	$\pm^{193.0}_{3.8}$	92.8	$\pm^{172.6}_{3.2}$	83.0	$\pm^{200.0}_{3.9}$	96.2	$\pm^{176.2}_{4.2}$	84.7

Table 7 The effect of drying, decorticating and the subsequent storage for 1yr. on free sugars in chickpeas

	RAW SEED	D		D - S		De		De - S	
	mg seed^{-1}	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention
Fructose	1.2	0.8	66.7	1.9	158.3	1.1	91.7	2.2	183.3
Galactose	1.4	0.5	35.7	0.7	50.0	1.2	85.7	1.8	128.6
Glucose	1.0	0.6	60.0	0.6	60.0	0.8	80.0	1.6	160.0
Sorbitol	T	T	—	0.1	—	T	—	0.1	—
Unknown	0.7	0.7	100.0	1.1	157.1	0.7	100.0	1.0	142.9
Sucrose	7.1	6.2	87.3	9.5	133.8	7.3	102.8	11.3	159.2
Maltose	1.3	1.0	76.9	3.4	261.5	0.9	69.2	3.0	230.8
Cellobiose	0.2	0.1	50.0	0.5	250.0	0.1	50.0	0.3	150.0
Mellibiose	0.2	0.2	100.0	0.7	350.0	0.1	50.0	0.9	450.0
Raffinose	1.3	0.9	69.2	0.2	15.4	1.1	84.6	0.3	23.1
Manninotriose	4.7	4.1	87.2	2.0	42.6	3.5	74.5	1.6	27.7
Stachyose	2.4	2.3	95.8	9.8	408.3	3.3	137.5	11.2	466.7
Total free sugars	21.5±0.5	17.4±0.3	80.9	30.5±0.8	141.9	20.1±0.4	93.5	35.3±0.7	164.2

The original seed coat must have contained most of these carbohydrates which were consequently lost on milling the seeds.

After one-year storage, a further loss of 11.5% total available carbohydrates brought the total loss to 15.3% corresponding to a net weight of 176.2mg seed^{-1} left in the seed.

Effects of drying and dried storage:

Total available carbohydrate retention after dried processing was 92.8% corresponding to 193mg seed^{-1} (Table 6). More loss has been incurred in the free sugar fraction (19.1%) than in the starch and higher oligosaccharides (5.8%).

There are two stages in which losses of carbohydrates may have occurred. The first is the soaking medium solution into which diffusion of nutrients is possible which would account for the leaching of soluble free sugars. This, however, cannot account for the reduction in starch and higher oligosaccharides. Over the long soaking period, a series of enzymic changes is likely to take place in the seeds during steeping.

When water is removed by dehydration, the dissolved are concentrated in the remaining water, unless they crystallise out. Moreover, the temperature is raised in order to supply the necessary energy to transform the water into the vapour phase. Thus, breakdown of starch or reaction of products with other nutrients could have accelerated during drying, as well as during storage of the dehydrated seeds.

Increases in sucrose, maltose, cellobiose, mellibiose and stachyose and decreases in raffinose and manninotriose (Table 7), strongly indicate a certain amount of degradation of carbohydrates during one year of storage. This has led to a reduction in amount of total available carbohydrate on storage.

Evidence for the occurrence of non-enzymic browning reactions is implied due to increases in fructose higher than in glucose and in galactose. This is in contradiction to reports by LEA (1949) and WILLIAMS (1976) who stated that the Maillard reaction is inhibited at very low moisture contents in foods.

Effects of freezing and frozen storage:

The amounts of total available carbohydrates in WF, MF, WPF and MPF chickpeas were 196.2, 200.5, 184.0, 188.0 mg seed^{-1} respectively (Tables 8 and 10).

There was no significant difference on the retention of total carbohydrates in raw or precooked frozen chickpeas or the amount between medium soaking and water soaked ones.

Losses incurred in total available carbohydrate in the freezing process range from 3.6% in raw seeds to 11.5% in precooked seeds. This greater loss in precooked seeds was expected as cooking aids further hydrolysis reactions.

MEINERS et al (1976) found that cooking chickpeas for 140 minutes in water brought approximately a 50% reduction in carbohydrates. Results in tables 9 and 11 show that they are not in agreement with those of RAO and BELAVADY (1978), who reported that 24 hour soaking of chickpeas in water brought about 50% reductions in stachyose and raffinose but cooking for 15 minutes at 15 lb pressure brought about 100% increases in stachyose, raffinose and sucrose.

Further losses in total available carbohydrates incurred after one year frozen storage ranged from 6-12% (Tables 8 and 10). These are similar to the processing losses themselves. Breakdown of starch, cellulose and higher oligosaccharides on storage has been indicated by increases in maltose, cellobiose and stachyose respectively.

Table 8 The effect of freezing and 1yr. frozen storage on total available carbohydrates in chickpeas

	RAW SEED	WF		WF-S		MF		MF-S	
	mg seed^{-1}	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention
FREE SUGARS	21.5 ± 0.5	15.2 ± 0.4	70.7	34.5 ± 0.1	160.5	19.0 ± 0.3	88.4	25.5 ± 0.2	118.6
STARCH + HIGHER OLIGOSACCHARIDES	186.5 ± 0.6	181.1 ± 1.0	97.1	141.5 ± 3.8	75.9	181.5 ± 1.1	97.3	162.3 ± 3.2	87.0
TOTAL AVAILABLE CARBOHYDRATES	208.0 ± 0.8	196.2 ± 1.1	94.3	176.0 ± 3.7	84.6	200.5 ± 1.2	96.4	187.8 ± 4.8	90.3

Table 9 The effect of freezing and 1yr. frozen storage on free sugars in chickpeas

	RAW SEED	WF		WF - S		MF		MF - S	
	mg seed^{-1}	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention
Fructose	1.2	0.9	75.0	2.6	216.7	0.5	41.7	1.1	91.7
Galactose	1.4	0.9	64.3	0.7	50.0	T	—	0.4	28.6
Glucose	1.0	0.7	70.0	1.3	130.0	0.2	20.0	0.7	70.0
Sorbitol	T	T	—	0.1	—	T	—	0.2	—
Unknown	0.7	0.6	85.7	1.4	200.0	0.3	42.9	0.9	128.6
Sucrose	7.1	5.4	76.1	12.4	174.7	6.0	84,5	7.8	109.9
Maltose	1.3	0.9	69.2	3.7	284.6	1.3	100.0	2.3	176.9
Cellobiose	0.2	0.2	100.0	0.6	300.0	0.3	150.0	0.4	200.0
Mellibiose	0.2	0.1	50.0	0.6	300.0	0.4	200.0	0.6	300.0
Raffinose	1.3	0.5	38.5	0.2	15.4	0.9	69.2	0.4	30.8
Manninotriose	4.7	3.5	74.5	1.5	31.9	6.2	131.9	1.6	34.0
Stachyose	2.4	1.4	58.3	9.4	391.7	2.9	120.8	9.4	391.7
Total free sugars	21.5±0.5	15.2±0.4	70.7	34.5±0.1	160.5	19.0±0.3	88.4	25.5±0.2	118.1

Table 10 The effect of freezing and 1yr. frozen storage on total available carbohydrates in precooked chickpeas

	RAW SEED	WPF		WPF - S		MPF		MPF - S	
	mg seed^{-1}	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention
FREE SUGARS	21.5 ± 0.5	12.5 ± 0.7	58.1	26.6 ± 1.1	123.7	11.3 ± 0.5	52.6	25.7 ± 0.5	119.5
STARCH + HIGHER OLIGOSACCHARIDES	186.5 ± 0.6	172.0 ± 3.0	92.2	132.0 ± 2.5	70.8	176.8 ± 2.3	94.8	141.2 ± 2.6	75.7
TOTAL AVAILABLE CARBOHYDRATES	207.0 ± 0.8	184.0 ± 3.2	88.5	158.5 ± 3.0	76.2	188.0 ± 2.5	90.4	166.9 ± 3.1	80.2

Table 11 The effect of freezing and 1yr. frozen storage on free sugars in precooked chickpeas

	RAW SEED	WPF		WPF - S		MPF		MPF - S	
	mg seed^{-1}	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention
Fructose	1.2	0.4	33.3	1.1	91.7	0.2	16.7	0.8	66.7
Galactose	1.4	0.3	21.4	0.3	21.4	T	—	T	—
Glucose	1.0	0.2	20.0	0.4	40.0	T	—	T	—
Sorbitol	T	T	—	T	—	T	—	T	—
Unknown	0.7	0.4	57.1	0.8	114.3	0.1	14.3	0.6	85.7
Sucrose	7.1	3.4	47.9	7.2	101.4	3.2	45.1	7.6	107.0
Maltose	1.3	0.9	69.2	3.5	269.2	0.7	53.9	3.0	23.1
Cellobiose	0.2	0.3	150.0	0.8	400.0	0.2.	100.0	0.5	250.0
Mellibiose	0.2	0.1	50.0	1.0	500.0	0.3	150.0	0.8	400.0
Raffinose	1.3	0.7	53.9	0.2	15.4	0.6	46.2	0.2	15.4
Manninotriose	4.7	3.6	76.6	1.4	29.8	3.7	78.7	1.6	34.0
Stachyose	2.4	2.1	87.5	10.1	420.8	2.3	95.8	10.9	454.2
Total free sugars	21.5±0.5	12.5±0.7	58.1	26.6±1.1	123.7	11.3±0.5	52.6	25.7±0.5	119.5

Effects of canning and canned storage:

The content of total available carbohydrate found in canned chickpeas ranged from 195.0 mg seed^{-1} corresponding to a loss of 6.2% for water soaked seeds to 189.8 mg seed^{-1} corresponding to a loss of 8.7% for medium-soaked seeds (Table 12). This shows that the effect of soaking the seeds in the medium solution caused no appreciable added loss as compared to being soaked in water.

The starch and higher oligosaccharides have been well retained in water-soaked seeds as the main loss of carbohydrates has been probably due to leaching of free sugars into the cooking water. The loss of starch and higher oligosaccharides is higher in medium-soaked seeds. The salts used in the medium are mainly responsible for imparting the quick-cooking characteristics to the seeds. This is due to the solubilising effect on the proteins. Higher oligosaccharides attached to the protein would be released and be susceptible to further hydrolysis to form free sugars. Further evidence pointing to this is shown by the higher level of free sugars found in medium-soaked seeds as compared to water-soaked seeds.

Storing canned chickpeas at 22^{o}C for 1 year resulted in further losses of total available carbohydrates but the new levels found in water-soaked seeds were 175.8 mg seed^{-1} while those were fairly similar in medium-soaked ones (177.4 mg seed^{-1}). This indicates that whether chickpeas were soaked in water or medium prior to canning, the remaining level of total available carbohydrates after one year storage would be the same.

Storing the cans for one year at 30^{o}C has more effect on the medium-soaked seeds where the resulting amount of total available carbohydrates were found to be 166.0 mg seed^{-1} (Table 14). This corresponds to additional losses in both

Table 12 The effect of canning and 1yr. canned storage at 22°C on total available carbohydrates in chickpeas

	RAW SEED	WC		WC 22		MC		MC	
	mg seed^{-1}	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention
FREE SUGARS	21.5 ± 0.5	9.3 ±0.1	43.3	16.6 ± 0.3	77.2	13.0 ± 0.9	60.5	19.8 ± 0.7	92.1
STARCH + HIGHER OLIGOSACCARIDES	186.5 ± 0.6	185.6 ± 4.2	99.5	159.2 ± 6.2	85.4	176.8 ± 0.9	94.8	157.6 ± 4.5	84.5
TOTAL AVAILABLE CARBOHYDRATES	208.0 ± 0.8	195.0 ± 4.6	93.8	175.8 ± 6.8	84.5	189.8 ± 1.0	91.3	177.4 ± 5.1	85.3

Table 13 The effect of canning and 1yr. canned storage at 22°C on free sugars in chickpeas

	RAW SEED	WC		WC 22		MC		MC 22	
	mg seed^{-1}	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention
Fructose	1.2	0.2	16.7	1.9	158.3	0.3	25.0	0.7	58.3
Galactose	1.4	T	—	0.2	14.3	T	—	T	—
Glucose	1.0	0.2	20.0	0.7	70.0	0.1	10.0	0.2	20.0
Sorbitol	T	T	—	T	—	T	—	0.2	—
Unknown	0.7	0.1	14.3	0.7	100.0	0.2	28.6	0.5	71.4
Sucrose	7.1	3.2	45.1	1.3	18.3	3.9	54.9	4.9	69.0
Maltose	1.3	0.6	46.2	1.4	107.7	0.8	61.5	2.0	153.9
Cellobiose	0.2	0.1	50.0	0.4	200.0	0.2	100.0	0.4	200.0
Mellibiose	0.2	0.2	100.0	0.7	350.0	0.3	150.0	0.7	350.0
Raffinose	1.3	0.4	30.8	0.2	15.4	0.7	53.9	0.2	15.4
Manninotriose	4.7	2.7	57.5	0.8	17.0	4.0	85.1	1.3	27.7
Stachyose	2.4	1.7	70.8	8.4	350.0	2.8	116.7	8.9	370.8
Total free sugars	21.5±0.5	9.3±0.1	43.3	16.6±0.3	77.2	13.0±0.9	60.5	19.8±0.7	92.1

Table 14 The effect of canned 1yr. storage at 30°C on free sugars in chickpeas

	RAW SEED	WC 30		MC 30	
	mg seed^{-1}	mg seed^{-1}	% Retention	mg seed^{-1}	% Retention
FREE SUGARS	21.5 ± 0.5	16.2 ± 0.5	75.4	17.8 ± 0.6	82.8
STARCH + HIGHER OLIGOSACCHARIDES	186.5 ± 0.6	156.9 ± 6.5	84.1	148.2 ± 5.6	79.5
TOTAL AVAILABLE CARBOHYDRATES	208.0 ± 0.8	173.1 ± 7.2	83.2	166.0 ± 6.3	79.8

Table 15 The effect of canning and 1yr. canned storage at 30°C on free sugars in chickpeas

	RAW SEED mg seed^{-1}	WC 30		MC 30	
		mg seed^{-1}	% Retention	mg seed^{-1}	% Retention
Fructose	1.2	0.6	50.0	1.2	100.0
Galactose	1.4	T	—	0.7	50.0
Glucose	1.0	T	—	1.1	110.0
Sorbitol	T	T	—	0.2	—
Unknown	0.7	0.5	71.4	0.6	85.7
Sucrose	7.1	2.9	40.9	2.8	39.4
Maltose	1.3	1.6	123,1	0.3	23.1
Cellobiose	0.2	0.3	150.0	0.5	250.0
Mellibiose	0.2	0.6	300.0	0.5	250.0
Raffinose	1.3	0.1	7.7	0.4	30.8
Manninotriose	4.7	0.9	19.2	2.7	57.5
Stachyose	2.4	8.7	362.5	7.0	291.7
Total free sugars	21.5±0.5	16.2±0.5	75.3	7.8±0.6	82.8

free sugars and starch and higher oligosaccharides. Table 15 shows (c.f. MC22 column in Table 13) that, for the above, stachyose breakdown has primarily been responsible for the resulting build up of manninotriose, raffinose, glucose, galactose, and fructose.

Conclusion

There was a significant loss of total available carbohydrates due to decorticating, drying, freezing, and canning of chickpeas when compared to the raw seed.

Further losses on one-year storage were also significant in all cases when compared to their respective processed samples (those immediately analysed).

An interesting point worthy of note is the fact that after one year of storage there was no significant difference between the carbohydrate levels in the processed samples and those in the stored untreated raw seeds.

Losses are significantly greater in the dried seeds than the ordinarily milled seed but this is not so after one year of storage.

In the deep-freeze processing the medium-soaked chickpeas retained higher levels of carbohydrates while cooking has significantly brought about a loss.

The effect of the medium or temperature has not significantly affected the stability of carbohydrates in canned seeds.

In general, due to hydrolysis of higher raffinose oligosaccharide molecules, the processing conditions studied may have brought an increase in the digestibilty of total available carbohydrates in chickpeas.

CHAPTER IV

EFFECT OF PROCESSING AND STORAGE ON PROTEINS

Introduction

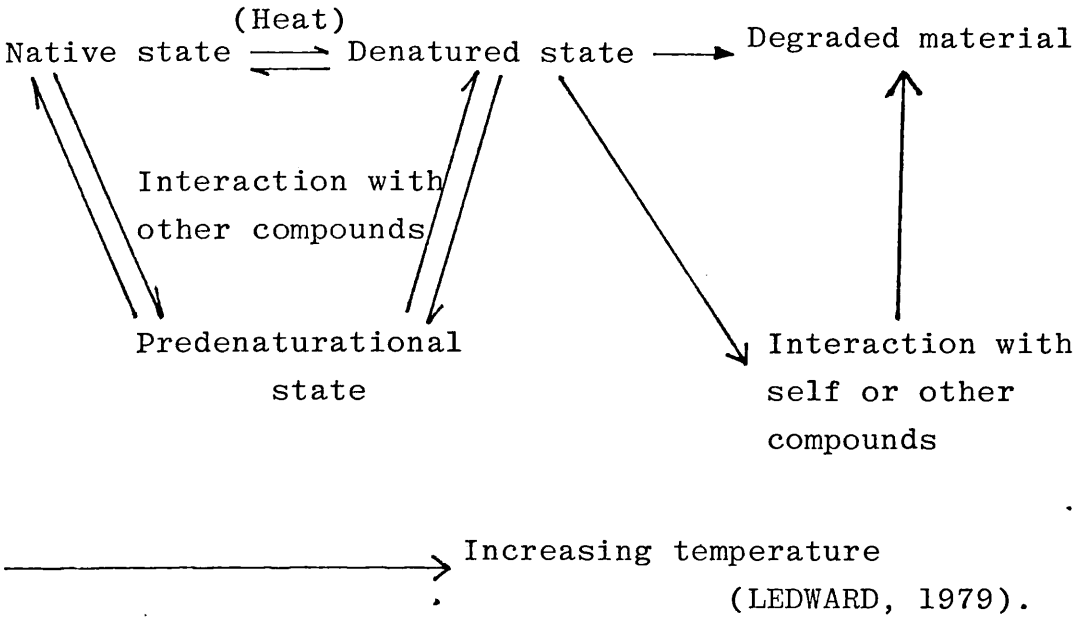
Proteins are polymers of amino acids which may exist in a variety of forms; namely primary, secondary and tertiary configuration. Food processing may have an effect on individual amino acid components, which in turn may lead to an alteration in the structure of the protein molecules. This sometimes renders the protein indigestible to enzymes in the human body.

Nutritional losses occur when individual amino acids are destroyed or become metabolically unavailable (OSNER and JOHNSON, 1968). Processing operations most likely to cause changes in the nutritive value of protein in foods are those entailing the application of heat (RICE AND BEUK, 1953).

Since hydrogen bonds play a major role in maintaining the different configurations of protein, it is not surprising that a radical change in structure of protein usually occurs on heating. This change, which occurs without the severance of any covalent links, is known as denaturation (TANFORD, 1968). However, minor conformational changes may occur prior to denaturation which are called predenaturational transition. These are normally of little importance in food systems as they have a minimal effect on the properties of the protein.

Both denaturation and predenaturational transitions are reversible. Often, following denaturation, the proteins may interact either with themselves, or with other molecules, to form aggregates (precipitates) and these reactions are virtually irreversible. If the heat supplied to the protein is excessive,

the covalent bonds may rupture, leading to thermal degradation of the molecules (Fig 10).



(LEDWARD, 1979).

Fig. 10

Schematic representation of stages in denaturation of proteins

The effects of heat treatments on proteins can be briefly explained as follows:

1. The first, which requires only mild heat, is an alteration of the tertiary structure (denaturation) and has no effect on the nutritive value. This is, however, of importance, since the physical and chemical properties of the proteins are changed (MAURON, 1975).

In general, provided the temperature does not exceed about 100°C and the period of heating is no more than 1 hour, little damage occurs. As these conditions are exceeded, lysine, arginine, methionine, cystine, leucine, tryptophan and histidine may become metabolically unavailable to some degree (OSNER and JOHNSON, 1968).

2. The second type of change is caused by mild heat in the presence of reducing substances and results in a linkage between the end amino group with a reducing group. This is the Maillard or 'non-enzymic browning' reaction (CARPENTER and BOOTH, 1973).

3. More severe heating reduces the availability of amino acids in the absence of reducing substances. Reactions can take place within proteins themselves, between the free amino groups of lysine and arginine. Furthermore, amino acids can react with sulphur groups, particularly those of cystine and to a lesser extent those of methionine (MAURON, 1976). Acid hydrolysis of these combinations will release the intact amino acids, hence they are not actually destroyed (EVANS and BUTT, 1949).

4. Damages are also caused by oxidation. At high temperature lipid peroxides can react with the amino acid residues of proteins and decrease their availability (CUQ et al, 1973)

Heating plays an essential role during the preparation of legume foods. These often contain heat labile anti-nutritional factors (trypsin inhibitors), which affect the efficient utilisation of the protein, hence these need to be destroyed.

Heated soybean meal is biologically more effective than un-heated meals and over-heating reverses this effect (RICE and BEUK, 1953). It seems probable that the two changes occur simultaneously. As the meal is heated, the factors opposing high biological utilisation are destroyed, with apparent increase in value. At the same time the protein itself may be undergoing some deterioration. The practical problem in food processing is to balance the gains and losses of nutritional quality in such a manner that a meal of optimum quality results. Success in this may aid the consuming public in that these conditions would already be standardised in commercial processing.

When considering the effects of dehydration, cooking, freezing, canning and storage, various proteins respond differently to these treatments depending upon the composition and properties of the protein and the type of mixture (food) in which they are included. Proteins are ordinarily mixed or chemically combined with other materials such as water, lipids, carbohydrates, fibre, minerals and vitamins. In considering the effects upon proteins, these non-protein materials frequently are of as much importance as the severity of the processing treatment.

Several workers have reported that when sucrose replaces starch in the diet there is a fall in the nutritive value of the protein (KREHL et al, 1946; HARPER and KATAYAMA, 1953)

This is explained by the rapid absorption of the sucrose compared with the more continuous supply of energy from starch; the latter will accompany the relatively slow and continuous release of the amino acids during digestion. Apart from the need for all amino acids to be present at the site of synthesis at the same time, energy is also necessary. If it is not available in sufficient quantity at the time needed, some part of the amino acids will be oxidised and the nutritive value of the dietary protein reduced (BENDER, 1972).

The presence of moisture and also the length of storage time have some effect on the damage done to proteins. Lowering of moisture has some effect on decreasing loss of portein, but no simple generalisation can be made.

Experiments of LEA and HANNAN (1949) established that heat damage is most severe at 10-14% moisture levels. CARPENTER et al (1962) found that the loss of available lysine was greatest at 4-14% moisture. The moisture level in the dehydrated chickpeas has been kept to approximately 4% in this study.

The nutritive value of a dietary protein is dependent on its 'essential' amino-acid composition and their relative percentages (VENKATARAMAN et al, 1976). Losses in these have a profound effect on the nutritional status of the food.

The essential amino-acids showed percentage cooking losses of between 5-40%; lysine and threonine being the most unstable (HARRIS, 1975).

In the evaluation of the protein in a food, the 'chemical score' determination of essential amino acids, is often used as a criterion. It is also well known that chemical scores correlate well with biological value as determined by several methods (RICE and BEUK, 1953). In the present study these values (chemical scores) were used as a guide to asses the losses in protein in chickpeas after processing and storage.

MATERIALS AND METHODS

Estimation of protein

Various methods were tried for the quantitative measurement of protein in chickpeas. These included the Folin-phenol method (LOWRY et al, 1975), Coomassie Brilliant Blue dye-binding method of BRADFORD (1976), and the Macro-Kjeldahl method (OSBORNE and VOOGT, 1978).

In the former two methods, where the dye-binding technique is used, the protein solutions gave a blue colouration for which the maximum absorbance was measured at the appropriate wavelenths. These techniques actually measure the phenol-containing amino acid tyrosine present in all proteins. An assumption is then made of its proportion in relation to other amino acids present in the proteins.

The results were found to be unsatisfactory due to inconsistency of readings obtained. Under different conditions, certain amino acids may have been destroyed hence this method overestimates the total protein. Conversely, some tyrosine may be destroyed hence an underestimated protein value is obtained.

The Kjeldahl method was chosen due to its consistency of results for protein value despite the fact that it is more cumbersome. It is basically employed for the determination of nitrogen (N) in foods, but various modifications have been devised to improve accuracy and speed. Its principles involve the oxidation of organic compounds by sulphuric acid (H_2SO_4) to form carbon dioxide and water, and the release of N as ammonia (NH_3). The NH_3 exists in the H_2SO_4 solution as ammonium sulphate, but the CO_2 and water are driven off. Alkali is added, and the liberated NH_3 distilled into an excess of boric acid solution. The amount of NH_3 produced is determined by titrating against hydrochloric acid (HCl). However part of this N is derived from non-protein sources e.g. free amino acids, amides, and nucleic acids which may be calculated by additional experiments.

Reagents:

i) Sulphuric acid (concentrated).

ii) Hydrochloric acid; 0.1 normal standardised.

iii) Boric acid solution; 40g boric acid (H_3BO_3) was dissolved in distilled water and diluted to 1l.

iv) Sodium hydroxide solution; 500g of sodium hydroxide (NaOH) was dissolved in 1l distilled water.

v) Copper sulphate pentahydrate ($CuSO_4 . 5H_2O$).

vi) Potassium sulphate (anhydrous, K_2SO_4).

vii) Mixed indicator solution; 2 g of methyl red and 1g of methylene blue were dissolved in 1l ethanol (96% v/v). Colour change occurs at pH 5.4. Stored in a dark and cool place.

viii) Glass boiling beads.

Digestion

A few glass beads were placed in a Kjeldahl flask to which 15g K_2SO_4 and 0.5g $CuSO_4$ was added. Approximately 1g of seeds were also transferred to the flask and 25 ml H_2SO_4 was carefully poured in. Gentle swirling of the liquid ensured sufficient mixing.

The flask was placed on an electrically heated hot-plate at an angle of approximately 40^{o} from the vertical. The flask was then gently heated until foaming ceased and the contents liquefied.

Digestion was completed by boiling the mixture vigorously until the liquid became clear and of a light blue-green colour (approximately 3 hours). The flask was occasionally rotated while heating but caution was taken as to avoid any spillage. The solution was then cooled to 40°C, and 50 ml water cautiously added. After mixing, the flask was allowed to cool.

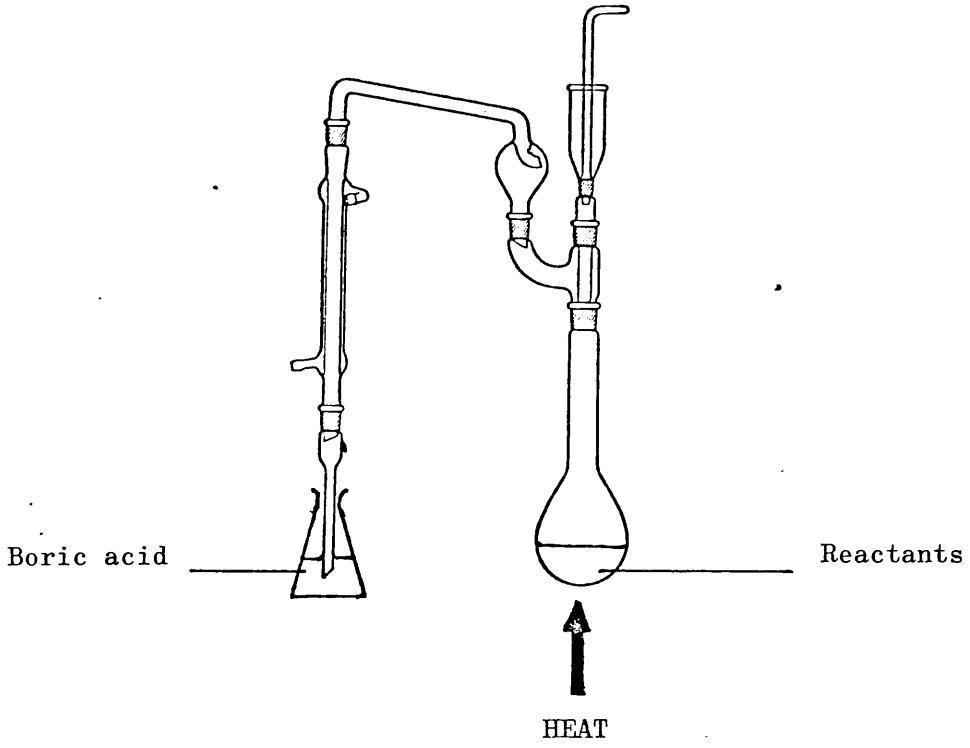


Fig 11 Distillation equipment used in the Kjeldahl procedure

Distillation and titration:

The contents of the Kjeldahl flask were diluted with approximately 300ml distilled water to which fresh boiling beads were added.

The distillation was set up as in Fig 11. Fifty ml of boric acid solution was added to a conical flask to which 4 drops of the indicator solution was also administered. The flask was placed under the condenser of the distillation apparatus so that the outlet dipped into the liquid.

After 15 minutes 100 ml of NaOH solution was added carefully down the inclined neck of the flask so as to form two layers. The mixture was distilled to give between a minimum distillate of 150 ml and a maximum of 250 ml. If bumping was irregular after 150 ml the distillation was discontinued.

The outlet was removed from the liquid just prior to terminating distillation. The outlet was rinsed with distilled water. To verify completion of ammonia distillation, red litmus was used to test the distillate from the condenser.

The contents of the conical flask were titrated with HCl and the volume of the latter used, was noted.

A blank test was conducted at the same time, following this procedure exactly, except that no sample was added here.

Calculation:

Weight (g) of test sample = W
Volume (ml) of HCl used for blank = V_1
Volume (ml) of HCl used for sample = V_2
Normality of HCl solution = H
Non-protein nitrogen = NPN
Correction factor = 6.25

$$\text{Total nitrogen (\%)} = \frac{(v_2 - v_1)H}{w} \times$$

Protein nitrogen = Total nitrogen - NPN

True protein (%) = Protein nitrogen x 6.25

Estimation of non-protein nitrogen (NPN)

As already mentioned (p98), part of the nitrogen obtained in the Kjeldahl digestion process is derived from non-protein sources which include free amino acids, amides, and nucleic acids.

The amount of NPN needs to be determined in order to calculate the true protein content in chickpeas.

Studies by BHATTY and FINALAYSON (1973) showed that NPN extraction from soybean and oilseed meal by the use of 80% ethanol was poor. SINGH and JAMBUNATHAN (1981) working on chickpeas, tested NPN extraction using different concentrations of trichloro-acetic acid (TCA). These ranged from 1-20% TCA. They concluded that the values obtained by direct extraction using 10% TCA represented the NPN of the sample.

Experimental:

NPN was determined by the method of SINGH and JAMBUNATHAN (1981).

Five hundred mg of ground and dried (50^{o}C overnight) chickpea seed, was dispersed in 15ml of10% TCA and shaken in a centrifuge bottle by using a shaker for 1 hour at room temperature. The insoluble material was sedimented by centrifuging at 20,000 rpm for 15 minutes. The residue was washed with another 10ml of the solvent with 30 minutes shaking and again centrifuged to separate the insoluble material. The supernatants were combined to give a volume of 25ml.

The nitrogen content was determined by the Kjeldahl method already described (p ^{97}). Instead of adding the ground seeds the 25ml supernatant (TCA) was directly mixed with the H_2SO_4. NPN was calculated using the same formula used previously.

Identification and determination of amino acids

Free amino acids:

Free amino acids were extracted by the method of SINGH and JAMBUNATHAN (1981) already described. The pH of the resulting TCA solution which contained free amino acids was adjusted to 2 $\pm$ 0.1 using 1 normal NaOH.

Total amino acids:

Total amino acid composition maybe obtained, by adding free amino acids to amino acids obtained by hydrolysing the protein of chickpea seeds. The mixture of amino acids will accurately reflect the composition of the original proteins to be studied; since the only reaction taking place during hydrolysis is the addition of water to the peptide link and the side-chain amide groups. In practice, this is seldom the case as a variety of other reactions will take place during the hydrolytic treatment. The amino acid analysis will therefore be influenced by side reactions, and corrections must be applied to obtain the true compositon.

Hydrolytic agents employed are of three kinds: acids, bases, enzymes. In the present study only acid and alkaline hydrolyses were carried out.

Acid hydrolysis using HCl showed good results but it is known that cystine, cysteine, methionine, and tryptophan are labile under these conditions (OSBORNE and VOOGT, 1978). These amino acids are more stable under alkaline conditions (BLACKBURN, 1968). In order to obtain a complete picture of the total amino acids, both acid and alkaline hydrolyses of proteins were carried out (MAURON, 1973).

Experimental:

Chickpea seeds were dried at 50^{o}C overnight and ground to a powder.

Acid hydrolysis:

To 20 mg of the dried powder 10ml of 6 normal HCl was added and hydrolysed in a boiling water bath for 20 hours. After filtration using Whatman No 1 filter paper, the pH of the acid hydrolysed protein was adjusted to 2 $\pm$ 0.1 with 5 normal NaOH (Fig 14).

Alkaline hydroloysis:

To 20mg of the dried powder was added 10ml of 5 normal NaOH. This was hydrolysed in a boiling water bath for 12 hours. After filtration using Whatman No 1 filter paper the pH of the solution was adjusted to 2 $\pm$ 0.1 with 4 normal H_2SO_4 (Fig 13).

Estimation of individual amino acids

The separation and quantitative determination of amino acids was carried out using an amino acid analyser. In principle, this instrument automatically records the value of the ninhydrin colour of the effluent after ion exchange columns. The influent buffer is pumped at a constant rate through a column of sulphonated polystyrene resin. The effluent is met by a capillary stream of ninhydrin reagent. The colour is developed by passing the mixture of reagent and effluent through a a spiral, capillary Teflon tubing immersed in a boiling water bath. The absorbance of the resulting solution is measured continuously at 570 and 440 nm as it flows through a cylindrical glass cell. The peaks on the recorded curves can be integrated with a precision of 100 $\pm$ 3% for loads from 0.1 to 3.0 umoles for each amino acid (SPACKMAN et al, 1958).

Experimental:

The separation and identification of the amino acids were carried out on a Jeol Model JLC 6AH fully automatic amino acid analyser. The amount of amino acid in each sample was calculated by comparison of peak areas with those obtained

Fig 12.

Amino acid analysis by automatic amino acid analyser (Standards).

Following key applies to Fig 12, 13, & 14.

1 Tryptophan
2 Lysine
3 Histidine
4 Ammonia
5 Arginine
6 Aspartic acid
7 Threonine
8 Serine
9 Glutamic acid
10 Proline
11 Glycine
12 Alanine
13 Cystine
14 Valine
15 Methionine
16 Isoleucine
17 Leucine
18 Tyrosine
19 Phenylalanine

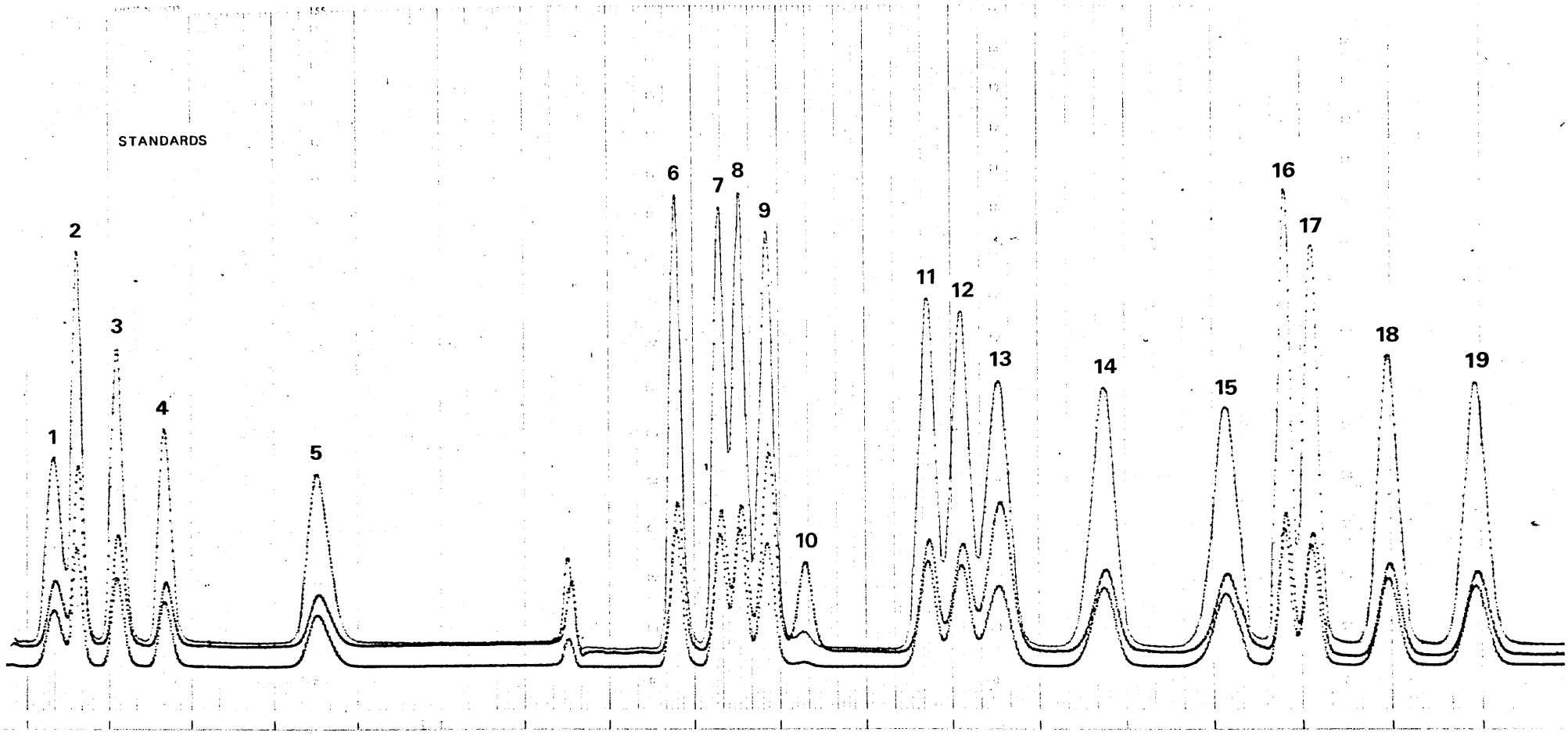

STANDARDS
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Fig 13.

Amino acid analysis of alkaline-hydrolysed raw seed protein.

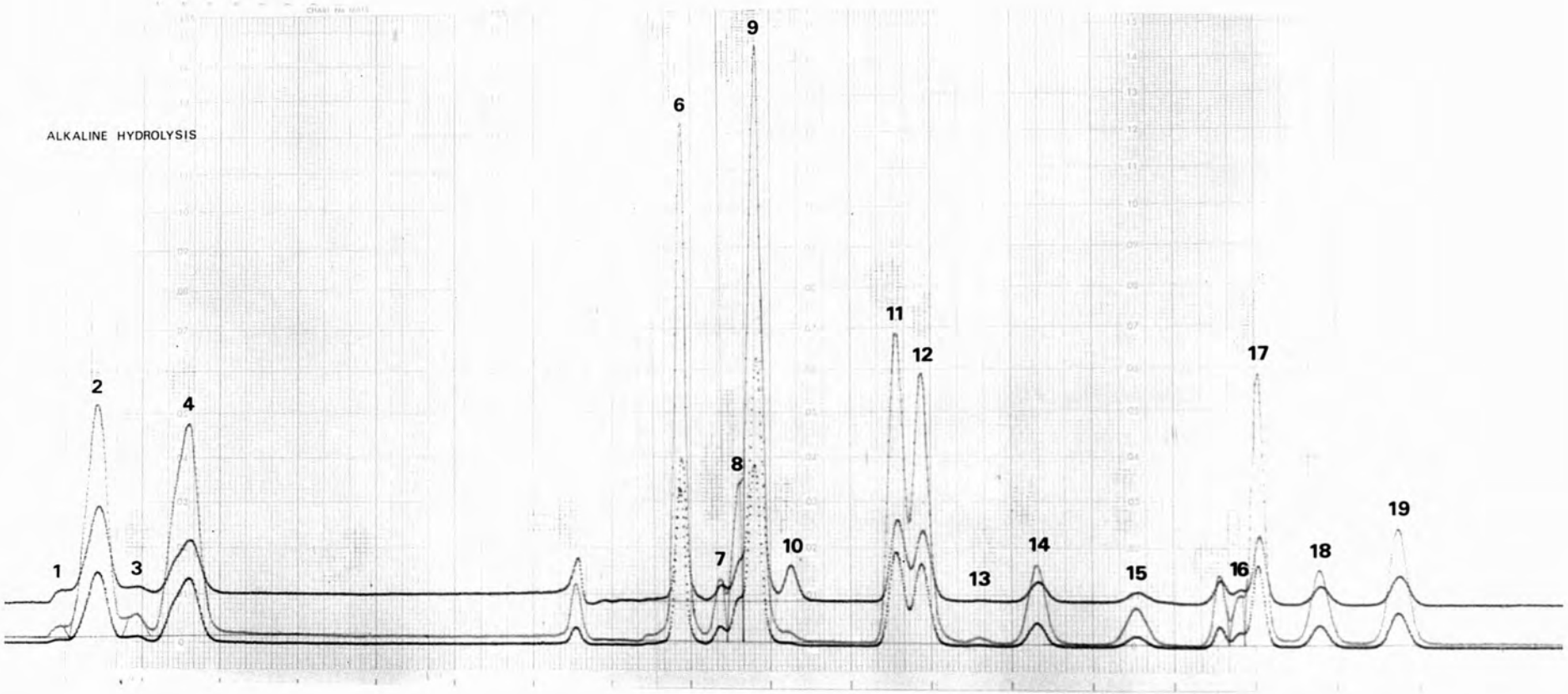
ALKALINE HYDROLYSIS
1
2
3
4
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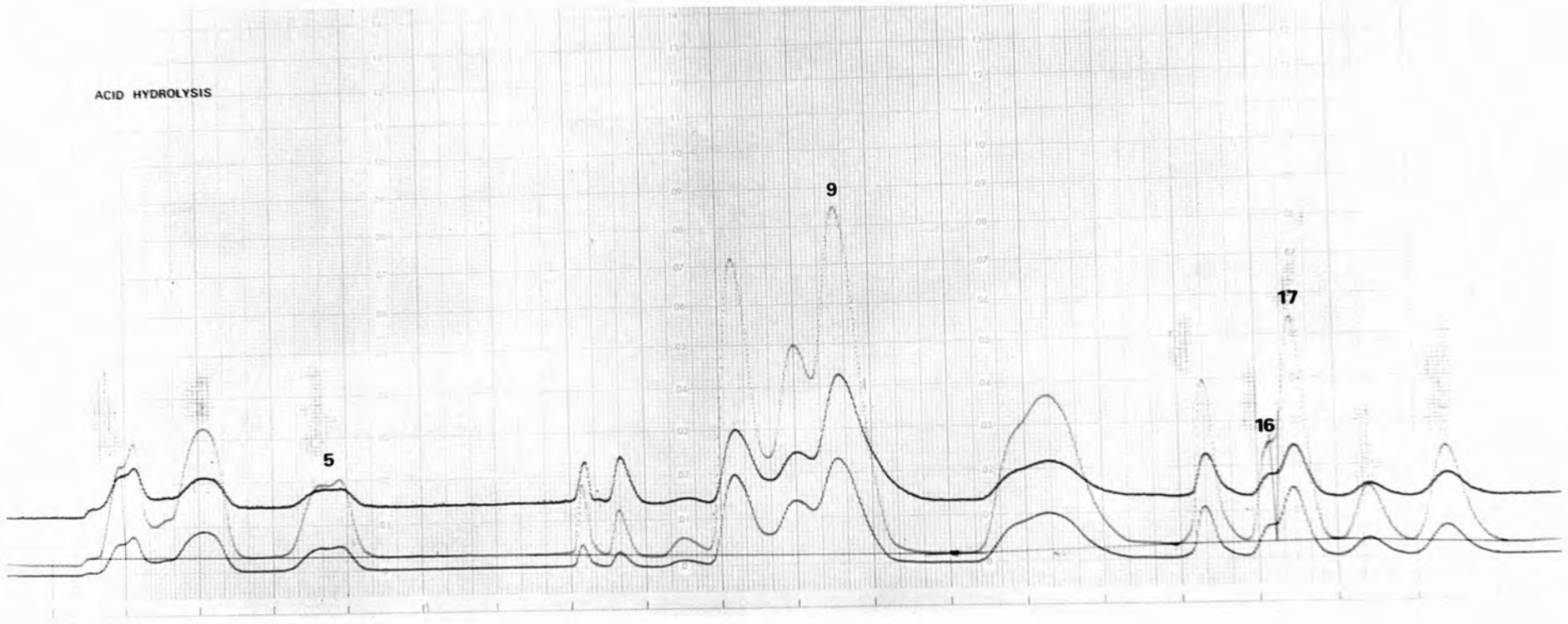
Fig 14

Amino acid analysis of acid-hydrolysed raw seed protein.


ACID HYDROLYSIS
9
17
16
5

using a calibration mixture as described by EVELEIGH and WINTER (1970), (Fig 12).

The calibration mixture of amino acids was expressed in umoles g^{-1} mixture. In order to convert the values for amino acids into mg weight, a modified formula was used to include the molecular weights of the individual amino acids.

$$\text{Weight of amino acid (g) in sample} = \text{umoles} \times M \times 10^{-6} \text{ amino acid}$$

where:
M = Molecular weight of amino acid.

Chemical score:

In order to calculate the chemical score of the amino acids, egg was used as the reference standard (FAO, 1970).

The content of each essential amino acid in a food protein (Ax) is expressed first as a ratio of total essential amino acids (Ex) in the food $\frac{Ax}{Ex}$. These ratios are then expressed as a percentage of ratios between each amino acid in egg (Ae) and the total essential amino acids of egg (Ee) using the formula:

$$\text{Chemical score} = \frac{Ax}{Ex} \div \frac{Ae}{Ee} \times 100\%$$

$$= \frac{AX}{Ex} \times \frac{Ee}{Ae} \times 100\%$$

(RAO, 1970)

Results and discussion

Total protein and amino acids in chickpea and effects of storage on the raw seeds:

In Table 16 the results show that the average total protein content in raw chickpea is 84.5mg seed^{-1}, decreasing to 83.5mg seed^{-1} on one year storage corresponding to a retention value of 98.8%. This shows that total proteins are not adversely affected by one-year storage at 10^{o}C. The increase in free amino acid content from 0.4 to 0.8 mg seed^{-1} indicates that some hydrolysis of the proteins has occurred. Non-protein nitrogen was classed under ammonia.

The presence of eighteen different amino acids was confirmed. These included tryptophan, lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine.

The most abundant amino acids found were glutamic acid, aspartic acid with leucine and lysine in relatively large amounts as well, while the sulphur-containing amino acids (methionine and cystine), tryptophan and histidine were present in small amounts. (Table 17).

These results on chickpeas are in agreement with a number of others (KANDE, 1967; RAO and SUBRAMANIAN, 1970; KHATTAB, 1972; SOSULSKI and HOLT, 1980; SINGH et al, 1981). In most of the cases, however, arginine was also found in relatively large amounts.

Of all the amino acids supplied in the diet eight of these are regarded as essential hence must be obtained directly from the food. These are tryptophan, lysine, threonine, valine, methionine, isoleucine, leucine, and phenylalanine. These amino acids cannot be synthesised in adequate amounts by the human body hence are regarded as essential. Not only does the status of a food protein depend

Table 16 The effect of processing and 1yr. storage on the protein and free amino acid content of chickpeas expressed as mg seed^{-1} or as % retention

	Total protein mg seed^{-1}	Free amino mg seed^{-1}	Ammonia mg seed^{-1}	% Retention of raw unstored seeds	
				Total protein	Free amino acids
Raw seed	84.5±2.2	0.4± *	2.2± *	—	—
Raw-S	83.5±2.7	0.9± *	3.1± *	98.8	225.0
D	83.5±1.9	0.5± *	1.5± *	98.8	125.0
D - S	82.4±0.6	0.6± *	2.1± *	97.5	150.0
De	80.3±1.7	0.3± *	2.0± *	95.0	75.0
De - S	78.8±0.8	0.9± *	3.1± *	93.3	225.0
WF	103.8±1.3	0.4± *	1.2± *	122.8	100.0
WF - S	100.1±2.5	0.8± *	3.6± *	118.5	200.0
MF	89.5±2.3	0.4± *	2.9± *	105.9	100.0
MF - S	86.3±1.2	0.8± *	2.3± *	102.1	200.0
WPF	95.3±1.8	0.3± *	1.6± *	112.8	75.0
WPF - S	92.0±2.5	0.7± *	2.5± *	108.9	175.0
MPF	83.1±2.4	0.3± *	2.5± *	98.3	75.0
MPF - S	80.3±1.7	0.5± *	2.2± *	95.0	125.0
WC	89.5±2.0	0.3± *	2.3± *	105.9	75.0
MC	90.8±1.2	0.2± *	1.8± *	107.5	50.0
WC22	87.5±1.0	0.6± *	2.5± *	103.6	150.0
MC22	87.5±0.9	0.5± *	2.8± *	103.6	125.0
WC30	85.2±1.1	0.6± *	2.8± *	100.8	150.0
MC30	91.3±1.6	0.7± *	3.1± *	108.0	175.0

* S.E. < 0.1 mg seed^{-1}

Table 17 The effect of drying, decorticating, and the subsequent storage for 1 yr. on total individual amino acids in chickpeas (mg seed^{-1} where applicable).

	Try	Lys	His	Arg	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Ileu	Leu	Tyr	Phe
Raw seed	0.8	6.2	1.7	5.4	10.9	4.0	2.7	13.8	3.4	4.4	3.8	0.3	5.4	1.4	4.4	7.0	2.6	4.5
Raw-S	0.7	5.4	1.7	6.5	10.6	3.5	2.5	13.8	3.0	4.0	4.5	—	5.2	0.9	4.4	6.8	2.6	4.5
% Retn.	87.5	87.1	100.0	120.4	97.3	87.5	92.6	100.2	88.2	90.9	118.4	—	96.3	64.3	100.0	97.1	100.0	100.0
D	0.7	5.2	1.8	5.6	11.8	3.7	2.9	14.3	3.5	4.7	3.9	—	5.0	1.3	4.1	6.2	3.0	4.1
% Retn.	87.5	83.9	105.9	108.3	108.3	92.5	107.4	103.6	102.9	106.8	102.6	—	92.6	92.9	93.2	88.6	115.4	93.3
D-S	0.5	4.1	1.0	5.2	12.3	2.9	2.4	15.1	3.2	4.6	4.2	—	5.1	0.8	5.8	6.1	3.1	4.1
% Retn.	62.5	66.1	58.8	96.3	112.8	72.5	88.9	109.4	94.1	104.5	110.5	—	94.4	57.1	131.8	87.1	119.2	91.1
De	0.7	5.8	1.6	5.5	9.9	3.3	2.3	13.7	3.1	3.7	3.0	0.2	5.4	1.4	5.0	6.7	2.1	4.2
% Retn.	87.5	93.6	94.1	101.9	90.8	97.5	85.2	99.3	91.2	84.1	79.0	66.7	100.0	100.0	113.6	95.7	80.8	93.3
De-S	0.6	5.2	1.5	5.0	10.2	3.2	2.5	15.1	2.5	2.4	3.2	—	4.0	0.8	5.5	6.7	2.7	4.3
% Retn.	75.0	83.9	88.2	92.6	93.6	80.0	92.6	109.4	73.5	54.5	84.2	—	74.1	57.1	125.0	95.7	103.8	95.6

on the presence of adequate amounts of these essential amino acids but also on a good balance between them; the latter is determined by chemical score evaluations. The nutritional value of the total protein is represented by the lowest chemical score among the essential amino acids.

The amount of essential amino acids in raw seeds is 33.7mg $seed^{-1}$ representing approximately 40% of the total amino acid (Table 18). This is in almost complete agreement with the results of FAO (1970).

The chemical scores for the individual amino acids ranged from 56 in methionine to 118 in lysine, indicating that chickpea is rich in lysine but limited by methionine. Tryptophan, with a score of 73, was the second limiting amino acid. FAO (1970) and KHATTAB (1972), have considered the chemical score of methionine together with cystine as that of total sulphur-containing amino acids, and have also found that these were first limiting while tryptophan was the second limiting amino acid. RAO and SUBRAMANIAN (1970) on the other hand have also found sulphur-containing amino acids together with tryptophan to be limiting in chickpeas; tryptophan was the first limiting one. It is to be noted that AHMAD et al (1975) have stated that apart from methionine and tryptophan, pulses contain all essential amino acids in sufficient quantities, especially lysine.

After one-year storage of raw seeds there were no notable changes in histidine, aspartic acid, valine, isoleucine, leucine, tyrosine and phenylalanine. There were decreases in all the others except for arginine and alanine which increased. These observations imply that the quality of the chickpea proteins decreased due to a lowering in the chemical scores for tryptophan and methionine, hence making them more seriously limiting.

Table 18 The effect of drying, decorticating and the subsequent storage for 1yr. on the chemical scores of essential amino acids in chickpeas expressed as a % of that found in egg

Sample		Try	Lys	Thr	Val	Met	Ileu	Leu	Phe	Lowest score	TEAA mg seed^{-1}	TEAA Retention %
Raw Seed	% TEAA	2.4	18.4	11.9	16.0	4.2	13.1	20.8	13.4			
	Chemical Score	73	118	104	105	56	93	105	105	56	33.7	
Raw-S	% TEAA	2.2	17.2	11.2	16.5	3.0	13.9	21.7	14.3			
	Chemical Score	67	110	97	108	40	99	110	112	40	31.4	93.2
D	% TEAA	2.3	18.0	12.0	16.2	4.1	13.3	20.4	13.7			
	Chemical Score	71	115	104	106	55	94	103	107	55	30.4	90.2
D-S	% TEAA	1.7	14.0	10.0	17.2	2.6	19.6	20.8	14.1			
	Chemical Score	52	90	87	112	34	139	105	110	34	29.4	87.2
De	% TEAA	2.2	17.5	11.7	16.3	4.3	13.0	21.2	13.8			
	Chemical Score	67	112	102	107	57	92	107	108	57	32.9	97.6
De-S	% TEAA	2.0	17.2	10.6	13.1	2.6	18.2	22.0	14.3			
	Chemical Score	62	110	92	86	35	129	111	112	35	30.3	89.9

General effects of processing:

Except for decorticated seeds the total protein content has either been unaltered or been increased by drying, freezing, and canning. The free amino acids have remained the same or else have generally been reduced. It appears that during the presoaking periods whether in medium or water, synthesis of more protein molecules may have occurred. This was not expected, especially since it was well known that the presence of water would promote the hydrolysis of larger molecules to smaller subunits.

One explanation could be that the imbibition of water during soaking has aided in activating certain enzymes in the cells which have then led to protein synthesis. This would probably have involved amino transferases which are responsible for converting keto acids into amino acids.

There was no general pattern in which individual amino acids behaved when chickpeas were processed hence it was difficult to assess all differences observed. The only consistent effect was that cystine is destroyed in all cases. MAURON (1982) stated that cystine is particularly susceptible to heat damage.

Apart from decorticating and drying the seeds, all other treatments have caused an increase in total essential amino acids. One-year frozen and canned storage have not caused net loss of total protein compared to raw seeds. Some hydrolysis of protein must have taken place during storage as there was an increase in free amino acid content in all cases studied.

The effect of processing and one-year storage on proteins are shown in Fig 15 .

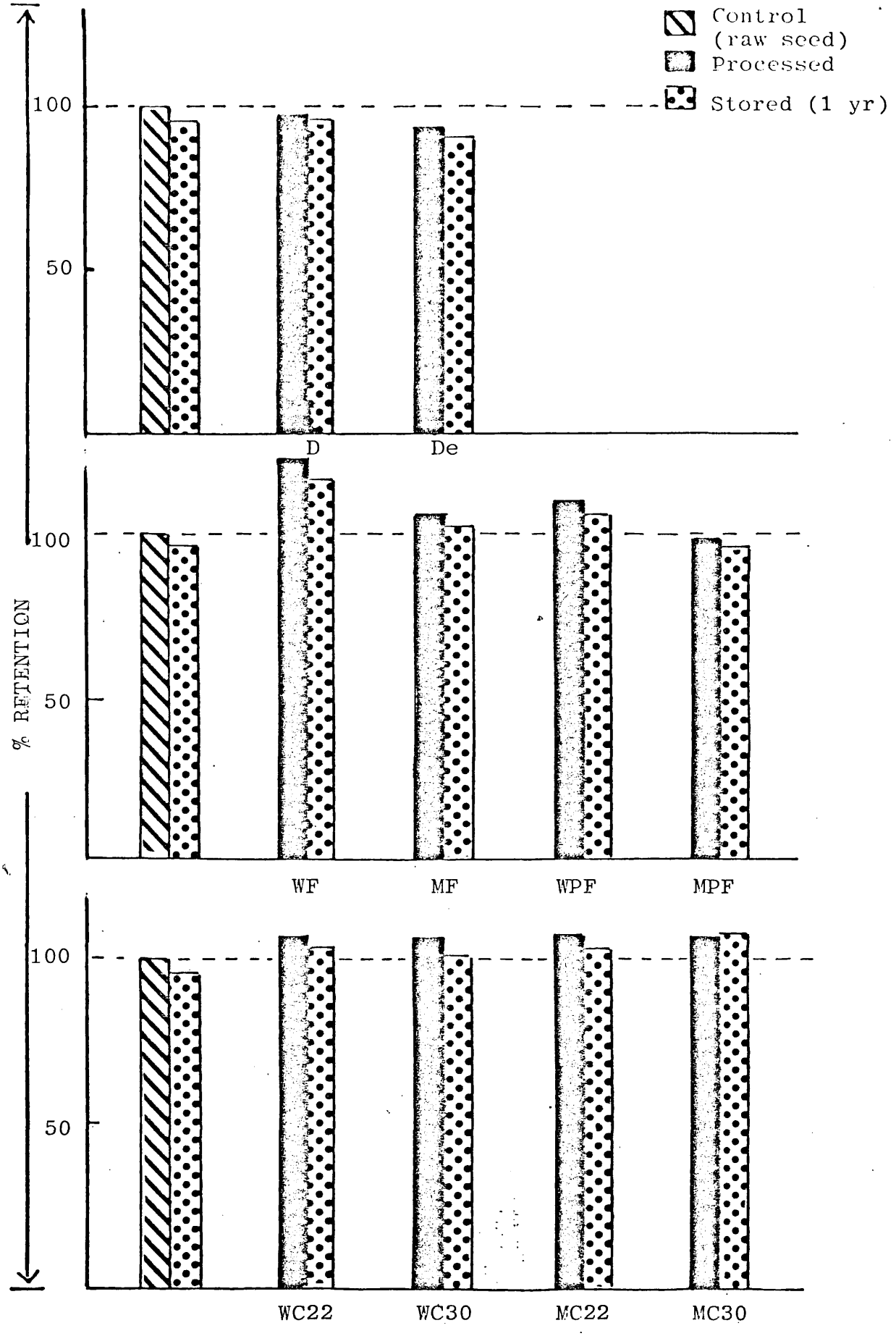


Fig 15 The effect of processing and 1-year storage on % retention of total protein in chickpeas.

Effect of decorticating and storage:

Decorticated seeds contained 80.3mg seed^{-1} of protein (Table 16) which when compared to the protein content of raw seeds indicates that 3.2mg seed^{-1} of protein (assumed to be in the seeds coat) was lost during the decorticating (milling) of the seeds. Another 1.5mg seed^{-1} protein loss occurred after one-year storage of the decorticated seeds.

Immediately after decortication no appreciable change was observed in arginine, threonine, glutamic acid, valine, methionine and leucine. There were slight losses in all other amino acids except for isoleucine which appeared to increase. Chemical scores showed that methionine was the first limiting amino acid. Apart from tryptophan there were adequate amounts of all the other essential amino acids.

After one-year storage, further losses occurred in tryptophan, lysine, histidine, arginine, threonine, proline, glycine, cystine, valine and methionine. There was approximately 90% retention of total essential amino acids.

The chemical scores of valine and methionine have severely decreased making methionine even more limiting.

The increase in free amino acid on storage does not fully account for the loss in protein but the increase in ammonia suggests that degradation of some amino acids may have taken place.

Effect of drying and storage:

Dried seeds contained 83.5mg seed^{-1} protein, which is not much different from that of raw seeds. One-year storage did not cause any substantial reduction with the new value being 82.4mg seed^{-1} of total protein.

The drying process has brought about 10% reduction in the essential amino acids to 30.4mg seed^{-1}. Cystine was also destroyed in the process. However, as no changes in the chemical scores have been noted, all must have been lost in approximately equal amounts.

One-year storage of dried seeds led to further losses in a few of the esstial amino acids e.g. tryptophan, lysine, threonine, methionine and also in a few non-essential ones e.g. histidine, serine, and proline.

MAURON (1982) has reported that the presence of auto-oxidising lipids increases damage to proteins in foods especially when heat is applied. Furthermore, heat damage in foods of low moisture content is quite high. This is due to the fact that decreasing the moisture content to very low levels (as found in the dried seeds) increases the formation of lipid peroxides in the seeds. As a result complex formation between protein-lipid molecules occurs. These complexes may then increase in size in further reactions or else be degraded, releasing ammonia. The scheme for this reaction is further discussed in chapter 5.

Increases in free fatty acid (see chapter 5) and ammonia after storage partially supports this view. Further search of the literature revealed that DESAI and TAPPEL (1963) found that the amino acids histidine, serine, proline, arginine,methionine, and cystine were most labile to peroxidative damage. Although, in this study, losses in arginine were not apparent, losses in all of the others mentioned above may also point to the occurrence

of peroxidative damage of proteins in chickpeas. It should be further noted that DESAI and TAPPEL (1963) studied model systems using casein as the protein material hence arginine may have been protected from lipid damage by other components found in the seeds.

Although not much further loss in total essential amino acids has occurred, the nutritional value of the protein has been decreased to an extent due to lowering of chemical scores of tryptophan, lysine, threonine, and methionine by large amounts.

Effects of freezing and storage:

Table 16 shows that total protein contents of WF, MF, WPF, and MPF seeds are 103.8, 89.5, 95.3, and 83.1mg seed^{-1} respectively. These results indicate an increase in total protein in chickpeas except for MPF seeds.

One-year storage of WF, MF, WPF, and MPF show values 100.1, 86.3, 92.0, and 80.3mg seed^{-1} respectively indicating approximately a 4% loss in total protein in each case when compared to seeds before storage. Except for MPF all the above mentioned values after storage are higher than those of protein in either raw stored or raw untreated chickpeas.

The results of individual amino acids of WF, MF, WPF and MPF are shown in Tables 19 and 21. It will be observed that WF MF processing of chickpeas does not bring about substantial reductions in individual amino acids. The nutritional value of the protein in either case is not affected in any way as compared to that of raw seed (Table 20).

In WPF and MPF samples however, noticeable differences in individual acids are observed. In the case of WPF slight losses of tryptophan and lysine, aspartic acid, serine, proline, and alanine were observed. Some protein losses do occur when food is cooked but it is obvious in the present study that immediate freezing of the seeds has a beneficial effect in that less protein losses were observed.

To explain why MPF has incurred more loss of individual amino acids than WPF the function of the medium treatment must be considered in more detail.

The relationship between salt concentration and solubility is quite complex. Types of proteins such as globulins and albumins are both soluble in dilute salt solutions (5-10%) but

Table 19 The effect of freezing and frozen 1 yr. storage on total individual amino acids in chickpeas (mg seed^{-1} where applicable).

Sample type	Try	Lys	Hys	Arg	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Ileu	Leu	Tyr	Phe
Raw seed	0.8	6.2	1.7	5.4	10.9	4.0	2.7	13.8	3.4	4.4	3.8	0.3	5.4	1.4	4.4	7.0	2.6	4.5
WF	0.8	6.2	2.1	8.5	13.7	4.0	3.6	21.3	4.7	6.0	4.5	–	5.5	1.4	4.5	7.3	3.6	4.7
% Retn	100.0	100.0	123.5	157.4	125.7	100.0	133.3	154.4	138.2	136.4	118.4	–	101.9	100.0	102.3	104.3	138.5	104.4
WF-S	0.8	5.1	0.9	5.4	14.7	3.9	2.8	18.9	4.3	5.5	4.7	–	5.8	1.3	4.9	7.2	3.6	4.7
% Retn.	100.0	82.3	52.9	100.0	134.9	97.5	103.7	137.0	126.5	125.0	123.7	–	107.4	92.9	111.4	102.9	138.5	104.4
MF	0.8	6.0	2.2	6.7	12.9	3.9	2.9	14.2	3.6	3.9	4.0	–	5.3	1.3	4.3	7.1	2.8	4.6
% Retn.	100.0	96.8	129.4	124.1	118.4	97.5	107.4	102.9	105.9	88.6	105.3	–	98.2	92.9	97.7	101.4	107.7	102.2
MF-S	0.8	4.9	1.3	4.9	10.9	3.5	2.7	16.0	3.6	4.6	3.8	–	6.0	1.2	4.4	6.7	2.9	5.6
% Retn.	100.0	79.0	76.5	90.7	100.0	87.5	100.0	115.9	105.9	104.5	100.0	–	111.1	85.7	100.0	95.7	111.5	124.4

Table 20 The effect of freezing & 1 year frozen storage on the chemical score of essential amino acids in chickpeas expressed as a % of that found in egg.

Sample		Try	Lys	Thr	Val	Met	Ileu	Leu	Phe	Lowest score	TEAA mg seed^{-1}	TEAA Retention %
WF	% TEAA	2.3	18.1	11.7	16.1	4.1	13.0	21.1	13.6			
	Chemical Score	70	116	102	105	55	92	107	106	55	34.3	101.8
WF-S	% TEAA	2.4	15.0	11.7	17.2	3.8	14.5	21.5	13.9			
	Chemical Score	73	96	102	112	50	103	109	109	50	33.7	100.0
MF	% TEAA	2.4	18.0	11.8	15.9	4.0	12.9	21.3	13.7			
	Chemical Score	73	115	103	104	53	92	108	107	53	33.4	99.1
MF-S	% TEAA	2.3	14.8	10.6	18.2	3.7	13.2	20.2	17.0			
	Chemical Score	70	95	92	119	49	94	102	133	49	33.2	98.5

Table 21 The effect of freezing and 1 yr. frozen storage on total individual amino acids in precooked chickpeas (mg seed^{-1} where applicable)

	Try	Lys	His	Arg	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Ileu	Leu	Tyr	Phe
Raw seed	0.8	6.2	1.7	5.4	10.9	4.0	2.7	13.8	3.4	4.4	3.8	0.3	5.4	1.4	4.4	7.0	2.6	4.5
WPF	0.7	5.8	2.2	6.7	13.3	3.9	3.5	16.7	4.1	5.9	4.7	-	5.4	1.4	4.6	7.3	3.1	5.1
% Retn.	87.5	93.6	129.4	124.1	122.0	97.5	129.6	121.0	120.6	134.1	123.7	-	100.0	100.0	104.6	104.3	119.2	113.3
WPF-S	0.3	3.9	1.0	5.0	15.1	2.7	2.8	15.6	3.6	5.0	4.2	-	5.4	0.7	5.9	7.2	3.2	5.1
% Retn.	37.5	62.9	58.8	92.6	138.5	67.5	103.7	113.0	105.9	113.6	110.5	-	100.0	50.0	134.1	102.9	123.1	113.3
MPF	0.7	5.8	2.1	5.5	9.1	4.0	2.4	14.3	2.9	4.3	3.5	-	5.3	1.4	4.3	7.3	2.7	4.9
% Retn.	87.5	93.6	123.5	101.9	83.5	100.0	88.9	103.6	85.3	97.7	92.1	-	98.2	100.0	97.7	104.3	103.9	108.9
MPF-S	0.3	3.6	0.2	4.8	12.0	2.8	1.9	14.8	3.0	4.3	3.4	-	5.0	0.7	5.4	7.8	2.8	5.2
% Retn.	37.5	58.1	11.8	88.9	110.1	70.0	70.4	107.2	88.2	97.7	89.5	-	92.6	50.0	122.7	111.4	100.0	115.6

Table 22 The effect of freezing and 1yr. frozen storage on the chemical score of essential amino acids in precooked chickpeas

Sample		Try	Lys	Thr	Val	Met	Ileu	Leu	Phe	Lowest score	TEAA mg seed^{-1}	TEAA Retention %
WPF	% TEAA	2.1	16.8	11.5	15.8	4.1	13.5	21.4	14.8			
	Chemical Score	64	108	100	103	55	96	108	116	55	34.2	101.5
WPF-S	% TEAA	1.1	12.5	8.7	17.2	2.3	18.8	23.1	16.3			
	Chemical Score	32	80	76	112	30	133	117	127	30	31.2	92.6
MPF	% TEAA	2.0	16.5	11.8	15.7	4.2	13.0	21.5	15.3			
	Chemical Score	61	106	103	103	56	92	109	120	56	33.6	99.7
MPF-S	% TEAA	1.0	11.7	9.0	16.1	2.4	17.6	25.3	16.9			
	Chemical Score	30	75	78	105	32	125	128	132	30	30.8	91.4

only albumins are readily soluble in water. However, in concentrated salt solutions, all proteins become less soluble. The increase in solubility of gobulins in dilute salt solutions, known as 'salting in', may be explained in terms of the relative affinity of the protein molecules for each other and for the solvent. The ions of the neutral salt will interact with bonds in the protein, thereby decreasing protein-protein interactions and consequently increasing the solubility of globulins.

Therefore in MF it appears that soaking in medium has effectively weakened the bonds between protein molecules in the seed coat hence more of the protein will have been lost after cooking.

There has, however, been a fairly good retention of total essential amino acids with little effect on the chemical scores (Table 22).

One-year storage of WF and MF shows losses in lysine, histidine, and methionine but not to any large extent. There is no change in the total essential amino acids except that there is a large lowering of the chemical score of lysine.

Losses of the essential amino acids in WPF and MPF after one-year storage have been slightly larger than in WF and MF. There has also been a large reduction in the chemical scores of tryptophan, lysine, threonine, and methionine. This has effectively reduced the protein value and has also made tryptophan and methionine equally limiting.

Effects of Canning and Storage:

The average total protein contents for WC and MC were 89.5 and 90.8mg seed^{-1} respectively (Table 16) indicating that there were no additional losses caused by the medium treatment compared to the water treatment of the seeds prior to canning. Apart from cystine being completely destroyed, only minor losses were noted and these were restricted to tryptophan and to lysine. There is, however, full retention of total essential amino acids, and the chemical scores have not been altered to any large extent. MAURON (1982) has already suggested that in food processing except for cystine, actual destruction of amino acids remains negligible.

After canned storage (22^{o}C) for one year, losses of total protein were fairly minimal, ranging from 2.3% to 3.9% when compared to that of the immediately canned seeds (WC and MC respectively); the final values for WC22 and MC22 both being 87.5mg seed^{-1} (Table 16).

The temperature of storage did not have any appreciable effects on total protein.

There was a substantial loss in the amino acids tryptophan, lysine, histidine, arginine, threonine, serine, and methionine (Table 23).

Changes in amino acids in chickpeas after cooking have been the subject of a number of studies, but conflicting results have been obtained. MORCOS et al (1976) have noted increases in tryptophan and lysine but decreases in all other amino acids on cooking chickpeas. AHMAD et al (1975) have observed increases in arginine, histidine, typtophan, and phenylalanine but decreases in leucine, isoleucine, valine, methionine and lysine in cooked chickpeas. These results are not unexpected in that a certain amount of denaturing would have occurred in the protein molecules

Table 23 The effect of canning and 1yr. canned storage at 22°C and 30°C on total amino acids in chickpeas (mg seed^{-1} where applicable)

	Try	Lys	His	Arg	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Ileu	Leu	Tyr	Phe
Raw seed	0.8	6.2	1.7	5.4	10.9	4.0	2.7	13.8	3.4	4.4	3.8	0.3	5.4	1.4	4.4	7.0	2.6	4.5
WC	0.7	5.8	2.0	5.7	12.1	4.0	2.9	14.8	3.7	4.6	3.9	-	5.3	1.4	4.3	7.3	2.8	4.9
% Retn.	87.5	93.6	117.7	105.6	111.0	100.0	107.4	107.0	108.8	104.6	102.6	-	98.2	100.0	97.7	104.3	107.7	108.9
MC	0.7	6.1	1.9	5.8	11.2	4.1	3.0	14.4	3.9	4.8	4.0	-	5.5	1.4	4.5	7.3	3.2	4.7
% Retn.	87.5	98.4	111.8	107.4	102.8	102.5	111.1	104.0	114.7	109.1	105.3	-	101.9	100.0	102.3	104.3	123.1	104.4
WC22	0.3	3.2	1.4	4.6	12.6	2.5	2.1	15.9	3.8	4.4	4.1	-	4.6	0.8	4.7	8.5	2.7	7.3
% Retn.	37.5	51.6	82.4	85.2	115.6	62.5	77.8	115.2	111.8	100.0	107.9	-	103.7	57.1	106.8	121.4	103.8	162.2
MC22	0.4	3.5	1.4	0.9	13.2	2.7	3.1	15.6	3.8	4.4	3.7	-	5.6	0.8	4.6	8.2	2.6	7.4
% Retn.	50.0	56.5	82.4	90.7	121.1	67.5	114.8	113.0	111.8	100.0	97.4	-	103.7	57.1	104.5	117.1	100.0	164.4
WC30	0.3	3.2	1.1	4.7	12.5	2.5	2.2	15.1	3.7	4.4	4.1	-	5.1	0.8	4.6	8.6	3.0	6.5
% Retn.	37.5	51.6	64.7	87.0	114.7	62.5	81.5	109.4	108.8	100.0	107.9	-	94.4	57.1	104.5	122.9	115.4	144.4
MC30	0.4	3.4	1.5	5.0	13.2	2.7	2.5	15.4	3.6	4.5	3.8	-	5.3	4.8	4.8	7.4	3.0	7.2
% Retn.	50.0	54.8	88.2	92.6	121.1	67.5	92.6	111.6	105.9	102.3	100.0	-	98.1	57.1	109.1	105.7	115.4	160.0

Table 24 The effect of canning and 1yr. canned storage on the chemical score of essential amino acids in chickpeas

Sample		Try	Lys	Thr	Val	Met	Ileu	Leu	Phe	Lowest score	TEAA mg seed	TEAA Retention %
WC	% TEAA	2.2	17.3	11.8	15.8	4.1	12.8	21.6	14.4			
	Chemical Score	67	111	103	103	55	91	109	113	55	33.7	100.0
MC	% TEAA	2.1	17.6	11.7	16.1	4.0	13.2	21.7	13.6			
	Chemical Score	64	113	102	105	53	94	110	106	53	34.8	103.3
WC22	% TEAA	1.0	9.8	7.5	17.1	2.3	14.3	25.8	22.2			
	Chemical Score	30	63	65	112	30	101	130	173	30	32.9	97.6
MC22	% TEAA	1.1	10.5	8.2	16.8	2.3	14.0	24.7	22.4			
	Chemical Score	32	67	71	110	30	99	125	175	30	33.2	98.5
WC30	% TEAA	1.0	10.0	7.9	16.2	2.5	14.6	27.2	20.6			
	Chemical Score	30	65	69	106	33	104	137	161	30	31.7	94.1
MC30	% TEAA	1.2	10.6	8.4	16.5	2.6	14.9	23.1	22.7			
	Chemical Score	35	68	73	108	34	106	117	177	34	31.9	94.7

due to the combined effects of pre-cooking and canning.

Total essential amino acids do not seem to have been affected very much. Tryptophan, lysine, threonine, and methionine became severely limiting as observed from the chemical score values hence there was a decrease in nutritive value of the protein (Table 24).

The increased water content of the seeds in the cans would make them susceptible to the browning reaction in the presence of reducing sugars. Three observations point to the fact that a certain amount of browning had, in fact, occurred.

Firstly, the observation in Chapter 2 (p 47), that the liquor in the cans was found to be darker in colour indicates that either colour is being leached from the chickpeas themselves or that non-enzymic browning has occurred forming coloured pigments.

Secondly, it was noted in Chapter 3(p 85), that on storage of canned seeds there was an increase in the free sugar content in all cases hence this together with increased moisture would also facilitate browning via the Maillard reaction.

Finally, HURRELL and CARPENTER (1977) have shown that Maillard damage can destroy large proportions of lysine, arginine and to a lesser extent tryptophan, cystine, and histidine. In this study losses in these amino acids during the storage of canned seeds has taken place.

The loss of threonine could be due to its higher susceptibility to heat damage. The occurrence of peroxidative damage to proteins by lipids cannot, however, be fully ruled out due to the observed reduction in level of serine which was suggested by DESAI and TAPPEL (1963) as being labile to peroxidation.

Conclusion

Significant increases in total protein occurred after freezing and canning except for medium - soaked precooked frozen seeds. MPF and dried seeds showed no significant change, whereas decorticated seeds showed a significant decrease in proteins.

Further protein losses due to storage of raw, dried, and decorticated seeds were not significantly different.

Medium treatment, precooking and one-year storage significantly reduced the total protein in chickpeas only when compared to water-soaked frozen (not stored) seeds. Not much change in nutritional value of protein occurred as determined by chemical score evaluations. Increased storage temperature did not significantly affect the protein of canned seeds.

Even though browning reactions have occurred in canned seeds, this does not necessarily mean that the nutritional properties of the remaining protein would be adversely affected by Maillard reaction products. ATKINSON and CARPENTER (1970), and BOCTOR and HARPER (1968) have shown that heated cod-glucose and egg white-glucose mixtures, that had lost more than 80% of their nutritive value after the Maillard reaction, when supplemented to well balanced diets did not depress the growth of rats.

The presence of auto-oxidising lipids in the chickpeas would also promote heat damage of proteins which will be discussed in Chapter 5.

C H A P T E R V

EFFECT OF PROCESSING & STORAGE ON LIPIDS

Introduction

The term ' lipids' is difficult to define precisely but their characteristics are summarised below.
Lipids:

(i) are insoluble in water
(ii) are soluble in organic solvents such as chloroform, ether, or benzene
(iii) contain long-chain hydrocarbon groups in their molecules.
(iv) are present in or derived from living organisms.

These properties cover a wide range of compounds and include long chain hydrocarbons, alcohols, aldehydes, fatty acids and derivatives such as glycerides, wax esters, phospholipids glycolipids, and sulpholipids.

Lipids may be sub-divided into two broad classes: Simple, which contain one or two hydrolysis products per molecule, and 'Complex' which contain three or more types (CHRISTIE, 1973).

In this present study simple lipid classes and complex lipid classes have been analysed separately.

In the processing of chickpeas, the operations involved may bring about changes in the total lipid content. Other changes may also take place which have a direct bearing on the storage life of the product in terms of increase in fatty acid and oxidative degradation of unsaturated lipids. In the present study these will be taken into consideration.

There is some evidence that undesirable changes which occur

in heated lipids may have a deleterious effect on human health (CRAMPTON et al, 1956 ; KAUNITZ, 1956).

The greatest changes in structure take place in those lipids which are the more unsaturated. In the heat processing of lipids, which may include drying and cooking treatments, three main types of reactions can occur.

(a) Hydrolysis results in the formation of free fatty acids which at high concentration may promote rancidity of the food hence giving a sour taste to the food.

(b) Reactions with oxygen (auto-oxidation) lead to the formation of hydroperoxides, epoxides, and hydroxides. All these may subsequently undergo further degradation into smaller molecules.

Lipid auto-oxidation is very important and of much interest because it results in the formation of off-odours, reduction or destruction of fatty acids, and the formation of brown pigments. The most common type of lipid oxidation is autocatalytic oxidation. The rate of an autocatalytic reaction increases with time, because the products which are formed during the reaction tend to catalyse the rest of the reaction and also reinitiate the cycle of events. Thus, as the reaction proceeds, the rate of hydroperoxide accumulation increases.

The rate of auto-oxidation of the fatty acids and their derivatives depends particularly on the amount of unsaturation of the fatty acids. Further, the rate of oxidation can be greatly accelerated by a number of other factors e.g. trace metals, oxidative enzymes, light, and temperature (LUNDBERG, 1962).

The free radical chain mechanism of auto oxidation reactions was determined by BOLLAND (1946) & BOLLAND & GEE (1946) as follows:

Initiation:

$$RH + O_2 \longrightarrow \text{Free radicals}$$

$$\left.\begin{matrix} ROOH \\ \\ (ROOH)_2 \end{matrix}\right) \longrightarrow \begin{matrix} \text{Free radicals} \\ (\text{e.g. } R^{\bullet}, RO^{\bullet}, RO_2^{\bullet}, HO^{\bullet}) \end{matrix}$$

Propagation:

$$R^{\bullet} + O_2 \longrightarrow RO_2^{\bullet}$$

$$RO_2^{\bullet} + RH \longrightarrow R^{\bullet} + ROOH$$

Termination:

$$\left.\begin{matrix} R^{\bullet} + R^{\bullet} \\ \\ R^{\bullet} + RO_2^{\bullet} \\ \\ RO_2^{\bullet} + RO_2^{\bullet} \end{matrix}\right) \longrightarrow \begin{matrix} \text{Stable (non-radical)} \\ \text{end products.} \end{matrix}$$

It has to be noted that the hydroperoxides by themselves do not contribute materially to the unwanted odours and flavours of auto-oxidised food materials. These off-flavours are caused by secondary substances formed during the various reactions and possibly through further oxidation of the peroxides and their products (PATTON et al, 1959).

The number and variety of hydroperoxide degradative compounds are indeed quite large. Further, oxygen can act on ethylenic bonds to yield other degradative products. In addition to these

alcohols, aldehydes, and other compounds formed during initial degradation are susceptible to further oxidation.

(c) Heating may cause cross-linking to form new carbon-carbon bonds between two triglyceride molecules, particularly in the absence of oxygen. If these bonds are formed within one fatty acid, cyclic fatty acids are obtained. New bonds between two different fatty acids lead to the formation of dimeric acids either within one triglyceride molecule or between two molecules. This latter reaction is the first step in the polymerisation of lipids (MORTON, 1977). This could lead to decreased digestibility of lipids in the diet.

Similar to the fact that certain amino acids are considered essential so are certain fatty acids. The human body cannot synthesize polyunsaturated fatty acids from saturated fatty acids, hence the former need to be obtained directly from the food in the diet.

The more common polyunsaturated fatty acids which are found in most plant materials include the 18:2 and 18:3 types which are regarded as 'essential'. The numbers after the colons denote the number of double bonds contained in the molecule. These unsaturated fatty acids can, however, be further converted into less saturated forms by enzymes in the body according to its needs.

Due to their important role in the diet the fatty acids of chickpeas have been investigated in detail in this study. Degrees of unsaturation in fatty acid molecules may be estimated by the Iodine Value which denotes the weight of Iodine absorbed per 100g lipid. Hence, this has also been carried out.

MATERIALS AND METHODS

Lipid extraction

There are several procedures for lipid extraction in which different organic solvents are involved. All these methods have their own advantages and disadvantages. When choosing a procedure, consideration needs to be taken on the nature of the sample and the purpose of the experiment. In general, the relevant procedure must take the following into account:

1) There must be complete recovery of all the lipids of the sample.
2) The non-lipid compounds must be eliminated from the extract.
3) Artefacts which may be produced under some circumstances, must be prevented.

Satisfactory methods for the preparation and purification of lipids have been described by FOLCH et al (1957) and BLIGH & DYER (1959). These methods have been widely used and will be used in this study, except that certain necessary modifications have had to be made.

CHRISTIE (1973) has shown that a mixture of chloroform-methanol (2:1; v/v) extracted lipids more exhaustively from plant, animal or bacterial tissues than most other simple solvent systems. BLIGH & DYER (1959) mentioned that this method recovered 95-99% of the lipid content of the sample. After comparing several methods, FISHWICK & WRIGHT (1977) found a chloroform-methanol mixture to be the most efficient.

LOUGH et al (1962) have found that if chloroform-methanol or any other alcoholic extract which contains lipids, is stored for long periods in the presence of very small amounts of tissue, transesterification takes place and large amounts of

methylesters are found in the extracts. Most extractions should be completed fairly quickly in order to avoid this occuring.

It is well known that most polar organic solvents used to extract lipids from tissues, also extract significant amounts of non-lipid contaminants (FOLCH et al, 1957) and a dilute salt solution (0.88% sodium chloride solution) at approximately ¼ of total solvent volume will help to remove almost all non-lipid contaminating compounds.

Experimental:

Raw chickpea seeds are hard and cannot be properly crushed in a blender, so they had to be ground in a mortar first. Processed chickpeas were relatively soft, hence could be extracted directly.

Approximately 10g chickpea seeds were homogenised in 200ml chloroform and 100ml methanol in a Waring blender for 2 minutes. The extract was then filtered through a sintered glass funnel and the above procedure repeated again. The volume of the filtrate was then measured accurately. A volume of 0.9% NaCl solution (in distilled water), corresponding to a ¼ of the total filtrate was used to wash the lipid extraction into a separating funnel. The lower chloroform layer contained total lipids, while the upper aqueous methanol layer containing lipid contaminant was discarded.

Estimation of total lipids

From the total chloroform extract a certain volume (100ml) was removed and evaporated under vacuum at approximately 25°C to determine the total lipid content. This was calculated using the following formula:

$$\text{Total lipid content (\%dry weight)} = \frac{w \times v_1}{a \times v_2} \times 100$$

in which,

w = weight of the oil after evaporation
v_1 = total volume of extract
v_2 = volume removed for evaporation
a = weight of the sample (dry weight).

The rest of the extract was evaporated to dryness and redissolved in 10ml benzene containing butylated hyroxy toluene (100mg/l), which acted as an antioxidant.

The lipid sample was stored at -18°C.

Fractionation and identification of simple lipid classes

The most common classes of simple lipids are monoglycerides, diglycerides, sterols, free fatty acids, triglycerides and to a lesser extent, hydrocarbons, methyl esters, wax esters and glyceryl esters. In general, plant lipids commonly contain triglycerides as the predominant simple lipid (HITCHCOCK & NICHOLS, 1971)

Three preliminary techniques used in an attempt to obtain the different lipid classes present in chickpeas were solvent partition, column chromatography, and thin layer chromatography (TLC).

Solvent partition (counter current distribution), involved a 4-step separation of simple lipids from complex lipids by partitioning the total lipid extract between petroleum ether (b.pt. 40-60°C) and 87% ethanol. GALANOS & KAPOULAS (1962) have shown that the ethanol fraction gives 97% recovery of complex lipids while nearly all the simple lipids would dissolve in the ether layer.

This was not very successful as the ethanol fraction still contained large amounts of simple lipids, so after a number of trials this technique was discarded.

Column chromatography using silicic-acid (100-200 mesh) by the procedures of ROUSER et al (1967), involving serial elution with different solvents, was also not satisfactory due to incomplete separation of lipid classes.

TLC using silica gel coated plates was found to be the most successful in terms of separating and identifying different lipid classes. For this purpose the solvent systems of CHRISTIE (1973), hexane: diethyl ether: formic acid (80:20:2, v/v/v) and those of STORRY & TUCKLEY (1967), benzene: diethyl ether: ethylacetate: acetic acid (80:10:10:0.2, v/v/v/v) were tried.

Since there was a better separation with the first solvent system (Fig 16), this was used routinely from then on.

Experimental:

Thin-layer plates (20 x 20 cm) plastic-backed, coated with a layer of 0.25 mm thick silica gel G, supplied by Mackery - Nagel & Co Ltd, were used.

The samples, together with a number of different standard compounds were applied to the chromatoplates by graduated micro-syringes as bands 1.5cm long with spaces of 0.5cm between them and 2cm from the edge of the plates.

Fig 16.

TLC analysis of simple lipids in raw and processed seeds.

Bands were identified as follows :

1 Sterol esters

2 Triglycerides

3 Free fatty acids

4 Unknown

5,6 'Other sterols'

7 β -sitosterol

8,9 Diglycerides

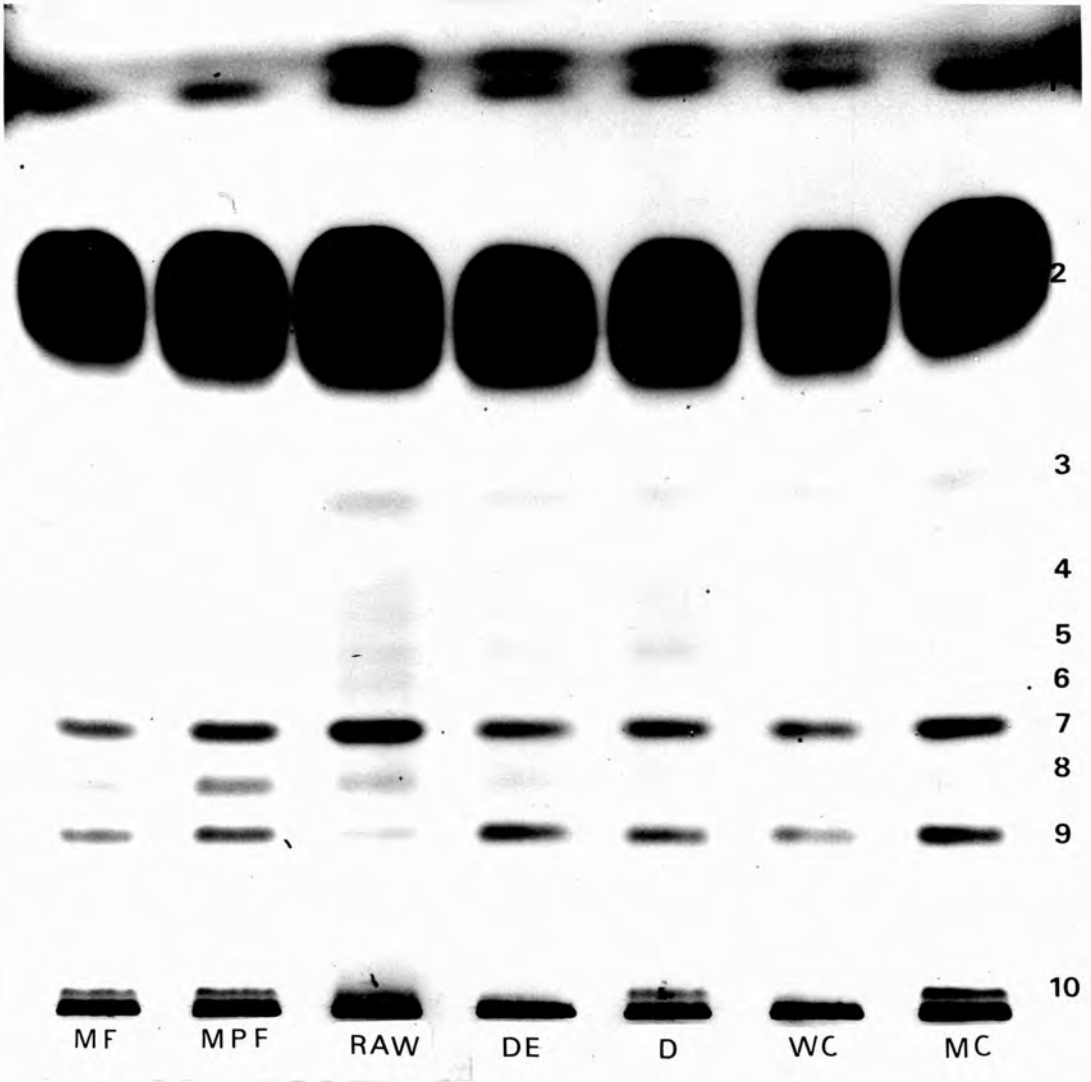
10 Monoglyceride

Developing solvent :

Hexane : diethyl ether : formic acid (80:20:2, V/V)

Spray :

Cupric acetate


2
3
4
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6
7
8
9
10
MF
MPF
RAW
DE
D
WC
MC

These were placed in airtight chambers containing the already mentioned solvent system of CHRISTIE (1973).

The best way of obtaining straight solvent fronts was by inserting plates in vertical position with the backs almost in complete contact with the chamber wall. It was found that if only one plate per chamber was used, good reproducibility could be obtained.

The solvent front was allowed to move to 4 cm from the top of the plate, which was then air-dried at room temperature for 4 hours.

The plates were ready for identification by using appropriate sprays (p 142).

Fractionation and identification of complex lipid classes

Complex lipids can be subdivided into three main classes:

i) Phospholipids (phosphoglycerides) which on hydrolysis yield glycerol, fatty acids, inorganic phosphate and an organic base or polyhydroxy compound.

ii) Glycolipids comprising glycosyldiglycerides, which on hydrolysis yield glycerol, fatty acids, sugars, and certain sphingolipids which contain a long chain base, fatty acids and inorganic phosphate, carbohydrate or other complex organic compounds.

Due to the difficulty of separating all complex lipid fractions by a single dimensional TLC procedure (CHRISTIE, 1973): glycolipids tending to overlap phospholipids, several methods were adopted which will be discussed presently.

Separation of glycolipids:

The technique used by GARDENER (1968) for isolating glycolipids gave good results hence this was adopted. The TLC plates were developed in the following solvent:-

acetone-acetic acid-water (100:2:1,v/v/v).

The simple lipids moved with the solvent front, while the phospholipids remained at or near the origin. The glycolipids were the compounds that separated out under these conditions, and these were identified as mentioned (p 144).

Separation of complex lipids other than glycolipids:

In order to separate these complex lipids, a two-step developing system was required. After trying various solvent systems it was found that those recommended by NICHOLS et al (1965) gave the best results.

The TLC plates were run first in the following solvent mixture:

Petroleum ether: acetone (2:1, v/v) and then air-dried for 2 hours.

The plates were then run in the same direction in the second solvent mixture of:

Chloroform-methanol-acetic acid-water (170:25:25:6, v/v/v/v)

The plates were allowed to air-dry overnight before spraying.

Two-dimensional TLC for complex lipids:

The problem of separating all phospholipids as well as glycolipids has previously been mentioned. In order to confirm that the separation was succesful, and that there were no lipids

Fig 17 .

TLC analysis of complex lipids in raw and processed seeds.

Bands were identified as follows :

1 Phosphatidic acid

2 Steryl glycoside

3 Phosphatidyl ethanolamine

4 Sulpholipid

5 Digalactosyl diglyceride

6 Phosphatidyl choline

7 Phosphatidyl inositol

8 Lysophosphatidyl choline

First developing solvent :

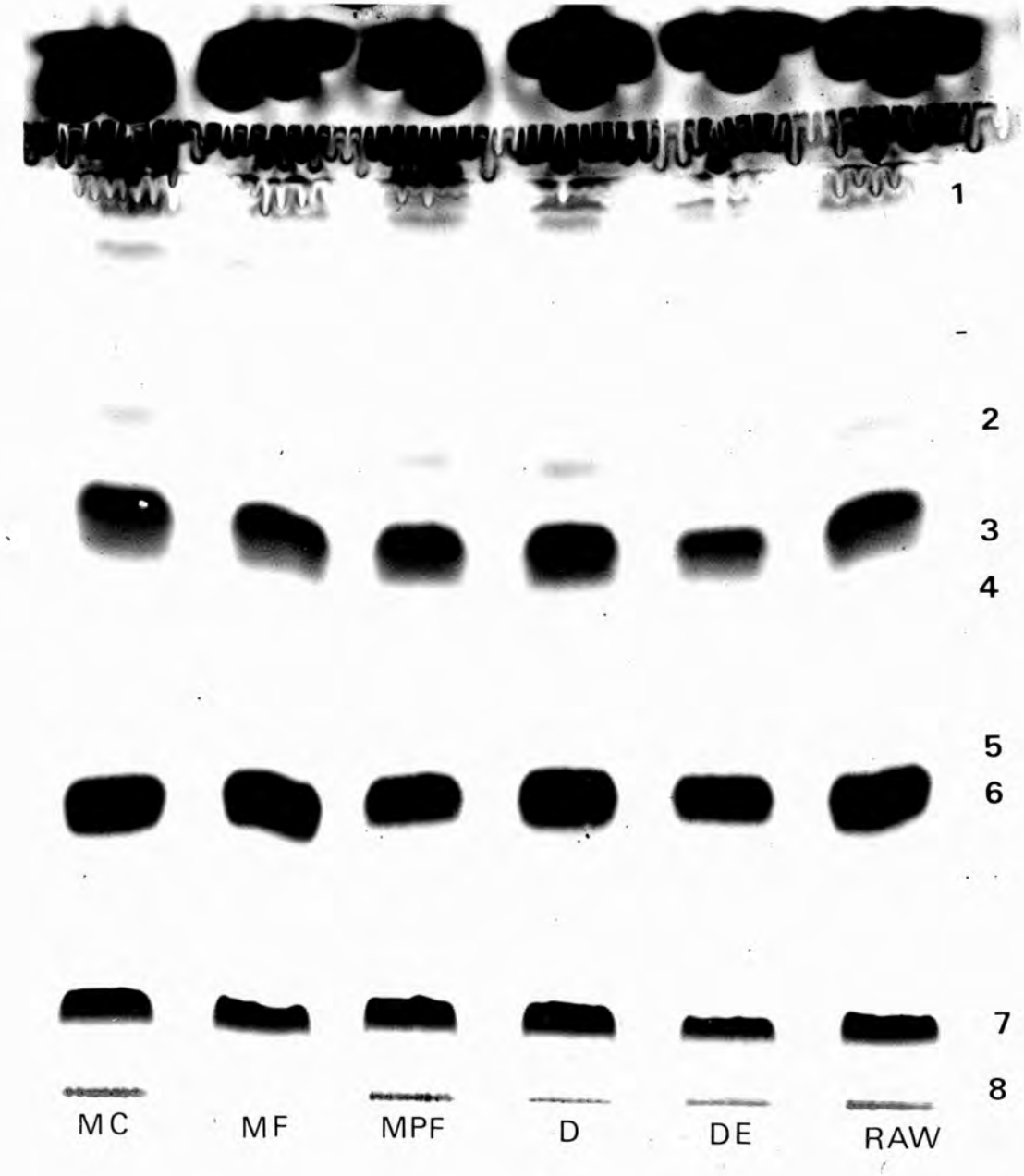
Petroleum ether : acetone (2:1, V/V)

Second developing solvent :

Chloroform : methanol : acetic acid : water (170:25:25:6, V/V)

Spray :

Cupric acetate


1
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7
8
MC
MF
MPF
D
DE
RAW

masked by others (overlap), two-dimensional TCL was adopted. Different combinations of solvents were used but those of NICHOLS (1964) were adopted as they gave the best separation possible.

The method was as follows: ten µl of lipid sample was applied to a corner of a silica gel G plate as a single spot 2cm from each edge. The plate was then developed in chloroform-methanol - 7 normal ammonia (65:30:4, v/v/v) for the first dimension. The plate was air-dried until all traces of ammonia had been removed (detected by odour). The plate was then rotated at right angles to the first direction and placed in a second solvent system of chloroform-methanol-acetic acid-water (170:25:25:6; v/v/v/v).

Detection of total lipid fractions

Lipid fractions can be detected and visualised on chromatoplates by several destructive (D) or non-destructive (ND) reagents. Some of the techniques which have been used in this study are described. All the following are non-specific reagents, hence give positive reactions with all lipid classes.

(i) Cupric acetate (D):

The chromatoplates were sprayed until the surface became wet, with cupric acetate solution (3g of cupric acetate in 100ml of 8% phosphoric acid). The plates were then heated at 180°C for 25 minutes. This procedure minimises the evaporation of the volatile material and therefore there is little difference in the intensity of spots between saturated and unsaturated compounds (PRIVETT et al, 1973). This procedure was used for the quantification of lipids in the scanning densitometer.

(ii) Sulphuric acid (D):

The chromatoplate was sprayed lightly with 50% sulphuric acid followed by heating at 180°C for 20 minutes. All lipids, as well as other organic non-volatile compounds formed dark brown spots on the white back ground (PRIVETT & BLANK, 1962)

(iii) 2', 7' - Dichlorofluorescein (ND):

The chromatoplates were sprayed with a 0.1% (w/v) solution of 2', 7' - dichlorcfluorescein in 95% methanol. The lipids showed up immediately as yellow spots under u.v. light (CHRISTIE, 1973)

(iv) Iodine vapour (ND):

The chromatoplates were hung in a developing tank containing a trough filled with crystals of iodine. After a few minutes most lipids appeared as brown spots on a pale yellow background (SKIPSI & BARCLAY, 1969).

Identification of different lipid classes

Different lipids present in lipid extracts can be identified on the developed chromatoplates either by one of the following two methods or by a combination of them:

1. By co-chromatography.
2. By using a specific reagent spray which only reacts with one of the compounds or group of compounds present in the sample, and which gives specific colour reaction.

Some of these specific reagent spray are as follows:-

(i) Free fatty acid spray:

The developed plates are sprayed in turn with a 2', 7' dichlorofluorescein (DCF) spray (0.1% DCF in 95% methanol, 1% alluminium chloride in ethanol, a 1% aqueous ferric chloride solution. The plates were warmed briefly after each spay. Free fatty acids gave a rose - violet coloration (CHRISTIE, 1973).

(ii) Sterols and sterol esters spray:

Fifty mg ferric chloride was dissolved in water (90ml) with acetic acid (5ml) and sulphuric acid (5ml). The developed plates were sprayed with this reagent, then heated at 100°C for 2 - 3 minutes when the presence of sterols and sterol esters were indicated by the appearance of a red - violet colour (CHRISTIE, 1973).

(iii) Molybdenum blue (phospholipid spray):

Reagent I : 40 mg of molybdic anhydride (MoO_3) was added to 11 of 25 normal sulphuric acid and was boiled gently until MoO_3 was dissolved. Reagent II : 1.79 g of powdered molybdenum was added to 500 ml of reagent I and the mixture was boiled gently for 15 minutes. Reagent III equal volumes of reagents I and II were mixed and the combined solution was mixed with 2 volumes of water.

The developed plates were lightly sprayed with reagent III. Phospholipids showed up immediately as blue spots on a white background (DITTMER & LESTER, 1964).

(iv) α- Napthol (glycolipid spray)

Half a gram α- naphthol was dissolved in methanol - water (1:1, v/v) and sprayed on the developed plates. After air-drying, the plates were sprayed lightly with 95% sulphuric acid and heated at 120°C for a few minutes, when glycolipids appeared as blue - purple spots (SIAKOTOS & ROUSTER, 1965).

(v) Ninhydrin (free amino group spray):

A solution of 0.2% ninhydrin in water saturated butanol was sprayed on the developed plates. All compounds with free amino groups such as phosphatidyl ethanolamine or phosphatidyl serine, appeared as red - violet spots after heating at 100°C for a few minutes (SKIPSI & BARCLAY, 1969).

The ninhydrin spray was also used as a non-lipid (impurities) detection test in the lipid sample extract. If any impurities were present in the sample, it would have given a positive reaction with the ninhydrin on the TLC plates, but a negative one with the cupric acetate spray (PRIVETT et al , 1973).

(vi) Clorox bleach (sphingolipids spray)

Reagent I: 50 ml benzene was mixed with 5ml clorox bleach (trade name of commercial bleach, active reagent sodium hypochloride) and 5ml of glacial acetic acid.

Reagent II: 200 mg benzidine dihydrochloride and a small crystal of potassium iodine were dissolved in 50ml of 50% ethanol and filtered.

The developed plates were sprayed with freshly prepared reagent I and were left at room temperature for 30 minutes. They were dried in hot air for 10 minutes and then sprayed with reagent II. Sphingolipids appeared as blue spots almost immediately (SKIPSKI & BARCLAY, 1969).

Quantitative analysis of lipid classes TLC

There are several different methods in which the amount of different simple and complex lipid compounds can be measured. The method which has been used is based on measurement of the intensity of light absorption in the charred lipid spot by photodensitometry. These measurements are performed by scanning the chromatoplates with a specially designed photodensitometer which automatically moves the plate over a narrow slit located between the light source and the photometer. The results are shown as a series of peaks on the recorder.

The areas of the peaks are proportional to the amount of lipids originally present (CHRISTIE , 1973).

So, by developing standard compounds and comparing their peaks with those of the unknown sample according to the following:

$$\frac{\text{Amount of lipid in standard}}{\text{Amount of lipid in sample}} = \frac{\text{Peak area of standard}}{\text{Peak area of sample}}$$

the amount of the lipid in sample can be obtained.

There is some doubt that there is a linear relationship between the density of a spot and the amount of material present (CHRISTIE, 1973).
Precision of the method was improved by using specific standards corresponding to each spot obtained in the TLC separations hence compensating for charring differences obtained by constituents differing in molecular weight. Any other error was further reduced by making analysis in triplicate on a single plate. In the present study, results with the densitometer scans were generally reliable as they compared favourably with those of the weighing method.

Experimental:

The developed plates were sprayed with 3% cupric acetate in 8% phosphoric acid and heated at 180^{o}C for 20 minutes . All lipid classes were charred (MARSH & WEINSTEIN , 1966).

The plates were then cut in such a way that each slice contained the starting line and the solvent front of each sample. The slices were scanned in a chromoscan double - beam recording and integrating densitometer (JOYCE - LOEBL & CO. LTD) with quartz iodine light source, blue filter, and 20cm x 1cm high slit and sample holder (drive in gear ratio was 1:1).

Fractionation and analysis of component fatty acids by gas - liquid chromatography

Gas-liquid chromatography is an extremely powerful tool for identification of fatty acids after the latter have been converted to their methyl esters. This has been used successfully in fatty acid analyses. The commonest chromatographic stationary phases used for fatty acid analysis are polyester - packing materials polyethylene - glycol adipate (PEGA) and diethylene - glycol succinate (DEGS) (HITCHCOCK & NICHOLS, 1971).

Polyester columns resolve fatty acid esters according to both chain length and their degree of unsaturation. The increase in chain length gives increased retention time. Two components with identical chain lengths elute according to the number of double bonds they contain; increasing number gives increasing retention times.

When components of methyl ester fraction are unknown, they can frequently be identified by a comparison of their retention volumes with those of reference substances analysed under the same conditions. PEGA was found to give superior resolution, hence was used in this study.

Experimental:

Total fatty acid esterification

Saponification of lipid classes leading to the release of fatty acid components and their subsequent methylation was carried out using the rapid technique of METCALFE et al, (1966), as modified by WIJNGÅRDEN (1967).

Approximately 150 mg of lipid was added to a 50ml round bottomed flask equipped with a ground glass joint. Two ml of 0.5 normal methanolic sodium hydroxide was added and the flask connected to a helicoil condenser. The mixture was boiled under total reflux on an electrically heated

hot - plate until the fat globules went into solution (2 - 5 minutes). Two ml of boron trifluoride - methanol (14% boron trifluoride in methanol) was added via the condenser and the boiling continued for 2 minutes. Two ml of heptane was then added via the condenser and the mixture boiled for another minute before removing the flask for cooling. Enough saturated sodium chloride solution was poured in to bring the liquid level up to the neck of the round bottomed flask, and 1 ml of the upper (heptane) layer was pipetted into a glass stoppered sample tube ready for direct injection into the gas chromatographic column.

Free fatty acid esterification

Essentially the same method as already described for total fatty acid esterification was used, with one modification, namely that the 2 - 5 minutes boiling of lipid with 0.5 normal methanolic sodium hydroxide was omitted.

Gas-liquid chromatgraphy

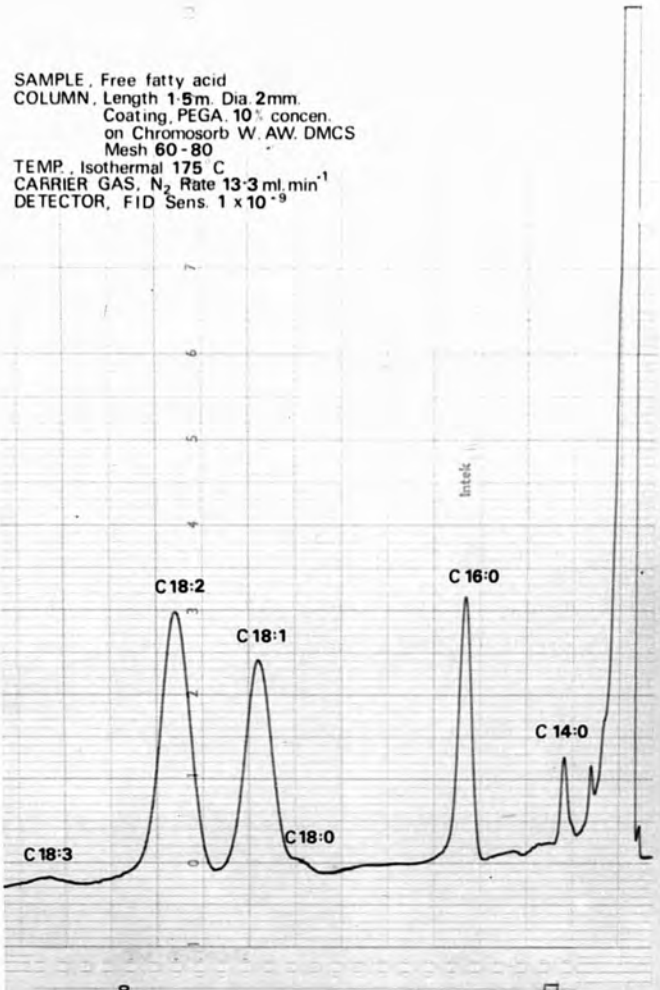
For the GLC of fatty acids, a PYE 'series 104' chromoatograph with flame ionisation detector, nitrogen carrier gas and full - scale drift of 10 - 12 per hour was used.

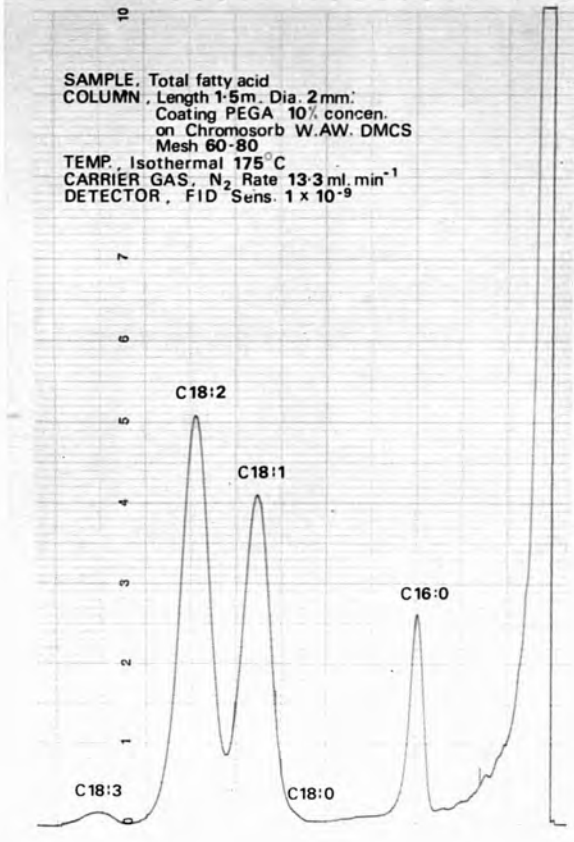
The glass colums (1.5m long and 2mm diameter) were packed with PEGA (on 10% chromosorb W.AW. DMCS, 60-80 mesh) which was supplied by Phase Separation Ltd., and separation carrried out under the following condition.

Temperature	: injection 170°C, isothermal
Carrier gas (nigrogen) flow rate	: 13.3 cm min^{-1}
Chart speed	: 76.2 cm h^{-1}
Hydrogen pressure	: 12 p s i
Air pressure	: 12 p s i

Fig. 18

GLC analysis of Free and Total fatty acids.


SAMPLE, Free fatty acid
COLUMN, Length 1·5m. Dia. 2mm.
Coating, PEGA. 10% concen.
on Chromosorb W. AW. DMCS
Mesh 60-80
TEMP., Isothermal 175 C
CARRIER GAS, N_2 Rate 13·3 ml.min^{-1}
DETECTOR, FID Sens. 1 x 10^{-9}
C18:2
C18:1
C16:0
C14:0
C18:3
C18:0

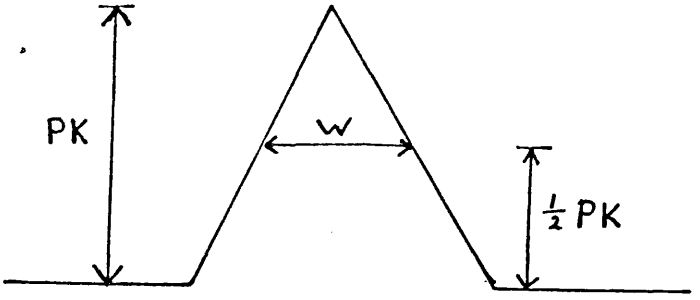

SAMPLE, Total fatty acid
COLUMN, Length 1·5m. Dia. 2mm.
Coating PEGA 10% concen.
on Chromosorb W.AW. DMCS
Mesh 60-80
TEMP., Isothermal 175°C
CARRIER GAS, N_2 Rate 13·3 ml.min^{-1}
DETECTOR, FID Sens. 1 x 10^{-9}
C18:2
C18:1
C16:0
C18:3
C18:0

Quantification of fatty acids

The areas of the peaks on the recorder trace are proportional to the amount of fatty acid (FA) originally present in the lipid sample and standards. Amounts of fatty acid ester were calculated as follows:

$$\text{FA in sample (ug)} = \frac{\text{Peak area of sample}}{\text{Peak area of standard}} \times \text{FA in standard (ug)}$$

Peak area = Peak height x width at half height
= PK x W



All experiments were repeated at least four times.
The results wherever possible were expressed as $\bar{x} \pm$ s. d.

Determination of iodine value (Hanus method)

The iodine value is a measure of the unsaturation of lipids and is expressed in terms of the number of grams of iodine absorbed per 100 gram of lipid (% iodine absorbed).

Reagents:

(1) Hanus iodine monobromide solution; 13.2g pure iodine was dissolved in one litre glacial acetic acid with slight heating. After cooling, 3 ml bromine was added to produce the double halogen.

(2) Potassium iodine solution; 15g potassium iodine was dissolved in 100 ml distilled water.

(3) Sodium thiosulphate solution; 0.1 normal and 0.01 normal accurately standardised.

(4) Starch indicator solution; 1% starch in distilled water.

(5) Carbon tetrachloride; high grade.

Procedure:

In a 500 ml glass - stoppered flask, approximately 200 mg was accurately weighed and dissolved in 10 ml carbon tetrachloride. To this 10 ml of the iodine monobromide solution was carefully added. The flask was stoppered and shaken carefully. This was left to stand for 30 minutes in the dark with occasional shaking. Ten ml of 15% potassium iodine and 50 ml of freshly boiled and cooled distilled water was then added, washing down any free iodine on the stopper. The iodine in the mixture was titrated with standard 0.1 normal and 0.01 normal thiosulphate which was added gradually with constant shaking until the yellow solution turned almost colourless. Half a ml of starch indicator was added and titration continued until the blue colour disappeared. Towards the end of the titration the flask was shaken vigorously so that any iodine remaining in the carbon tetrachloride layer was taken up by the potassium iodine solution. Blank determinations were carried out using carbon tetrachloride only.

Calculation:

The number of ml sodium thiosulphate required by blank, minus ml used in the determination on the sample, gives sodium thiosulphate equivalent of iodine absorbed by oil.

$$I\,Br \text{ (left in solution)} + I^{-} \text{ (from KI)} \longrightarrow I_2 + Br^{-1}$$

$$I_2 + 2\,Na_2S_2O_3 \longrightarrow 2Na\,I + Na_2S_4O_6$$

$$\text{Iodine number} = \frac{(B-S) \times N \times 12.7}{W} \qquad \text{where:}$$

B = Blank titration (ml)
S = Sample titration (ml)
N = Normality of sodium thiosulphate
W = Weight of sample (g)

Results and discussion

Total Lipids

The term 'total lipids' refers to the cumulative value of all lipid classes extracted by the procedures mentioned in the section 'materials and methods'. Although the procedures used are reported to be exhaustive, small amounts of lipids may be tightly bound to other molecules (e.g. protein)which makes their complete extraction difficult. This would lead to slight under-estimation of values obtained.

Table 25 shows the amount of total lipid in chickpeas before and after processing and after one-year storage. The value found in raw seeds was 25.1 mg seed $^{-1}$, which corresponds to approximately 6.8% of the dry weight. The values reported in the literature for total lipids in chickpeas ranged from 4.5% found by KANDE (1967), through 5-6% by SHARMA and GOSWANI (1971), to 7-7.9% stated by MURTHY and URS (1979). Simple lipids make up most of the lipids (see also fig.18a).

Storing raw seeds at 10% for one year reduced the total lipid content significantly to 20.7mg seed $^{-1}$.

Total lipid values for dried, decorticated, and all freeze processed samples ranged from 21.6 - 27.5 mg seed $^{-1}$. Values in this range were not statistically significant.

Total lipid in water-soaked and medium - soaked canned chickpeas was 20.7 and 21.7 mg seed $^{-1}$ respectively. The decrease in the former, when compared to raw seeds, was

TABLE 25 The effect of processing and 1 year storage on simple, complex and total lipids in chickpeas (mg seed^{-1} where applicable).

SAMPLE	TOTAL SIMPLE LIPIDS mg seed^{-1}	TOTAL COMPLEX LIPIDS* mg seed^{-1}	TOTAL LIPIDS mg seed^{-1}	TOTAL LIPID RETENTION (%)	% LIPID RECOVERY
Raw seed	23.2 ± 2.0	1.9	25.1 ± 1.9	—	93.6
Raw-S	19.5 ± 1.5	1.2	20.7 ± 1.7	82.5	95.3
D	24.0 ± 2.0	2.2	26.2 ± 2.0	104.4	95.1
D-S	18.9 ± 1.4	2.0	20.9 ± 1.9	83.3	93.9
De	22.8 ± 1.8	2.1	24.9 ± 1.7	99.2	96.2
De-S	19.1 ± 1.9	0.8	19.9 ± 1.6	79.3	93.1
WF	25.3 ± 1.2	2.2	27.5 ± 1.8	109.6	97.2
WF-S	23.4 ± 2.0	1.0	24.4 ± 1.9	97.2	97.8
MF	20.5 ± 2.0	1.8	22.3 ± 1.9	88.8	98.1
MF-S	21.7 ± 1.7	1.5	23.2 ± 1.6	92.4	94.9
WPF	20.6 ± 1.2	1.6	22.2 ± 1.6	88.4	89.7
WPF-S	16.5 ± 2.0	0.6	17.1 ± 1.9	68.1	91.3
MPF	20.2 ± 1.9	1.4	21.6 ± 1.7	86.0	89.9
MPF-S	16.1 ± 1.4	1.3	17.4 ± 2.1	69.3	93.5
WC	19.4 ± 1.9	1.3	20.7 ± 1.8	82.5	95.3
MC	20.3 ± 1.8	1.4	21.7 ± 2.0	86.5	92.9
WC22	12.0 ± 1.9	0.4	12.4 ± 2.0	49.4	86.7
MC22	12.1 ± 1.5	1.6	13.7 ± 2.0	54.6	91.9
WC30	12.5 ± 1.7	1.1	13.6 ± 2.1	54.2	84.6
MC30	13.7 ± 1.8	0.6	14.3 ± 1.9	56.1	92.7

*Standard error $<$ 0.1 mg seed^{-1}

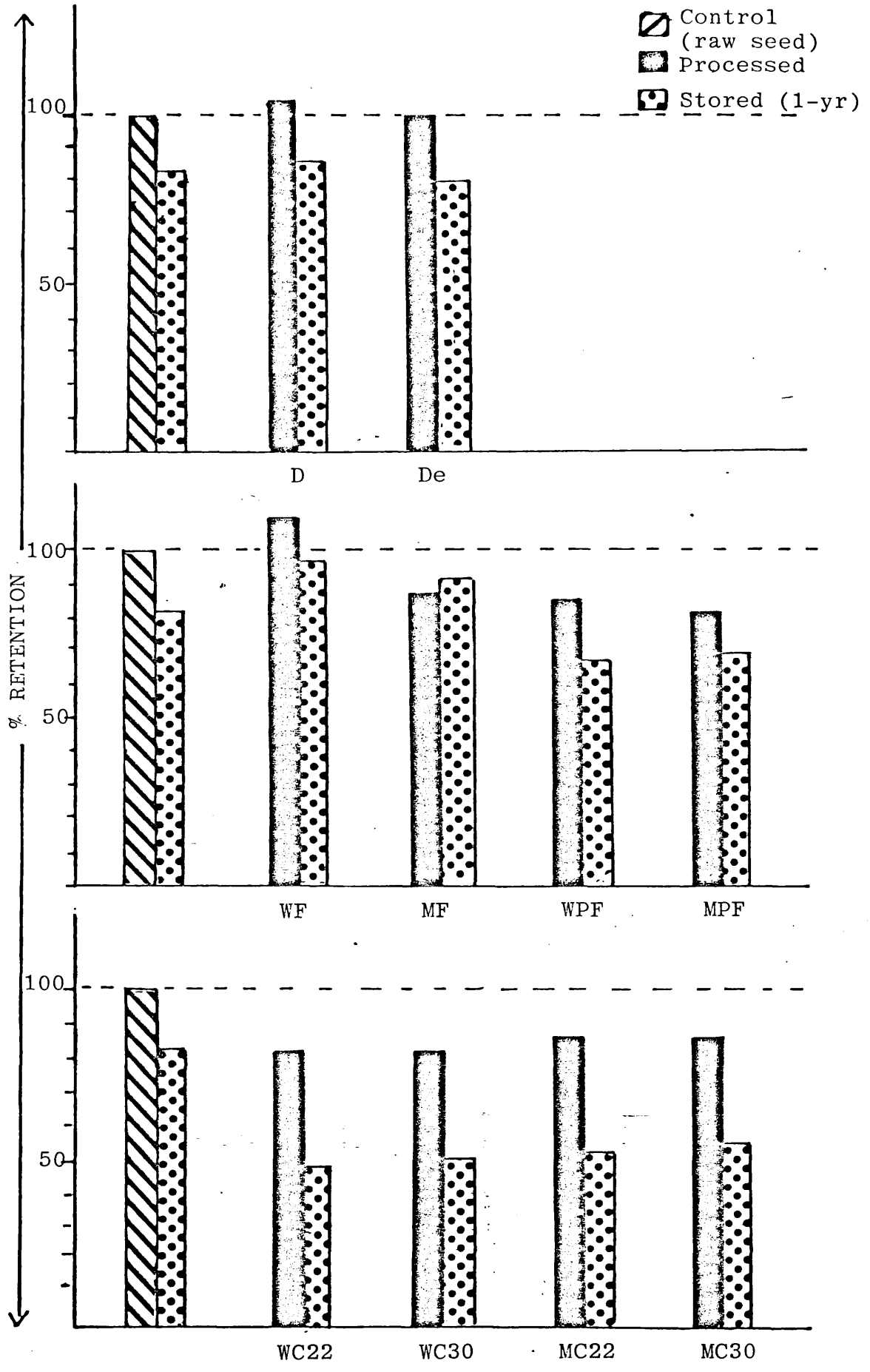


Fig 18a The effect of processing and 1-year storage on % retention of total lipid in chickpeas.

significant whereas that in the latter was not, implying that the medium treatment has aided in total lipid preservation.

Storage of WF and MF for one year brought about reductions in their total lipid contents to 23.2 and 24.4 mg seed^{-1} respectively, which did not represent significant decreases when compared to unstored controls, nor to the values in the raw seed.

Further significant losses in total lipid, however were incurred in dried, samples after one-year storage. These losses varied from approximately 20% in dried and decorticated seeds approximately 30% in pre-cooked frozen seeds to approximately 50% in canned seeds (Table 25). Increased temperature of canned storage had no significant effect on this loss.

In general it can be said that drying, decorticating, freezing, and canning processes do not significantly reduce the total lipid content of chickpeas.

Except for WF and MF samples, one-year storage of all the above mentioned processed samples brought about significant reductions in lipids. The highest losses, of approximately 50%, were found in canned samples irrespective of their storage temperature.

Simple lipids

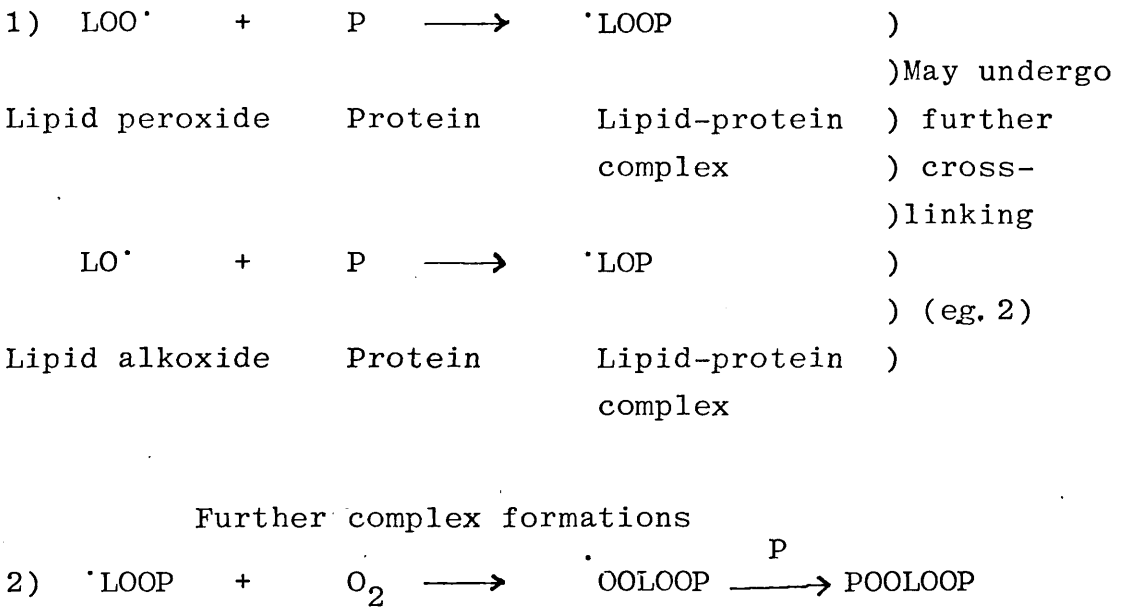
Table 25 shows that the total simple lipid content of chickpea was 23.2 mg seed^{-1} corresponding to 92.4% of total lipid. After one-year storage this content was reduced to 19.5 mg seed^{-1} corresponding to 94.3% of total lipid. This reduction in lipid content, however is only just statistically significant. Results of ARYA (1980) show that little change in total simple lipids occurred in chickpea flour, with a moisture content less than 10.8%, stored at room temperature for one year although hydrolysis of certain fractions did occur. Flours with greater than 13.7% moisture content did, however, give substantial reductions in total simple lipids.

Total simple lipid contents in dried, decorticated, all frozen, and all canned processed chickpeas ranged from 19.4 (in WC) to 25.3 mg seed^{-1} (in WF), which were statistically different from each other although, individually, none was significant when compared to the contents in raw seed.

One-year storage of WF, and MF gave no significant decrease in simple lipids when compared to the raw seed, whereas drying the seed led to slight losses.

As stated by MAURON (1982), reducing the moisture content in foodstuff also reduces the occurrence of non-enzymic browning reactions between protein and sugar molecules but increases oxidation changes within the lipid fractions of the food. These oxidative changes may have been responsible for the loss of lipids after one-year dried storage. It is known that complex formation also occurs between oxidised lipids and proteins (NARAYAN and KUMMEROW, 1963) following the reactions shown in Fig 19.

Fig 19



Scheme of reactions between peroxidised lipid free radicals with protein

Results of DESAI and TAPPEL (1963) suggest that the amino acids histidine, serine, proline, arginine, methionine, and cystine were most labile to peroxidative damage. If peroxides or other degraded products of lipid damage are involved in reactions with protein, reductions in the above six amino acids may then ensue. Results in chapter 4 for effects of dried storage on individual amino acids show reductions in histidine, and serine but arginine, proline and methionine remain fairly constant. Several inferences may be drawn from this observation. The results in this study partially support the stated observation. Due to the fact that these authors studied model reaction systems containing linolenic acid and cytochrome C, the results might not be generally applicable to all food products. It is possible that other constituents in the chickpea may aid in protecting proteins, to some extent, from peroxidative damage by lipids. This would lead to the supposition that very little complexing between lipids and proteins had, in fact, taken place and that most damage to the lipid was due

to direct oxidative degradation.

One-year storage of decorticated seeds led to marginally significant losses in simple lipids.

Pre-cooked frozen and canned chickpeas after one-year storage underwent significant decreases in total simple lipids. These losses were 4.1 mg seed^{-1} in WPF and MPF while those in canned samples were about 7 mg seed^{-1}. Temperature of storage of cans did not affect the losses in simple lipids. The latter results are not altogether surprising as in WPF, MPF, and canned chickpea the seeds were subjected to a temperature of 90^{o}C for 13 minutes (Ch. 1) Lipid destruction may not have been apparent on immediate analysis but it is possible that certain chemical bonds within or among lipid molecules may have been weakened by heat treatment. This unstable state even in non-oxidative conditions as specified by NAWAR (1969), can lead to dehydration, decarboxylation, hydrolysis of ester bonds, occurrence of double bond conjugation, polymerisation, dehydrogenation, and degradation by carbon-carbon cleavage in lipid molecules.

The following simple lipid classes: monoglycerides, 1,2 diglycerides, 1,3 diglycerides, sterols, free fatty acids, triglycerides, and steryl glycerides were identified in the raw seed. Among the sterols the presence of β-sitosterol was confirmed.

Two points of observation can be made immediately. Firstly, that the presence of all the above mentioned simple lipids are confirmed in raw and all processed samples before and after storage (Tables 26,27 and 28). Secondly, that the majority of simple lipids (in fact, of total lipids) exists as triglycerides which are a major form of storage lipid in most seeds.

Table 26 The effect of drying, decorticating & the subsequent storage for 1yr. on individual simple lipids in chickpeas (mg seed^{-1} where applicable)

Lipid Classes	Raw seed	Raw-S	D	D-S	De	De-S
Monoglycerides	0.3	0.5	0.5	0.8	0.1	0.8
1,2 Diglycerides	0.6	0.1	0.6	0.2	0.6	0.5
1,3 Diglycerides	0.3	0.3	0.8	0.6	0.4	0.2
β-Sitosterol	1.3	0.9	1.1	0.7	1.2	0.8
Other sterols	0.2	0.4	0.3	0.4	0.3	0.3
Unknown	0.7	0.3	0.8	0.2	0.6	0.4
Free fatty acids	0.1	0.2	0.1	0.1	0.1	0.1
Triglycerides	18.2	15.5	18.5	14.5	18.4	14.8
Steryl esters	1.5	1.3	1.3	1.4	1.2	1.2
Simple Lipid Wt., (mg seed^{-1})	23.2	19.5	24.0	18.9	22.8	19.1
% of Total Lipid	92.4	94.3	91.7	90.3	92.0	96.0

Table 27 The effect of freezing & 1 yr. frozen storage on individual simple lipids in chickpeas (mg seed $^{-1}$ where applicable).

Lipid Classes	WF	WF-S	MF	MF-S	WPF	WPF-S	MPF	MPF-S
Monoglycerides	0.3	1.4	0.2	0.1	0.2	0.2	0.2	0.2
1,2 Diglycerides	1.9	0.8	1.4	1.1	1.2	0.6	1.3	0.8
1,3 Diglycerides	0.5	0.5	0.5	0.6	0.5	0.3	0.6	0.4
β-Sitosterol	2.0	1.2	1.2	0.9	1.3	0.8	1.3	0.8
Other Sterols	0.4	0.4	0.3	0.5	0.3	0.3	0.4	0.2
Unknown	0.8	0.4	0.5	0.2	0.6	0.1	0.6	0.2
Free Fatty Acids	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.2
Triglycerides	17.7	16.8	15.1	15.9	15.7	13.0	14.8	12.1
Sterylesters	1.6	1.7	1.2	1.4	0.9	1.0	0.9	1.2
Simple Lipid Weight(mg seed $^{-1}$)	25.3	23.4	20.5	21.7	20.6	16.5	20.2	16.1
% of Total Lipid	92.2	96.0	92.1	93.4	93.0	96.7	93.4	92.4

Table 28 The effect of canning & 1 yr. canned storage on individual simple lipids in chickpeas(mg seed^{-1} where applicable)

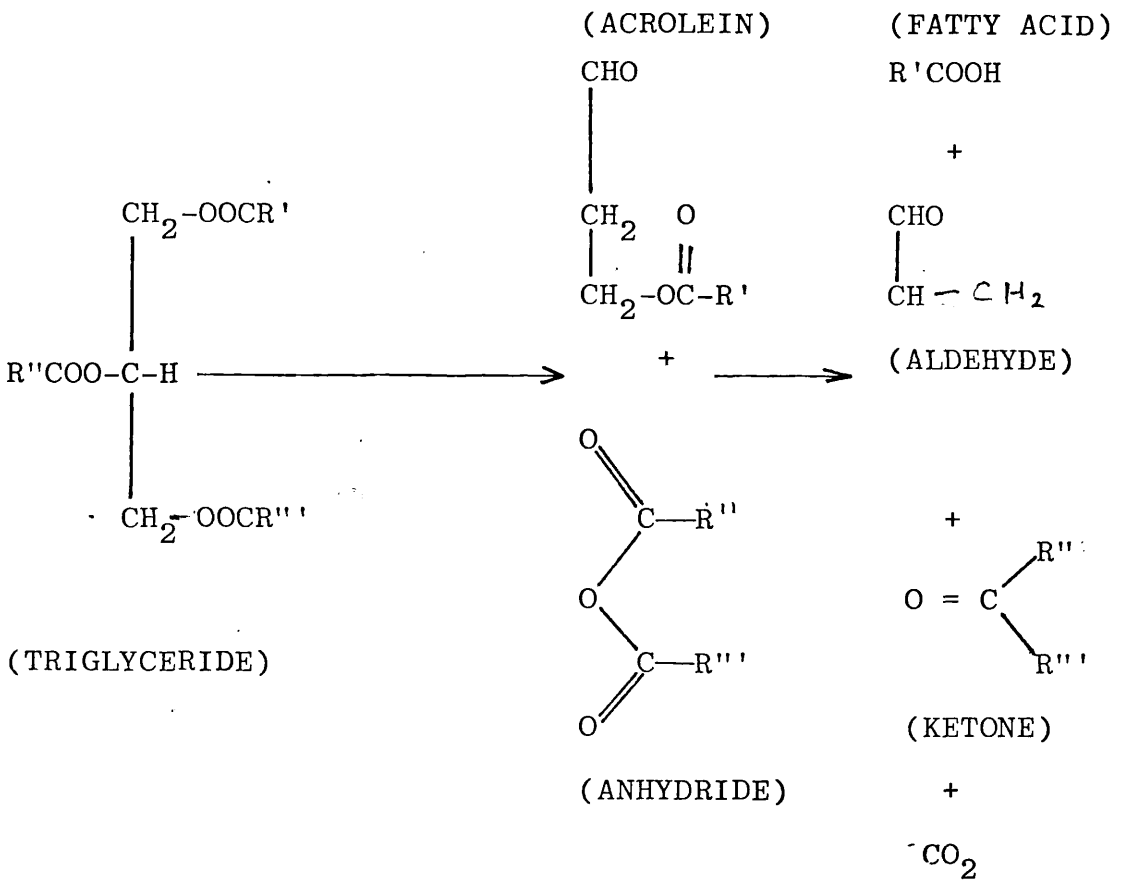
Lipid Classes	WC	MC	WC22	MC22	WC30	MC30
Monoglycerides	0.1	0.1	0.1	0.1	0.1	0.2
1,2 Diglycerides	0.5	0.5	0.2	0.3	0.3	0.3
1,3 Diglycerides	0.8	0.9	0.4	0.6	0.6	0.7
β-Sitosterol	1.1	1.1	0.6	0.6	0.5	0.7
Other Sterols	0.3	0.3	0.2	0.2	0.1	0.2
Unknown	0.5	0.8	0.2	0.2	0.2	0.2
Free Fatty Acids	0.1	0.1	0.1	0.1	0.8	0.9
Triglycerides	15.5	15.2	9.4	9.1	9.1	9.6
Sterylesters	0.5	1.3	0.8	0.9	0.8	0.9
Simple Lipid Weight (mg seed^{-1})	19.4	20.3	12.0	12.1	12.5	13.7
% of Total Lipid	93.6	93.4	96.5	88.6	92.0	95.9

Drying and decorticating the seeds does not alter the composition of simple lipids (Table 26) to any large extent.

There is substantial reduction of triglycerides with a corresponding increase in diglycerides in all frozen and canned samples. (Tables 27 and 28).

On one-year storage of raw seeds the decrease in triglyceride is not coupled with a corresponding increase in diglycerides although monoglycerides increase slightly. A similar observation is made for one-year stored dried and decorticated seeds. This may indicate the types of reactions occuring in the lipids. For instance, the fact that an increase in diglycerides is not observed, raises the psssibility that they are degraded further fairly rapidly or else there might be a direct degradation of triglycerides as shown in Fig 20. (NAWAR, 1969).

Fig 20



Decomposition of triglycerides by heat in absence of moisture

(Modification of scheme by NAWAR, 1969).

Not much further effect is observed on simple lipid composition in one-year stored WF and MF samples wher~~e~~ in MPF and MPF, triglyceride loss is 2.7 mg seed^{-1} in both cases.

In one-year stored canned seeds further losses are noted in diglycerides, β - sitosterol, and triglycerides. Steryl esters increase in water-treated seeds whereas they decrease in medium-treated seeds. Slight changes in fatty acid content are noted in storage temperatures of 22°C whereas large increases are noted at storage temperatures of 30°C, indicating the initiation of rancidity.

Fatty acids

Free fatty acids:

Tables 29, 30 and 31 show the composition of the free fatty acids and their unsaturation ratios. In total six fatty acids were separated and identified. These are myristic (14:0), palmitic (16:0), stearic (18:0). oleic (18:1), linoleic (18:2), linolenic (18:3).

Raw seeds contain 6.5% of 14:0, 18.1% of 16:0, 0.5% of 18:0, 32% of 18:1, 40.5% of 18:2, and 1.8% of 18:3, VIOQUE and MAZA (1970) found values of 10.3% for 16:0, 2.2% for 18:0, while those found by GHIRARDI et al (1973) were 6.8 - 10.2% for 14:0, 11% for 16:0, 3.5% for 18:0, 22 - 27% for 18:1, 38 - 46% for 18:2, and 4 - 5% for 18:3 in chickpeas.

It is quite obvious that 18:1 and 18:2 are the most abundant fatty acids. This is found to be the case even after processing, and after one-year storage.

The ratio of unsaturated to saturated fatty acids, defined as desaturation ratio by HITCHCOK and NICHOLS (1971), was calculated to indicate if processing had any effects on unsaturated fatty acids in terms of hydrogenation and on saturated fatty acids in terms of their degradation.

Drying, decorticating, and all the freezing processes used have not seriously affected the total free fatty acid content. Fluctuations in percentage fatty acid composition are not significant, but evidence for the occurrence of hydrogenation of fatty acids is offered by slight but consistent decreases in unsaturation ratio value for all the above names samples. The fact that some degradation has also occurred is reflected by slight increases in 14:0 of frozen samples.

Table 29 The effect of drying, decorticating and the subsequent storage for 1yr. on total free fatty acids and their unsaturation ratios in chickpeas

Sample	% of Total Free Fatty Acids						Total Weight ug seed^{-1}	Unsaturation Ratio
	14:0	16:0	18:0	18:1	18:2	18:3		
Raw Seed	6.5	18.1	0.5	32.0	40.5	1.8	115.8	3.0
Raw-S	-	12.4	0.4	17.1	64.8	5.3	234.7	6.8
D	6.6	19.7	0.6	29.5	42.7	0.9	110.3	2.7
D-S	-	16.2	0.5	19.5	60.7	3.2	146.6	5.0
De	6.2	19.2	1.3	27.9	44.1	1.3	112.7	2.7
De-S	-	13.5	1.6	21.4	59.8	3.5	143.05	5.6

Table 30 The effect of canning & 1 yr. canned storage on total free fatty acids & their unsaturation ratios in chickpeas

Sample	% of Total Free Fatty Acids						Total Weight ug seed^{-1}	Unsaturation Ratio
	14:0	16:0	18:0	18:1	18:2	18:3		
WC	4.6	17.8	0.7	28.6	47.2	1.2	103.6	3.3
MC	3.3	18.0	0.5	27.5	49.0	1.8	101.4	3.6
WC22	—	13.7	0.7	29.2	52.8	3.6	104.3	5.9
MC22	—	13.8	0.6	24.0	56.5	5.1	105.4	5.9
WC30	—	13.9	0.4	26.4	53.8	5.5	807.2	6.0
MC30	—	12.1	0.5	19.0	57.2	11.3	923.9	6.9

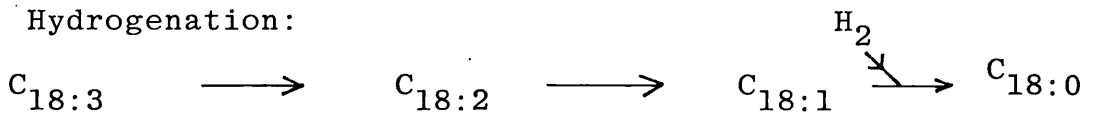
Table 31 The effect of freezing & frozen 1 yr. storage on total free fatty acids & their unsaturation ratios in chickpeas

Sample	% of Total Free Fatty Acids						Total Weight	Unsaturation
	14:0	16:0	18:0	18:1	18:2	18:3	ug seed^{-1}	Ratio
WF	8.5	17.1	1.8	29.9	40.7	1.4	113.5	2.6
WF-S	–	13.5	1.2	36.7	46.8	1.8	160.7	5.8
MF	11.6	14.6	2.3	26.9	39.8	4.9	113.6	2.5
MF-S	–	14.3	1.0	32.1	49.7	2.9	141.0	5.5
WPF	7.8	18.5	1.3	28.4	40.8	3.2	112.4	2.6
WPF-S	–	15.1	0.5	29.5	52.1	2.8	151.9	5.4
MPF	7.5	21.9	1.7	25.2	41.2	2.5	112.7	2.2
MPF-S	–	13.4	1.1	34.7	47.8	3.0	152.1	5.9

In canned samples some loss of free fatty acids has occurred. 18:1 appears to have been most affected in that it has decreased from a value of 32.0% in raw seed to approximately 28% in canned seeds. The fact that the unsaturation ratio has been slightly increased suggests that despite hydrogenation occurring followed by a sequential degradation process (Fig 21) some dehydrogenation to form linolenic acid has also occurred.

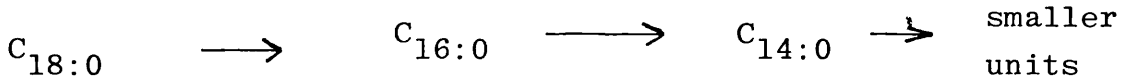
Fig 21.

1) Hydrogenation:



followed by

2) Degradation:



Hydrogenation and subsequent degradation of linolenic acid

Raw seeds stored for one year at 10°C show a substantial increase in free fatty acid, in fact a doubling in quantity. Degradation or hydrolysis reactions are definitely occuring which is further supported by the decrease in triglyceride content reported earlier (Table 26). The mechanism of this reaction could be the fact that no notable increase in diglycerides has been noted.

Reductions in 18:1, 18:0, 16:0, and complete loss of 14:0 further supports the occurrence of the reactions shown in Fig 21. This has obviously led to an increase in 18:2 as a percentage value, hence an increase in the unsaturation ratio. This increase in fatty acid may lead to a lowering of the pH in raw seeds, affecting flavour and also increasing rancidity.

Increases in free fatty acid content have also been noted in dried, decorticated, and all frozen samples, which are not as high as those in raw seeds, after one-year storage.

If hydrolysis had taken place, an increase in mono-and di-glycerides would have been noted with an increase in fatty acids and a decrease in triglycerides. In WPF and MPF seeds stored for one year, decreases of triglycerides were noted without the expected increases in mono-and di-glycerides. This therefore suggests that the increases in free fatty acids may have been due to the oxidation reaction pathway shown in Fig 20 . It appears that although there is a lower net increase in free fatty acids in the above mentioned one-year stored samples, a lot of the fatty acids may have also followed the reaction sequence of Fig 21.

The unsaturation ratio has also increased on one-year storage of these samples for the same reasons already mentioned.

In one-year stored canned seeds there is no further net increase in free fatty acids at 22°C. Degradation has obviously occurred as 14:0 fatty acid is no longer present and 16:0 fatty acid has been reduced. However, some hydrolysis or degradation of other lipid classes must have occurred, and the rate of this breakdown must have been equal to the degradation of the fatty acids, due to the fact that there is no net loss of total free fatty acids. The increase in unsaturation ratio further implies that the hydrogenation of 18:3, 18:2, and 18:1 fatty acids in the sequence of events is slower than the subsequent further degradative reactions into smaller units.

Substantial degradation and hydrolysis has occurred in the lipids of one-year stored cans at 30^{o}C as the net increase in free fatty acid is almost four times that in the one-year stored raw seeds. The acidity in the seeds themselves would be greatly increased hence severe impairment in flavour of the seeds would be the result. Direct loss by peroxide formation and subsequent degradation of 18:1, 18:2, and 18:3 fatty acids is also suggested due to the fact that very little net fluctuation in 18:0 fatty acid value is noted in canned chickpeas.

Total fatty acids

As observed for free fatty acids, six fatty acids were separated and identified among the total fatty acids of lipids of chickpeas. These were, again, myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic acids (18:3). All these fatty acids as a percentage of total fatty acids, their unsaturation ratios, and their Iodine values in raw and processed chickpeas are given in Tables 32, 33, and 34.

Results of the raw seed show that there were traces of 14:0 which were completely lost on storage in all cases. 16:0, 18:0, 18:1, 18:2, 18:3 as a percentage of total fatty acids are 11.0%, 0.2%, 32.0%, 54.2%, and 2.2% respectively. These percentages have remained almost unchanged for 18:0 and 18:1 when compared to those of free fatty acids whereas 16:0 has been substantially reduced and 18:2 and 18:3 fatty acids increased. These observations plus the fact that the unsaturation ratio is more than double that of raw seeds point to the facts that the majority of the fatty acids esterified to other lipid classes are unsaturated. This would also explain the increase in 18:2 and 18:3 fatty acids in the free fatty aiid fraction after one-year storage. This is also supported by the high Iodine value observed of 130.0g iodine absorbed per 100g lipid in seeds. This indicates that the raw seeds contain high quantities of polyunsaturated fatty acids essential to the human diet.

On one-year storage of the raw seeds there is no apparent change in the percentage of individual fatty acids but there is an increase in unsaturation ratio coupled by a very large decrease in Iodine value. This directly points to the fact that there is a very high degree of hydrogenation or oxidative reactions occurring which involve esterified fatty acids. This is in direct contradiction to the results obtained earlier in that there was an increase in the unsaturation

Table 32 The effect of drying, decorticating and the subsequent storage for 1yr. on total fatty acids, their unsaturation ratio and iodine value (g I_2 100g $lipid^{-1}$) of lipids in chickpeas

Sample	% of Total Fatty Acid						Unsaturation	Iodine
	14:0	16:0	18:0	18:1	18:2	18:3	Ratio	Value
Raw Seed	T	11.0	0.2	32.0	54.2	2.2	7.9	130.0
Raw-S	-	10.4	0.4	32.0	54.9	2.4	8.3	89.7
D	T	11.6	0.5	33.7	52.0	2.2	7.3	96.9
D-S	-	11.1	0.3	36.2	50.4	2.1	7.8	79.5
De	T	10.6	0.4	30.0	55.8	3.2	8.1	129.2
De-S	-	10.2	0.6	37.2	51.2	0.7	8.3	73.9

Table 33 The effect of freezing and frozen 1yr. storage on total fatty acid, their unsaturation ratio and iodine value (g I_2 100g $lipid^{-1}$) of lipids in chickpeas

Sample	% of Total Fatty Acid						Unsaturation Ratio	Iodine Value
	14:0	16:0	18:0	18:1	18:2	18:3		
WF	T	11.0	0.2	35.7	50.8	2.3	7.9	96.7
WF-S	–	10.5	0.4	37.5	48.9	2.7	8.2	88.2
MF	T	12.0	1.2	32.6	51.7	2.5	6.6	125.4
MF-S	–	11.8	0.5	33.0	52.6	2.1	7.1	88.9
WPF	T	11.8	0.8	34.6	51.2	1.6	6.9	102.8
WPF-S	–	11.8	0.5	37.2	48.8	1.8	7.1	88.5
MPF	T	11.7	0.5	35.1	50.0	2.6	7.2	118.0
MPF-S	–	10.5	0.7	35.0	51.7	2.2	7.9	88.0

Table 34 The effect of canning and 1yr. canned storage on total fatty acid, their unsaturation ratio and iodine value (g I_2 100g lipid^{-1}) of lipids in chickpeas

Sample	% of Total Fatty Acid						Unsaturation Ratio	Iodine Value
	14:0	16:0	18:0	18:1	18:2	18:3		
WC	T	11.4	0.6	36.0	50.5	1.4	7.3	103.6
MC	T	10.8	0.7	33.4	53.4	1.7	7.7	102.9
WC22	-	11.4	0.4	32.1	53.4	2.7	7.5	88.6
MC22	-	10.1	0.2	35.6	52.2	1.8	8.7	87.1
WC30	-	10.7	0.3	36.4	50.6	2.0	8.1	83.7
MC30	-	8.8	0.7	36.3	48.3	2.9	9.2	83.2

ratio. Hence, the latter must be due to considerable degradation of the saturated fatty acids. This is disadvantageous nutritionally. Decorticated and one-year stored decorticated seeds showed similar effects to that of raw seeds.

Dried seeds showed a decrease in unsaturation ratio without any compositional change in the fatty acid ratios indicating loss of unsaturated fatty acids. This also caused a large decrease in Iodine value. This was not unexpected due to the fact that decreasing the moisture content facilitates the access of oxygen to the seed contents. Lipid molecules being sensitive to oxygen are then liable to oxidative changes. The introduction of oxygen to the fatty acid molecules brings about the formation of peroxides which, by free radical mechanisms, may undergo considerable degradation.

One-year storage of the dried seeds brought about a further decrease in Iodine value and an increase in unsaturation ratio indicating further saturation of the double bonds in the fatty acids but larger decreases in saturated fatty acids.

On the whole, drying does not appear to be a good preservation method in terms of maintaining essential fatty acids in lipids.

Among the frozen processed seeds in MF and MPF the Iodine value was not appreciably affected hence saturation of double bonds in the lipids has been limited. The decrease in unsaturation ratio and observation of the fatty acid compositional table indicates that saturation has preferentially affected the 18:2 fatty acid in MF and MPF. In comparison to the latter, WF and WPF showed a more pronounced decrease in Iodine value. In particular, WPF showed a relatively large decrease in unsaturation ratio as well.

In general then, it can be said that the medium treatment in the case of frozen chickpeas has aided in maintaining good retention values of essential fatty acids. After one-year storage, however, the changes observed in Iodine value and unsaturation ratio follow very similar trends as those of one-year stored raw seeds.

Canning of chickpeas, irrespective of water or medium treatment, has led to substantial decrease in iodine value and slight decrease in unsaturation ratios. The decrease in Iodine value is indicative of a great deal of hydrogenation and peroxide formation of unsaturated fatty acids in canned seeds. The decrease in unsaturation ratio suggests that smaller amounts of fatty acid chain degradation reaction have also occurred (Fig 21). After one-year storage there have been further decreases in Iodine value indicating that there has been a continual hydrogenation and peroxide formation process occurring during storage. The unsaturation ratio, however, has increased substantially when compared to the values found in cans before storage, implying the occurrence of increased amount of fatty acid chain degradation. The temperature of storage has no further effect. On the whole, canning processes conducted in this study have had adverse effects from the point of view of maintaining essential fatty acids

Complex lipids

Eight individual lipids were separated and identified among the complex lipids. These were phosphatidyl inositol (PI), lysophosphatidyl choline (LPC), phosphatidyl choline (PC), digalactosyl diglyceride (DGDG), sulpholipid (SQ), phosphatidyl ethanolamine (PE), sterol glycoside (SG), and phosphatidic acid (PA).

The results found for all these individual components in the raw and processed seeds and after one-year storage are shown in Tables 35, 36 and 37. It can be seen that lysophosphatidyl choline and digalactosyl diglyceride are in trace or minute quantities in each of the samples studied.

From the results it is observed that complex lipids amount to 2.2 mg seed^{-1} corresponding to 7.8% of total lipid. Phospholipids cumulatively make up the major portion of the complex lipids, PC being the most abundant.

After one-year storage of raw seeds decreases in all individual and total complex lipids are observed, complex lipids being now only 1.2 mg seed^{-1}. Complex lipids as a percentage of the total lipids have also decreased.

Decorticating the seeds has not brought any significant change in total complex lipid while a slight but significant increase of this same fraction has been observed in dried seeds. Reductions in PA with increases in PI may have been responsible for this total increase of complex lipids. There is a possibility that, on drying, a condensation reaction has occurred between PA and free inositides in the seeds to produce PI. This has also led to an increase in the complex lipid fraction as a percentage of total lipids.

Table 35 The effect of drying, decorticating and the subsequent storage for 1yr. on individual complex lipids in chickpeas (mg seed^{-1} where applicable)

Lipid Classes	Raw Seed	Raw-S	D	D-S	De	De-S
Phosphatidyl inositol	0.4	0.2	0.6	0.3	0.5	0.1
L-phosphatidyl choline	T	T	T	T	T	T
Unknown	T	0.1	T	0.1	T	T
Phosphatidyl choline	0.7	0.5	0.7	0.7	0.7	0.4
Digalactosyl diglyceride	T	T	0.1	T	T	T
Sulpholipid	0.1	T	0.1	T	T	T
Phosphatidyl ethanolamine	0.4	0.3	0.4	0.5	0.4	0.2
Sterol glycosides	0.1	T	0.2	0.1	0.1	T
Phosphatidic acid	0.2	0.1	0.1	0.3	0.4	0.1
Complex lipid Wt., mg seed^{-1}	1.9	1.2	2.2	2.0	2.1	0.8
% of Total Lipid	7.6	5.7	8.3	9.7	8.0	4.0

Table 36 The effect of freezing & 1yr. frozen storage on individual complex lipids in chickpeas (mg seed^{-1} where applicable)

Lipid Classes	WF	WF-S	MF	MF-S	WPF	WPF-S	MPF	MPF-S
Phosphatidyl inositol	0.6	0.2	0.5	0.2	0.3	0.1	0.2	0.2
L-phosphatidyl choline	T	T	T	T	T	T	T	T
Unknown	T	T	T	0.1	T	T	T	T
Phosphatidyl choline	0.7	0.3	0.6	0.5	0.6	T	0.6	0.4
Digalactosyl diglyceride	T	T	T	T	T	T	T	T
Sulpholipid	0.1	T	0.1	T	T	0.2	T	0.1
Phosphatidyl ethanolamine	0.5	0.3	0.4	0.5	0.5	0.2	0.4	0.4
Sterol glycosides	0.2	0.1	0.1	0.1	0.1	T	0.1	0.1
Phosphatidic acid	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1
Complex lipid Wt., mg seed^{-1}	2.2	1.0	1.8	1.5	1.6	0.6	1.4	1.3
% of Total Lipid	7.8	4.0	7.9	6.6	7.0	3.3	6.6	7.6

Table 37 The effect of canning and 1yr. canned storage on individual complex lipids in chickpeas (mg seed^{-1} where applicable)

Lipid Classes	WC	MC	WC22	MC22	WC30	MC30
Phosphatidyl inositol	0.3	0.3	0.1	0.3	0.2	0.1
L-phosphatidyl choline	T	T	T	T	T	T
Unknowns	T	T	T	0.1	0.1	0.1
Phosphatidyl choline	0.5	0.6	0.2	0.6	0.4	0.2
Digalactosyl diglyceride	T	T	T	T	T	T
Sulpholipid	T	T	T	T	T	T
Phosphatidyl ethanolamine	0.4	0.4	0.1	0.5	0.3	0.1
Sterol glycosides	T	T	T	T	T	T
Phosphatidic acid	0.1	0.1	T	0.1	0.1	0.1
Complex lipid Wt., mg seed^{-1}	1.3	1.4	0.4	1.6	1.1	0.6
% of Total Lipid	6.4	6.6	3.5	11.4	8.0	4.1

Very similar observations to that of dried chickpeas are noted in water-treated frozen seeds while there is no apparent change in medium-treated samples. Precooking the seeds prior to freezing them as in WPF and MPF has also brought about significant decreases in complex lipids but without an accumulation in PA suggesting that a direct oxidative breakdown may have occurred as opposed to simple hydrolysis reactions.

One-year storage of all frozen samples has brought about a significant decrease in total complex lipids of water treated seeds whereas these decreases are not obvious in medium-treated ones. This further supports the observations made concerning total fatty acids (p.178) about frozen seeds.

Canning of chickpeas brings about significant decreases in total complex lipids ranging from 0.3mg to 1.5 mg seed^{-1} compared to that in raw seeds. These decreases are independent of water versus medium treatment, also of temperature. This also supports the observations made on total lipids and fatty acids where canning has the most adverse effect on lipids in both cases.

C H A P T E R VI

Scanning Electron Microscope Studies of Raw and Processed Chickpeas.

The scanning electron microscope (S.E.M.) has been used extensively in studies of the ultrastructure of various plant seeds: rye grain (HALLAM et al , 1973), stored pea seeds (HARMAN and GRANETT,1972), and french beans (OPIK,1966). SRIVASTAVA et al (1970) and LINEBACK and DE (1975) have carried out S.E.M. studies of starch of chickpeas, but not of whole cells. To the author's knowledge, this has yet to be carried out.

The present study so far has been mainly concerned with chemical analyses of the constituents of chickpeas. Further knowledge of the effects of processing on chickpeas was gained from S.E.M. studies by which structural changes occurring within the cells were observed.

MATERIALS AND METHODS

Fixation of Seed Material

This procedure generally depends on the type of material to be looked at. If the material is soft and membraneous, a complex fixation using glutaraldehyde or osmium tetroxide is required. If the material is fairly tough a less powerful fixative such as formaldehyde or even acetone would suffice. Although the raw chickpea is quite dry and hard while canned seeds are relatively soft in texture, the glutaraldehyde was used as a fixative to maintain consistency in the procedure.

A 2mm thick layer of the cotyledon was sliced, using a sharp blade, and washed with distilled water. This was transferred first into a solution of 1% glutaraldehyde in phosphate buffer (pH 4) for 30 minutes and then into a 3% solution of glutaraldehyde for 20 minutes. On removing and washing again in distilled water the sample was ready for dehydration.

Critical Point Drying

This process dries the material without causing deformation of the cell membranes due to water surface tension effects.

The Critical Point (C.P.) of a liquid is reached at a certain temperature and pressure when the gas and liquid phases of substance reach instant equilibrium.

For water the C.P. is reached at impossibly high temperatures and pressures, therefore, in material to be dried, water is first substituted with a suitable intermediate such as acetone, and then this is exchanged for liquid carbon dioxide which has a much lower C.P.

The sample was dehydrated by placing for 15 minutes in each of a graded sequence of acetone concentrations from 20 to 90%, and then twice for 30 minutes in 100% acetone. The sample was then transferred to an E3000 Polaron CP-dryer and flushed with carbon dioxide to remove the acetone. The carbon dioxide was then removed by heating to 36^{o} C, maintaining pressure below 1400 $lb\ in^{-2}$. The sample was ready for coating.

Coating and Viewing

The dried sample was fractured using tweezers, mounted on a stub by means of double - sided scotch tape, and coated with gold-palladium, 50mm thick, using a Polaron E5100Series II 'Cool' Sputter Coater.

The cells were examined and photographed using a Joel SM 25 S.E.M. at a 15 KV accelerating voltage.

Results and Discussion

Raw Seed and Effect of Storage

The average cell size observed in raw seeds was approximately 90 um x 60 um in longitudinal section (Fig 22). The individual cells were observed to be densely packed which contributed to the overall hard texture of the seed. The cell contents which were also densely packed were bounded by a membrane which had retracted slightly away from the cell wall with which it was originally in close contact. This may occur as a result of the dehydration procedure during sample preparation.

Among the cell contents were observed spherical and ovoid structures which were identified as starch granules (Fig 22). The size of these ranged from 9 to 17 um. The granules occupied a large volume of the cell and resembled the isolated starch from chickpea shown in the photomicrographs of LINEBACK and DE (1975). In that study chickpea starch ranged from large oval-shaped (17 to 29 um) to small spherical (6 - 7 um) granules; they all appeared to have quite smooth surfaces. Tight packing within the seed probably caused the irregularities in the shape of individual granules. SRIVASTAVA et al (1970) reported that chickpea starch had 'round' granules, diameter 3 to 4 um. No mention was made of the large granules although they appeared to be present in one photomicrograph of the starch.

The remainder of the cell in raw chickpeas, in this study, appeared to consist of a packing matrix 'thread-like' in appearance. Due to the fact that protein content of the seeds ranged from 20 to 30% , it would be expected that a large proportion of this matrix would be proteinaceous material.

L.S. of cell

(x 1381)

Fig 22.

Scanning electronmicrographs from cotyledon cells of raw chickpeas.

The following key is to be used in all S.E.M. figures (from Fig 22 to Fig 31) :

P = Proteinaceous matrix (cytoplasm)

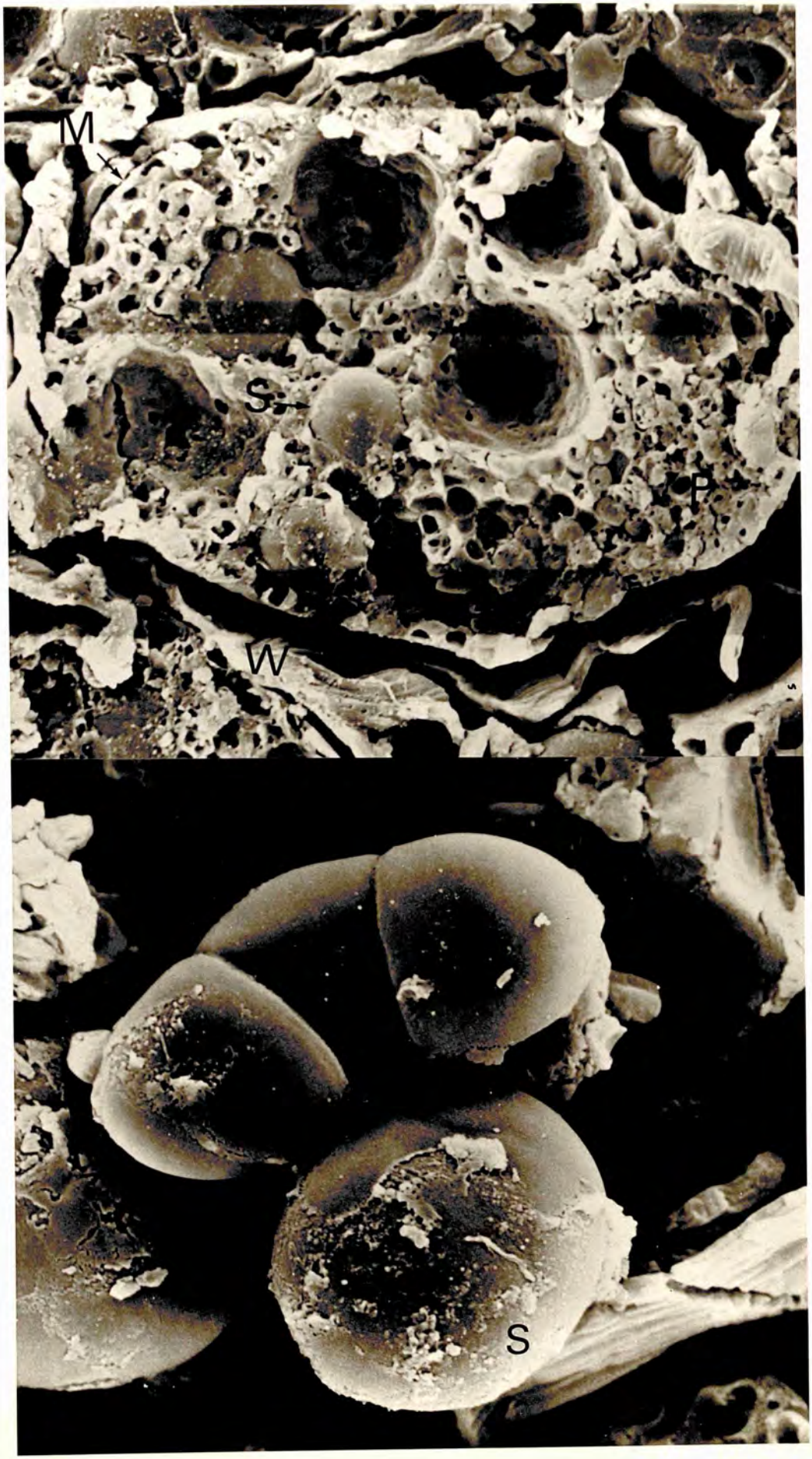
S = Starch granules

W = Cell wall

M = Cell membrane

Starch granules

(x 2762)


M
S
P
W
S

ROCKLAND and JONES (1974) working on Lima beans stated that although protein constitutes a high proportion of the bean solids, its presence was not obvious. They, however, mentioned that raw, dry-fractured cotyledon frequently contained small amorphous particles, presumably protein. An apparent thin film, bridging some of the intracellular starch granules was also suggested to be cytoplasmic protein which coated the granules during drying. So, in all probability the packing-matrix here was proteinaceous.

After one-year storage there appeared to be an increase in the size of the cells (now 105 um x 63 um longitudinal section) while there was also an increase in the size of starch granules (range 13 to 27 um) (Fig 23). The membrane containing the cell contents appeared to retract further away from the cell wall as compared to the raw control. VARRIANO-MARSTON and JACKSON (1981) have also, observed a similar phenomenon in Black beans (Phaseolus vulgaris) which when stored for 55 days showed a retraction of the cytoplasm from the cell wall in most places. Similar observation have been reported for embryo cells from non-viable rye grains(HALLAM et al , 1973),for cells in the embryonic axis of stored pea seeds (HARMAN and GRANETT, 1972) and for cotyledons of germinated french beans (OPIK,1966).

The proteinaceous matrix then seemed to have lost its compact appearance and was more loosely distributed.

Decorticated Seeds

The individual cells of decorticated seeds are still observed to be well packed together as no air voids were apparent between them (Fig 24). The cell contents were a little less compact than those found in the raw seeds as controls, but the cytoplasm had retracted further away from the cell wall.

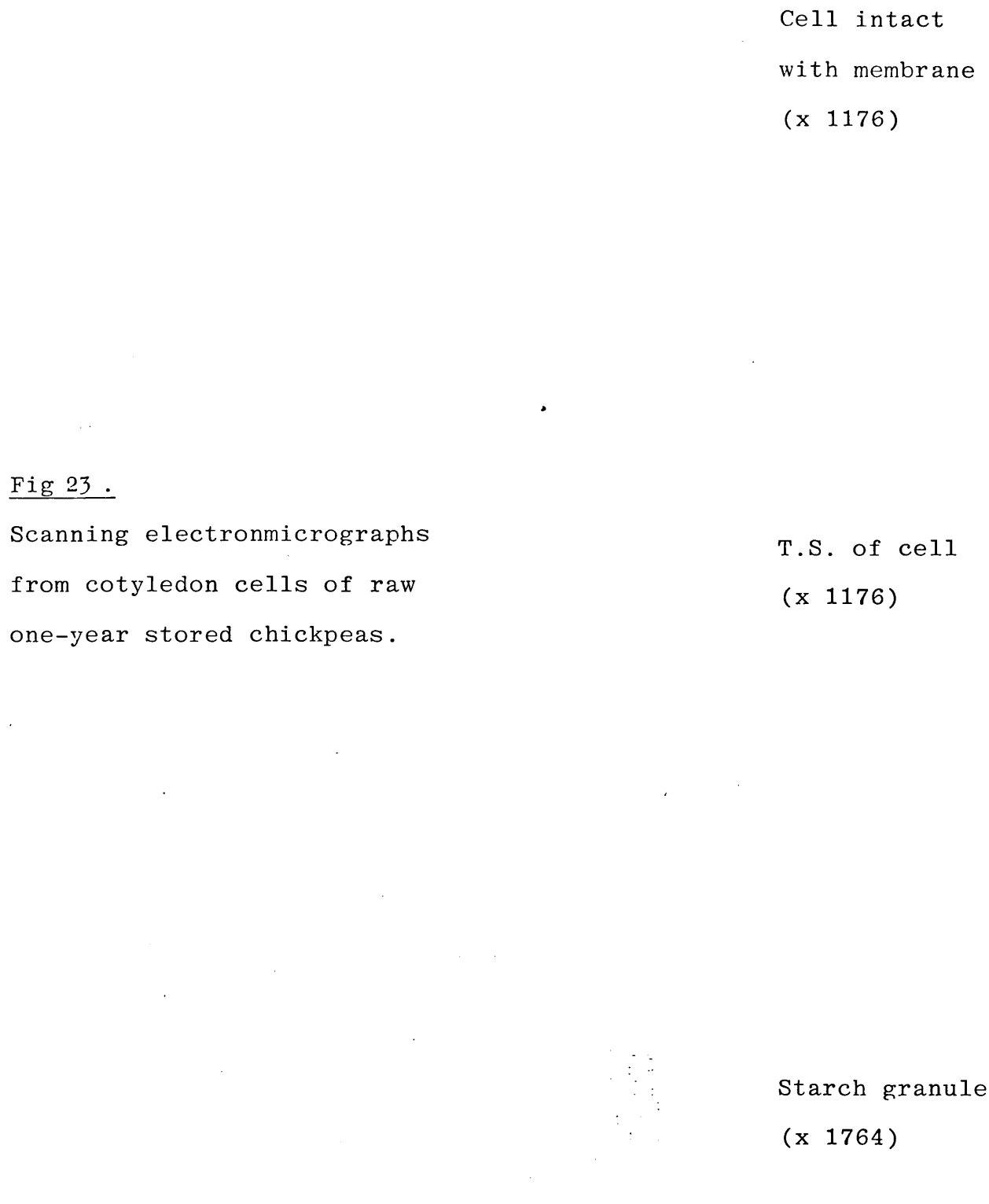
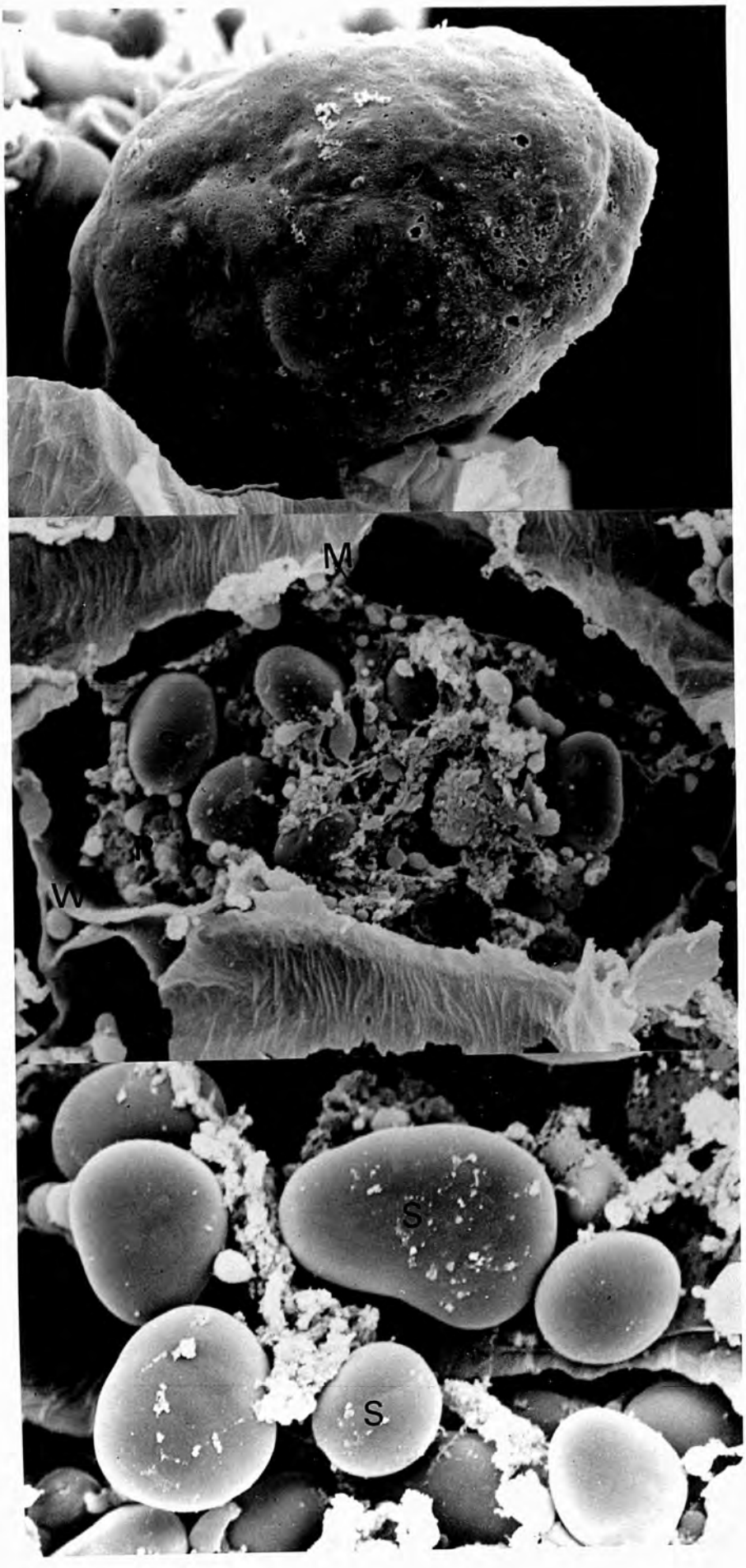
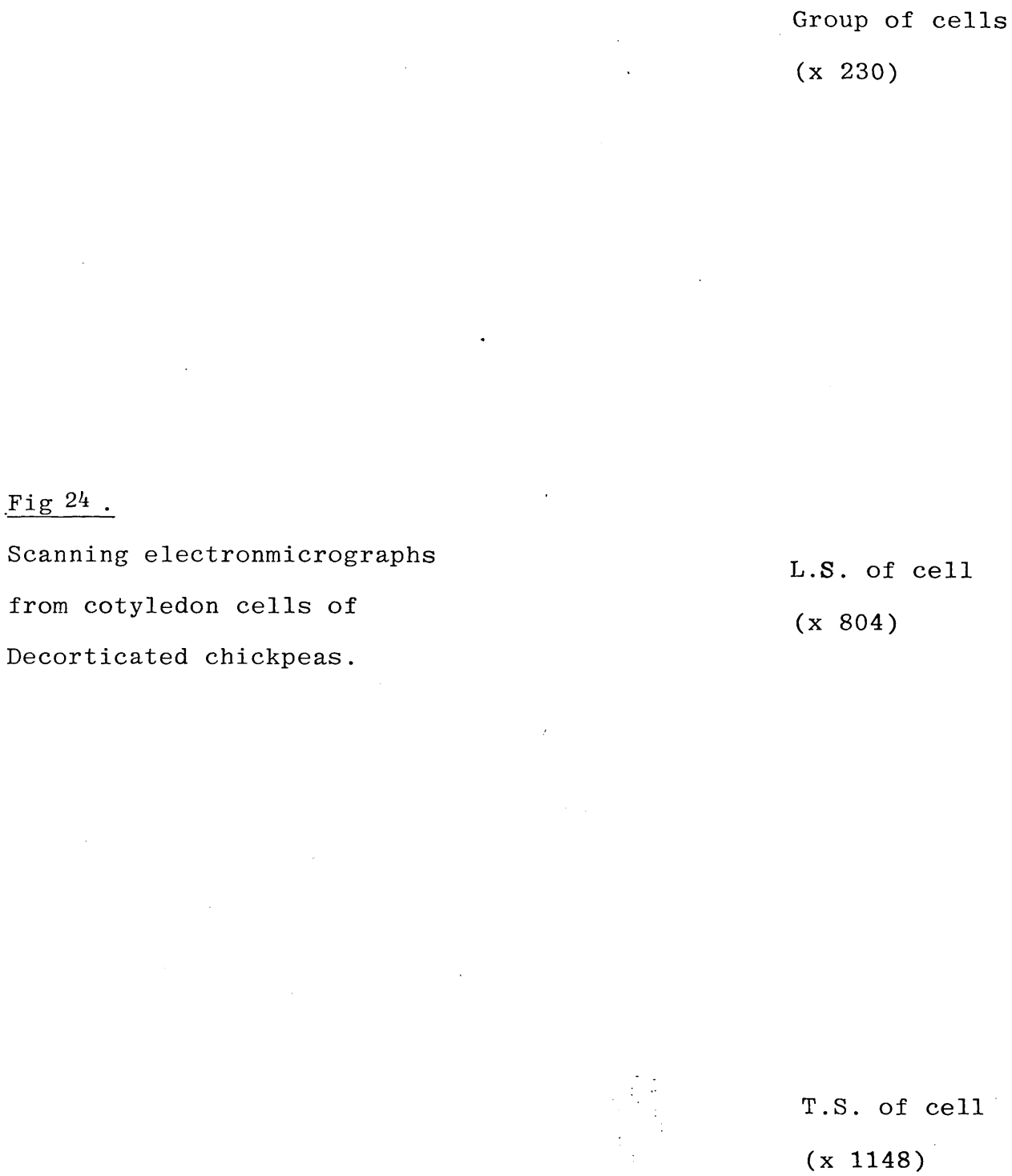


Fig 23 .

Scanning electronmicrographs from cotyledon cells of raw one-year stored chickpeas.


M
W
S
S

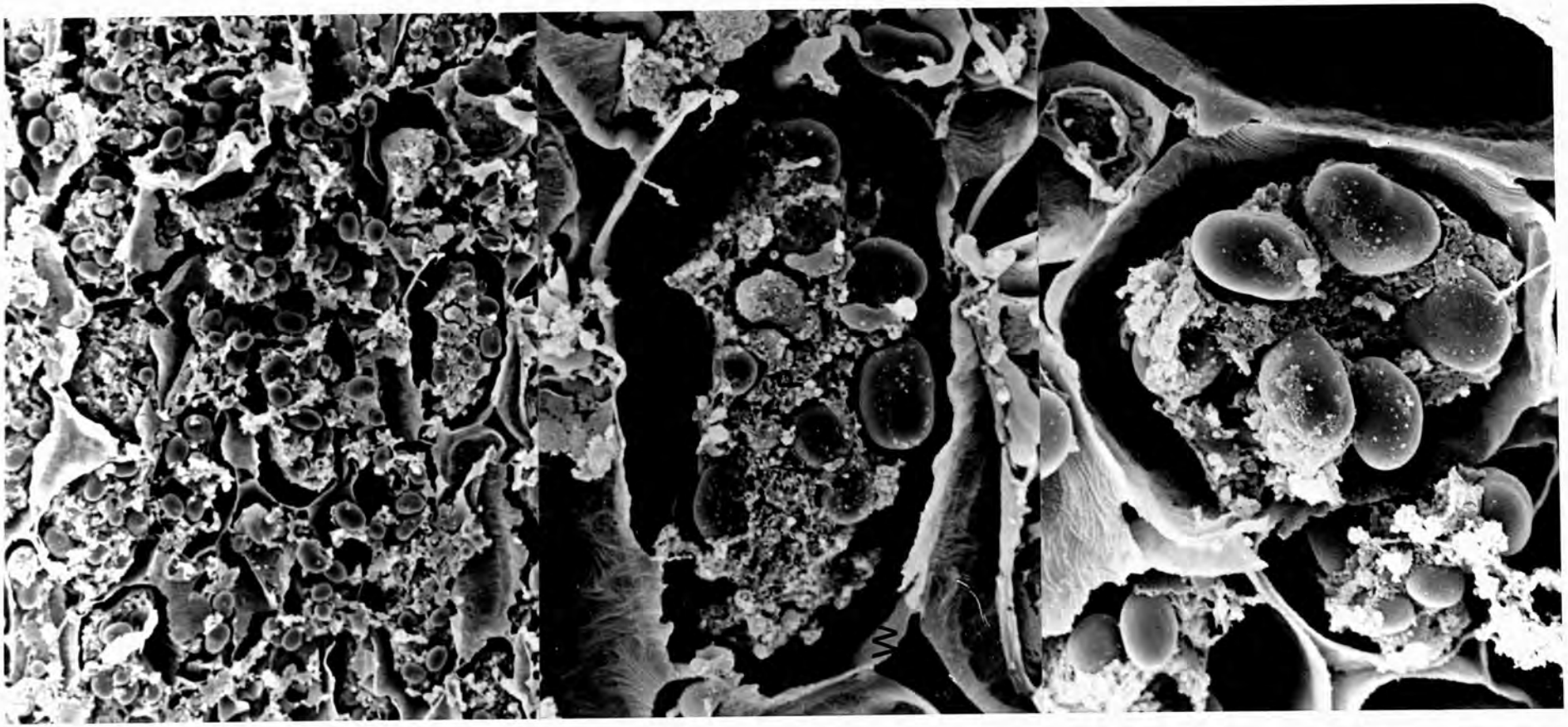


Group of cells
(x 230)

L.S. of cell
(x 804)

T.S. of cell
(x 1148)

Fig 24 .
Scanning electronmicrographs
from cotyledon cells of
Decorticated chickpeas.



Group of cells

(x 319)

Fig 25 .

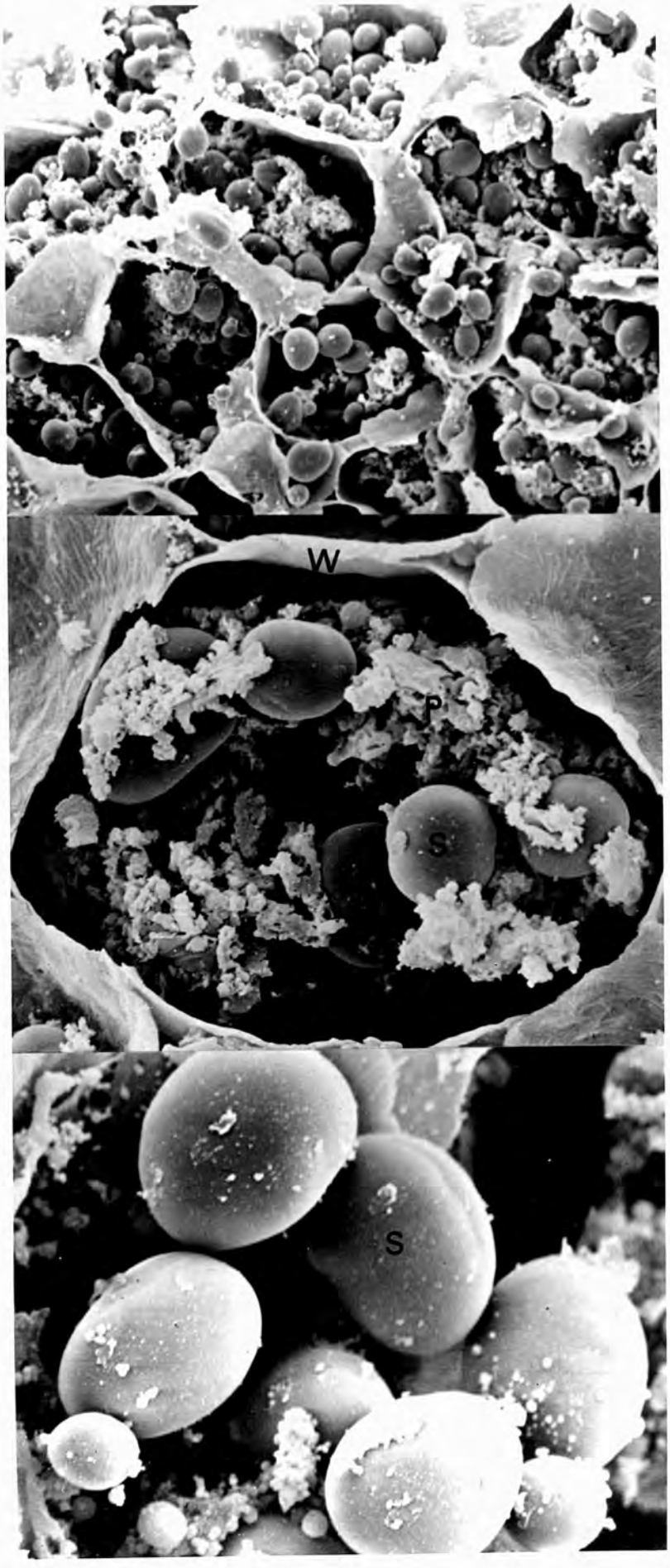
Scanning electronmicrographs of cotyledon cells of Dried chickpeas.

T.S. of cell

(x 1064)

Starch granules

(x 1596)


W
P
S
S

The starch granules and proteinaceous matrix were still apparent and constituted the major portion of the cell. There was not much change in the dimensions of the starch grains (range 10 to 20 um) when compared to those of raw controls.

Dried Seeds

Quite obvious changes were noted in the dried seeds (Fig 25) directly due to the drying process itself.

Generally, the average length of the individual cells had increased to 110 um without change in average width. The cell contents did not appear to be bound or restricted by any membrane but were loosely distributed throughout the cell. The starch granules still occupied the major volume of the cells but a certain amount of loss of the proteinaceous material was apparent. Many cells were found devoid of contents suggesting that some alteration in the cells had occurred in response to the shearing action of the blade during fracturing. There was no visible change in the dimensions of the starch granules which ranged from 10 to 21 um when compared to those of raw controls.

Frozen Seeds

Photomicrographs of frozen seeds are shown in Figures 26, 27, 28 and 29.

It is quite apparent that the water - and medium - soaking treatments had brought about increases in cell size due to increased moisture content.

For both WF and MF the dimensions of the individual cells were approximately 200 um x 100 um. This had also caused the cell contents, which still appeared densely packed, to be pushed tightly against the cell wall. Starch and proteinaceous matrix were abundant.

It was also interesting to note that in WF and MF the starch

Group of cells

(x 112)

Fig 26.

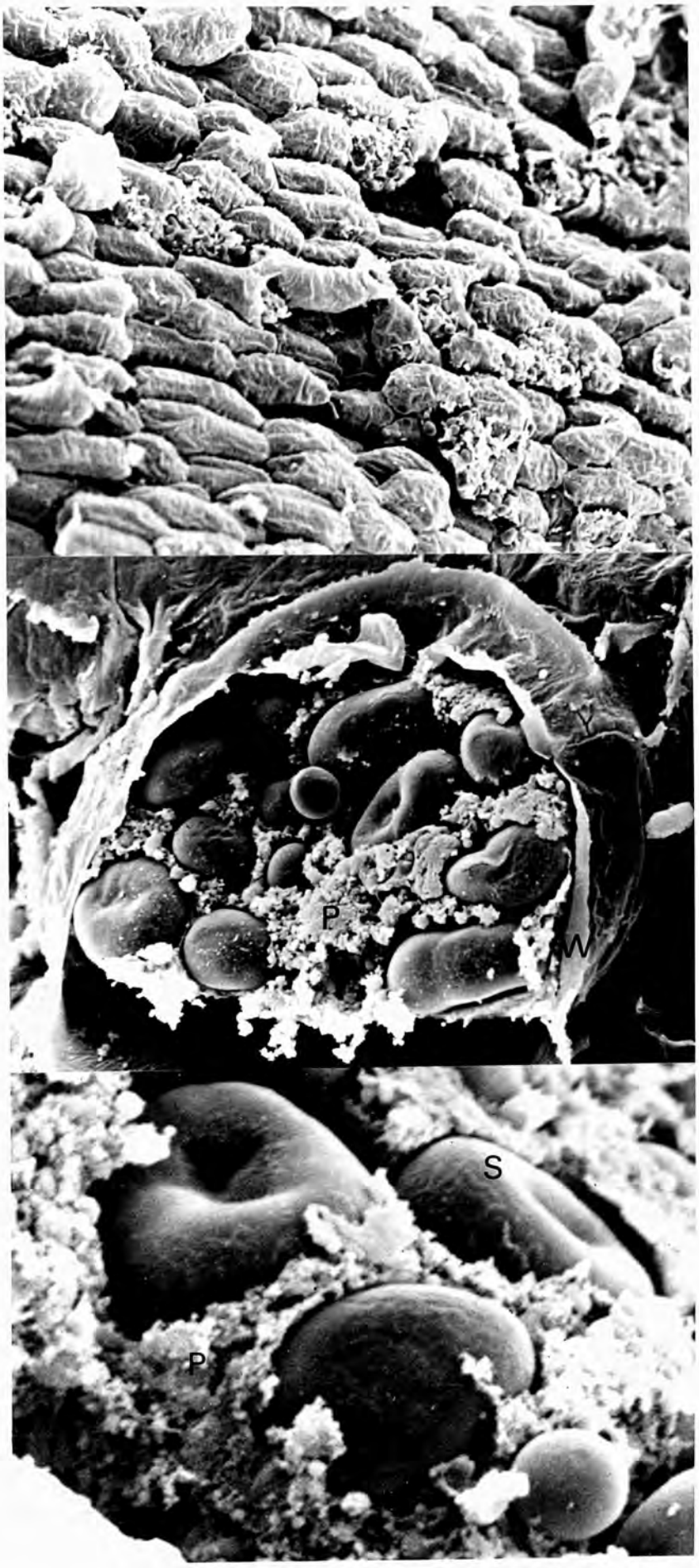
Scanning electronmicrographs from cotyledon cells of WF chickpeas.

T.S. of cell

(x 1120)

Starch granules

(x 2240)


P
W
S
P

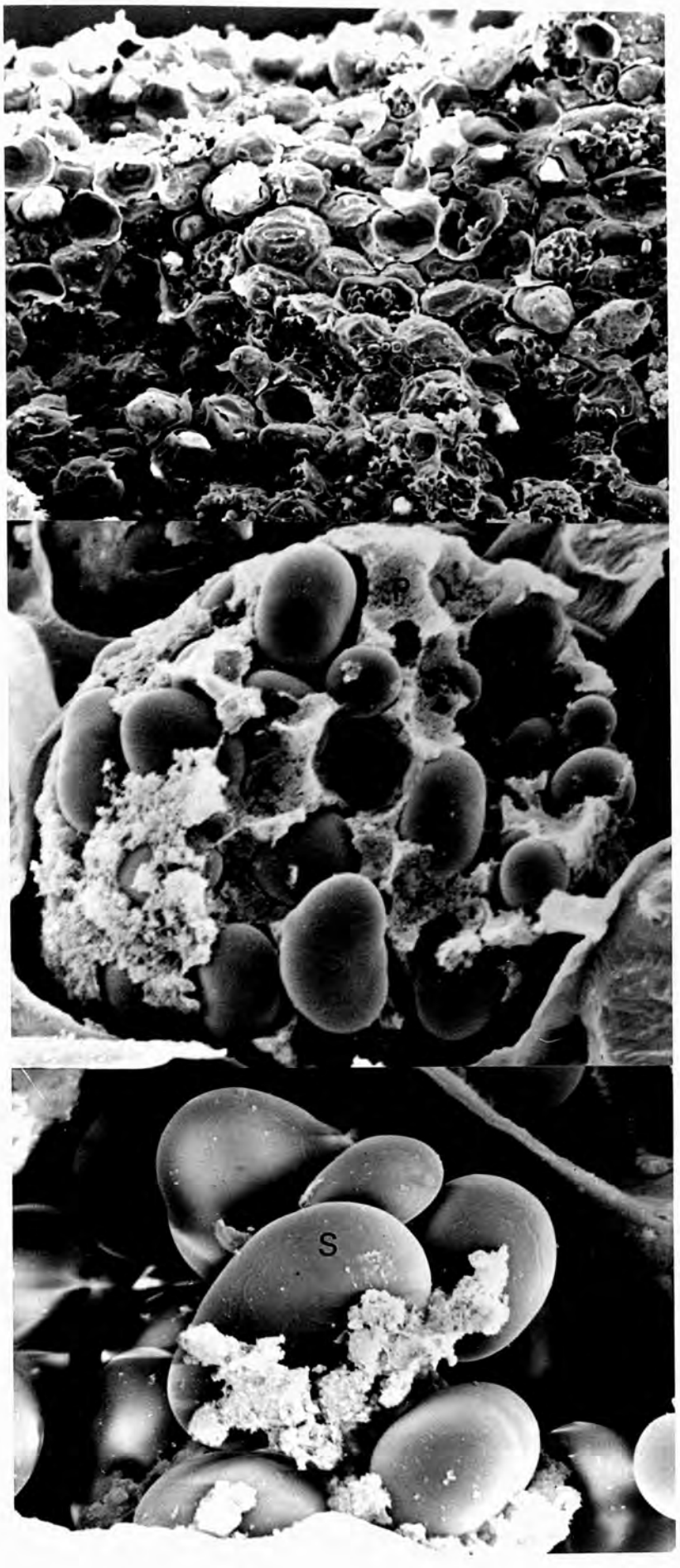
Group of cells
(x 107)

Fig 27 .

Scanning electronmicrographs from cotyledon cells of MF chickpeas.

T.S. of cell
(x 1073)

Starch granules
(x 2147)


P
S

Group of cells

(x 111)

Fig 28.

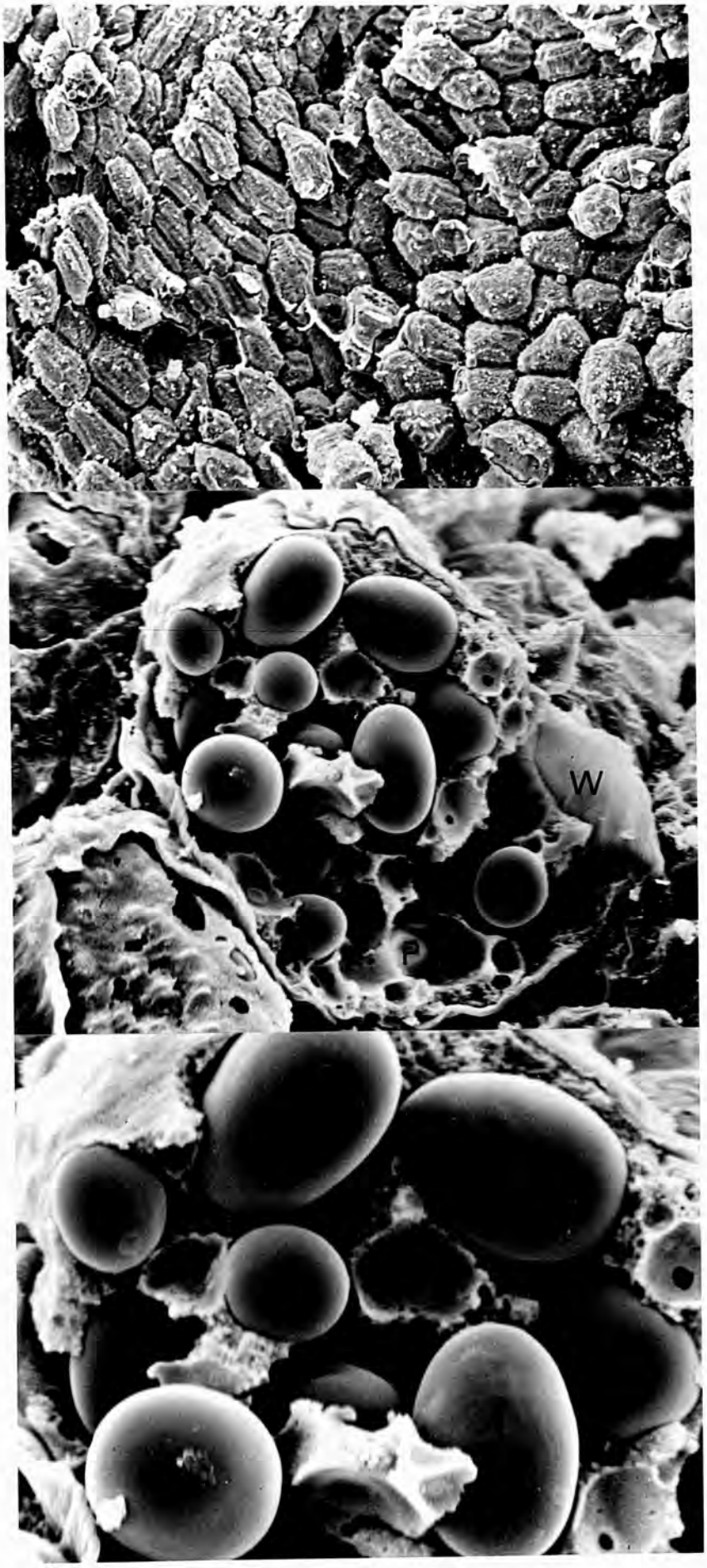
Scanning electronmicrographs from cotyledon cells of WPF chickpeas.

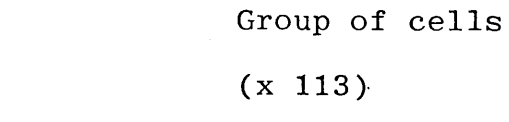
T.S. of cell

(x 1110)

Starch granules

(x 2220)

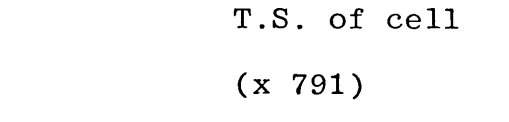

W
P



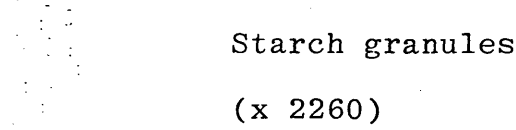
Group of cells
(x 113)

Fig 29 .

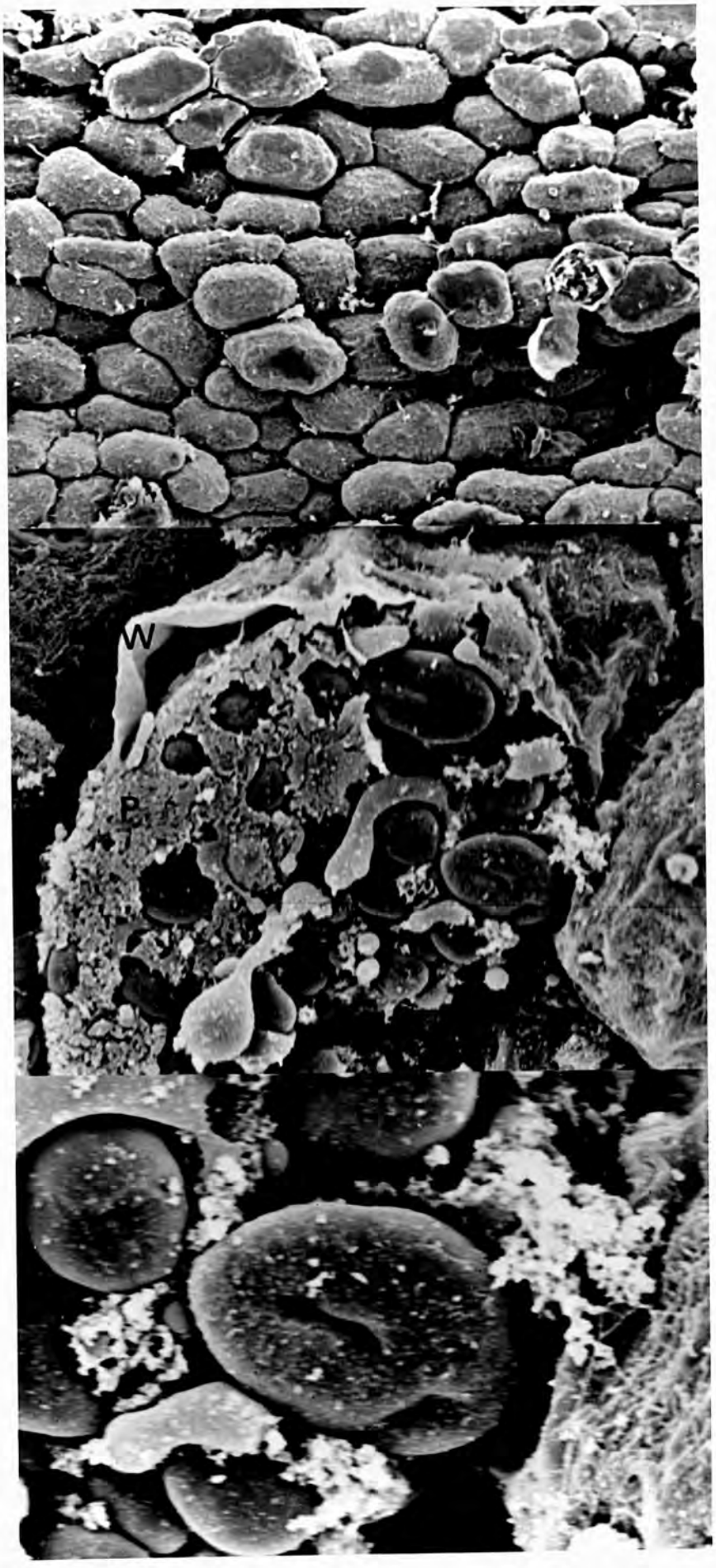
Scanning electronmicrographs from cotyledon cells of MPF chickpeas.



T.S. of cell
(x 791)



Starch granules
(x 2260)


W
P

granules had only altered their dimensions slightly as they ranged from 8 to 23 um. A large amount of swelling of the starch due to the imbibition of water would have been expected to occur but this was not so.

One small difference between cell contents of WF and MF was, however, observed. The starch granules in WF showed hollow dips in the centre giving a ''doughnut '' type appearance whereas in MF this was not so marked. ROCKLAND and JONES (1974) suggested that this hollowing was an indication of the initiation of starch hydrolysis. Water-soaking would, in fact, initiate the first stages of germination hence this observation was expected. The medium, it would seem, has prevented this occuring to any large extent.

Pre-cooking the seeds prior to frozen storage has also brought about changes in the appearance of the cells. In WPF the cell size has been substantially reduced to 109 um x 36 um whereas in MPF this reduction has not been as marked (150 um x 80 um). The cell contents were still compact and tightly pressed against the cell wall.

The size of the starch granules has slightly increased, with a range of 9 to 27 um. The '' doughnut'' appearance was not apparent in the WPF cells hence during the cooking further swelling of the starch grains may have occurred hence masking the effect of starch hydrolysis. On the other hand, the starch granules in MPF had developed this "doughnut" type appearance suggesting that starch hydrolysis was in progress there.

Results in chapter 5 (Tables 8 and 10) support the view that some hydrolysis has taken place in all the frozen samples. The cooking has not, however, brought about apparent change in the overall appearance of the cell contents.

Canned Seeds

The individual cells in the canned samples were not as tightly packed as those found in the seeds of all other processed samples already mentioned (Fig 30 and 31)..

The cells in canned samples appear to be smaller than those of frozen seeds. The dimensions are approximately 90 um x 50 um in longitudinal section for both WC and MC. The moisture contents already reported in Table 1 (Ch.2) show that canned seeds have a higher value than those of frozen seeds hence the cells in WC and MC would be expected to be larger. This suggests that the increased moisture content is due to a higher amount of water accumulation in the air spaces between the individual cells of canned seeds. This also implies that the canning process has altered the cell walls in some way, hence reducing adhesiveness between cells. These alterations may also have involved changes in the properties of the middle lamellae.

Further, it is apparent that certain intracellular changes have occurred due to the almost complete disappearance of defined starch granules. Although all these changes in cell structure appeared significant no major difference was observed between water- and medium-soaked seeds.

Group of cells

(x 110)

Fig 30.

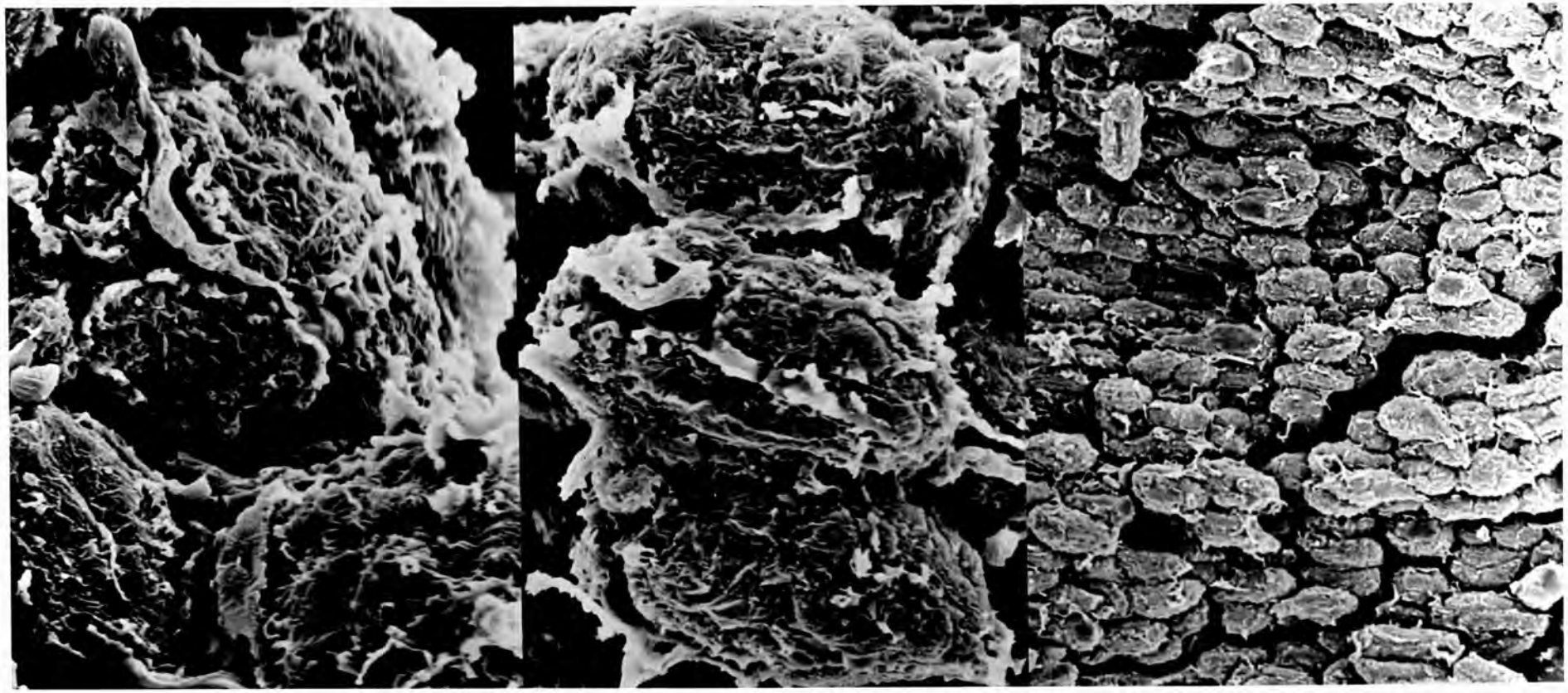
Scanning electronmicrographs from cotyledon cells of WC chickpeas.

L.S. of cell

(x 770)

Cell interior

(x 1100)



Group of cells

(x 322)

Fig 31.

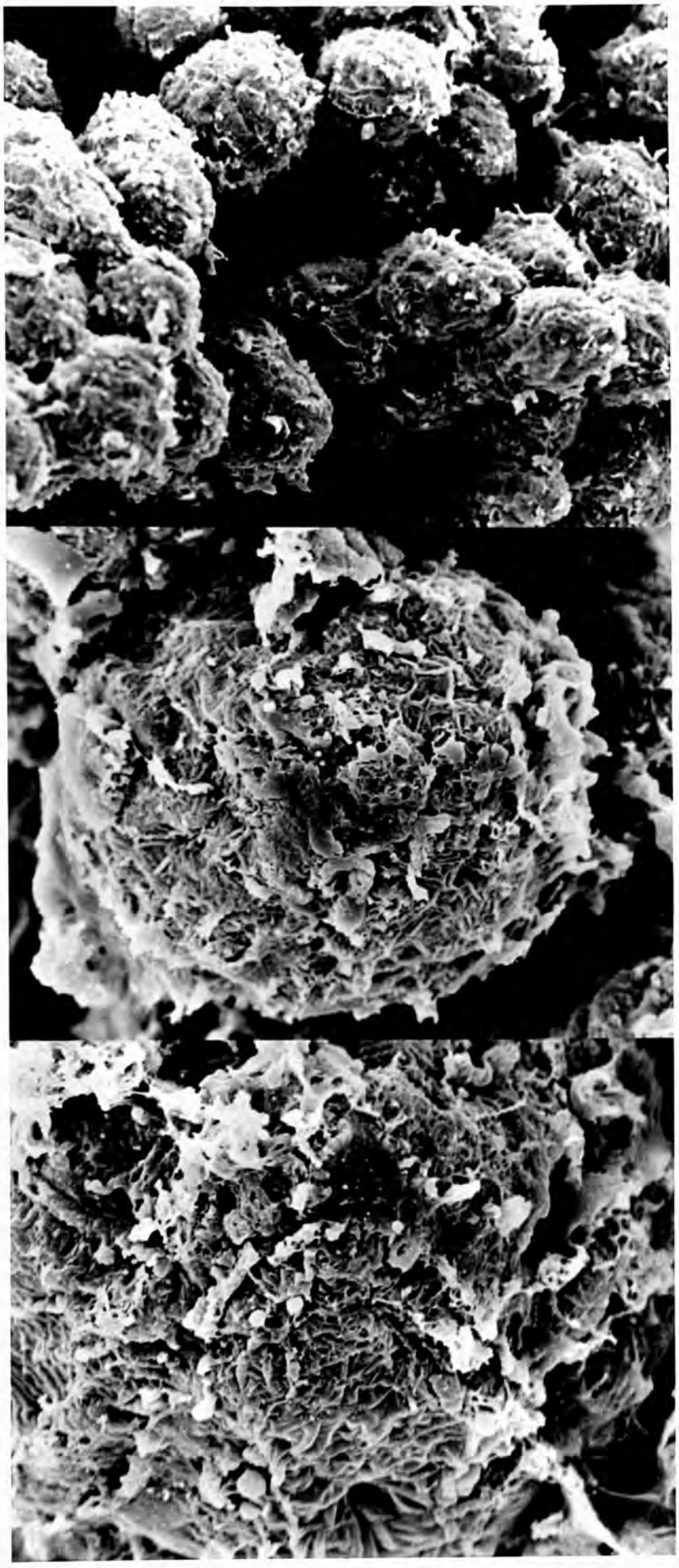
Scanning electronmicrographs from cotyledon cells of MC chickpeas.

T.S. of cell

(x 1073)

Cell interior

(x 2146)



CHAPTER VII

GENERAL DISCUSSION AND CONCLUSION

In the general discussion, separate accounts of the composition of raw and decorticated seeds and the effects of storage on them will be given. This will be followed by the effects of drying, freezing, canning and storage on medium-soaked seeds (raw or pre-cooked).

Raw seeds

a) Raw seeds were found to contain 208 mg seed^{-1} total available carbohydrate consisting of 186.5 mg of starch + higher oligosaccharides and 21.5 mg free sugars per seed. The free sugars were: fructose, galactose, glucose, sorbitol, sucrose, maltose, cellobiose, mellibiose, raffinose, manninotriose and stachyose.

After one-year storage the content of total available carbohydrate was 175.7 mg seed^{-1} (84.5% total retention).

b) Total protein, free amino acids, and ammonia contents were 84.5, 0.4 and 2.2 mg seed^{-1} respectively. Eighteen amino acids were identified: tryptophan, lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine.

The chemical score of the protein was 56 and the limiting amino acid was methionine.

After one-year storage the values for total protein, free amino acids, and ammonia were 83.5, 0.9 and 3.1 mg seed^{-1} respectively implying that there was no significant loss of

total protein. There was, however, a reduction in protein chemical score with tryptophan as well as methionine being limiting factors.

c) The total lipid content of raw seeds was 25.1 mg $seed^{-1}$ which showed a significant reduction to 20.7 mg $seed^{-1}$ after one-year storage. The simple lipids consisted of monoglycerides, diglycerides, triglycerides (the major fraction), sterols, steryl esters, and free fatty acids, while the complex lipids were phosphatidyl inositol, lysophosphatidyl choline, phosphatidyl choline, digalactosyl diglyceride, sulpholipid, phosphatidyl ethanolamine, sterol glycoside, and phosphatidic acid.

Free fatty acid contents were observed to increase on storage.

The raw seeds were found to be a rich source of essential fatty acids; C18:2 being the most abundant.

Decorticated seeds

a) Decorticated seeds contained 200 mg $seed^{-1}$ of total available carbohydrate consisting of 179.9 mg starch + higher oligosaccharides and 20.1 mg free sugars per seed. This represents a 3.8 % loss of total available carbohydrate due directly to the milling of the seeds.

Losses in total carbohydrates of decorticated seeds after one-year storage were less than those of raw seeds. However, the final amount remaining in decorticated seeds was approximately equal to that remaining in the raw seeds.

This implies that the storage of seeds in the decorticated state does not reduce the total available carbohydrate content compared to storing raw (whole) seeds.

b) Total protein, free amino acids, and ammonia contents of decorticated seeds were 80.3; 0.3 and 2.0 mg seed^{-1} respectively. This significant loss in total protein was due directly to seed coat removal.

After one-year storage the values for total protein, free amino acids, and ammonia were 78.8, 0.9 and 3.1 mg seed^{-1} respectively. This also indicated significant losses in total protein when compared to raw seeds stored for the same time period.

c) There was no significant loss in total lipid content due directly to the effect of decorticating the seeds. This appliedequally well to both simple and complex lipids.

One-year storage brought about losses in total lipid and in both simple and complex lipids which were only just significant.

The free fatty acids have not been affected directly by decortication but have increased on storage, this increase being less than that in stored (whole) raw seeds.

In general, from the point of view of retaining total protein, decorticating chickpeas is not an effective method of processing or storing the seeds.

It is also found that this method cannot be completely ignored, for several reasons. Firstly, that carbohydrates and lipids have been relatively well retained even after one-year storage. The removal of the seed coat also aids in reducing the hydration time required in soaking of the seeds and subsequently the total time required for cooking the seeds. At the same time the fibrous waste material (seed coats) may be used for fodder and other uses.

Dried seeds

a) Dried seeds contained 193 mg of total available carbohydrate per seed indicating a significant loss when compared to the raw seeds. This implies that soaking the seeds in the medium and subsequent drying have been directly responsible for this loss. The free sugar fraction has suffered more loss then the starch + higher oligosaccharides fraction.

After one-year storage a further loss has occurred but the total carbohdrate content now (172.6 mg $seed^{-1}$) was approximately equal to that of stored raw seeds.

b) Total protein, free amino acids, and ammonia contents found in dried seeds were 83.5, 0.5, and 1.5 mg $seed^{-1}$ respectively. There was no significant change in total protein due to medium-soaking and subsequent drying. After one-year storage there was still no significant change in total protein but the nutritional value of the protein was slightly reduced due to the lower chemical scores for tryptophan, lysine, threonine, and methionine.

c) Total lipid content remained almost unchanged after soaking and drying but there was a significant loss after one-year storage in both simple and complex lipids.

Amounts of free fatty acid were not affected at first, but increased slightly on storage. Furthermore, total fatty acid was affected in that there was a decrease in the unsaturation ratio indicating a loss of essential fatty acids.

Two views may be taken on the advantage of soaking chickpeas in the medium solution and subsequently drying them to low moisture contents.

1. If the chickpeas are needed for almost immediate consumption this method of processing offers the advantage of reducing cooking time, of retaining total protein content without change in nutritional value, and also of retaining the essential fatty acids in the lipid fraction.

2. If the seeds need to be stored for one-year, their advantage over raw untreated seeds stored for the same period is that there is no further loss of total carbohydrate, total protein or total lipid. At the same time, the seeds are more acceptable to the consumer by being faster to cook. On the other hand, raw seeds may have developed 'hard-shells' during this storage time and take longer to cook.

Frozen seeds

Uncooked seeds;

a) The content of total available carbohydrates in medium-soaked, uncooked frozen seeds was 200.5 mg seed^{-1} which represented a significant loss, due directly to this processing technique.

After one-year storage there was a further loss bringing the content of carbohydrates down to 187.8 mg seed^{-1}. This corresponded to 90.3% retention which was higher than the 84.5% retention of carbohydrates in raw seeds stored for one year.

b) Total protein, free amino acids, and ammonia were 89.5, 0.4 and 2.9 mg seed^{-1} respectively, which corresponded to a significant increase in total protein. Some protein synthesis may have occurred during soaking in the medium but the nutritional value of the protein has not been reduced in any way.

After one-year storage the total protein content was 86.3 mg seed^{-1}, this value being still higher than that in the raw seed.

The nutritional value of the protein was also well retained, except for methionine which was still limiting.

c) Total lipid content of uncooked, frozen seeds was significantly different from the raw seed even after one-year storage. The increase in free fatty acid after storage was not as high as that in stored raw seeds. The total fatty acids were not affected immediately after processing as indicated by high Iodine value, but after one-year storage the Iodine value showed a slight decrease.

The freezing process of uncooked seeds used here, would be a very suitable method for the preservation of quick-cooking chickpeas. This is due to the fact that total carbohydrate, total protein, and total lipids were better retained than in one-year stored raw seeds.

Cooked seeds:

a) Total available carbohydrates of medium-soaked, pre-cooked frozen chickpeas before and after storage were 188.0 and 166.0 mg $seed^{-1}$ respectively. These results are significantly lower than those of raw seeds, but are not significantly different from those of one-year stored raw seeds.

b) Total protein contents before and after storage were 83.1 and 80.3 mg $seed^{-1}$ respectively, indicating no significant loss of total protein due to the pre-cooking, but a significant loss due to one-year storage. The nutritional value of the protein was not affected in the first instance but after one-year storage there were large reductions in chemical scores of tryptophan, lysine, threonine, and methionine.

c) Cooking did not appear to significantly reduce the total lipid content but after one-year storage a significant loss was observed. There was a greater significant loss in simple lipids than in complex ones.

There was an increase in free fatty acids after one-year storage but the effect on total fatty acids (as measured by Iodine value) was less than that on the raw seed stored for one year.

One point needs stressing if, nutritionally, one comes to the conclusion that pre-cooked frozen seeds are inferior to uncooked frozen ones. Although there is a significant reduction in total lipids and a lowering of the nutritional value of the protein after one-year storage, the pre-cooked seeds may yet offer one advantage over uncooked ones.

The cooking here has been carried out under standardised conditions which has minimised loss of nutrients. In order to prepare for the table, a reduced amount of reheating is necessary. On the other hand, uncooked seeds are likely to incur greater subsequent losses when cooked by the consumer at non-standardised temperature and time periods.

Canned seeds:

a) Canned seeds which were previously medium-soaked were found to contain 189.8 mg seed^{-1} total available carbohydrates and 177.4 mg seed^{-1} after one-year storage at 22^{o}C. These values indicated a significant loss when compared to that of the raw seed but were not significant when compared to raw seeds stored for the same time.

b) Total protein content was 89.5 mg seed^{-1} before and 87.5 mg seed^{-1} after storage at 22^{o}C. These values were both higher than those of the total protein content of raw seeds.

There was, however, a reduction in the nutritive vale of the protein-in which tryptophan, lysine, threonine, and methionine became severely limiting after one-year storage. Temperature of storage (be it at 22 or 30^{o}C) did not cause any additional losses.

c) No significant loss of total lipid was noted as a direct result of the canning procedure but after one-year storage there were highly significant losses; approximately a 50% reduction. These occurred both in simple and in complex lipids.

There was, however, a slight loss of free fatty acids due to canning but there was no net increase after one-year storage at 22°C indicating that considerable fatty acid degradation had occurred. Substantial decreases in Iodine values after storage, indicated that a high amount of hydrogenation had also occurred in the lipids.

In general, a great deal more research will need to be carried out on the combined effects of medium-soaking, pre-cooking, and canning on chickpeas in order to improve the retention of the major nutrients. Although total available carbohydrates have not been adversely affected, there has been a considerable loss in the nutritional value of the protein and an even greater loss of total lipids, due to the destruction of essential amino acids and essential fatty acids respectively.

It appears that the medium-soaked seeds when kept in the can for one year, may have lost some of the nutrients into the liquor due to leaching. No further investigation was carried out on this liquor hence a firm conclusion on the fate of these nutrients cannot be made, but it is likely that some of the nutrients may have been leached into the liquor.

Final Conclusion

Quite a lot has been learnt in this study concerning the effects of the processing of chickpeas. Further studies on other pulses will have far reaching implications. With less arable land available in the world due to increased building and increased population, there will have to be a reduction in meat availability and therefore increased use of pulses will be the order of the day. It is well known that 50 times more land is required to produce the same amount of meat as of pulses. There will come a time when growing pulses will have to be increased to meet the world requirements.

In the meantime it is to be noted that world trade in pulses has shown a rising trade in recent years and international trade in 1970 totalled two million tonnes (AITKIN, 1973); Japan and the Netherlands being the largest importing countries.

Fresh peas and beans are the most commonly eaten pulses, but as a result of changes in dietary habits there is a growing demand for a number of other pulses which may be sold in the dried, frozen or canned forms. This is especially so in Australia where there is a growing demand for pulses, 80% of which have to be imported.

With the increased use of pulses, more will have to be known about processing techniques used for their preservation and their effects on the major nutrients contained in the food will have to be studied. This will serve to increase the varieties available on the market, hence increasing the choice available to the consumer. At the same time, a number of these processing techniques e.g. canning and freezing, will make transport of these pulses more economical and in the end, this will be advantageous to the consumer.

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APPENDIX 1

The effect of drying on the moisture content of hydrated chickpeas.

DRYING TIME (h)	AVERAGE MOISTURE CONTENT (nearest 0.5%)
0	60.0
1	55.0
2	42.0
3	34.0
4	30.5
5	25.0
6	21.5
7	17.5
8	15.5
9	12.5
10	9.0
11	7.0
12	6.0
13	5.0
14	4.5
15	4.0
16	4.0
17	3.0
18	3.0
19	3.0
20	3.0

APPENDIX 2 Essential amino acids (E.A.A.) of whole egg from hens (FAO,1970)

	Try	Lys	Thr	Val	Met	Ileu	Leu	Phe	Total E.A.A.
Weight mg $100g^{-1}$ egg	184	863	634	847	416	778	1091	709	5522
Fraction of total E.A.A.	0.033	0.156	0.115	0.153	0.075	0.141	0.198	0.128	———

APPENDIX 3 Standard error for amino acids in raw and processed chickpeas

	Try	Lys	His	NH_3	Arg	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Ileu	Leu	Tyr	Phe
Raw	0.30	0.15	0.06	0.98	0.20	0.46	0.08	0.02	0.38	0.33	0.32	0.14	0.05	0.06	0.05	0.01	0.32	0.11	0.24
D	0.61	0.04	0.06	0.40	0.10	0.18	0.05	0.04	0.45	0.09	0.29	0.08	0.03	0.04	0.03	0.03	0.15	0.06	0.60
De	0.06	0.02	0.03	0.06	0.12	0.19	0.04	0.03	0.32	0.09	0.25	0.05	0.01	0.02	0.02	0.02	0.19	0.04	0.11
WF	0.30	0.13	0.07	0.03	0.35	0.23	0.12	0.29	0.61	0.20	0.27	0.17	0.01	0.05	0.02	0.03	0.15	0.01	0.04
MF	0.09	0.12	0.09	0.04	0.60	0.09	0.28	0.14	0.24	0.06	0.57	0.24	0.01	0.14	0.10	0.09	0.56	0.21	0.01
WPF	0.07	0.01	0.04	0.05	0.35	0.23	0.16	0.05	0.39	0.19	0.38	0.11	0.01	0.05	0.08	0.03	0.43	0.06	0.16
MPF	0.07	0.07	0.07	0.07	0.27	0.09	0.18	0.33	0.14	0.01	0.30	0.20	0.01	0.07	0.07	0.06	0.31	0.09	0.20
WC	0.27	0.06	0.03	0.03	0.27	0.11	0.13	0.36	0.04	0.18	0.09	0.01	0.10	0.06	0.05	0.33	0.14	0.26	0.19
MC	0.04	0.07	0.05	0.14	0.32	0.20	0.36	0.02	0.10	0.02	0.24	0.40	0.01	0.08	0.02	0.08	0.34	0.05	0.16