The Botany and Proximate Analyses of some edible species of the New Zealand Flora

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Abstract

1. The edible organs of some New Zealand plant species have been assessed at light microscope level for their botanical basis, and for their nutritional basis by proximate analysis and Plasma Emission Spectrometry.

2. The species investigated, listed by Colenso (1880) as the most valued plant foods of the pre-European Maori, were:
Pteridium esculentum (rhizome), *Corynocarpus laevigatus* (kernel), *Elaeocarpus dentatus*, (pericarp), *Sonchus asper* (herb), *Calystegia sepium* (rhizome), *Cyathea medullaris* (frond stipe), *Cordyline australis* (leaf bases, root), *Rhopalostylis sapida* (apical shoot), *Typha orientalis* (pollen and rhizome), *Beilschmiedia tawa* (kernel), *Marattia salicina* (rhizomal scale), *Porphyra columbina* (frond), *Auricularia polytricha* (basidiocarp), *Arthropodium cirratum* (rhizome), *Bolboschoenus fluviatilis* (rhizomal tuber), *Gastrodia cunninghamii* (rhizome) and *Asplenium bulbiferum* (immature frond).

3. Specimens were collected at the appropriate traditional seasons (except for *Gastrodia cunninghamii*) and samples prepared by freeze-drying and milling. Samples were also prepared of the cooked organs of *Corynocarpus laevigatus*, *Elaeocarpus dentatus*, *Sonchus asper*, *Cyathea medullaris*, *Beilschmiedia tawa* and *Porphyra columbina*.

4. Analytical determinations were made for lipid, by extraction with di-ethyl ether; nitrogen, by micro-Kjeldahl method and colorimetric measurement of ammonia using indophenol; protein, by Bradford procedure using Coomassie Brilliant Blue and colorimetry; dietary fibre, by Englyst procedure using enzymatic digestion and colorimetry; soluble sugar, by acid hydrolysis and colorimetry; and starch, by enzymatic digestion and colorimetry.

5. Botanical investigations were made following histological procedures and microtechnique using paraffin wax embedding and staining with safranin and fast green; and by differential staining of hand-cut sections using Sudan Blue, iodine and Coomassie Brilliant Blue.

6. Analytical determinations were made for 23 trace, minor and major constituent elements, using inductively-coupled argon plasmas in a simultaneous emission spectrometer.

7. Proximate analyses showed high levels of lipid in *Corynocarpus laevigatus*, *Cyathea medullaris*, and *Sonchus asper*; of protein in *Corynocarpus laevigatus*, *Sonchus asper*, *Rhopalostylis sapida*, *Typha orientalis* (pollen) and *Asplenium bulbiferum*; of dietary fibre in *Auricularia polytricha*, *Beilschmiedia tawa*, *Marattia salicina* (root) and *Porphyra columbina* (uncooked); of soluble sugar in *Cyathea medullaris*, *Cordyline australis* (leaf bases and root), *Typha orientalis* (rhizomes and pollen) and *Pteridium esculentum*; and of starch in *Corynocarpus laevigatus*, *Elaeocarpus dentatus*, *Marattia salicina*, *Calystegia sepium* and *Gastrodia cunninghamii*.

8. High levels of essential minerals and trace elements were measured in many samples, and some excess levels of toxic metals were recorded.

9. The nutritional and ethnobotanical aspects of a pre-European Maori diet were related to the analytical and botanical findings of the investigation.

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Table of Contents

List of Plates

List of Tables

List of figures

Abbreviations

DMSO	di-methyl-sulphoxide
DNS	di-nitrosalicylate
FAA	formalin-acetic-acid
NSP	non-starch polysaccharides
PES	Plasma Emission Spectrometry
rcf	relative centrifugal force
TBA	tertiary butyl alcohol

Introduction

A group of 18 New Zealand indigenous food plants has been researched for this thesis, these plants having been listed and described by William Colenso, FLS, in his paper, "On the Vegetable Food of the Ancient New Zealanders before Cook's Visit" (1880). Parts I and II of this paper deal with plants formerly cultivated; it is from Part III, subtitled "Of the Wild or Uncultivated Food-producing Plants of the Ancient New Zealander" that this list of plants has been drawn, and this has provided the framework of the research.

The 18 plant organs, and in some cases groups of plants, were taken by Colenso "as they (the pre-European Maori) valued them and used them" (1880:20). For each, he described the pertinent botanical characteristics and distribution, the seasonal collection times and traditional methods of collection, the use made of the particular edible organ and traditional methods of treatment for food preparation.

References to these plants by Colenso and other early writers of the 18th and 19th centuries contain many comments acclaiming their "highly nutritious" value, or "wholesomeness" as foods, but there has been scant analytical data to verify these claims. Conversely, it has often been assumed that diets of indigenous people have been nutritionally inadequate. The New Zealand Maoris are no exception to this group and indeed, to quote the Maori anthropologist, Makereti Papakura, writing in the 1920s, "Much nonsense has been written about the starving Maori" (Makereti 1938:157).

This thesis seeks to examine the value of this group of uncultivated plants in the New Zealand flora in the pre-European Maori diet, by analysing the edible plant organs for their nutrient components and further by examining them at the light microscope level to gain knowledge of their structural and analytical basis. Some species have additionally been tested after cooking, bringing the total number of samples to 26. The proximate analyses carried out have been conventionally performed for plant food data, namely for the determination of moisture, lipids, nitrogen, proteins, dietary fibre, sugars and starch. The standard analysis for ash determination, having less relevance in this investigation, has been omitted. Samples were also analyzed by Plasma Emission Spectrometry (PES) for the determination of 23 elements.

These 18 food plants belong to diverse groups of the plant kingdom, and include fungi, algae, pteridophytes and angiosperms; of the latter there are trees, shrubs and herbs. There is also a variety in the type of plant organ utilized for food, and this includes roots, rhizomes, tubers, stem pith, leaves and shoots, pollen and fruits, of the latter both flesh and kernels.

This group certainly by no means exhausts the wild plant foods of the early Maori. There were many other uncultivated species in New Zealand's flora that formed some part of the early New Zealanders' diet, and these included the many seasonal fruits of the forest flora (Colenso 1880).

Plant species under investigation.

The following plants are those named and numbered by Colenso in order of his assessment of their value to the Maori:

1. *Pteris esculenta*, fern-root, *aruhe*, *roi*, *marohi*
2. *Corynocarpus laevigata*, *karaka*
3. *Elaeocarpus dentatus*, *hinau*
4. *Sonchus oleraceus* var., common sow-thistle, *puwha*
5. *Convolvulus sepium*, common convolvulus, bindweed, *pohue*
6. *Cyathea medullaris*, black tree-fern, *korau*, *mamaku*
7. *Cordyline australis*, cabbage tree, *tii*, *kouka*, *whanake*
8. *Areca sapida*, southern palm-tree, *nikau*
9. *Typha augustifolia*, common bulrush, *raupo*
10. *Nesodaphne tawa*, *tawa*
11. *Marattia salicina*, *para*
12. *Laminaria* sp., *karengo*
13. Several fungi:
    - *Lycoperdon fontanesei*, *pukurau*
    - *L. giganteum*, *pukurau*
    - *Agaricus adiposus*, *harore*
    - *Hirneola auricula-judae*, *hakekakeka*
    - *Ileodictyon cibarium*, *paruwhatitiri*
14. *Arthropodium cirratum*, New Zealand lily, *rengarenga*
15. *Typha augustifolia*, common bulrush, *raupo*

16. *Scirpus maritimus*, tall sedge, *riiriiwaka*
17. *Gastrodia cunninghamii, perei*
18. Several smaller plants, as leafy vegetables:

    Solanum nigrum, raupeti

    Barbara australis, toi

    Taraxacum dens-leonis, tohetake

    Asplenium bulbiferum

    A. lucidum

Some of the plants have since been renamed; presently classified, the plant list reads as follows:

1. *Pteridium esculentum* (Forst.f.) Kuhn; Dennstaedtiaceae
2. *Corynocarpus laevigatus* J.R. *et* G.Forst.; Corynocarpaceae
3. *Elaeocarpus dentatus* (J.R. *et* G.Forst.) Vahl; Elaeocarpaceae
4. *Sonchus asper* (L.) Hill; Asteraceae
5. *Calystegia sepium* (L.) R.Br.; Convolvulaceae
6. *Cyathea medullaris* (Forst.f.) Swartz; Cyatheaceae
7. *Cordyline australis* (Forst.f.) Endl.; Agavaceae
8. *Rhopalostylis sapida* Wendl. *et* Drude in Kerch.; Palmae
9. *Typha orientalis* C.B.Presl.; Typhaceae
10. *Beilschmiedia tawa* (A.Cunn.) Benth. *et* Hook.f. ex Kirk; Lauraceae
11. *Marattia salicina* Smith in Rees; Marattiaceae
12. *Porphyra columbina* Montagne; Bangiaceae
13. *Auricularia polytricha* Montagne (Saccardo); Auriculariaceae
14. *Arthropodium cirratum* (Forst.f.) R.Br.; Liliaceae
15. *Typha orientalis* C.B.Presl.; Typhaceae
16. *Bolboschoenus fluviatilis* (Torrey) Soják; Cyperaceae
17. *Gastrodia cunninghamii* Hook.f.; Orchidaceae
18. *Asplenium bulbiferum* Forst.f.; Aspleniaceae

Further taxonomic discussion of numbers 1, 4 and 12 is presented in the respective sections of plant data. Of the groups of fungi and leafy vegetables listed previously under numbers 13 and 18 respectively, only one species from each has been chosen for investigation . The five cited species of fungi are presently classified as follows:

Calvatia utriformis (Bull. ex Pers.) Jaap; Lycoperdaceae

C. gigantea (Batsch ex Pers.) Lloyd; Lycoperdaceae

Pholiota aurivella (Batsch ex Fr.) Kummer; Cortinariaceae

Auricularia polytricha (Montagne) Saccardo; Auriculariaceae

Clathrus cibarius Micheli ex Pers.; Clathraceae

Of these, *Auricularia polytricha* has been collected and used for analysis; this choice has been based on the relative abundance and ease of identification of the species. The five cited species of smaller plants are presently classified as follows:

Solanum nigrum L.; Solanaceae

Barbarea australis J.D.Hook.; Brassicaceae

Taraxacum megellanicum Comm.ex Sch.Bip.; Asteraceae

Asplenium bulbiferum Forst.f.; Aspleniaceae

A. lucidum Forst.f.; Aspleniaceae

Colenso gives no botanical information on these species. The plant named as *Barbara australis* (*sic*), or *toi*, may have been *Barbarea intermedia*, as the only (other) record of *B. australis* from New Zealand (Hooker 1852) is based on a specimen of *B. intermedia* (Webb *et al.* 1988). *Taraxacum dens-leonis* may refer to the indigenous *T. megellanicum* or possibly the introduced *T. officinale*; both are known now by the same Maori name, *tohetake*, which Colenso used for *T. dens-leonis*. *Asplenium bulbiferum* has been chosen from this group for use in this investigation; this choice has been based on the ease of identification of the species and a lack of ambiguity regarding its classification.

The accuracy of Colenso's records, both botanical and historical, has been viewed and assessed by comparison with records of other early writers. These include the early discoverers Cook (Beaglehole [Ed] 1955) and Banks (Beaglehole [Ed] 1962); explorers and botanists Dieffenbach (1842), Wakefield (1845), Angas (1847), Hooker (1852), Bauke (1928) and Best (1942); and some of the many missionaries, Yate (1835), Wade (1842), Taylor (1855) and Stack (Reed [Ed] 1935).

In his study of the New Zealand flora, Colenso was in regular contact with other botanists of his day. Notable amongst these were Cunningham and Hooker. Allan Cunningham, as colonial botanist of Sydney, visited Colenso in the Bay of Islands and was influential in encouraging the amateur botanist to further his study of plants (Bagnall and Petersen 1948). Dr Joseph Hooker, as assistant surgeon and naturalist to the

"Erebus", also visited Colenso while making botanical investigations in that area. Both professional botanists partook of field trips with Colenso who was considered by Hooker to be the foremost New Zealand botanical explorer of the time (Hooker 1852).

Colenso had come to New Zealand in 1834 and between then and his death in 1899 recorded his findings in detail and sent numerous specimens to Kew for identification, confirmation and herbarium preservation.

During this 65 years in New Zealand Colenso became intimately acquainted with the Maori, and made detailed recordings of their knowledge and use of plant species. In this respect the validity of his contribution to the ethnobotanical literature is held in high esteem (Best 1942; Bagnall and Petersen 1948).

The use of the term "Maori" in the text refers only to the New Zealand Maori regardless of their individual or collective origins in the Pacific and should not be confused for example with indigenous peoples of the Cook Islands.

Part 1
Procedures and analytical methods

Specimen collection and sample preparation

Prior to the analyses, two important areas of procedure were the identification and collection of species, and the preparation of samples.

Identification and collection.

All plant species were carefully identified and compared with herbarium specimens. Plants were located and chosen for collection from sites where specimens were healthy and relatively abundant. Minimal interference with the flora was observed. All collection sites were natural habitats except for *Arthropodium cirratum* and *Marattia salicina*. Both these species were also located and photographed in their natural habitats, coastal rocks and bush respectively. Those species from which organs were to be collected at a season when the plant was not in flower were studied for identification in the previous flowering season.

Herbaceous specimens were collected using a stainless steel knife or scissors, and with minimum handling. Grip-seal plastic bags, containing moistened paper towels in smaller perforated plastic bags, were used for immediate storage. Fruits and underground organs were similarly stored. For non-herbaceous specimens, cut ends were covered with plastic.

The time interval between field collection and laboratory preparation of samples was kept to a minimum.

Preparation of samples

Specimens were sorted and cleaned as appropriate in the laboratory. Roots and rhizomes were cleaned thoroughly to remove all traces of soil, rinsed in distilled water and blotted dry. Leaves and stems were checked for contamination and foreign bodies, and cleaned where necessary using a lens blower. Fruits were wiped clean using a cloth dampened in distilled water. The removal of fruit flesh from kernels, and all cutting of specimens, was carried out using clean stainless steel blades. Minimal handling of the samples at this stage was observed to avoid finger grease (J. Lee pers. comm.)and other contamination, bruising of tissues and water loss.

I Proximate analyses

Determinations for the following components are described:

A Moisture

B Lipid

C Nitrogen

D Protein

E Dietary fibre

F Soluble sugar

G Starch

A Moisture

For the determination of water content, three cleaned fresh sub-samples, of approximately 0.5 g, were weighed accurately on a digital screen balance correct to 4 decimal places. The samples were put into an electric oven at 85°C, on small glass petri dishes. They were reweighed after 24 hours and again, after further drying, at 48 hours. Drying was continued until constant weights were reached. Moisture content by oven drying was expressed as a percentage with percentage deviation calculated as the mean of three replications. The length of time to secure maximum drying varied for different plant types, particularly between different organ types and between mucilaginous and non-mucilaginous tissues.

Moisture content was also assessed using fresh weights and freeze-dried weights of the two sub-samples prepared for use in the following proximate analyses.

Proximate analyses B to G

Material for these analyses was sub-sampled, weighed and frozen in sealed plastic bags. Two sub-samples were taken, of between 10 and 100 g fresh weight. Frozen samples were freeze-dried to constant weight then milled to a fine particle gauge using a Braun domestic mill with stainless steel rotary blades; this was adapted for use with small samples by fitting into it a removable perspex screen. Care was taken to prevent

overheating during the milling.

For measurement of fresh weight:dry weight ratios of the treated samples, fresh weights were recorded prior to cooking, and dry weights recorded after freeze-drying the prepared material, before milling.

Prepared material was put in screw-top glass bottles and these were stored over silica in lidded tins, and kept in the freezer until required for analysis.

For conformity in weighing, samples were removed from the freezer two hours beforehand and stood in a lidded silica desiccator.

As the amounts used in the following analyses are relatively small, every care was taken to ensure that these were representative of the original material, and homogenous sampling was observed throughout.

All procedures except the lipid determination were carried out in duplicate, provided by the primary whole specimen sub-sampling. Secondary stage replication, in the weighing of samples for determination, was done in duplicate for the dietary fibre and nitrogen determinations, and in triplicate for the protein assessment. A tertiary replication was carried out in the removal and analysis of aliquots in both the nitrogen and protein determinations.

In all procedures requiring the use of distilled water, Millipore de-ionized water was substituted for greater accuracy.

B Lipid determination

Choice of procedures

Methods for lipid determination fall into two groups, extraction methods and saponification methods. The former depend on the solubility of lipids in suitable organic solvents, and three considerations are involved. Firstly, oxidation of unsaturated fatty acids during the preliminary drying process must be minimised. This has been a more critical consideration in the past, and with efficient freeze-drying procedures, the risk of oxidation is low. Secondly, the choice of a good fat solvent to give complete extraction is crucial. Single solvents may be used or, alternatively, a blend. Thirdly is the importance of choosing a solvent which will extract the minimum amount of substances other than lipid. Ethyl ether is one of the best solvents for fat, but it must be anhydrous, and the sample must be finely ground and thoroughly dry. Different forms of apparatus have been used in an attempt to obtain the most efficient extraction; the Soxhlet continuous extraction is the apparatus most frequently used, but there are numerous other modifications (Gunstone and Norris 1983).

In the saponification methods, the lipid is saponified by alkali, and fatty acids are subsequently set free from the soap by treatment with concentrated acid. This is based on the original method of von Lieberman in 1898. The fatty acids, dissolved in petroleum ether, are estimated either gravimetrically or volumetrically. Saponification methods have been criticized because petroleum ether is not a good solvent for fats, but McCance and Shipp (1933) tested samples for completeness of extraction with satisfactory results. The same authors then employed the Soxhlet and saponification methods for different plant foods, and their results showed a wide variation in values for the two methods. The Soxhlet method gave lower results for some cereals, but much higher results for spice plants, ginger and pepper, containing volatile oils.

The procedure followed by Visser and Burrows (1983), based on the method of Folch *et al.* (1957), is the exhaustive extraction with di-ethyl ether, using Soxhlet extractors, and weighing after evaporation of all solvent. This has been the method of choice in this investigation, being considered by contemporary workers (J. Lee pers. comm.) to be the most refined and reliable.

Interferences in this procedure may be from some components which are partially soluble in ether and slowly extracted, oils which may be at least partially volatile in some plants, and the possible contamination from finger grease or apparatus grease (J. Lee pers. comm.). The hazards involved in this procedure relate to the low boiling point (37°C) of di-ethyl ether and the extreme toxicity of its highly inflammable vapour.

Lipid determination by extraction with di-ethyl ether

Equipment:

Soxhlet extractors and condensers; round bottomed flasks; extraction thimbles (lipid-free); filter paper cones; electromantle heater.

Reagent:

analytical grade di-ethyl ether.

Method:

A flask and two boiling chips were weighed and fitted to the extractionbody. A thimble containing 0.5 g of free-flowing freeze-dried sample was placed in the extraction body and topped by a small filter paper cone to catch solvent drops and prevent splashing. The condenser was attached to the extraction body and fume cupboard switched on. Using a funnel, di-ethyl ether was poured through the top of the condenser, sufficient to cover the sample. The top of the condenser was covered with a beaker, and left to soak overnight. The level of the solvent was topped up next morning so that it siphoned over into the flask, then a further 20 ml added. Water was adjusted to flow through the condenser, the heating adjusted so that solvent recycling proceeded at 3-5 drops per second, and the extraction continued for 6 hours from the onset of the siphoning.

At the end of extraction the ether was allowed to siphon over prior to disconnection of the condenser from the extraction body, and the thimble removed. The flask was disconnected and remaining ether in the extraction body added to that in the flask. This was left in the fume cupboard for complete evaporation of

solvent, then removed to a 70°C oven overnight, cooled in a desiccator and weighed. All moving of the flask was done using tongs or gloves, but strictly with no hand contact.

Following this procedure, one sample was assayed. Extraction cycles were also performed using both a reagent blank and a standard of known lipid composition (4.4 - 4.6 g%). For the reagent blank a thimble without sample was used. The standard reference material was the one used for routine food research and commercial determinations by Dr J Lee, New Zealand Institute of Agricultural Research Limited and comprised protein (10%), minerals and vitamins, cellulose and starch, and fat (4.4 - 4.6%). A 0.5 g sample of this was extracted with di-ethyl ether as above.

The difference in flask weight before and after extraction was taken to represent the lipid component of the sample.

C Nitrogen determination

Choice of procedures

The conversion of organic nitrogen to ammonia and its subsequent estimation is the basis of the well-known Kjeldahl method. This method of wet combustion was developed by Johan Kjeldahl in 1883, and has since been the method of choice for the determination of nitrogen in plants. Many publications, including that of Bradstreet (1965), have dealt with the different aspects of this procedure.The principle of the method depends on the decomposition of nitrogenous compounds, by boiling with concentrated sulphuric acid. In this digestion, the carbon and hydrogen compounds of the organic material are oxidised to carbon dioxide and water. Part of the acid is reduced to sulphur dioxide and this in turn reduces the nitrogenous compounds to ammonia.

Many modifications to the procedure have been made over the century since its inception, but the principle and method remain essentially the same. One important modification has been in the hastening of digestion. Wilfarth (1885, in Bradstreet 1965) suggested the use of mercury or mercuric oxide as a catalyst to act as an oxygen carrier and thus hasten digestion. Other catalysts have since been used to this end. Selenium was suggested by Lauro (1931) but it appeared to have no advantage over mercury. However, selenium is currently preferred by some analysts and is the relevant ingredient in commercially available catalyst tablets. Vanadium pentoxide has been used by Lees (1968) and the possibilities of other elements, such as molybdenum, titanium and chromium have been well explored (Bradstreet 1965).

The hastening of digestion is also achieved by raising the boiling point of the sulphuric acid, as was suggested by Gunning (1889, in Pomeranz and Meloan 1980), who used potassium sulphate. This addition is included in current procedures, and is used concurrently with one of the catalysts mentioned above.

The second area of modification in the procedure has been in the introduction of micro-methods. The chief advantages of the small scale are the short time and the size of sample required. Macro-methods use a 10 g sample compared with a few milligrams in the micro-method.

The third major area of modification has been in the measurement of ammonia. In the original method, this was liberated by the addition of concentrated alkali. The free ammonia was distilled into a known excess of standard acid and the excess then back-titrated with standard alkali. This distillation method has been refined and amounts as low as 10 μg of nitrogen can be measured with a precision of ± 1%. It is the method of choice of many analysts, and suitable apparatus is available commercially.

An alternative to distillation is the use of a colorimetric method. With the advent of more sophisticated equipment, colorimetric methods have recently been widely used as a reliable alternative. The Nessler method is the more sensitive of the two colorimetric procedures but it is less suitable for direct use with the Kjeldahl digests of plant material. For these analyses, the indo-phenol blue procedure is the one of choice. Although this reaction has long been known it has been slow to supplant distillation and titration, mainly because of the difficulty of obtaining reproducible colour development. Automated versions are highly satisfactory. The manual alternative is also satisfactory but care must be taken not to exceed the time of colour development because of fading. Also the final pH value of the reaction mixture affects the intensity of colour (Bradstreet 1965). Other critical factors and interferences are discussed by Bradstreet (1965), and Pomeranz and Meloan (1980). In this method, ammonium nitrogen is oxidised by sodium hypochlorite and then coupled with phenol to produce the indo-phenol blue colour. The sensitivity is further improved by the addition of nitroprusside and with care a precision of ± 2% is possible and is comparable to the distillation-titration system in this respect (Apostolatos 1984).

The choice of procedure in this investigation has been a modified micro-digestion, and the measurement of ammonium nitrogen by manual indo-phenol blue colorimetry. In the digestion stage the choice of catalyst has been mercuric oxide used with potassium sulphate. The use of copper sulphate with the catalyst had been found by Apostolatos (1980) to result in coloured blanks, and was not satisfactory for use in colorimetric procedures.

Strictly this method only estimates total organic nitrogen and ammonium nitrogen. Nitrate is not included, but amounts normally present are very small. A further possible modification is the addition of salicylic acid at the digestion stage , so that nitrate is

reduced to ammonia (Scoville in Bradstreet 1965). This addition has not been included in the present method, assuming that nitrate in unfertilized, uncultivated vegetation would be insignificant.

Recipes for reagents have been based on those used by Apostolatos (1984), using a ratio of sample to catalyst to acid of 1:20:80. The block digester recommended by Apostolatos was replaced by a bunsen rack and condenser, holding a set of micro-Kjeldahl flasks, as used by Kerr (1986) and having given satisfactory results with bean seed samples.

Nitrogen determination by a modified micro-Kjeldahl procedure

Equipment:

micro-Kjeldahl flasks; bunsen racks (to hold 6 flasks) with condensers; volumetric flasks (25 cm^3); glass test tubes (20 ml); water bath (37°C); vortex mixer; spectrophotometer.

Reagents and stock solutions:

Reagents used were sodium hydroxide solution (50% w/v), concentrated sulphuric acid (96% w/v), ammonium sulphate (analytical grade, 20.98 - 21.38%N), phenol crystals, mercuric oxide, sodium nitroprusside, ethylenediamine tetracetic acid disodium salt (EDTA), commercial "Janola" bleach solution, glycine paratoluene sulphonic acid (glycine PTSA).

The following stock solutions were made:

Solution "A": Nine parts of 0.75 M NaOH solution were mixed with 70 parts of 150 ppm EDTA solution in de-ionized water and adjusted to pH 10.0.

Solution "B": Five grams of phenol crystals and 25 mg sodium nitroprusside were made to 500 ml with de-ionized water. This was stored in an amber bottle to avoid instability of the solution; the storage life at 40°C is approximately 2 months.

Solution "C": Two parts of commercial "Janola" bleach solution were mixed with 98 parts buffer immediately prior to the assay. This precaution is necessary as solution "C" rapidly deteriorates even when kept cold.

Buffer for solution "C": 2.5 g NaOH, 1.87 g Na_2HPO_4, and 15.9 g $Na_3PO_4.12H_2O$ were dissolved in 500 ml of de-ionized water. The storage life at 40°C is 2 months.

Ammonium sulphate stock solution: 0.472 g NH_4SO_4 was dissolved in ionized water and made up to 1 litre (1 ml ≡ 0.1 mg NH_4^+-N). A working standard (1 ml ≡ 0.001 mg NH_4^+-N) was made by diluting the stock solution 100 times, and this prepared freshly every day.

Method:

50 mg samples were well mixed with one gram of catalyst in micro-Kjeldahl flasks. These were heated with 4 ml concentrated sulphuric acid and 2 boiling chips on a bunsen rack. Flasks were rotated at intervals to aid rapid digestion, and heating was continued until solutions became colourless. Flasks were cooled for a few minutes and, before the suspension solidified, a little de-ionized water was added, and the flask contents further cooled until just warm. The solutions were transferred to volumetric flasks and, using several washes of de-ionized water to ascertain removal of the whole digest, made up to 25 cm^3. The solutions were mixed in a vortex mixer, capped with rubber stoppers, and kept at room temperature until further use. Sample digests were performed in duplicate. Glycine paratoluene sulphonic acid was used as a standard of known nitrogen content (5.665% nitrogen) and one standard was digested with each run of 12 samples. Blank reagent digests were also prepared, using one gram of catalyst and 4 ml of concentrated sulphuric acid.

The ammonia was assayed by adding 100µl of the diluted digest to 7.9 ml of solution "A", and this was followed immediately by adding 1 ml each of solutions "B" and "C". The product was well mixed on a vortex mixer, and colour developed in a 37°C water bath for 30 minutes. Absorbance was read at 630 nm. Standards were made from the ammonium sulphate standard solution and de-ionized water in the range 0 to 100µg of nitrogen, and a calibration curve constructed.

D Protein determination

Choice of procedures

A conventional method of protein estimation is to assess it from a generally accepted ratio of nitrogen per quantity of protein. It is generally assumed that a mixture of pure proteins will contain 16% nitrogen (Pomeranz and Meloan 1980). Thus the protein content of a sample is obtained by multiplying the determined nitrogen by the factor 6.25 (=100/16). This conversion is inaccurate if the use of the factor 6.25 is applied to all plant material, since the total nitrogen may not be wholly protein nitrogen (McCance and Widdowson 1978). Such is shown in Appendix 2.1 (p197). The availability of better methods for protein isolation and characterization (including amino acid composition) provides the basis for a continuous re-examination of the conversion factors (Tkachuk 1966). Hence the need in this study for a specific method of protein measurement. The use of the Folin phenol reagent in the standard Lowry procedure (Lowry *et al.* 1951) is rapid and sensitive, but is subject to interference by certain compounds. These include the ions of potassium and magnesium, EDTA, Tris, thiol reagents and carbohydrates (Bradford 1976). The biuret reaction, described by Mokrasch and McGilvery (1956) is less sensitive and also subject to interferences. Later authors in the 1970s used the technique of dye-binding. Bradford (1976) described a procedure involving the binding of Coomassie Brilliant Blue G-250 to protein. This was based on the observation that this dye exists in two different colour forms, red and blue. The red form is converted to blue form upon binding to protein, requires only two minutes for development, and is stable for up to one hour, with timing not critical within this range. The binding of the dye to protein causes a shift in the absorption maximum of the dye from 465 nm to 595 nm, and it is the increase in absorption which is monitored. This method eliminates most of the problems involved in the previous procedures. Work following shortly after this, by the authors Spector (1978) and Sedmak and Grossberg (1977), included variations in procedure. The colour reagent used by the latter authors can be stored indefinitely at room temperature. The standard Bradford colour reagent, stored at 20°C, is stable only for about two weeks, after which time there may be a decreased extinction coefficient when bound to protein.

In order to determine the comparative suitability and accuracy of these procedures (Bradford 1976, and Sedmak and Grossberg 1977) with the plant samples, test runs were performed using both colour reagents. There was no significant advantage in using

the longer lasting reagent, and the choice of procedure was that described by Bradford, using freshly made filtered reagent.

Protein determination by the Bradford procedure, utilizing the principle of protein-dye binding.

Equipment:

Set of 1.5 ml lidded Eppendorf capsules and plungers; 10 ml screw-cap propanol tubes; vortex mixer; Eppendorf centrifuge (10 000 rcf); spectrophotometer.

Reagents:

Coomassie Brilliant Blue G-250 (Sigma B 1131); ethanol 95%; phosphoric acid 85% (w/v).

Stock solutions:

0.15 M phosphate buffer, pH 7.0, was prepared by mixing 122 ml of 0.15 M sodium phosphate dibasic (anhydrous grade) Na_2HPO_4 (9.47 g dissolved and diluted to 1 litre), with 78 ml of 0.15 M potassium phosphate monobasic, KH_2PO_4, = potassium dihydrogen orthophosphate (9.08 g dissolved and diluted to 1 litre).

Bradford colour reagent was prepared by dissolving 100 mg Coomassie Brilliant Blue G-250 in 50 ml 95% ethanol and 100 ml 85% (w/v) phosphoric acid. The solution was diluted to a final volume of 1 litre and filtered through a Whatman No. 1 filter paper.

Stock solutions of 1 M NaOH and 3 M NaOH were prepared.

BSA stock solution. To 0.01 g soluble protein bovine serum albumin was added 10 ml de-ionized water, the solution mixed to dissolve and stored in the freezer.

Method:

Triplicate samples of 0.02 g of dry milled material were weighed into Eppendorf capsules and homogenized with 1 ml phosphate

buffer. Pellets were precipitated using an Eppendorf centrifuge for 5 minutes (9,800 xG) and the supernatants decanted and mixed. (Pellets were retained for further extraction). 100µl aliquot were withdrawn immediately after mixing, before formation of protein gradients, and placed in 10 ml tubes. 5 ml Bradford reagent was added and mixed by vortexing, and the absorbance read at 595 nm after 2 minutes and before 1 hour.

The pellets retained above were mixed with 1 ml 1 M NaOH for further extraction of protein, and the above procedure followed to absorbance reading.

For closer conformity, absorbances were read at between 15 and 25 minutes for all samples, using reagent blanks of buffer and reagent respectively for the two extractions. A calibration curve was constructed for each, using standard BSA solutions from 0 to 100µg of protein.

For samples containing mucilage, the protein bound in this way was solubilized by the addition of 50µl 3 M NaOH to the decanted supernatant from the buffer extraction (Fountain 1985). An additional calibration curve was constructed for these readings.

E Dietary fibre determination

Choice of procedures

The term "dietary fibre" was coined by Hipsley (1953) and is presently defined as "the sum of the polysaccharides and lignin which are not digested by the endogenous secretions of the human gastro-intestinal tract" (Trowel 1985), or simply as NSP, "non-starch polysaccharides".

Specific methods for dietary fibre determination are all relatively recent. Detergent methods (van Soest 1963) were based on the principle that detergent solutions solubilize fats, nitrogen-containing compounds, simple sugars and some starches.

Enzymatic procedures were first developed by Williams and Olmstead (1935), using pancreatin and acid hydrolysis. The great advantage of the enzymatic methods is that the assessment of the fibre component in nutrition can only be valid after undergoing a breakdown procedure mimicking that in the digestive system. Procedures have been modified and improved (Southgate 1976), leading to the Englyst (1982) method using the enzymes alpha-amylase, pancreatin and pullulanase. Alpha-amylase is an endoenzyme, attacking multiple starch grains in a random way in the digestive process. It occurs in most plants and has an optimal pH range from 6.0 to 7.0. An activator such as chloride is essential, and for stability both chloride and calcium ions are required (Dreher 1987). Amyloglucosidase can replace pullulanase as the other endoenzyme. These hydrolyse alpha linkages, and the combined use of either of these with alpha-amylase should completely digest starch (Dreher 1987).
Amyloglucosidase preparations have pH optima in the range of 4.5 - 5.5, and hydrolysis is carried out in acetate buffer (Southgate 1976).

An important modification to the original method was the use of di-methyl-sulphoxide (DMSO) for the dispersion of all starch, including resistant starch (RS). Enzymes will not attack intact starch granules and it is essential to gelatinize the starch in the sample prior to addition of the enzymes (Southgate 1976).

The addition of ethanol after the enzymatic digestion results in the precipitation of most polysaccharides and has the additional value of removing the added enzyme at the same time (Southgate 1976).

In the Englyst procedure, starch is gelatinized and then removed by enzymatic digestion as above, and the non-starch polysaccharides are hydrolysed by sulphuric acid, releasing neutral sugars and uronic acids which are measured by colorimetry. The technique is simple, rapid and reliable. This procedure has been used in this investigation, using modifications by John Monro (pers. comm.).

Determination of total, soluble and insoluble dietary fibre by the Englyst procedure

Equipment:

10 ml polypropylene screw-cap tubes; 20 ml glass test tubes; water baths (100°C; 35-50°C); vortex mixer; centrifuge (1560 rcf); spectrophotometer.

Reagents:

Ethanol 80%; di-methyl-sulphoxide (DMSO); alpha-amylase ("Termamyl"; Sigma A 5426); amyloglucosiderase (from *Aspergillus niger*; Sigma A 9913) 12 M H_2SO_4 (637 ml concentrated H_2SO_4/litre); pancreatin (Sigma P 7545).

Stock solutions:

Sodium acetate buffer 0.1 mol/l pH 5.2. 13.6 g sodium acetate tri-hydrate $CH_3.COONa.3H_2O$ was dissolved and made up to 1 litre with de-ionized water. The pH was adjusted to pH 5.2 with acetic acid, 0.1 mol/l. To stabilize and activate enzymes, 4 ml calcium chloride, 1 mol/l, was added to 1 litre of buffer.

Phosphate buffer, pH 7.0: (a) 0.2 M sodium phosphate, $Na_2HPO_4.2H_2O$ was mixed with, (b) $NaH_2PO_4.2H_2O$, 31.21 g/litre in the proportion (a) to (b) of 61 to 39 ml.

Di-nitrosalicylate solution, DNS: the following proportions were mixed, 10 g 3,5 DNS solution, 16 g NaOH, 300 g Na/K tartrate or in a final volume of 1 litre de-ionized water, and kept for two days before use.

Polysaccharide standard: cellulose/xylan/arabinogalactan/pectin, 1:1:1:1. The polysaccharide standard is a reference for both soluble and insoluble fibre and provides ranges of monosaccharides typically encountered in the cell wall. Cellulose and xylan are the insoluble, pectin and arabinogalactan the soluble, components.

Method:

100 mg portions of dry-milled samples were weighed into screw-cap tubes, in quadruplicate, duplicates for both insoluble and total fibre measurements. To each was added 5 ml 80% ethanol, and tubes were heated, with lids on, at 50°C for 1 hour. Samples were centrifuged, supernatants discarded (see below) and residues washed with 80% ethanol and again centrifuged. The decanted supernatants contained the soluble sugar component of the samples, 3 ml aliquots of each were reserved for sugar determination (F). For conformity throughout this procedure all centrifugation was performed at 2500 revolutions per minute for three minutes.

0.4 ml di-methyl-sulphoxide (DMSO) was added to the samples for dispersion of all starch, including resistant starch, and these heated for 30 minutes at 100°C. Stirring with a glass rod was necessary to ensure complete dispersion as the starch gelatinized.

1.6 ml alpha-amylase in acetate buffer (0.5 ml/50 ml) was added, and heated for 10 minutes at 100°C

100µl of amyloglucosidase (diluted 250µl in 2.5 ml de-ionized water) was added, and tubes heated for 30 minutes at 55°C, then cooled to room temperature.

In the next stage the samples for insoluble NSP and total determinations are treated separately.

For insoluble NSP determination

10 ml phosphate buffer pH 7.0 was added and tubes heated for 30 minutes at 100°C. Residues were washed firstly in 80% ethanol, then absolute alcohol and finally acetone, centrifuging

and decanting supernatants at each stage. Tubes with the final residua were laid flat in an oven at 70°C for 10 minutes for removal of residual acetone. 1 ml 12 M H_2SO_4 was added, tubes heated for 1 hour at 35°C, 5 ml water added then heated for a further hour at 100°C. Tubes were cooled and centrifuged, and 0.5 ml aliquot of supernatant removed to glass test tubes. To these were added 50µl glucose (1 mg/ml), 0.6 ml 4 M NaOH and 0.1 ml DNS. Samples were heated for 15 minutes at 100°C for colour development. They were cooled, 10 ml de-ionized water added, and absorbance read at 530 nm.

For total NSP determination

8 ml absolute ethanol was added to the enzymatic digest and extracted for 1 hour at 0°C. residues were centrifuged, washed with 80% ethanol and dried with acetone. The next stages of acid hydrolysis and the addition of ingredients for colour change were followed as above to absorbance reading. Polysaccharide standards were used in these measurements. For the standard, a 100 mg sample of the 4-component polysaccharide mixture was treated as above by acid hydrolysis and enzymatic digestion, and absorbance read at 530 nm.

F Soluble sugar

Choice of procedure

The isolation of soluble sugar from the dried sample is performed most successfully using an alcoholic extraction. Although these sugars are extremely soluble in aqueous solutions such extractions cannot be used satisfactorily due to the accompanying solubility of some lipids, pigments and free amino and organic acids. Free sugars are generally soluble in aqueous alcoholic solutions and, as proteins and virtually all polysaccharides are insoluble at alcoholic strengths above 75% (v/v), these reagents have formed the basis of most extraction procedures (Southgate 1976). The proteins are coagulated in this extraction and removed by centrifugation.

The choice of other extraction media include the use of a lower alcohol strength (50% [v/v] ethanol) which is increased to 95% (v/v) or absolute alcohol; this extraction brings large amounts of free sugars into solution (Southgate 1976). An alternative is extraction with hot 85% (v/v) methanol by refluxing the sample with the solvent, this having a high capacity as a lipid solvent (Southgate 1969). Isopropanol at room temperature has also been used successfully for extraction (Friedemann 1967). Measurement of the soluble sugars proceeds with an acid hydrolysis followed by a colorimetric method. In this investigation, to avoid duplication of procedure, the ethanol extraction from the previous analysis (E) was used for the soluble sugar determination.

Determination of soluble sugar by acid hydrolysis and colorimetric measurement.

Equipment:

Rotary incubator; centrifuge (1500 g); water baths, 100°C, 50°C; spectrophotometer; 10 ml polypropylene screw-cap tubes; 20 ml glass tubes; glass marbles.

Reagents:

Ethanol 80%; 2 M HCl; 4 M NaOH; di-nitrosalicylic acid (DNS)(for recipe, see dietary fibre determination, analysis E); glucose 1 mg/ml.

Method:

200 mg samples, in graduated tubes, were mixed with 10 ml ethanol (80%) and warmed for a few minutes in a water bath at

50°C. The tubes were shaken and inverted frequently to ensure adequate mixing, then transferred to a rotating rack in the incubator at 50°C for 30 minutes. Tubes were then cooled, centrifuged, and supernatants decanted. From each, 1 ml aliquots were taken, 0.5 ml 2 M HCl added, and these brought slowly to the boil; glass marbles were inserted onto the tops of the tubes and boiling continued for 30 minutes. After cooling, centrifugation and decanting of the supernatants, aliquot were taken for the colour procedure. To 0.5 ml aliquot were added 50µl glucose (1 mg/ml), 0.6 ml 4 M NaOH and 1 ml DNS. These were boiled for 15 minutes, cooled and diluted with 10 ml distilled water. Absorbance was read at 530 nm using a reagent blank (0.5 ml water and colour stage ingredients). Standards were made using 0.5 ml glucose (4 mg/ml) and colour stage ingredients.

G Starch

Choice of procedure

Most analytical procedures for the measurement of starch involve hydrolysis of the polysaccharide and the measurement of the products of hydrolysis, usually as glucose (Southgate 1976). From a nutritional aspect, the choice of method must closely simulate the extent of hydrolysis and utilisation in man; there is good evidence that enzymatic methods meet this criterion most closely (Bolton 1960). Enzymatic methods are considered by Southgate (1976) to be the most specific for measuring starch as 1,4 and 1,6 α-glucans.

Di-methyl-sulphoxide is used to bring starch into solution as the basis for the subsequent hydrolysis.

Free sugars must be extracted previously from the sample before proceeding with acid hydrolysis, or untrue readings will be obtained.

Samples containing protein and, to a lesser extent, fat, may form condensation products between amino acids and carbohydrates when subjected to acid hydrolysis. This is minimized if a high ratio of acid to sample is used. The strength of acid used for hydrolysis may be varied; 0.4 N H_2SO_4 will convert starch quantitatively to glucose after 4 hours under reflux (Southgate 1976). The use of a more concentrated acid for a shorter hydrolysis time is satisfactory (J Monro pers. comm.).

In this investigation, to avoid duplication of procedure, the supernatant formed by enzymatic digestion in Analysis E was used for starch measurement.

Determination of starch by enzymatic digestion, acid hydrolysis and colorimetric measurement.

Equipment:

as for dietary fibre determination (Proximate analysis E).

Reagents:

as for dietary fibre determination.

Method:

For stages of ethanol extraction, starch gelatinization using (DMSO) and enzymatic digestion, see method for dietary fibre determination, page ..

Following enzymatic digestion and centrifugation, a 1 ml aliquot of supernatant was used for acid hydrolysis using 0.5 ml 8 N HCl. These were hydrolysed for 30 minutes at 100°C. The cooled hydrolysates were diluted and 0.5 ml aliquot taken in a glass test tube for colour procedure; 50µl glucose (1 mg/ml), 0.6 ml 4 M NaOH and 0.1 ml DNS were added.

Tubes were heated for 15 minutes at 100°C. After cooling and dilution with 10 ml distilled water, absorbance was read at 530 nm, using reagent blanks.

Standards were prepared using the polysaccharide standard cellulose/xylol/arabinogalactan/pectin, 1:1:1:1, (for detail see dietary fibre determination, analysis E).

Results of all proximals in the foregoing analytical procedures are presented individually for each plant species in Part 2. All results are expressed as g/100 g dry weight.

II Elemental analyses

Elemental analyses of the plant organ samples were performed by Plasma Emission Spectrometry (PES), an automated elemental analysis system for precise quantitative analysis and monitoring of trace, minor and major constituents in liquids (DSIR 1991).

The basic principle underlying spectroscopic element analysis was demonstrated by Kirchoff and Bunsen in 1859. Salts of various alkali metals were heated in a Bunsen flame which vaporized the samples. Each metal was observed to give a characteristic pattern of colours, which was a unique atomic spectrum, and the colours appearing at different positions in the spectrum corresponded to different wavelengths.

Each band of colour in the spectrum extends over an extremely narrow wavelength range, and this is known as the spectral "line"; the presence of these "lines" at specific wavelengths indicates the presence of a given element. This relationship is the basis of atomic emission techniques. As the intensity of the emission varies with concentration of the element, the technique provides a means of quantitative analysis. Inductively-coupled argon plasmas (ICP) are employed to generate high temperature atomic vapours, up to 10 000°C. Plasma formation occurs in the plasma torch, consisting of a quartz tube surrounded by an inductive coil. Argon flows upwards through the coil and when radio frequency energy (r.f.) is applied to the coil an intense magnetic field develops. A spark from a tesla coil is applied to provide seed electrons, the argon becomes conductive, and argon ions and electrons flow in a circular path. Charged ions and electrons are accelerated in the oscillating magnetic field and collisional heating occurs. The plasma forms almost instantaneously.

The radiation emitted by the plasma source is analyzed by the spectrometer. Radiation is dispersed by a grating and individual lines are isolated. Their intensities are measured by photomultiplier tubes, allowing measurement of many analytical lines at the same time.

The spectrometer used was an Applied Research Laboratories 34 000 simultaneous emission spectrometer. Plant organ samples were freeze-dried and finely ground, and presented for analysis in solution of 2 M HCl. The polychromator was configured for 23 elements. Values given (in Parts 2 and 3 and Appendix 3.1) are the calculated means of duplicate analyses.

III Histological methods and Microtechnique

Plant specimens were cut into suitable pieces and these were killed and fixed in formalin-acetic-alcohol (FAA) immediately, or within the shortest possible time, after collection. In some cases, collection sites were distant from the laboratory, and individual jars of FAA were used on site for immediate preservation of the plant organs. The recipe for the preservation fluid, FAA, was based on that given by Johansen (1940) and contained 900 cc ethyl alcohol (50%), 50 cc glacial acetic acid, and 50 cc formalin.

The following procedures were those carried out in sequence in the preparation of stained, mounted sections.

1. Dehydration

The fixed specimens, in small glass phials, were transferred through a rising series of alcohol concentrations using the tertiary butyl alcohol (TBA) method of Johansen (1940). Dehydration was gradual with small increments of alcohol strength, from 50% TBA to 100% (75/25) TBA, thus avoiding tissue damage. Pure TBA was renewed repeatedly over several hours to ensure absolute dehydration, this stage lasting for 42 hours.

2 Infiltration.

In this stage TBA was gradually replaced by pure paraffin wax. The paraffin oven was used throughout, set at 60°C; this was warm enough to maintain the paraffin wax in liquid form but not hot enough to damage plant structures. After the initial TBA/paraffin oil mixture (50:50), samples were repeatedly covered with fresh liquid paraffin; at 2 to 4 hourly intervals the molten wax was decanted and renewed. The whole sequence of infiltration took 30 hours after which there was no trace of TBA odour in the phials.

3. Embedding.

Each phial was taken from the oven and quickly transferred to a bath of molten wax. A "foil boat" was made and hot wax dropped into its base; this was allowed to solidify. The sample was transferred immediately from the bath to the "boat", covered with liquid wax and this was made to solidify quickly. Prepared "boats" at this stage were stored until required.

4.Microtoming.

A wax block was cut to size around the specimen; a metal "stamp" was prepared

and the wax block positioned onto it. Great care was taken to examine the specimen for alignment of surfaces for the chosen cutting plane. The stamp stem was connected to the microtome, the gauge set at 10μm, and ribbons of sections cut. Strips of these were transferred to hot water bath then onto glued (PVA) glass slides, and these left to dry.

5. Paraffin removal (pre-staining schedule).

   The slides were taken through a series of baths, each for about 5 minutes, containing in sequence xylol, absolute alcohol/xylol (1:1) and down to the strength of alcohol close to that used in the staining, in this case 70% alcohol. This re-hydration was carefully timed, with 6 changes in the series.

6. Staining.

   The first stain to be used was safranin, 0.5-2% in 60% alcohol. Sections were left in this overnight to overstain. This was followed by a rapid change through 95% and absolute alcohol, to the second stain, fast green in clove oil and absolute alcohol. To prevent loss of safranin differentiation by overstaining, slides were held in fast green stain for only a few seconds. Washings were done with clove oil and changes of xylol.

7. Mounting.

   Stained sections were mounted in D.P.X. Mountant and covered with glass cover slips.

8. Photography.

   Sections were examined using a Zeiss research microscope equipped with a Zeiss MC63 photomicrographic camera, and were photographed at x100 magnification.

The above routine was followed for all specimens with the exception of *Typha orientalis* pollen and *Porphyra* sp. thallus. Specific treatments were employed for these specimens, as below.

Typha orientalis pollen. In this case, a preservative and killing fluid was not required as mature dry pollen grains can be stored successfully without structural disintegration or deterioration (under anaerobic conditions). For microscopic examination, a stain was applied to highlight the gross characteristics of the grains. This stain was made up of glycerol 16.0% w/v, ethyl alcohol 33.0% w/v, and basic Fuchsin 0.02% w/v.

For the examination of finer detail, electron photomicrographs were prepared at magnifications of from 740x to 3200x.

Porphyra sp. thallus. In this case, a preservative and killing fluid was not appropriate for the delicate consistency of the algal thallus. Thoroughly air-dried specimens can be stored successfully without deterioration, and are readily rehydrated with the addition of cold water. For microscopic examination, the moist thallus was displayed flat on the slide, without sectioning, and covered with a glass slip.

Part 2
Plant species

For each of the 18 plants described in this section, data has been presented under the following headings:

(i) description and distribution

(ii) history

(iii) collection and organ preparation

(iv) results and discussion.

1 *Pteridium esculentum* (Forst. f.) Cockayne

family: Dennstaedtiaceae

bracken

aruhe

(i) Description.

There is immense variation in the size of bracken fern, from about 20 cm high in subalpine areas, to 4 metres or more in warm lowland areas and on forest margins. The fronds are broadly ovate and very stiff. The frond growth pattern is characteristic: the midrib rotates as it uncoils, resulting in the lower pinnae pairs projecting in different directions. The colour varies from green to brownish green, and the stems are brown. Fronds are 3-4 pinnate. The pinnae are rigid and curved, with the final segments linear, and their veins are forked and free. The sori form a band along the margins; they are covered by a delicate indusium which is obscured when the sporangia mature. There are characteristic lamina hairs and by these, as well as the elongated marginal sori, this species is distinguished from other close members in the Dennstaedtiaceae.

The underground organs consist of long creeping rhizomes, 1-2 cm in diameter. These may be fleshy or hard, depending on depth, age and season. Externally there is an uneven black covering and fine, dark hair-like roots.

The distribution of *Pteridium esculentum* is restricted to Australasia and the Pacific Islands. In New Zealand it is plentiful in all islands, but becoming more so towards the north where there are large tracts of land covered with fern. It attains its greatest height and most luxuriant growth in warm wind-sheltered areas, in full sun, forming miniature forests to the exclusion of other vegetation. It seems unable to thrive if overtopped by shrubs and trees.Spread is mainly by rhizomal growth, and the fern readily invades land after forest or scrub clearance.

The placing of the genus *Pteridium* in the family Dennstaedtiaceae is in accordance with Brownsey and Smith-Dodsworth (1989); Allan (1961) places *Pteridium* in Pteridaceae.

References:

Allan 1961; Brownsey and Smith-Dodsworth 1989; Dobbie 1930; Cheeseman 1925; Hooker 1864; Laing and Blackwell 1964.

(ii) History

Pteridium esculentum was found on Cook's first voyage to New Zealand (1769), and named *Pteris esculenta* by its botanical discoverer, Forster (Colenso 1880). Banks recorded it in his journal (Ed. Beaglehole 1962, Vol. 1:416) as "...a kind of Fern *Pteris crenulata* very like that which grows upon our commons in England, and is called indifferently Fern, Bracken, or Brakes". Beaglehole's footnote to this reads "The common New Zealand bracken fern, *Pteris aquilinum* var. *esculentum* (Forst.) Kuhn".

Taylor (1855) refers to the edible fern as *Pteris esculentis*, and Hooker (1864) classifies it as *Pteris aquilina*, Linn., var. *esculentum*. The fern description in Hooker's Flora concludes that the var. *esculentum* is distinguished chiefly by the decurrent linear pinnules, often hairy below. The distribution of *P. aquilinum* var. *esculenta* is restricted to the south temperate zone, where it is common, whereas the ordinary state (*sic*) of the plant is found all over the world (Hooker 1867).

It is apparent from the preceding notes that there was some degree of uncertainty as to the classification of *Pteridium esculentum*. Colenso commented more than a century after its first recording in New Zealand that there were still many botanists who considered the edible New Zealand species to be identical to the common British one, or that both plants were but variations of one species. He wrote (1880) that 25 years earlier, experiments had been carried out in England on roots (*sic*) of *P. aquilina* but these experiments had signally failed. The specimens had been dug up and used fresh, and there had been no experimental comparisons made between rhizomes of the two species collected and prepared in the same way; no further details of these experiments have become available. The English cryptogamist, Berkeley, recorded that the roasted rhizomes of *P. aquilina* had the"slimy consistency, taste and smell of ill-ripened brinjals (*Solanum melangena*), and the nauseous mucilage made this objectionable as an item of food" (in Colenso 1880:25). Colenso wrote that he did not believe the two species were the same, or varieties, as they differed in several important particulars (*sic*), particularly in the root itself.

Unlike the rhizomes of *P. aquilina* in England, those of *P. esculentum* in New Zealand were a valued source of food, and Banks (Beaglehole [Ed] 1962 Vol.II:19) wrote that it was "...to the Maori what bread is to us". This was considered by Colenso and other writers to have been the most important New Zealand native food-plant to the pre-European Maori.

Traditional methods of collection, preparation, cooking and storage have been well recorded. Called *aruhe* by the Maori, "fern-root" had long ago been observed to differ according to locality. The best were *motuhanga* (meaning brittle), and *maahunga* (meaning mealy), both found in the north; and *kaitaa* (meaning gentleman's food) and *renga* (meaning mealy), found on the East Cape. The first of these, *motuhanga*, has been described by Colenso as a really splendid fine-looking black-skinned smooth root, 8-10 lines in diameter, with scarcely any woody fibres, and these were small like a very fine rush, lustrous, hollow and white (Colenso 1880). The rhizome would snap readily, "like good biscuit". This was the test for a good edible rhizome: it should break easily revealing white starchy tissue with very few, or no, fibres. Poor root was hard to break and very fibrous, or was wet internally, and considered useless as food. Hildreth (1962) suggested that the starch content was determined by the richness of the land, and Makereti (1938) wrote that the proportion of starch to fibre increased when the fern grew near the bush edge and the soil was rich. Then, she added, there was very little fibre.

The rhizomes were harvested in the spring and early summer months according to Colenso (1880), but there are slight variations recorded by other writers. Thomson (1859) wrote "August to November inclusive"; Johnson (1847), recorded "August and September"; Bauke (1928) that it was "dug in the summer and dried in its heat"; Fuller (1978) recorded that "roots were dug up in early summer when the fresh growth had attained full height"; and Crowe (1990), "from late winter to early summer".

Good rhizome is not found in shallow soil. Taylor (1855) wrote that the upper roots are stringy, hard, and harsh to the palate. It is collected from a depth of 50 - 65 cm under ground where the rhizome diameter is greater and may reach 2.5 cm. The soil in which good rhizomes are produced is loose and rich, as is volcanic soil, and where there has

been no disturbance for many years. Areas of fern growth were traditionally burned in August, every 3 years. Various ideas have been recorded to explain this practice. Colenso (1880) commented simply that the burning "improves them"; Crowe (1990) that "it improves the flavour"; Fuller (1978) that it "makes them white". It has also been suggested that burning is to prevent the establishment of manuka and other small trees. Traditionally the burning was fired using only wood of the two species *Rhipogonum scandens* and *Melicytus ramiflorus*.

The harvested pieces of rhizome, about one foot long (Angas 1847), were cleaned and wind-dried (not sun-dried) for two weeks until completely dry (Colenso 1880); these could be stored for many years. To prepare as food, the dried lengths were soaked slightly, roasted in the fire embers, then beaten on a large smooth stone using a stone or wood pestle. Banks (Beaglehole [Ed] 1962) wrote that when the bark had come off, the inside consisted of a small proportion of a glutinous pulp mixed with many fibres. The inside part then became as "soft as a bit of tough dough" (Colenso 1880:21); it soon, however, became "stiff and hard, when it snapped like glass or good biscuit". For making into cakes, *komeke* or *kohere*, any fibres were pulled out of the warm beaten rhizome, it was shaped into round sticks and these were roasted. An alternative preparation was to leave the prepared rhizomes underwater in baskets for 2 to 3 years, as with maize.

The taste of "fern-root" has been variously described; by Thomson (1859), like ship's biscuits; by Stack (Reed [Ed] 1935), like the crust of newly baked bread; and by Taylor (1855:379) as by "no means unpalatable, neither is it an astringent, as is generally supposed, but rather the contrary". On Cook's first voyage, both he, Anderson (Cook's surgeon) and Parkinson (Banks' draughtsman), wrote very favourable comments on it. Stack (Reed [Ed] 1935) wrote that it was very nourishing, and Taylor (1855) that it was especially prized by the sick, who often preferred it to other food. It was always taken by persons going on a voyage as the best antidote for sea sickness (Taylor 1855).

More recently, less favourable claims have been made regarding fern-root. Investigations into the toxicity of *Pteridium aquilinum* fronds to grazing stock led to the finding that these contain radiomimetric and carcinogenic toxins and enzymes (Connor 1951). These include caffeic acid (Somogyi 1973), shikimic acid (Evans and Osman 1974), pterosins and pterosides, and the enzyme thiaminase (Evans *et al.* 1975). Although the bulk of

research has been carried out on *P. aquilinum*, and on fronds rather than rhizomes, Hirono *et al.* (1973) found the carcinogenic activity to be even greater in rhizomes than in the curled fronds, although this differed geographically. These writers showed that this activity was reduced, but not eliminated, from the fronds by cooking them. After preparing the rhizomes, however, by traditional methods (in Japan) of pounding and washing, the starch was found to be free of carcinogenic activity. No parallels have yet been drawn between the rhizome pharmacology of *P. esculentum* and *P. aquilinum*, and the importance of the traditional cooking procedure. Although Evans and Galpin (1990) claim that all parts of bracken (*P. aquilinum*) are carcinogenic, including the spores, it has been pointed out (Brooker *et al.* 1987) that there is no record of stomach cancer in the pre-European Maori. At that time not only was the prepared rhizome eaten as a regular staple food, but the fresh young shoots were also eaten. None of the early 19th century writers recorded anything suggestive of ill-health resulting from the diet of fern-root, apart from an observation that it caused constipation (Best 1942), and the apparent wearing down by rhizomal fibres of dental enamel.

On fern-root, Banks (Beaglehole [Ed] 1962 Vol.II:21) made the pertinent comment that

> "strange and unheard of as it must appear to an European to draw nourishment from a class of Plant which in Europe no animal, har(d)ly even insects, will taste, I am much inclined to think that it affords a nourishing and wholesome diet; these people eat but little and this is the foundation of their meals".

(iii) Collection and organ preparation.

Specimens of *Pteridium esculentum* rhizomes were obtained from a site 9 k WNW of Palmerston North (GR092028, NZMS 260 Sheet S23). This was an area of waste land between a minor road and cultivated farm land, and the bracken formed a dense swathe approximately 5 m across and 20 m long, reaching to about 3 m from the road. This land had apparently been undisturbed for many years. The soil was a dark, well-drained, sandy loam.

Collection was made on 20.10.91. The young fronds had uncoiled and reached a height of 2.7 m, but sori had not matured. A wide trench was dug to a depth of 1 m at the base

of the larger specimens. Rhizomes were found throughout the soil transect, from just below the surface, and formed prolific branching underground systems. Specimens of these were collected from 60 - 65 cm depth. Rhizome size ranged from 4 to 7 cm circumference. Specimens were cleaned and photographed. Distal ends (about 5 cm) of rhizomes were observed to be much less fibrous than the woodier and harder proximal lengths. The former snapped easily and were white internally, while the latter were not easily broken and were darker internally. Consequent to this observation, only the distal 5 cm (approximately) of each rhizome was used for the analytical samples.

Samples for oven drying and freeze-drying were prepared by stripping off the black outer layers.

For the botanical investigation a transverse section of the rhizome was cut between 4 and 5 cm from the distal end. This was further reduced by cutting to an extended arc, to include central and superficial tissues, and preserved in FAA.

(iv) Results and discussion.

Oven-dried rhizome samples lost nearly all moisture during the first 24 hours, and further traces continued to be lost over the next six days. The dried samples were hard and had none of the sponginess suggestive of retained mucilage. Freeze-dried rhizome samples required a prolonged drying time, and constancy of weight was not reached until the sixth day. Results of these weighings showed moisture contents of 92.39 $\pm$ 0.23% (three replications) by oven drying, and 92.09% by freeze-drying.

The lipid component was relatively low, at 2.95 g/100 g.

Both nitrogen and protein components were also low, at 1.31 and 8.18 g/100 g respectively. In the protein analysis, the procedure was complicated by the presence of mucilage at the buffer extraction stage; centrifugation produced a highly mucilaginous and indistinct supernatant. The addition of 50 µl of 3 M sodium hydroxide solubilized most of the mucilage giving 3.75 g protein/100 g; the greater proportion was that solubilized from the residuum by extraction with 1 M sodium hydroxide.

The total dietary fibre component was measured at 36.68 g/100 g, of which 24.35 g/100 g was insoluble fibre and 12.33 g/100 g was soluble fibre.There was a high recorded content of soluble sugar, 31.38 g/100 g and a relatively low figure of 2.67 g/100 g for starch.

A summary of proximate analyses is shown in Table 1.1 (p43).

Several elements were found, by Plasma Emission Spectrometry (PES), to be present at unusually high levels in the *Pteridium* rhizome sample. These included both nutritional minerals and toxic elements. Of the former, silicon, iron and chromium levels were high, and very high figures were recorded for aluminium and strontium.

Data obtained by PES is shown in Appendix 3.1 (p198).

The section across the rhizome (see Plate 1a, p40) shows the distribution of bundles within the tissues.

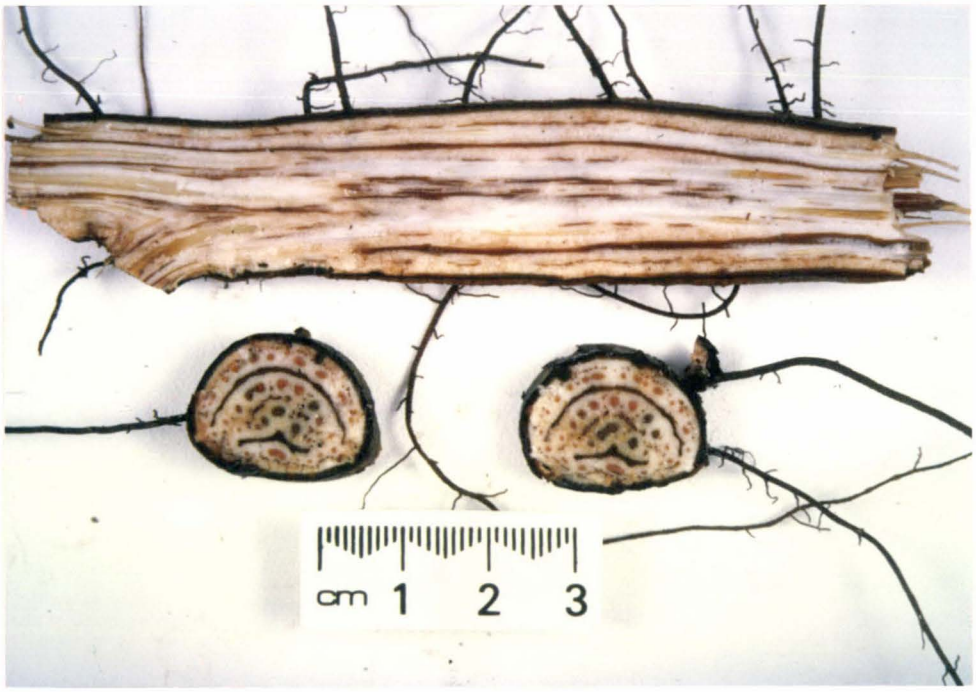
The microscopical analysis of the sections shows the outer cortical layers to be sclerenchymatous (see Plate 1b, p40). The several layers of superficial cells appear to be relatively much smaller, although tissue examination of *P. aquilina* rhizomes has shown the outer sclerenchymatous cells to be longitudinally elongated. A hand-cut section of *P. esculentum* was prepared for examination of the superficial cells, and these appeared as distinct fibres with scalariform thickening.

Beneath the sclerenchymatous tissue the parenchymal cortical cells contain abundant grains suggestive of starch grains. These appear as very pale pink elliptical grains, having retained some of the safranin stain.

To confirm the identity of these grains, a fresh hand-cut section was prepared, using material from the same collection sample. The iodine test was positive for starch in some grains only.The majority of grains, very abundant in this fresh section, remained uncoloured. A hand-cut section of the preserved specimen was also used to test with iodine, and this also gave a negative reaction in all but a few grains. It is suggested here that these unstained bodies are in fact amyloplasts, these perhaps remaining colourless

Plate 1a. *Pteridium esculentum*: rhizomes, cut longitudinally and transversely, showing arrangement of vascular bundles.

Plate 1b. *Pteridium esculentum*: rhizome, transverse section, stained with safranin and "fast green"; **f**, fibres; **s**, sclerenchymatous cells of outer cortex; **p**, parenchymatous cells of cortex; **t**, tannin-like substances.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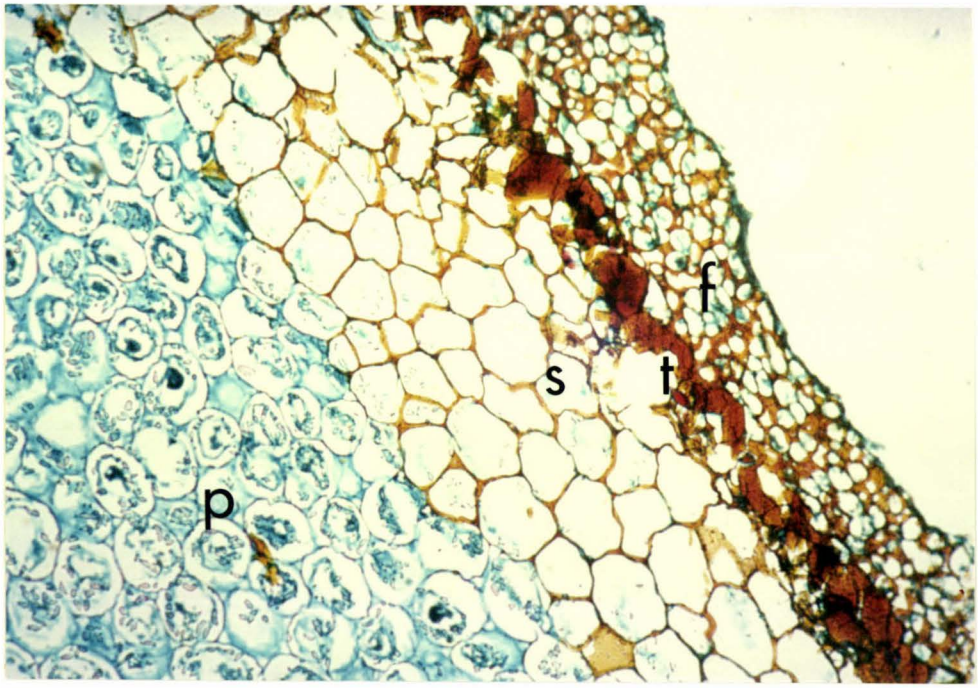
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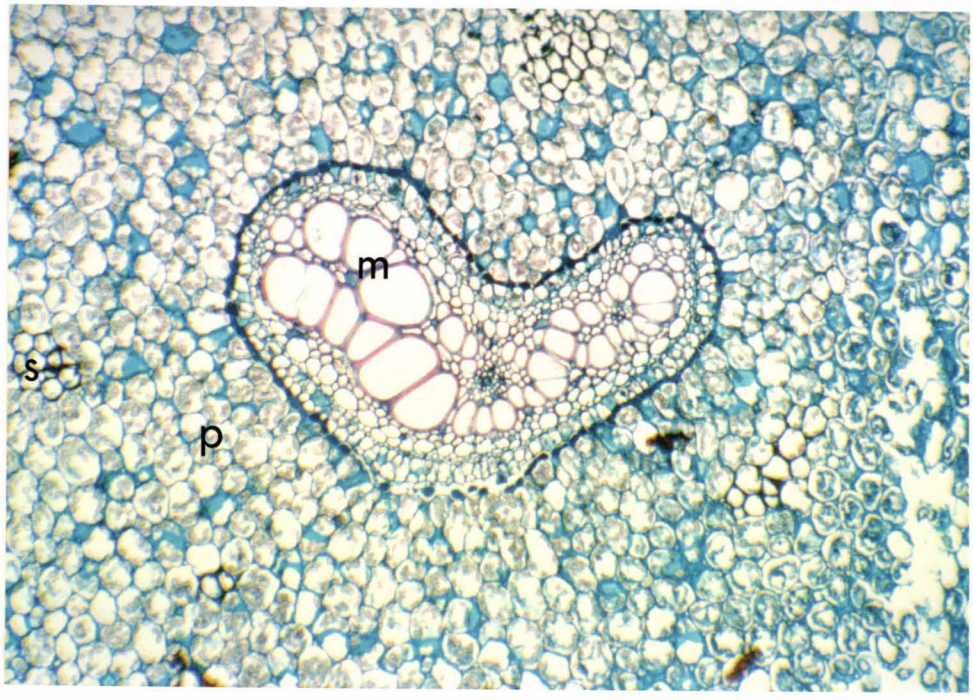
Plate 1c. *Pteridium esculentum*: rhizome, transverse section, stained with safranin and "fast green"; **m**, meristele; **p**, parenchymatous cells of cortex; **s**, sclerenchymatous cells of cortex.

Plate 1d. *Pteridium esculentum*: rhizome, transverse section, stained with safranin and "fast green"; **e**, endodermis; **mx**, metaxylem; **px**, protoxylem; **v**, vascular bundle.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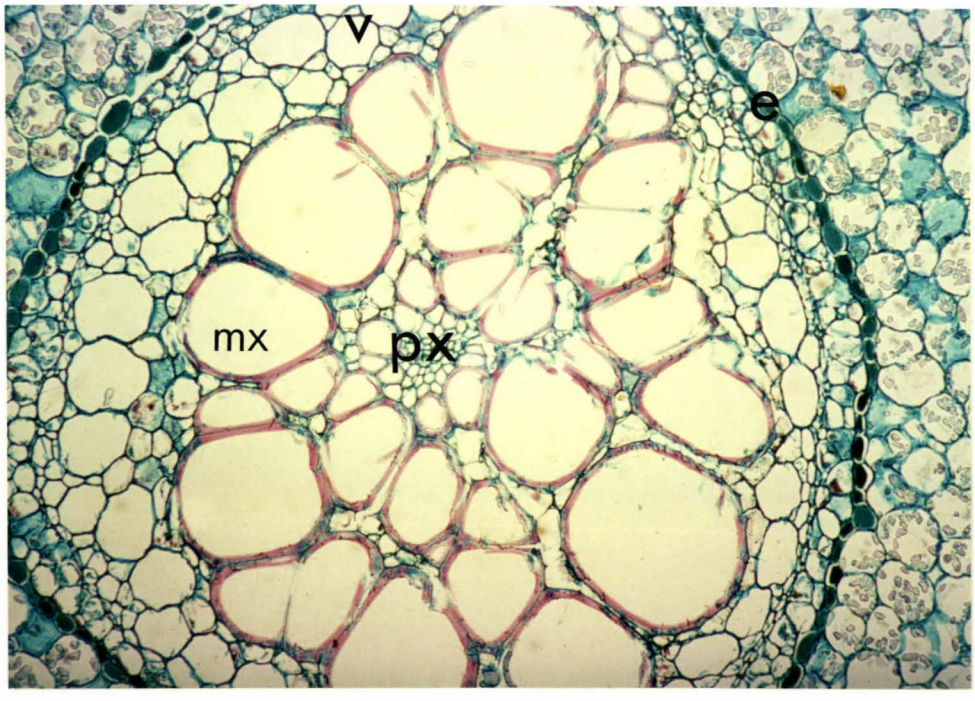
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when not active in the conversion of soluble carbohydrate into granules of storage starch. This finding is in keeping with the analytical estimation which showed a low starch figure of 2.67 g/100 g. Amyloplasts are characteristically small and abundant in meristematic plant cells and in underground organs (Sharp 1943).

The vascular bundles appear as either cylindrical or angled fibres. Examination of the cylindrical bundle (see Plate 1d, p41) shows a well-defined endodermal layer around the pericycle. The vessels are numerous within the bundle. The vessel walls of the young metaxylem are evenly, and fairly heavily, lignified, and these completely enclose the young protoxylem vessels. On the basis of anatomical similarity, the phloem is seen as an abundant tissue external to the column of xylem vessels and completely surrounding it peripherally.

The angle-shaped vessels show a similar picture histologically, but the vessels are arranged with the metaxylem in two rows along the arms of the bundle and protoxylem at the angle (see Plate 1c, p41). Protoxylem also appears occasionally between the two rows of vessels in the metaxylem.

Thickened bands of sclerenchymatous cells appear occasionally in the ground tissue; these are specific and well-defined, with cell walls fairly heavily thickened and stained with safranin.

Table 1.1 *Pteridium esculentum*: rhizome
Proximate analyses per 100 g dry weight.

A.Moisture:	
oven-dried	92.39
freeze-dried	92.09
B.Lipid	2.95
C.Nitrogen	1.31
D.Protein	8.18
E.Dietary fibre:	
insoluble	24.35
soluble	12.33
F.Soluble sugars	31.38
G. Starch	2.67

(For details of replications and analytical procedures see Part 1, Procedures and analytical methods).

2 *Corynocarpus laevigatus* J.R. *et* J.G. Forst.

family: Corynocarpaceae

karaka

(i) Description and distribution

Corynocarpus laevigatus is a large canopy tree which may reach a height of 15 to 20 metres. The bark is smooth and grey, and the branches are stout. The leaves are dark green, shiny and smooth-edged, from 7 to 18 cm long, with recurved margins. The flowers are 4 to 5 mm in diameter and are borne in panicles; the petals are a greenish yellow. The fruit is a pendulous drupe, ovoid and about 4 cm long; the immature pericarp is green, changing to orange when ripe (see Plate 2a, p50). The endocarp is fibrous, enclosing the kernel which has an adherent membranous testa. The kernel is 2 to 2½ cm long. The ripe fruit has a distinctive bitter-almond smell.

The natural distribution of the tree is in coastal and lowland forest areas of the North Island and the northern parts of the South Island.

References:
Allan 1961; Salmon 1980; Cheeseman 1925; Kirk 1889; Hooker 1867.

(ii) History

The kernel of the fruit was of inestimable value to the Maori as an article of vegetable food. Colenso (1880) wrote that he would have placed it before the fern root only it was not so common and confined to the vicinity of the sea. Best (1942) wrote that the tree was often planted close to settlements, accounting for the *karaka* now often being found isolated in inland spots away from its own natural habitat near the sea. Clarkson (1984) noted that even after 150 years, trees apparently cultivated by the Maori remain prominent around abandoned *pa* sites in Taranaki. Although Hooker (1867) had recorded *karaka* as being abundant near the sea, this has since been greatly limited by deforestation.

The *karaka* fruit was described as having a "coating of soft yellow pulp, sweet and satisfying, whose kernel baked and steeped in water to extract its bitter alkaloid poison,

was eagerly gathered and stored" (Bauke 1928:16). Of the kernel in its raw state, only "a small quantity suffices to throw one into convulsions and great and permanent distortions of the limbs, and to kill; but prepared and cooked it is perfectly innocent and wholesome" (Colenso 1880:25). A full description was quoted by Skey (1871) on the symptomatology of *karaka* poisoning. This same writer, as Colonial Analyst, had found that the poisonous principle resided in an essential oil which was intensely bitter and which, under treatment crystallized out into beautifully radiating acicular forms (Skey 1871).

Easterfield and Aston (1901) later isolated karakin from the fruits of *C. laevigatus*, and the chemistry of the glycoside has since been investigated by other writers. Carter (1951) established that its formula was $C_{15}H_{21}O_{15}N_3$, that is a 1, 4, 6-tris-(ß-nitropropionyl)-D-glucopyranose, and it could be hydrolysed to 3 molecules of ß-nitropropionic acid. This aglycone of karakin is known as hiptagenic acid and has a formula $O_2.N.CH_2.CH_2.COOH$. Easterfield and Aston (1901) observed that the quantity of karakin in fresh *karaka* kernels (0.3%) diminished (to 0.1% and less) over three months. It is now known that karakin is hydrolysed in boiling water to ß-nitropropionic acid, and that the acid decomposes at 100°C (Connor 1977).

A possible explanation for the symptoms of *karaka* poisoning would be competitive antagonism between hiptagenic acid and succinic acid ($HOOC.CH_2.CH_2.COOH$) which has been demonstrated in vitro (Hylin and Matsumoto 1964). It would therefore seem suitable to treat *karaka* poisoning by giving sodium succinate (Fastier and Laws 1975).

The ripe fruits were traditionally collected from the trees in the autumn, and placed in large heated ovens in the sea-beach above high water mark. They were baked and steamed for a "considerable time" (Colenso 1880). This cooking was followed by a thorough washing; the baked kernels were put in loosely woven baskets, laid in running water, and shaken frequently to detach the outer skin and pulp. This left the large kernel intact, with its own shell of fibrous endocarp.

Several writers, both early and contemporary, have given a variety of timings for the steaming and washing stages, as shown below. It is now considered safe to simmer the kernels for several hours and rinse them well (Arohia Durie pers.comm.).

Table 2.1 Recorded lengths of time for cooking and washing *karaka* kernels:

	Cooking	**Washing**
Thomson (1859)	10 days	several weeks in running stream
Skey (1871)	several hours	1 or 2 days
Colenso (1880)	several hours to one day or more	some time, a day or two at least
Kirk (1889)	18 hours	rinsed
Makereti (1938)	24 hours	-
Best (1942)	3 days and nights	some months
Crowe (1990)	4 hours	4 changes of water

Traditionally, after steeping, the kernels were cleaned, dried and stored away. They were very hard and thus could be kept for years (Colenso 1880). They would be steamed again to soften them for eating, and so prepared they were known as *kopia* (Best 1942). The endocarp was cut round the middle and each half was pressed between finger and thumb to get the soft substance out (Makereti 1938).

This food received poor praise from Nicholas (1817) who described it as of unctuous consistency and extremely ill-flavoured, an opinion seconded by Best (1942). Wade (1842) described the taste as nauseous; and Wakefield (1845) found the odour of the prepared kernels so offensive that he could never prevail on himself to eat them.

(iii) Collection and organ preparation.

Whole ripe fruits of *Corynocarpus laevigatus* were collected from coastal bush near Kerikeri in the Bay of Islands (GR967646, NZMS 260, Sheet P05), on 2.3.91. Both pericarp and endocarp were removed, using a scalpel, exposing the testa-covered kernel. Sub-samples were taken for oven-drying and freeze-drying.

A further weighed sample of 20 kernels was taken for cooking. These were simmered in water for a day (12 hours); they were removed from the pan, rinsed in several changes of fresh water, and cooled. Sub-samples of these were oven-dried and freeze-dried as before.

For the microscopy specimen, the testa was broken, the kernel halves separated, and these cut transversely in the centre, for preservation in FAA.

(iv) Results and discussion

The moisture loss during oven-drying was rapid, and weights were stable within the minimum monitoring time of 24 hours. Testas became detached from the kernels and flaked off.

A problem arose in freeze-drying the kernels as they proved to be extremely hydroscopic. It was necessary to further reduce the temperature of the samples by submerging them in a freezing bath for two hours prior to continuation of freeze-drying. The kernels then lost their pliability and became crisp and dry. Moisture loss was still less, however, in the freeze-dried samples; this was 44.01% compared with 48.19 ± 2.6% (three replications), in the oven-dried samples.

A study of *karaka* kernels by McCurdy (1947) showed a mean moisture loss of 45.8%. This figure was the mean of twelve estimates which ranged from 41.8% to 48.0% water loss; the method used in that study was direct distillation.

The moisture retention of the cooked kernels was to a large extent persistent. The weight of the cooled, cooked sample showed an increase of 11.45% above the fresh weight. This was slowly reduced by freeze-drying over a period of seven days, to give a moisture content of 4.76%. The value recorded by McCurdy (1947) for moisture content of the

treated sample was 11.85% by direct distillation.

The lipid content of the untreated kernels was very high, at 19.23 g/100 g. McCurdy (1947) recorded a comparable figure of 17.48%. The lipid content of the cooked sample was also high at 14.51 g/100 g. It had been noted during the cooking of the kernels that there was a strong bitter-almond smell, and that there were oily droplets on the water at the end of the cooking time. The latter could explain the difference in lipid content between the untreated and cooked samples.

McCurdy (1947) recorded a very slight decrease in lipid content on cooking (from 17.84 to 17.69 g/100 g). The procedure used in that case was the saponification method mentioned earlier.

The nitrogen determination showed high percentages for both samples, 2.62 g/100 g in the untreated kernels and 2.79 g/100 g in the cooked kernels. Results of McCurdy (1947) were 3.73 and 1.99 g/100 g for these analyses.

The protein values from that author are less useful for comparison, being calculated from the percentage nitrogen so the difference is exaggerated even further. In this analysis, protein contents were 15.91 and 15.94 g/100 g respectively for the untreated and cooked samples. In the procedure for protein determination, the supernatants of both buffer and hydroxide extractions rapidly developed protein gradients. These were characteristic in having, when allowed to form, an upper clear portion over an equally large indistinct opaque layer; the poorly defined residual pellet was white, and on the surface of the supernatant was a distinct layer of lipid. The immediate withdrawal, after mixing, of the test aliquot was crucial in this procedure to gain good replication with the three test samples.

The relative nitrogen and protein components in both samples suggests the presence of non-protein nitrogen, with calculated protein to nitrogen ratios of 6.07 and 5.71 respectively.

The dietary fibre percentages were 35.35 and 39.11 g/100 g respectively, and were made up of a relatively high proportion of soluble fibre. Insoluble and soluble fibre

percentages were both higher in the treated sample, but this difference was more marked in the insoluble component. The difference in the soluble sugar and starch content, between the untreated and treated samples, was most marked. The relatively high sugar level of the untreated sample (13.82 g/100 g) was lost in the cooking, leaving a low figure of only 1.13 g/100 g. No parallel can be drawn between this and other treated and untreated samples in this study. The only other kernel food, that of *Beilschmiedia tawa*, shows a totally dissimilar relationship. The hydrolysis and decomposition of the glycoside in cooking could explain this reduction. The almond smell during cooking could be explained by the decomposition of the amygdalin-related glycoside.

The starch component, conversely, was relatively higher (per gram dry weight) after cooking. The values were high,in terms of this study,for both untreated and cooked samples, 26.19 and 36.93 g/100 g respectively. The latter measurement was surpassed by only one other organ sample (see Appendix 1.1, p191).

The results of all proximate analyses for *Corynocarpus laevigatus* kernels are shown in Table 2.2 (p52).

Elemental analysis of the untreated *Corynocarpus* kernel sample showed high levels of nickel and copper and a very low level of sodium. After cooking, many of the mineral levels were significantly altered, for which no explanation has been elucidated. Magnesium was lower by a factor of 3, sodium higher by a factor of 3, boron higher by a factor of 4, and nickel lower by a factor of 5, all factors given as approximate whole numbers. For complete results of elemental analyses by PES see Appendix 3.1 (p198).

The sectioned kernel shows the cells to be small and close, and to a large degree similar in size and shape. The outstanding feature of this section is the abundance of a cellular component which has markedly retained the safranin stain (see Plate 2b, p50). Close examination shows these to be droplets within the cell spaces. In order to identify this component, hand-cut sections were prepared, using kernels from the same collection sample. These were stained (separately) with Sudan Blue and Coomassie Blue to differentiate lipid and protein respectively.

Plate 2a. *Corynocarpus laevigatus:* whole fruit.

Plate 2b. *Corynocarpus laevigatus*: kernel, transverse section, stained with safranin and "fast green"; **c**, cotyledonous tissue; **e**, epidermis; **l**, lipid, stained red with safranin; **v**, vascular trace.


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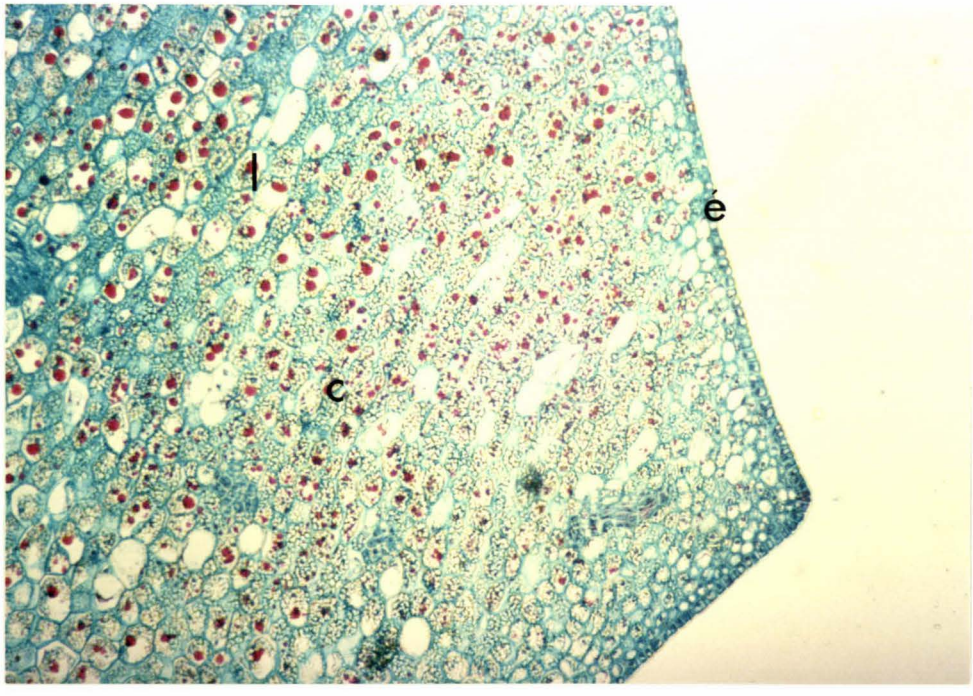

l
é
c

The Sudan Blue stain was readily absorbed without heating, by the large droplets, showing these to be composed of lipid.

The cells containing lipid droplets appear to be arranged in files, particularly in the more central portion of the kernel. These cells also appear to be flattened and elongated in the longitudinal plane of the kernel (see Plate 2b, p50).

Between the files of lipid-containing cells are rows of cells packed with inclusions suggestive of starch grains. The Coomassie Blue stains showed differential absorption into the cytoplasm of these cells, a further test was applied using iodine stain. This was differentially absorbed by the grains, showing them to be composed of starch.

The levels of these three depicted components, lipid, nitrogen and starch, had all been found by the analytical procedures to be relatively abundant, and in particular the lipid.

The epidermis is seen in the section as a single layer of small isodiametric cells, thickened on the inner anticlinal cell walls.

Occasional discrete vascular bundles appear throughout the parenchymatous tissue.

No further tests have been carried out to depict the storage sites within these tissues of the elements nickel and copper, both of which were shown by PES to be present at unusually high percentages.

Table 2.2 *Corynocarpus laevigatus*: kernels
Proximate analyses per 100 g dry weight.

	untreated	**cooked**
A.Moisture:		
oven-dried	48.19	-
freeze-dried	44.01	4.76
B.Lipid	19.23	14.51
C.Nitrogen	2.62	2.79
D.Protein	15.91	15.94
E.Dietary fibre:		
insoluble	8.26	11.09
soluble	27.09	28.02
F.Soluble sugars	13.82	1.13
G.Starch	26.19	36.93

(For details of replications and analytical procedures see Part 1, Procedures and analytical methods).

3. *Elaeocarpus dentatus* (J R et G Forst.) Vahl

family: Elaeocarpaceae

Hinau

(i) Description and distribution

Elaeocarpus dentatus is a medium sized forest canopy tree up to 15 to 20 metres high, with a trunk diameter of up to 1 metre. The bark is rough and grey. The leaves arise on stout petioles, about 25 mm long, at the tips of the branchlets. They are up to 12 cm long by about 2 to 3 cm wide, toothed and rolled back along the edges; the upper surfaces are bright green and the undersides white with silky down, and frequently pitted where the veins join the midrib. Juvenile leaves are longer and narrower. The flowers are white and droop from long racemes arising from the leaf axils, or directly from the branches; these appear in October or November. The fruit, produced from December to May, is a drupe, 15 - 18 m long, almost round and resembling a small olive (see Plate 3a, p57). The stone is relatively large and is deeply furrowed. Fruits grow in small loose racemes; immature fruit is a dull olive green colour, ripening to a reddish-purple to purple-brown colour, and firm even when ripe. The skin is hard, dry, brittle, and shining.

Elaeocarpus dentatus is distributed throughout the North and South Islands of New Zealand in lowland and montane forest, from sea level to 600 m, but not in coastal areas. It does not occur in Stewart Island.

Hinau fruit has also been given the common name New Zealand damson, being about the size of a sloe or small damson, but is otherwise dissimilar.

Salmon (1980) lists two endemic New Zealand species of *Elaeocarpus*, although there are nearly 200 other species of this genus distributed throughout Asia, Australasia and the Pacific.

References:

Allan 1961; Salmon 1980; Cheeseman 1925; Hooker 1867.

(ii) History

It was the flesh of the *Hinau* fruit which was eaten after traditional preparation. Colenso (1880:26) qualified the term "flesh" by adding "...if such it may be termed...is dryish, small in quantity, austere, and altogether uneatable in its fresh and raw state." It forms only a thin layer around the relatively large kernel and is enclosed superficially by the hard, brittle skin. Hooker (1867) wrote that the pulp is astringent but eatable, and Colenso (1880) likened the taste to that of acorn.

The fruits were collected when ripe from under trees, and placed in water for steeping, in the hull of a canoe or similar wooden trough. Taylor (1855) wrote that the steeping was prolonged for nearly a year in running water, to get rid of their bitter and astringent quality. They were well rubbed in the hands, the kernels, stalks and skins being strained out and discarded, leaving a coarse grey meal (Colenso 1880).

A variation to this method omitted the steeping in water, the fruits being merely pounded using a club and mortar, and then sifted. The coarse meal was allowed to settle in the water and made into a kind of huge cake or bread (*pua*) which was cooked for several hours (Makereti 1938). In colour it was a blackish grey, darker than barley or rye bread. The rough, unpalatable taste of the fruit in its raw state was wholly lost in the cooking (Colenso 1880).

Angas (1847:23) wrote of the "black, filthy mass of the fruit, compressed together and kept till quite rotten and musty"; and the "unwholesome look of the cakes, to which the children were so partial and ate with avidity", adding, "they tell me it is good for them" (Angas 1847:75). Crayon (1842) wrote of it as a substance like brown bread, with a slight acidity in its flavour, far from disagreeable, and the Maori were very fond of it. Colenso (1880) wrote that this food was greatly esteemed and always made a first-rate dish.

Some recent writers have wrongly stated that the *hinau* kernel was the part traditionally used. Moore and Irwin (1978) and Salmon (1980) both made this error; it is noted that in his bibliography Salmon does not cite Colenso or any of the other nineteenth century writers who have recorded the traditional preparation of *hinau*, but he does cite Moore and Irwin from whence this error was probably perpetrated.

(iii) Collection and sample preparation

Hinau berries were collected from Kahutarawa Road Reserve, 4 k south of Palmerston North (GR322810, NZMS 260 Sheet T24), on 25.4.91. One tree beside Kahutarawa Stream was laden with fruit and the ground strewn with freshly fallen fruit (following an overnight storm).

For the botanical samples, fresh, ripe whole berries were preserved in FAA.

Samples for proximate analyses were prepared by removing all flesh from the kernels using a scalpel.

A weighed sample for cooked pulp analysis was prepared by first soaking the whole ripe berries in distilled water. The container was agitated regularly. On two occasions during the first month, the water became filmy and was changed, but during the whole steeping period there was no sign of active fermentation or decomposition. the smell was quite distinct and not unpleasant, and the berries remained hard and intact throughout. Steeping was continued for four months. A sub-sample was taken, drained and weighed; this comprised 80 fruits. These were rubbed by hand and most required firm pressure to break them down; three berries totally resisted breakdown by manual pressure. Debris was strained out and rinsed using the steeping liquid; the mash was allowed to stand overnight and the water then drained off. The settled grey-green residuum was firm and this was baked in a covered dish for two hours. The cake was cooled overnight then frozen, freeze-dried, weighed and milled.

(iv) Results and discussion

The moisture content of samples was notably low for this type of organ, and similar figures were obtained by oven-drying and freeze-drying; these were 49.81 ± 1.7% (three replicates), and 50.38% respectively.

The lipid component was low at 3.04 g/100 g, and both nitrogen and protein levels were found to be very low; these were 0.69 and 4.32 g/100 g respectively. This latter was entirely supplied by the hydroxide extraction of protein from the residuum; none was solubilized in the buffer extraction.

The dietary fibre percentage was very low, made up of almost equally of insoluble and soluble fibre components, 15.88 and 16.53 g/100 g respectively.

Soluble sugar was also very low at 1.85 g/100 g. The starch level was found to be high (34.89 g/100 g), and was the only component measured here which was present in large proportions, both in relation to the other components measured here and in relation to other samples in the study.

The cooked sample showed a moisture percentage of 53.83% which was very similar to that of the untreated sample. The result of lipid extraction showed this to be twice as much after treatment (6.08 g/100 g). This has been interpreted to suggest that either the higher extraction from the cooked pericarp was composed of substances other than lipid (these may have been made available by cooking), or the lower extraction from the untreated sample was due to incomplete solvency in the di-ethyl ether.

The levels of nitrogen and protein are again very low and values are similar to those for the untreated samples (0.71 and 4.72 g/100 g respectively). Their relative proportions are likewise very similar, these results suggesting that the nitrogen is almost wholly protein nitrogen, with calculated ratios of protein to nitrogen being 6.26 to 1 and 6.65 to 1 respectively.

The dietary fibre results were lower for the cooked sample than the untreated samples; for the insoluble and soluble components these figures were 11.39 and 11.62 respectively.

The soluble sugar level had also decreased (per gram dry weight), to 0.089 g/100 g, but the starch component was greater (per gram dry weight), by 25%, after cooking, measuring 44.01 g/100 g, the highest starch level in this study.

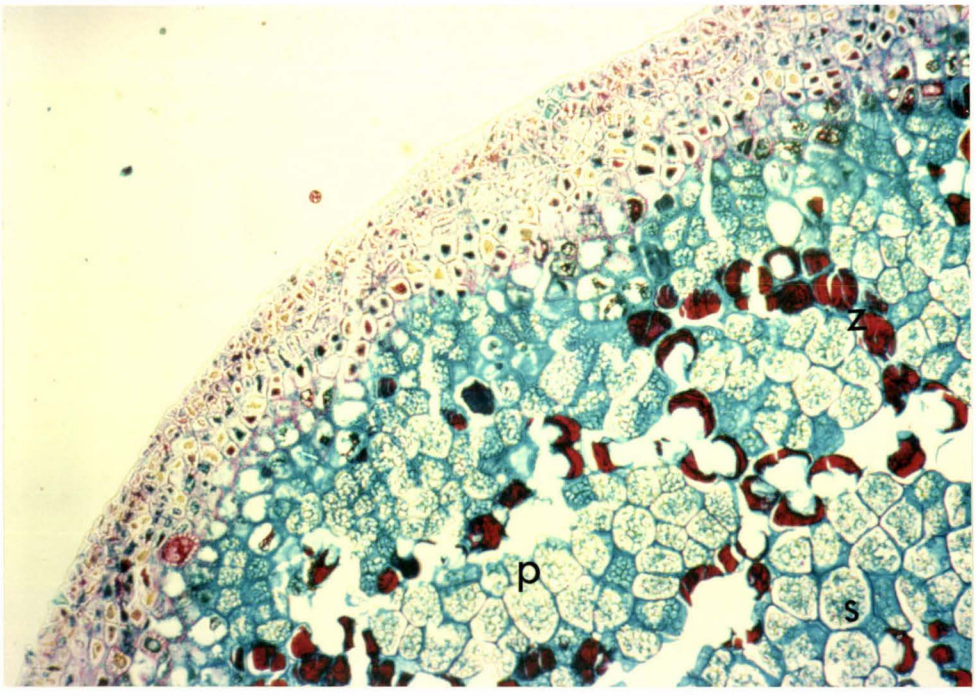
A summary of proximate analyses is shown in Table 3.1 (p59).

Elemental analysis of the untreated sample showed no unusually high levels. Some figures, however, were notably low, including those of magnesium, zinc,iron and sulphur. Aluminium was also low.

Plate 3a. *Elaeocarpus dentatus*: whole fruit.

Plate 3b. *Elaeocarpus dentatus*: pericarp, transverse section, stained with safranin and "fast green"; **p**, parenchymatous cells of cortex; **s**, starch grains; **z**, suberized cells.


cm 1 2 3


z
p
s

The effect on mineral levels of cooking gave substantial increases (per gram dry weight) in calcium, silicon, zinc, copper, iron and nickel. The increase (per gram dry weight) in copper put the percentage of that element at a relatively high level, 15.1 μg/g.

Conversely magnesium, sodium, potassium, manganese, phosphorous and boron levels were all substantially reduced (per gram dry weight) by cooking.

Data obtained by PES is shown in Appendix 3.1 (p198).

Microscopic examination of the pericarp section shows the tough outer skin to be composed of densely packed stone cells. These are typically small and irregular in shape; they have very thickened walls and prominent radial cracks extending through the walls. The brilliant interference colours, produced by double refraction, are also typical of stone cells (see Plate 3b, p57).

The cortex of the pericarp is seen to be composed of thin-walled parenchyma cells interspersed by wholly suberized cells. The latter, deeply stained by safranin, appear to be arranged in rings to circumscribe the former in groups of 20 to 40 cells. The thin-walled cells are densely packed with small polygonal grains suggestive of starch grains. To establish the identity of these grains a hand-cut section of pericarp was prepared, using fruit from the same collection sample, and iodine stain applied. This showed differential absorption by the grains, which were small, spherical, and densely packed in the cells confirming the presence of starch. The abundance of these grains reflects the very high level of starch found by proximate analysis. "Fast green" stain has been taken up and retained to some degree by non-suberized cortical cells.

Table 3.1 *Elaeocarpus dentatus*: pericarp
Proximate analyses per 100 g dry weight.

	untreated	cooked
A.Moisture:		
oven-dried	49.81	-
freeze-dried	50.38	53.83
B.Lipid	3.04	6.08
C.Nitrogen	0.69	0.71
D.Protein	4.32	4.72
E.Dietary fibre:		
insoluble	15.88	11.39
soluble	16.53	11.61
F.Soluble sugars	1.85	0.89
G.Starch	34.89	44.01

(For details of replications and analytical procedures see Part 1, Procedures and analytical methods).

4. *Sonchus asper* (L.) Hill.

family: Asteraceae

Prickly sowthistle

Raururoa puwha

(i) Description and distribution

Sonchus asper is an annual or biennial herbaceous plant, very variable in height from 20 cm to 1.5 m or more. The stem is erect, glabrous, and finely ribbed. The leaves have a firm texture, with dentate margins and prickly-spinose teeth. They are lanceolate, shiny and green above and paler below; the lower surface is often also glaucous. The lower stem leaves taper to an expanded auriculate base.

The involucre is about 1 to 1½ cm in length, and the florets exceed this by about one and a half times. The achenes are elliptical, pale brown, and ribbed on each face, with smooth spaces between the ribs.

The underground system comprises a stout, much branched tap root. All parts of the plant contain a white latex.

Sonchus asper differs from *S. oleraceus* in three features; it is identified by the characteristic achenes, also in having the rounded auriculated leaf-bases, and glossy upper leaf surfaces.

This plant is distributed throughout New Zealand where it is a successful colonizer of cleared land; it also appears on coastal sand, and in bush where the canopy is not too dense.

References:

Allan 1961; Webb *et al.*, 1988; Cheeseman 1925; Hooker 1867.

(ii) History

There are four species of *Sonchus* in New Zealand (excluding the Chatham Islands species); all are edible and all are known as *puwha* or sowthistle. Of these, *S. asper* (*raururoa puwha*) is the one which has the longest recorded history here. It was collected by the first European explorers and so was regarded as indigenous; now it is considered most likely to have been brought here by the Maori (Webb *et al.* 1988). *S. oleraceus* (*pororua, puwha*) was not recorded on Cook's first voyage, but was found by Banks on the second visit. *S. arvensis* (perennial sowthistle) is a more recently introduced species from Europe. The other species is *S. kirkii* (Webb *et al.* 1988), treated as *S. littoralis* (*S. asper* var. *littoralis*) by Allan (1982), and this is endemic. Webb *et al.* (1988) note that *S. asper* has been recorded in New Zealand as *S. oleraceus* var. *asper*, and that some very early records were based on material of *S. kirkii*. *S. kirkii* was apparently not collected by Banks or Solander.

In discussing *puwha* as a food plant, Colenso (1880:27) cited this as "...*puwha*, or common sowthistle, *Sonchus oleraceus* var., or two varieties, exclusive of the later introduced British one". Early literature suggested that *S. asper* was the species formerly used for food, but *S. oleraceus* was later favoured, being less bitter. Hooker (1867) differentiated between these two species as *S. oleraceus* var. α, which he called the introduced variety, and *S. oleraceus* var. ß, otherwise *S. asper*, Vill., which he considered to be certainly indigenous.

Based on the preceding records, the species chosen for use in this study has been *S. asper*.

Puwha was only used fresh as a vegetable and gathered daily, or twice a day as required, then steamed with other food in the earth ovens (Colenso 1880). Only the tender young leaves were used; the succulent stems of these were roughly bruised and washed in running water to get rid of the bitter milky juice before cooking. This plant was largely eaten in the spring and summer, and was greatly liked (Colenso 1880). It was one of the plants eaten by the crew of the Resolution on Cook's second voyage, eaten both boiled and in salad (Cook in Beaglehole [Ed] 1955).

Research by Chapman (1943), into the vitamin C content of *puwha*, initiated the

recommendation to New Zealanders that the vegetable be eaten during the Second World War. He recorded a level of vitamin C as similar to that found in oranges. Various species of sowthistle are eaten as vegetables throughout the world, on all continents (Crowe 1990).

(iii) Collection and organ preparation.

Collection of *S. asper* specimens was made on 3.11.91 from cultivated cropping land at Woodville (GR553889, NZMS 260 Sheet T24). The plants were robust and about 1 metre in height.

Small samples were cut from the plants to include leaves, stems and young flower buds.

The samples for microscopy were both young leaves and young flower heads, and these were preserved whole.

Samples for oven-drying and freeze-drying were thoroughly mixed for constancy of sub-sampling.

A further weighed sample was cooked gently for 15 minutes in a stainless steel pan with a little distilled water. The sample was strained and the cooking liquid reduced; both components were cooled, freeze-dried and weighed prior to milling.

(iv) Results and discussion

The moisture percentage obtained by oven drying for the untreated samples was 90.10 ± 0.13% (three replications). These required 48 hours drying time. The freeze-dried samples lost marginally less moisture (89.03%). The analyses conducted by Visser and Burrows (1983) using *S. oleraceus* showed a comparable moisture percentage (91% ±1) for the cooked sample.

The lipid component was found to be higher (per gram dry weight) in the cooked sample than in the untreated one: 10.25 g/100 g dry weight compared with 8.75 g/100 g dry weight. These are both relatively high values in terms of this study.

The value given by Visser and Burrows (1983) for a treated sample of *S. oleraceus* was 0.6 g lipid/100 g edible part; this equates to 6.67 g lipid/100 g dry weight, which is rather

lower than the present figure obtained for *S. asper*. The method used by these authors was also a di-ethyl ether extraction, based on the method of Folch *et al.* (1957).

McLaughlin and Wilson (1945), also using *S. oleraceus*, recorded 0.83 g lipid/100 g edible part, equating to 9.2 g/100 g dry weight.

The nitrogen determination showed the untreated sample to contain 3.58 g/100 g dry weight; this was slightly lower, 3.33 g/100 g dry weight in the cooked sample. The former figure was the highest of all plants analyzed in this study.

Comparative results obtained for cooked *S. oleraceus* samples by other authors were 4.8 g nitrogen/100 g dry weight (Visser and Burrows 1983), 3.56 g nitrogen/100 g dry weight (Shanley and Lewis 1969), and 4.8 g nitrogen/100 g dry weight (McLaughlin and Wilson 1945).

The total dietary fibre content was higher (per gram dry weight) in the cooked than in the uncooked sample; the insoluble fibre measurement was 10.58% higher and the soluble fibre measurement was 5.1% higher. The proportions of insoluble to soluble dietary fibre, (g/100 g dry weight) were 22.18 to 14.74 for the untreated and 24.80 to 15.52 for the cooked sample.

Visser and Burrows (1983) recorded 31 g total dietary fibre/100 g dry weight for *S.oleraceus*. This is slightly lower than the present result based on the sugar standard, ie 35.94 g/100 g dry weight, although considerably lower than the result 44.71 g/100 dry weight based on the polysaccharide standard.

Soluble sugars were lower in the cooked sample, 4.43 g/100 g dry weight compared to 5.44 g/100 g dry weight. Starches were also lower, 2.10 g/100 g dry weight compared to 2.39 g/100 g dry weight in the untreated sample.

S. asper is rather unique in this study; it is the only species of which the edible organ is predominantly photosynthetic, except for the red seaweed, *Porphyra* sp. To this it bears a close resemblance in proportions of starch and total dietary fibre. The partially photosynthetic young frond of *Asplenium bulbiferum* is very similar in its nitrogen content

and also the soluble sugar and total dietary fibre contents.

A summary of proximate analyses is shown in Table 4.1 (p68).

Elemental determination of these samples, both untreated and cooked, showed several very high values. These were calcium, zinc, copper, sodium, potassium, iron, manganese, silicon, sulphur, phosphorous, boron, cadmium, aluminium and strontium. For each of these elements, except for phosphorous and potassium, the levels were higher in the cooked samples. The levels of potassium, in both samples, were the highest by far in this study.

Data obtained by PES is shown in Appendix 3.1 (p198).

Microscopical analysis of the *Sonchus asper* leaf in transverse section (result not shown) shows the safranin to have been taken up by the cuticle layers of both upper and lower epidermal cells, defining the areas of cutinization. Between the epidermal layers are loosely-packed parenchymatous cells of the spongy mesophyll, these cells containing abundant chloroplasts. The longitudinal section through an immature bud is shown in Plate 4a (p65).Plates 4b, (p65) 4c and 4d,(p66) show sections of the immature stigma, styles and ovaries; pollen grains are seen in Plate 4c.

In order to test for the presence of starch in the various tissues, freshly prepared hand-cut sections were stained with iodine. In the leaf section, iodine was only absorbed differentially by the epidermal cell walls, showing no starch present in the mesophyll. similarly in the stem and petiole sections, iodine absorption was restricted to the epidermal cell walls. The author knows of no other occurrence where this staining absorption suggests extracellular starch. In the bud, no colour development was detected in any tissues.

As no starch grains are detectable in the prepared sections, and no positive staining obtained with iodine, this can be related to the very low starch measurement obtained in the analytical determination.

Plate 4a. *Sonchus asper*: bud, longitudinal section, stained with safranin and "fast green"; **b**, bract; **f**, petals of florets; **o**, ovaries; **r**, receptacle.

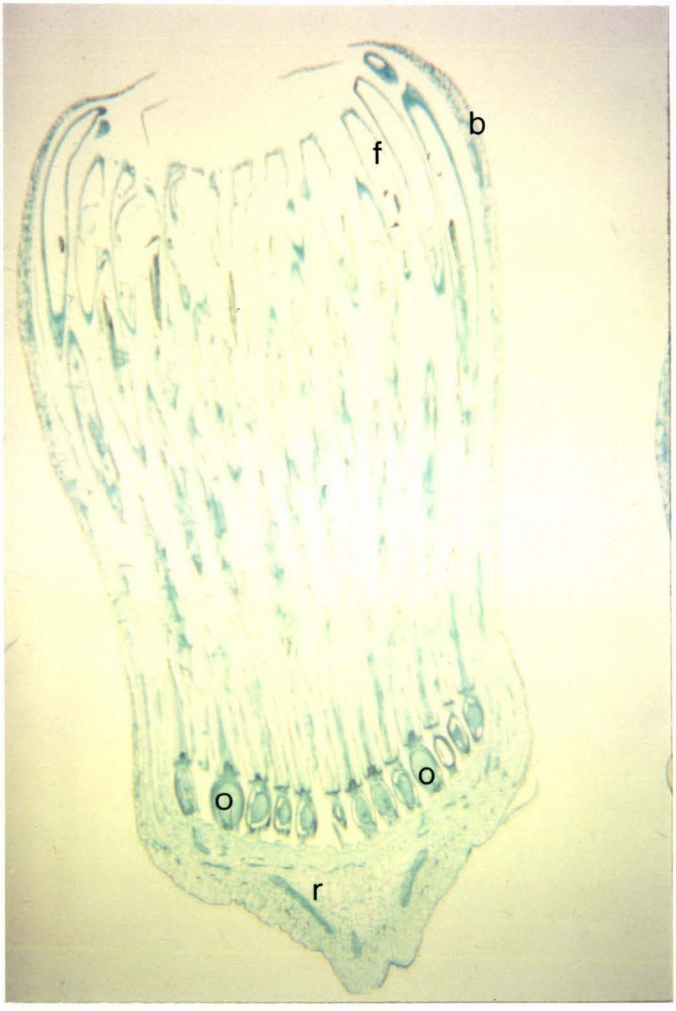
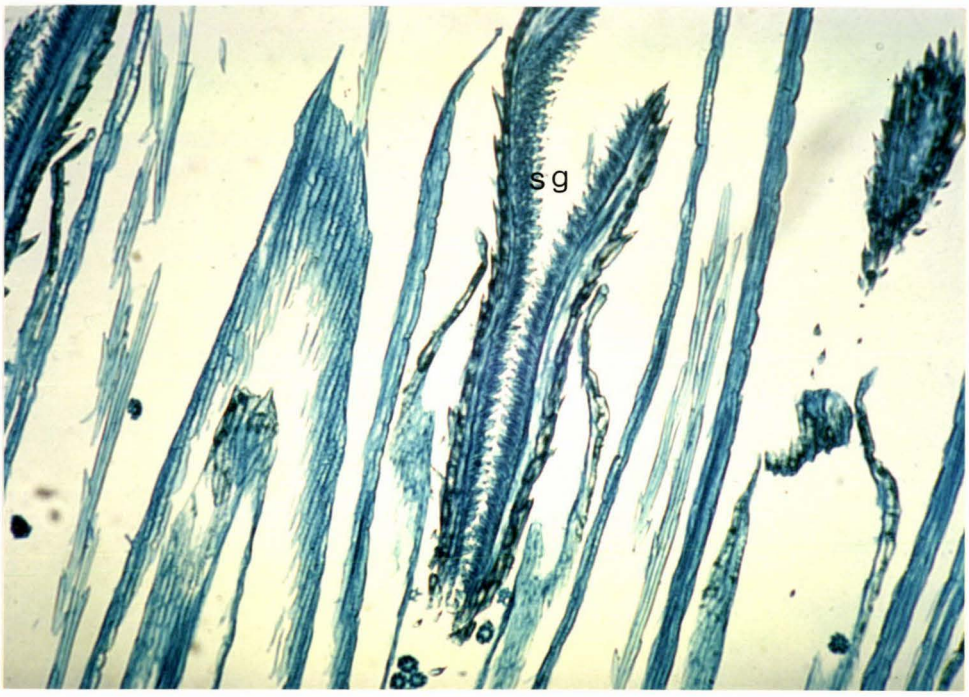
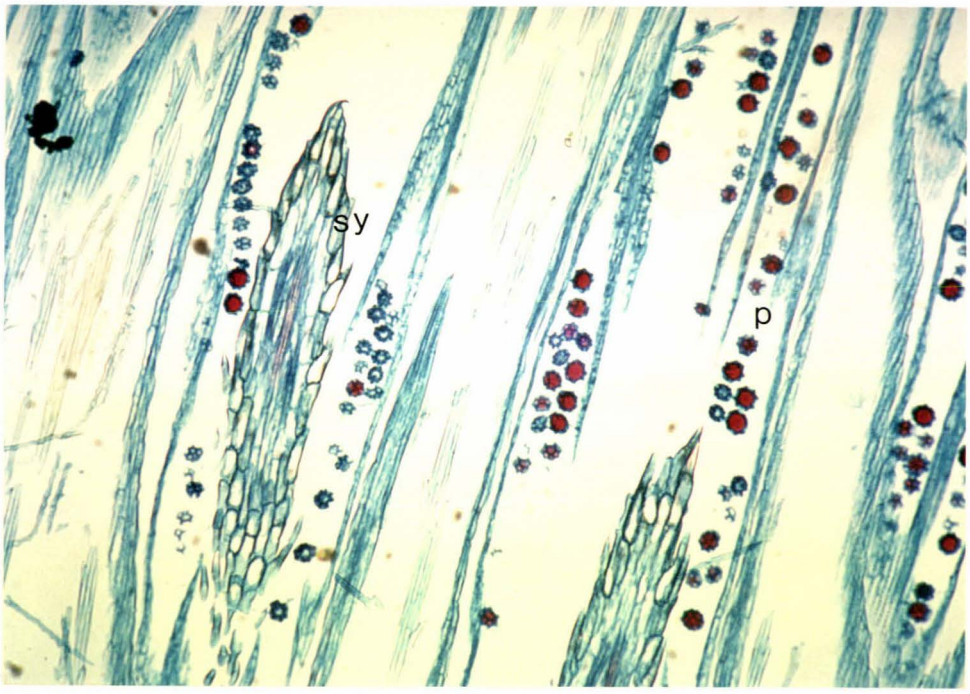

b
f
o
o
r

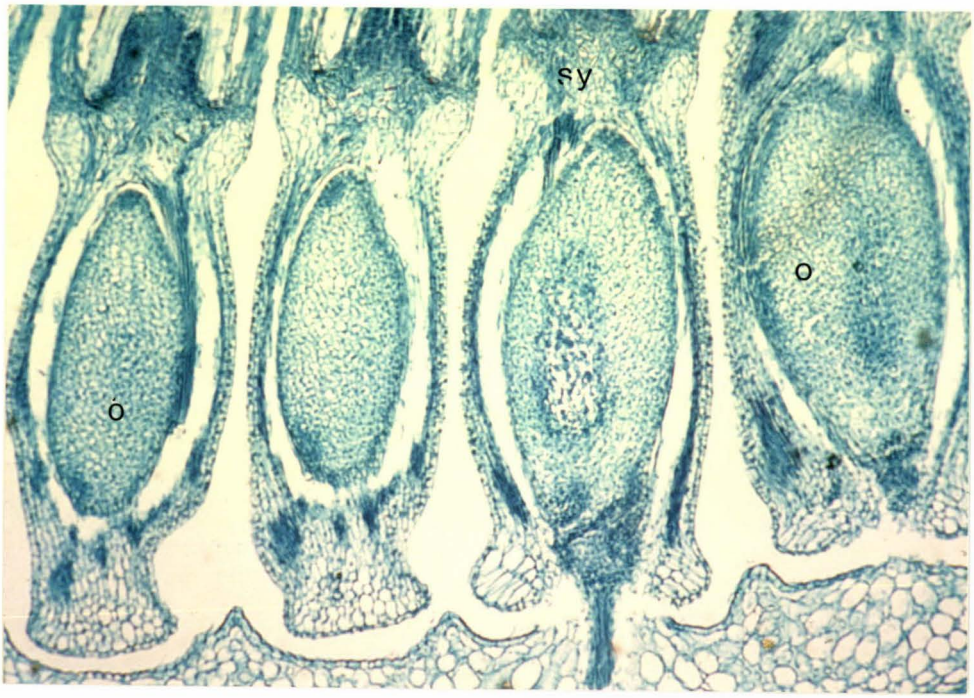
Plate 4b. *Sonchus asper*: longitudinal section of flower bud, stained with safranin and "fast green"; **sg**, immature stigma.

Plate 4c. *Sonchus asper*: longitudinal section of flower bud, stained with safranin and "fast green"; **sy**, style, cut obliquely; **p**, pollen grains.

Plate 4d. *Sonchus asper*: longitudinal section of flower bud, stained with safranin and "fast green"; **o**, ovaries; **sy**, base of style.


sg


sy
p


sy
o
o

The presence of latex is characteristic of leaves and roots in the Asteraceae, and latex tubes accompany the vascular bundles. Winton and Winton (1935) consider that proximate analysis of latex-containing plants is of little practical importance as it fails to take account of this component. It is to the presence of latex and tannin compounds that over-developed specimens of *puwha* owe their bitter taste.

Specific storage sites of the minerals which were found to be present at unusually high levels have not been identified. As magnesium is found both in meristematic tissues of most plants and also in latex (Winton and Winton 1935), these two sites could have high levels of this mineral to account for its high recorded percentage.

Table 4.1 *Sonchus asper*: leaves, shoots.
Proximate analyses per 100 g dry weight

	untreated	**cooked**
A.Moisture:		
oven-dried	90.10	91.05
freeze-dried	89.03	91.69
B.Lipid	8.75	10.25
C.Nitrogen	4.44	4.25
D.Protein	27.00	26.68
E.Dietary fibre:		
insoluble	24.60	27.51
soluble	16.33	17.20
F.Soluble sugars	5.44	4.43
G.Starch	2.39	2.10

(For details of replications and analytical procedures see Part 1, Procedures and analytical methods).

5. *Calystegia sepium* (L.) R.Br

family: Convolvulaceae

Pink bindweed

Pohue

(i) Description and distribution

The rhizomes of this plant are long, slender and branched. This is a twining species, with slender stems up to 3 m long. Leaves are oblong-sagittate, and cordate at their base; they are membranous, up to 10 cm long, and have long petioles 8 to 10 cm in length. The flowers are pink, or white and pink; the corolla is large, 4 to 8 cm in diameter and 5 to 6 cm long, and appears between October and March.

Calystegia sepium occurs throughout New Zealand in the North and South Islands, the Chathams, Kermadecs and the Three Kings. It is very common in many areas in the North Island; in the South Island it occurs in lowland situations as far south as Canterbury.

References:

Allan 1961; Webb *et al.* 1988; Cheeseman 1925; Hooker 1867

(ii) History

Calystegia sepium, the pink-flowered bindweed, was found in New Zealand by Banks and Solander (Banks in Beaglehole [Ed] 1962). Colenso (1880) described this plant as *Convolvulus sepium*, the common convolvulus or bindweed. The classification of this plant has been described by Cockayne and Allan (1926), who described the "species" as a linneon, appearing in New Zealand as an indigenous variety or varieties. In addition to this are introduced varieties. The pink-flowered bindweed is the commonest form and is considered to be indigenous, and Ogden (1978) wrote that the indigenous variety did not exactly match any European sub-species. The small white-flowered variety, similar to the European sub-species *sepium*, is probably introduced (Webb *et al.* 1988). The situation is further complicated by the occurrence of hybrids; these occur between *C. silvatica* and *C. tuguriorium*, and between the indigenous native *C.sepium* and *C. soldanella* (Webb *et al.* 1988). *C. soldanella* is also highly variable, and both this and *C.*

sepium are found in two variety forms, one from European stock with strongly purgative properties and one a native variety which can be safely eaten (Allan 1961).

Colenso (1880) was aware that the different varieties were pharmacologically distinct. He wrote that the roots (*sic*) of the New Zealand species, said to be identically the same species as the British one, were, in New Zealand, edible and wholesome, while in England (and elsewhere) were highly purgative. Other early writers and botanists wrote of the New Zealand plant as being the same as the English species; Hooker (1867) recorded *Convolvulus sepium* as the common convolvulus or bindweed of England. English common names are ambiguous and misleading in this respect. Colenso also used the names common convolvulus or bindweed. Of recent writers, Crowe (1990) uses the name greater bindweed, but in New Zealand the name great bindweed is applied to *Calystegia silvatica*, and for the New Zealand variety *C. sepium* Webb *et al.* (1988) use the name pink bindweed.

The rhizomes of this plant, the Maori *pohue*, were carefully dug up and cooked for food (see portion of rhizome, Plate 5a, p73). Colenso (1880), however, although rating them highly in his listing of these plants, wrote that they were not greatly esteemed, probably on account of the small quantity obtained for the amount of labour expended in digging them.

They were stacked to dry and packed for storage. For eating, dried lengths were soaked in water then steamed. They have been described as being floury as a potato with a slight bitter taste (Potts 1882), and as a delicacy in the Maori cuisine (Crayon 1842). Makereti (1938) records the cooked rhizome as quite good to eat.

Because of the variability of this species, and introduction and hybridizations during the last 100 to 150 years, rhizomes of *C. sepium* are not now regarded as safe to eat as they may contain the purgative principle (Crowe 1990).

(iii) Collection and organ preparation

Rhizomes were collected on 15.12.91 from an area of disused farmland 3 k east of Palmerston North (GR364908, NZMS 260 Sheet T24).

The rhizomes formed a dense underground system; none measured more than 5 mm in diameter.Material was washed carefully to remove all soil particles, then rinsed in distilled water. A 2 cm length of rhizome was preserved in FAA for the botanical section.

Sub-samples for proximal analyses were chopped into short lengths with scissors, and prepared for analytical sampling.

(iv) Results and discussion

The moisture content of this organ was found to be relatively high, and similar values were obtained by oven-drying and freeze-drying; these were 74.94 ± 0.36%,(three replications), and 74.55% respectively.

The value obtained by solvent extraction for lipid determination cannot be viewed without qualification; the weight recorded was 33.30 g/100 g. The solvent evaporation following the extraction procedure was continued to constant flask weight although the flask still contained residual fluid. This was measured to be 1.6 ml, had a very thin oily consistency, and a faintly detectable odour.

As already mentioned in the procedure for lipid determination, interferences in extraction may be due to constituent plant oils which volatise during heating; these condense and are siphoned into the extraction flask with the solvent. No research literature has been sighted on the pharmacology of *Calystegia* rhizomes and, as noted by Brooker *et al.* (1988) there is scope here for chemical investigation of the apparent differences between varieties. However, other members of the Convolvulaceae do exhibit pharmacological characteristics in their rhizomes; purgative resins are extracted from some species and used medicinally. These include *Ipomoea purga* and *I. orizabensis*, both of which contain ether-soluble resins (Trease and Evans 1966). The suggestion is made here, to explain the anomalous result obtained above, that *Calystegia sepium* rhizomes also contain an ether-soluble resin and this has masked the true lipid measurement.

The nitrogen and protein results were 2.02 and 11.55 g/100 g respectively, suggesting from this low protein to nitrogen ratio of 5.72 to 1, that the rhizomes may contain some non-protein nitrogen. An alternative explanation may be that all protein was not solubilized in the buffer extraction of the Bradford method. This seems less likely ,

however, as the supernatant was thin and clear, and not apparently mucilaginous as in most other rhizome samples where this ratio was low. Also the amount of protein extracted in this stage was quite high relative to the 1 M alkali extraction.

The insoluble and soluble dietary fibre measurements were 16.41 and 23.09 g/100 g respectively. Proportionally the soluble fibre component was the major component of these. Soluble sugar and starch were recorded to be 5.28 and 28.42 g/100 g respectively, the latter being a notably high figure.

A summary of proximate analyses is shown in Table 5.1 (p74).

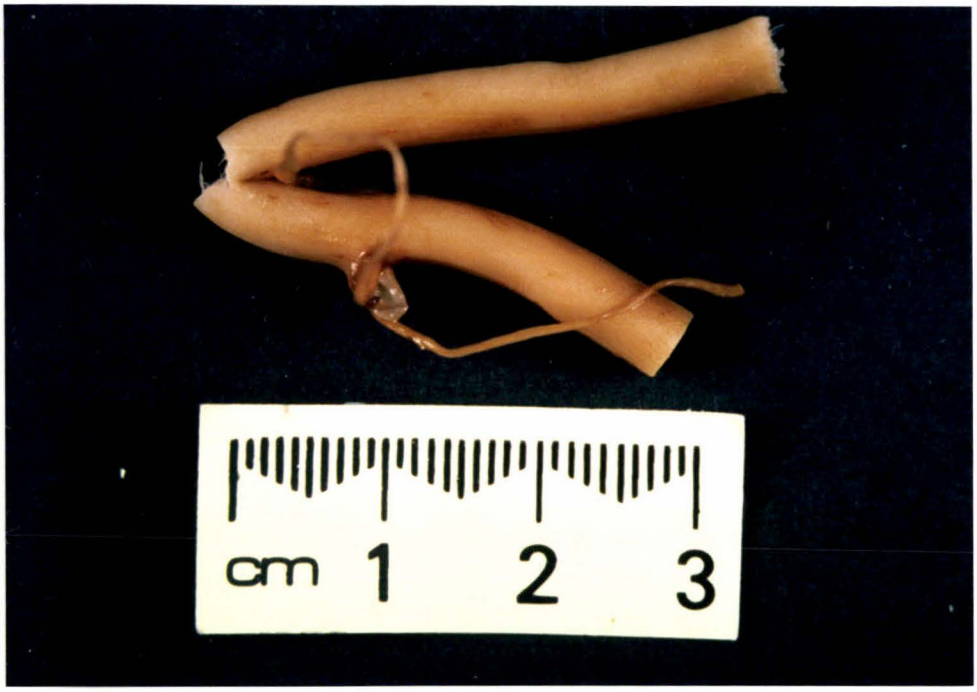
Elemental analysis showed only two outstanding levels for this sample, and these were high values for silicon and strontium. (No silicon storage cells have been identified in the section).

Data obtained by PES is shown in Appendix 3.1 (p198).

The rhizome transverse section (see Plate 5b, p73) shows abundant grains in the cortical parenchyma cells. These appear to be round and are very small, grouped in irregular clusters, some quite densely, and are suggestive of starch grains. To further identify these grains, a fresh hand-cut section of the rhizome was prepared and stained with iodine. This test was positive for starch, showing an abundance of small, densely packed round grains and confirming the high starch reading of 28.44 g/100 g, obtained by proximate analysis.

Plate 5a. *Calystegia sepium*: rhizome.

Plate 5b. *Calystegia sepium*: rhizome, transverse section, stained with safranin and "fast green"; **c**, cortex; **s**, starch grains; **v**, vascular bundles.


cm 1 2 3

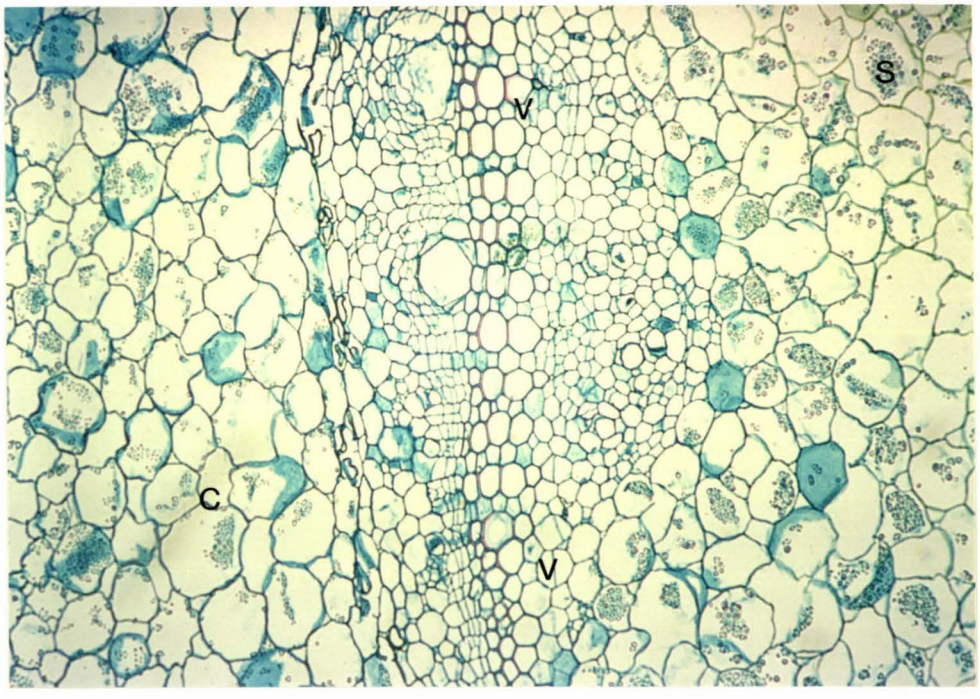

S
V
C
V

Table 5.1 *Calystegia sepium*: rhizomes
Proximate analyses per 100 g dry weight.

A.Moisture:	
oven-dried	74.94
freeze-dried	74.55
B.Lipid	33.30
C.Nitrogen	2.02
D.Protein	11.55
E.Dietary fibre:	
insoluble	16.41
soluble	23.09
F.Soluble sugars	5.28
G.Starch	28.42

(For details of replications and analytical procedures see Part 1, Procedures and analytical methods).

6. *Cyathea medullaris* (Forst. f.) Swartz

family: Cyatheaceae

Black tree fern

Mamaku

(i) Description and distribution

This is a large tree fern with a trunk up to 20 metres tall; it is the largest and most handsome of our tree ferns. The trunk is black, covered with hexagonal stipe scars, and these are a distinguishing feature. The stipes are thick, black and slightly rough, having scales with spiny margins. The fronds are 5 metres long, arching upwards from the crown; they are 3-pinnate and rather leathery. Dead fronds are abscised, except in immature plants where they may persist. The longest primary pinnae are 40 to 100 cm long, the undersurface bearing scales with spiny margins. These frond scales can be seen with a hand lens and are a distinguishing feature, not seen in any other mature tree fern. The indusia completely cover the sori at maturity and split irregularly.

Cyathea medullaris occurs in both North and South Islands, Stewart Island, the Chathams and the Three Kings. In the North Island it is common in lowland forest where it is prominent as a tall emergent tree species; in the South Island it is mainly coastal and is absent from the drier parts of Canterbury and Otago. It is frost tender, and prefers damp areas.

References:

Allan 1961; Brownsey and Smith-Dodsworth 1989; Dobbie 1930; Cheeseman 1925; Hooker 1867

(ii) History

Colenso (1880) recorded that the stipes and trunk of this fern, the *korau* or *mamaku*, provided an excellent sago-like substance. This was considered by Colenso to be one of their very best wild vegetable productions. The edible section of the trunk was cut into short lengths and their tough skin hewn off. This was cut into slices and the soft white pith steamed for 48 hours. The cooked slices were threaded on flax string and hung up to dry in the sun, and eaten cold.

Whereas using the "pithy core" of the trunk (*koata*) was destructive to the plant, the frond stems were alternatively used without harm to the fern. The first 50 cm or so of the stipes was used, and the pith prepared as for the *koata*.

Forster (1777), the first European who discovered and named this fern, spoke very highly of it; he wrote that it was "rather better than a turnep" (*sic*). Potts (1882) described the prepared pith as soft with a very sweet flavour. Taylor (1855:380) wrote "really an agreeable article of food, slightly sweet when cooked". Makereti (1938) said that it was nice to eat, and more recent writers have compared it, favourably, to marrow or turnip (Crowe 1990) and dried apples (Hildreth 1962).

The *mamaku* is extremely mucilaginous, and a cut surface allows a copious flow of clear, thick, sticky fluid. Only one reference to this component has been read, by Bauke (1928). He described how the exposed rods of slimy white pith, after removal of the bark, were left for the juices to run out in runnels, because the Maori knew that those juices were bitter.

(iii) Collection and organ preparation

A young frond specimen (see Plate 6a, p79) was collected on 30.9.91, cut from the crown of a mature plant in the Tiritea Reserve, Manawatu (GR358842, NZMS 260 Sheet T24). The frond was cut off near its base where its transverse measurements were 6 by 4 cm.

The lower portion of the specimen was rubbed free of dark frond scales, and cut for analysis. It cut very easily, with no resistance. The outer layer of skin could be peeled off like rhubarb, and the pith was cut into pieces with the release of copious mucilage. The cut surfaces browned readily on exposure.

A section of the cut specimen was preserved in FAA for botanical analysis. Sub-samples of cubed pith were weighed for oven drying and freeze drying. A cooked sample was prepared by steaming a weighed sub-sample of cubed pith for 1 hour in distilled water. Both cooked solids and reduced liquid were cooled, freeze-dried and weighed prior to milling and mixing.

(iv) Results and discussion

The moisture content was found to be 95.47 ± 0.15% (three replications) which was the highest value recorded for moisture content in the present study. By freeze-drying the measured value was 94.59%

Other measurements (for the non-treated stipe samples) were 15.40 g lipid/100 g, 1.70 and 8.33 g/100 g for nitrogen and protein respectively, 11.73 and 15.49 g/100 g of insoluble and soluble dietary fibre respectively, 40.11 g soluble sugar/100 g and 3.93 g starch/100 g. Of these measurements, the lipid and soluble sugar components are both seen to be high. In the protein determination, mucilage in the buffer extraction necessitated the addition of 3 M NaOH to solubilize the mucilage-bound protein. The nitrogen to protein ratio is seen to be high, which may suggest that all protein was not in fact solubilized and measured in that procedure. Alternatively this high ratio might reflect the presence in the nitrogen component of some non-protein nitrogen.

The soluble sugar measurement is one of the highest in the present study.

After cooking the stipe sample, these values were not greatly changed except in the case of the lipid component which was reduced to 3.53 g/100 g, and the insoluble dietary fibre component which increased (per gram dry weight). The reduction in lipid represented a loss of 77.08%. No satisfactory explanation can be given here, as the cooking water was reduced and included in the final analysis; also no oiliness had been observed during cooking. It is possible that the higher measurement obtained for the untreated sample included some volatile oils which gave an unrealistically high lipid measurement.

The level of insoluble dietary fibre after cooking was 18.43 g/100 g, which was higher (per gram dry weight) by 57.10% than in the untreated sample.

The other values obtained after cooking were 94.57% moisture, 1.52 and 7.81 g/100 g for nitrogen and protein respectively, an even higher (per gram dry weight) soluble sugar content of 46.23 g/100 g, and 3.41 g starch/100 g. These show that the nitrogen to protein ratio was slightly higher, (per gram dry weight) than in the untreated sample, unless, again, all protein was not solubilized for measurement; treatment with

unless, again, all protein was not solubilized for measurement; treatment with concentrated alkali was also carried out for these samples in the Bradford procedure.

A summary of proximate analyses is shown in Table 6.1 (p80).

Elemental analysis showed very high levels of both potassium and aluminium, the latter by far the highest in this study. There was also a measured very low level of iron.

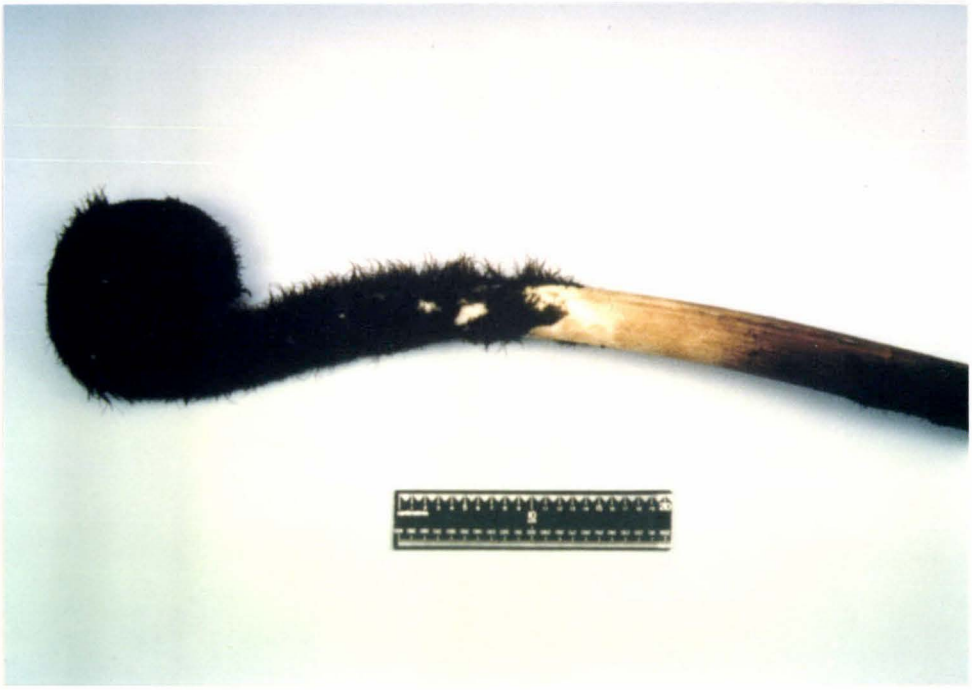
Data obtained by PES is shown in Appendix 3.1 (p198).

The prepared section (see Plate 6b, p79) shows the cortical region to be composed of fairly large thin-walled cells, with small intercellular spaces. Some relatively larger cells in the cortex are suggestive of mucilage cells. No starch grains are detectable in the section; this may reflect the low value for starch in the proximate determination, 3.93 g/100 g.

The vascular bundles are few and horse-shoe shaped. The walls of the metaxylem vessels show minimal absorption of safranin and do not appear thickened. An endodermal layer is detectable, with thickening on the medial and lateral walls of some, but not all, cells.

Plate 6a. *Cyathea medullaris*: unexpanded frond.

Plate 6b. *Cyathea medullaris*: stipe, transverse section, stained with safranin and "fast green"; **c**, cortex; **mc**, mucilage canal; **m**, meristele.



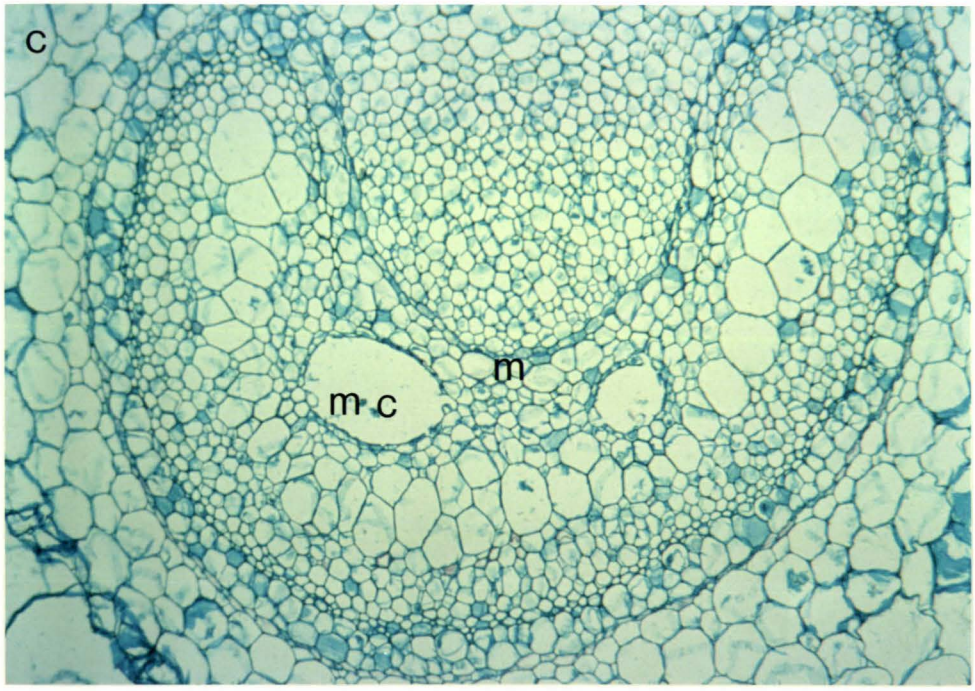

c
m
m c

Table 6.1 *Cyathea medullaris*: stipe pith.
Proximate analyses per 100 g dry weight.

	untreated	**cooked**
A.Moisture:		
oven-dried	95.47	-
freeze-dried	94.59	94.57
B.Lipid	15.40	3.53
C.Nitrogen	1.70	1.52
D.Protein	8.33	7.81
E.Dietary fibre:		
insoluble	11.73	18.43
soluble	15.49	13.94
F.Soluble sugar	40.11	46.23
G.Starch	3.93	3.41

(For details of replications and analytical procedures see Part 1, Procedures and analytical methods).

7. *Cordyline australis* (Forst. f.) Endl.

family: Agavaceae

Cabbage tree

Ti kouka

(i) Description and distribution

Cordyline australis grows to 20 metres in height, with a straight trunk and characteristic rounded heads of foliage; this shape is formed by the bending down of the older leaves from their bases. Leaves are long and narrow, up to 100 cm long by 3 to 6 cm wide; they are thick with indistinct midribs, and have fine longitudinal, parallel veins on both upper and lower surfaces.

Flowers are produced in late spring or early summer, in large panicles which may be up to 1.5 metres long. Panicle branches are at right angles to the main axis of the panicle, and flowers are crowded along the ultimate branches of these. They are strongly scented, and are a white or creamy colour when open but pink tinged when in bud. The sweet scented nectar is produced around the ovary bases.

The root of *Cordyline australis* is a thick large tap root.

This species grows throughout the North and South Islands, and on Stewart Island where it is rare. It occurs along forest margins and in regenerating bush, often in clearings and around swamps, but also frequently seen as isolated specimens or forming spinneys on exposed cleared land. It grows from sea level to 600 metres.

References:
Salmon 1980; Cheeseman 1925; Hooker 1867.

(ii) History

The inside of both the trunk and the root of the cabbage tree were eaten. The former was the blanched "heart shoot", called *korito*, which was eaten either raw or roasted. The very bases of the young inner leaves were also used; these are soft and white. Colenso (1880) considered this was an important plant to the Maori, particularly when travelling,

as the tree was very common. He described the taste as slightly bitter but not unpalatable. Makereti (1938) likened the taste to that of an artichoke.

To prepare the *korito*, the head of the tree was cut off to allow the sap to rise, then the outer bark chipped off, leaving the core to sun-dry.

The use of the tap root involved digging, splitting, and removal of the hard exterior. Colenso (1880) recorded that it was very fibrous and took a long time to cook. Best (1942) commented that there was always a very large proportion of fibrous refuse. Sweet, starchy meal was separated from the fibres by twisting and rubbing, and the meal mixed with water to form a sweet paste (Tregear 1926). Colenso (1880:28) wrote that the root contained a large amount of both "saccharine and farinaceous matter". It has since been shown to contain a glucofructo furanan polysaccharide (Fankhauser 1985), also present in the stem. Fleshy tap roots have been described as having a sweetish sugary taste, compared to that of stick liquorice (Cheeseman 1900). White (1890) wrote that it was a very good food indeed, and as sweet as European sugar. In fact, Fankhauser (1985) found the high-fructose syrup to be almost twice as sweet as sucrose syrup.

(iii) Collection and organ preparation

A young specimen of *Cordyline australis* was selected, growing close to Palmerston North (GR332882, NZMS 260 Sheet T24), and collection was made on 2.10.91. Samples were taken of both tops and tap root. For the top sample, leaves were stripped from the upper portion, exposing the soft heart; this was cut into pieces, and the youngest leaf bases included in the sample.

For the root sample, the outside was removed and the inner portion cut into small pieces. This was white and contained many fibres.

For the botanical specimens, a cut cube of inner tap root, and the very base of a young leaf were preserved in FAA.

(iv) Results and discussion

For the leaf-base samples, moisture content was found to be 81.18 ± 0.41% (three replications) by oven drying. Moisture loss occurred mainly in the first 24 hours of drying. The moisture content measured by freeze-drying was 83.92% and constant weights reached after 48 hours drying. Moisture loss occurred mainly in the first 24 hours of drying and constant weights were reached after 48 hours in the case of the freeze-dried samples.

The lipid measurement was 4.38 g/100 g. Nitrogen and protein levels were 1.49 and 8.91 g/100 g respectively, showing a protein to nitrogen ratio of 5.98 to 1.

Insoluble dietary fibre was relatively high, 34.08 g/100 g, and soluble fibre very low, 3.58 g/100 g which was the lowest soluble fibre measurement in the study.

The soluble sugar level was very high, 40.20 g/100 g, second only to the high level in the treated *Cyathea* stipes.

The starch level was also very low, 1.93 g/100 g.

For the root samples, the moisture content was 67.42 ± 0.69% by oven-drying, and four days were required for complete moisture loss. By freeze-drying the moisture content measurement was 68.79%.

The lipid component was very low, measuring only 1.17 g/100 g.

Nitrogen and protein figures were 0.48 and 3.22 g/100 g respectively, showing a high protein to nitrogen ratio of 6.70 to 1.These were the lowest nitrogen and protein figures in the study.

Of the carbohydrate measurements, only that of soluble sugar was found to be relatively high. Insoluble and soluble dietary fibre were measured to be 20.27 g/100 g and 14.57 g/100 g respectively.The soluble sugar level was high, measuring 20.86 g/100 g and starch level was relatively lower, at 3.20 g/100 g.

A summary of proximate analyses is shown in Table 7.1 (p86).

Carbohydrate analyses were carried out by Fankhauser (1985) on both roots and stems of *C. australis*. These results had shown that there was a high degree of seasonal variation in the sugar and polysaccharide components of both organs. The fructose component was notably higher in July than in January, particularly in the root. Total carbohydrate figures, however, showed higher levels in January, in both organs, the increase being more pronounced in the stem.

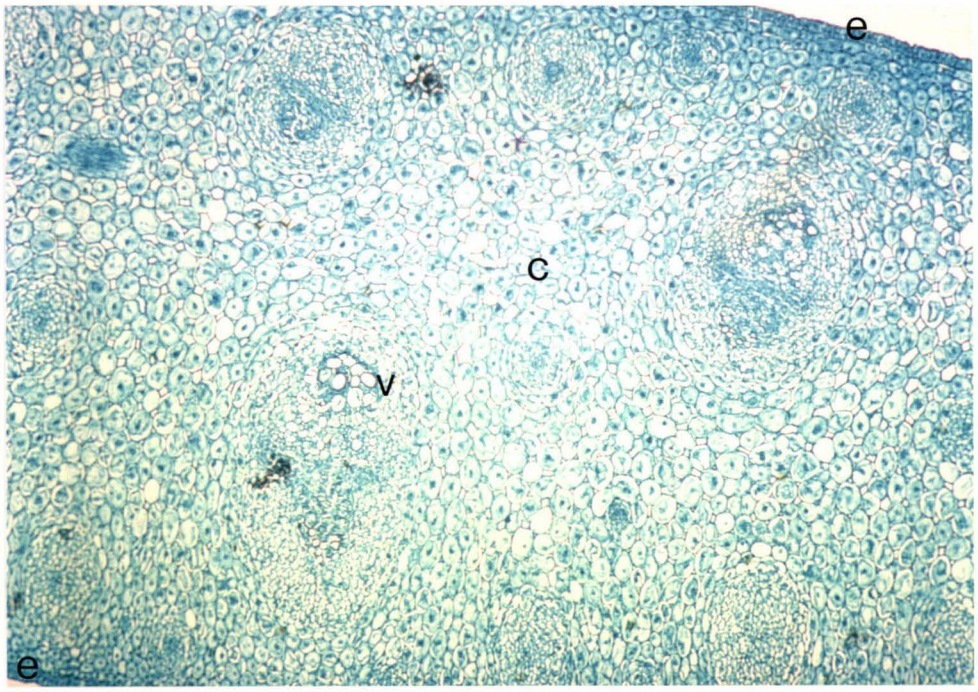
Elemental analysis showed that there were high mineral levels, in the leaf-base sample, of potassium, manganese, phosphorous, nickel and strontium; there was also a notably low level of iron. There were no similarities at all in the root sample which showed no unusually high elemental levels, but low levels of zinc and phosphorous.

Data obtained by PES is shown in Appendix 3.1 (p198).

The leaf-base section in the transverse plane shows a central mesophyll tissue of round parenchyma cells without apparent starch grains. The epidermal cells are much smaller, and are isodiametric with thickened walls. Safranin has not been well retained although it is visible in the vessel walls of the xylem. Bundles are fairly abundant across the leaf-base section, with prominent fibrous bundle sheaths aspected towards one epidermis (see Plate 7a, p85). The root section shows regular rows of isodiametric cells punctuated by vascular bundles. These are composed of between 60 and 70 thickened vessels, well stained with safranin (see Plate 7b, p85).

Plate 7a. *Cordyline australis*: leaf base, transverse section, stained with safranin and "fast geen"; **c**, cortex; **e**, epidermis; **v**, vascular bundle.

Plate 7b. *Cordyline australis*: root, transverse section, stained with safranin and "fast green"; **g**, thin-walled cells of ground tissue; **v**, tracheary elements of vascular bundle.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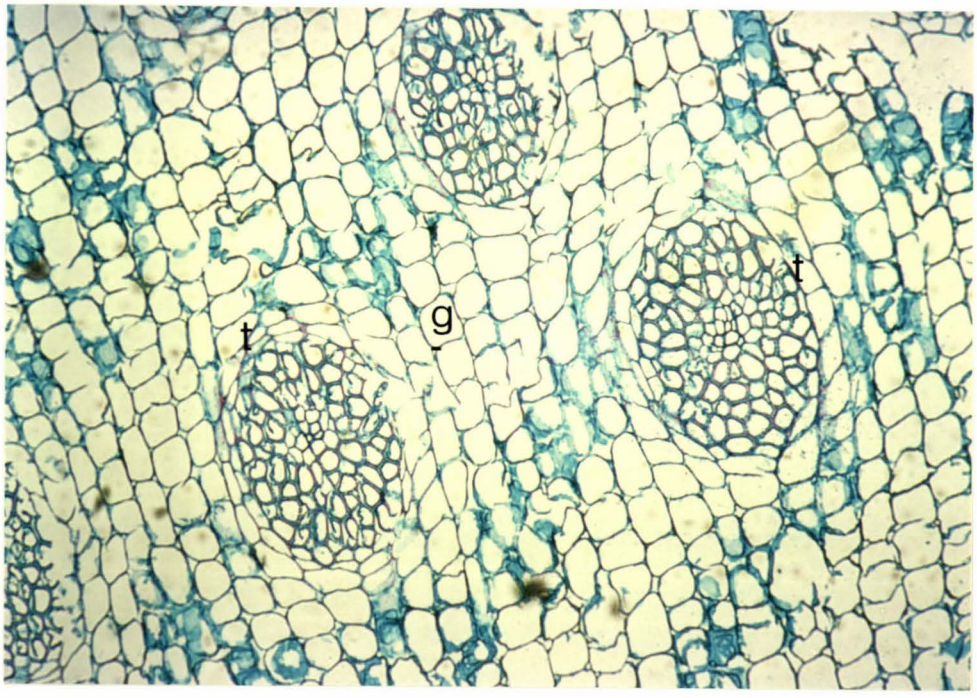
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Table 7.1 *Cordyline australis*: leaf bases; roots.
Proximate analyses per 100 g dry weight.

	leaf bases	**tap root**
A:Moisture:		
oven-dried	81.18	67.42
freeze-dried	83.92	68.79
B.Lipid	4.38	1.17
C.Nitrogen	1.49	0.48
D.Protein	8.91	3.22
E.Dietary fibre:		
insoluble	34.08	20.27
soluble	3.58	14.57
F.Soluble sugar	40.20	20.86
G.Starch	1.93	3.20

(For details of replications and analytical procedures see Part 1, Procedures and analytical methods).

8. *Rhopalostylis sapida* Wendl. et Drude

family: Palmae

Nikau

(i) Description and distribution

Rhopalostylis sapida grows to a height of 10 metres or more. The trunk is smooth and has closely spaced leaf scars; when young, the trunk between the scars is green. During the first few decades of growth, the palm is composed of a crown of fronds and a root system. The latter consists of a large tap root (underground stem) and a dense network of woody lateral (adventitious) roots (Corner 1968). The age at which palms show above-ground stem development appears to be in the vicinity of 40 to 50 years (Enright 1985). There are 13 to 15 fronds, and palms showing above-ground stems shed two fronds per year on average. Fronds are 3 to 4 metres long and about 2 metres wide. Palms normally reach reproductive maturity when they have 60 to 80 frond scars, at about 80 to 90 years old, or a stem height of more than 2.5 metres (Enright 1985).

Inflorescences are produced within two spathes; these arise above the leaf scar just below the oldest leaf. They measure about 30 cm by 15 cm, and these fall away as the inflorescence starts to expand. The flowers are small and packed closely together in groups of three; each group comprises a small female flower between two males. The males open first, with mauve petals and 6 stamens, and the females open later after the males have fallen off. The fruit is a brilliant red, containing a single large hard seed.

Rhopalostylis sapida grows inland forests throughout the North Island and in the South Island as far as Banks Peninsula on the east and Greymouth on the west.

References:

Allan 1961; Salmon 1990; Enright 1985; Corner 1968; Laing and Blackwell 1964; Cheeseman 1925.

(ii) History

Colenso (1880:28) recorded that a "very capital article of food was the blanched heart (*korito*) of the southern palm-tree, *nikau* (*Areca sapida*)". He added that as a fine tree only afforded a single dish, and the tree was killed, it was not very commonly used. The taste he described as "excellent, even in a raw state, juicy, succulent and nutty, with an agreeable taste, and very wholesome" (Colenso 1880:28). Angas (1847:16) described this as "refreshing and tasting rather pleasant than otherwise, its flavour somewhat resembling that of the cocoa nut". Banks (Beaglehole [Ed] 1962) described it as a delicious meal,and Thomson (1859) said that epicures gloated over the tender shoots.

Leaves were stripped from the tree and the young heart was eaten either raw or cooked in the earth oven for several days. When the oven was opened the sugar was encrusted on the pith of the tree in the form of soft brown crystals which were removed by knocking the fibres against a piece of wood (Fuller 1978).

(iii) Collection and organ preparation

A specimen of the *nikau* palm was chosen in an area of bush in the Manawatu (GR307780, NZMS 260 Sheet T24), where there were approximately 2 dozen palms within its close proximity. The upper portion of the palm, consisting of the fronds and heart, were removed, cutting just below the heart. Fronds were removed at their base using a knife, and the remaining portion was sawn longitudinally to reveal the woody base, the heart, and the soft parts at the base of the new inner leaves (see Plate 8a, p90). All these were white; the cut surfaces browned rapidly on exposure to air.

The heart was cut out using a knife; the top portion only was soft, and inner leaf bases were very soft.

Sub-samples were taken of the diced heart, for oven drying, freeze-drying, and for preserving in FAA.

The term "heart" used here and elsewhere in the text refers to the apical shoot.

(iv) Results and discussion

The moisture content was measured to be 89.27 ± 0.77% (three replications) by oven-drying, and constant weights were reached in 24 hours. By freeze-drying the measured moisture content was 89.71% and constant weights were reached in 30 hours.

The lipid component was measured to be 4.75 g/100 g, a significant but not high value.

Nitrogen and protein were both present in above average percentages in terms of this study. Values for these were 2.52 and 14.75 g/100 g respectively, showing a protein to nitrogen ratio of 5.85 to 1.

The insoluble and soluble dietary fibre components were measured to be 21.78 and 12.67 g/100 g respectively. Of all carbohydrate values, soluble sugar alone was relatively high, and this was measured to be 18.64 g/100 g. The relatively lower starch measurement was 5.95 g/100 g.

A summary of proximate analyses is shown in Table 8.1 (p92).

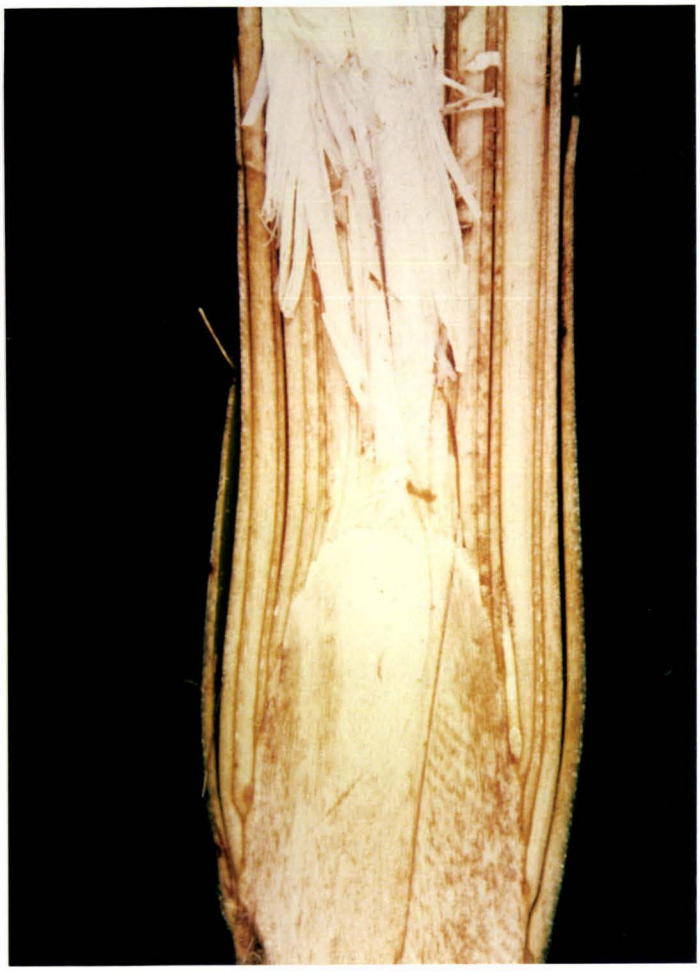
Elemental analysis showed high levels of magnesium, silicon and copper, and very high levels of zinc and potassium. There was also an extremely high level of strontium recorded, this being the highest value for strontium of all plants analyzed here.

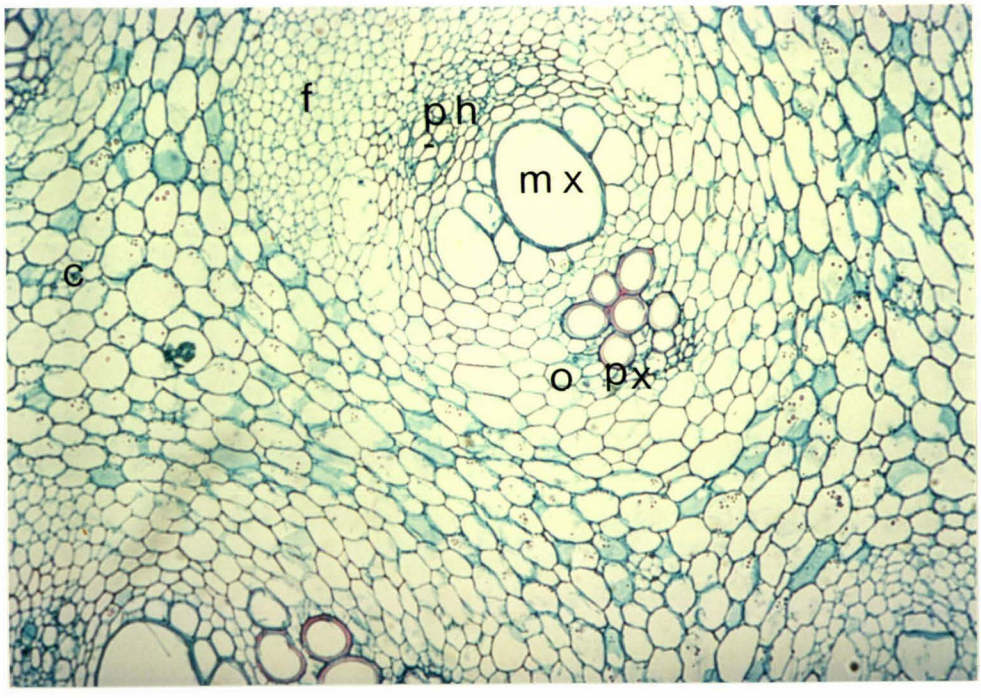
Data obtained by PES is shown in Appendix 3.1 (p198).

The botanical section shows numerous vascular bundles dispersed through a mass of ground tissue. The latter is seen to be composed of thin-walled parenchymatous cells (see Plate 8b, p90). Starch grains, of the very small circular type, can be seen in some of the parenchyma cells; they are not overly abundant and this finding is matched by the fairly low starch measurement by proximate analysis.

Plate 8a. *Rhopalostylis sapida*: apical shoot, cut longitudinally.

Plate 8b. *Rhopalostylis sapida*: apical shoot, transverse section, stained with safranin and "fast green"; **c** cortical parenchymatous cells with starch grains; **f**, fibrous sheath; **mx**, metaxylem; **px**, protoxylem; **ph**, phloem; **o**, stegmata containing opal phytolith.




f
ph
m x
c
o px

The vascular bundles show a regular arrangement of xylem, phloem and extended fibrous sheaths. These sheaths form a strand adjacent to, and partly enclosing, the phloem, and always widest on the phloem side. The xylem includes well-defined vessels of metaxylem and protoxylem. Vessels are uniformly and fairly heavily thickened, and well-stained with safranin. It could be assumed that the relatively high level of insoluble dietary fibre is composed largely of these numerous thickened vessels.

Some stegmata can be seen associated with the protoxylem; these are small isodiametric cells enclosing amorphous opal phytoliths. The high level of elemental silicon, the mineral component of opal phytoliths, may be largely due to the presence of these stegmata. Larger crystals can also be seen in occasional cells of the cortex, but no specific histological tests have been carried out to determine the identity of these.

Table 8.1 *Rhopalostylis sapida*: apical shoot
Proximate analyses per 100 g dry weight.

A:Moisture:	
oven-dried	89.27
freeze-dried	89.71
B.Lipid	4.75
C.Nitrogen	2.52
D.Protein	14.75
E.Dietary fibre:	
insoluble	21.78
soluble	12.67
F.Soluble sugar	18.64
G.Starch	5.95

(For details of replication and analytical procedures see Part 1, Procedures and analytical methods).

9. *Typha orientalis* C. B. Presl.

family: Typhaceae

Bulrush

Raupo

(i) Description and distribution

Typha orientalis is an erect marsh herb with long narrow leaves; these may be as much as 2 metres in length. They are thick, flat, and spongy, expanded at the base into a broad sheath. The single seed stalks (scapes) often exceed the leaves in height. These bear the male and female cylindrical catkins at the top; the upper or terminal one is male, usually from 5 to 15 cm long and this may be separated from the female catkin by a short interval of 2 or 3 cm. Sometimes the two are contiguous. The female catkin is longer, from 7 to 20 cm, and about 10 to 20 mm across, and at the base is a linear bracteole (see Plate 9b, p96).

When in flower, in January, the male catkin appears yellow with pollen.

The distribution of *Typha orientalis* is fairly widespread in the North and South Islands. It grows in marshes (see Plate 9a, p96), along river banks, and beside lakes and lagoons spreading "in a raft-like mass from the margins into the open water" (Esler 1978). It has formerly been described as abundant (Cheeseman 1925; Hooker 1867; Colenso 1880), but has become much more localised with the widespread practice of draining agricultural land, hence the loss of many natural marshland environments. This ecological shift was commented on by Esler (1978) in detailing the distribution of the plant in the Manawatu, and additional sites have been lost since that time.

The underground organ of *Typha* is a stout creeping rhizome.

References:

Moore and Edgar 1976; Esler 1978; Cheeseman 1925; Hooker 1867.

(ii) History

Colenso described the use of 2 distinct edible organs of this species. It was the pollen, discussed in this section, which was rated as of considerable importance; the use of the rhizome is discussed later (p132).

Called *pungapunga*, or *pua*, the pollen was collected in the summer when the plant was in full flower. To harvest the pollen, flower stalks were collected in early morning and late evening to avoid day-time wind loss. The stalks were left in the sun for several days to dry, being brought in at night to avoid dampening by dew. The pollen was then removed from the stem by gently beating it out of the male catkins, then sifting to separate other flower structures and debris. Colenso (1880:28) wrote, "in appearance, in its raw state it exactly resembles the ground yellow mustard of commerce". Pollen was mixed with water into cakes and baked. The taste has been described as sweetish and light, reminiscent of London gingerbread (Colenso 1880). Makereti (1938) also records the cooked *pua* as having a sweetish taste.

Large quantities of pollen are obtained from the flowering spikes. *Raupo* has been considered to be the only plant found in New Zealand from which enough pollen can be gathered to give a substantial supply of food (Hildreth 1962).

(iii) Collection and organ preparation

Specimens were collected from Gullery's Swamp, in the south Manawatu (GR246809, NZMS 260 Sheet S24), on 24.1.92. Ripe flowering stalks were cut and the heads inverted into bags. The specimens were air-dried for 5 days then shaken over sheets of paper, releasing pollen, anthers, hairs and other flower inclusions. The pollen was easily separated from this mixture by stirring through a fine mesh tea strainer.

Sub-samples of the strained pollen were taken and weighed for oven drying and freezing, and a sample stored in a glass bottle for botanical analysis.

(iv) Results and discussion

The moisture percentage of the pollen samples was low, measuring only 30.82 ± 1.08% (three replications) by oven drying, and 30.80% by freeze-drying. This low value is seen to reflect the maturity of the ripe male catkins, and there may also have been some moisture loss during the 5 days of laboratory air-drying prior to releasing the pollen from the catkins.

The lipid content was low, measuring 2.81 g/100 g dry weight.

The nitrogen and protein levels were relatively high, at 2.95 and 15.28 g/100 g respectively, showing a protein to nitrogen ratio of 5.18 to 1.

The total dietary fibre level was the lowest in the study, at 16.06 g/100 g. The insoluble fibre component, 3.95 g/100 g, was also the lowest in the study, but the level of soluble dietary fibre was relatively higher (per gram dry weight), measuring 12.11 g/100 g.

A summary of proximate analyses is shown in Table 9.1 (p99).

The elemental analysis of the pollen sample showed only one mineral to be present at an unusually high level. This was phosphorous, which was recorded at 6,501 µg/g, and this was by far the highest value for this mineral of all plant samples analyzed. Also none of the nutritional minerals or trace elements were recorded at unusually low levels. The presence of heavy metals was found to be either minimal or they were below the detection limit.

Data obtained by PES is shown in Appendix 3.1 (p198).

For the microscopy analysis, treatment of this sample was specific (see Histological methods and Microtechnique Part 1, III). As no sectioning was performed, microscopic botanical analysis was limited to examination of surface features. Plates 9c and d (p97) show the form of the grains to be alete and monoporate, and Plates 9d reveals the sculpturing of the exine to be rugulate.

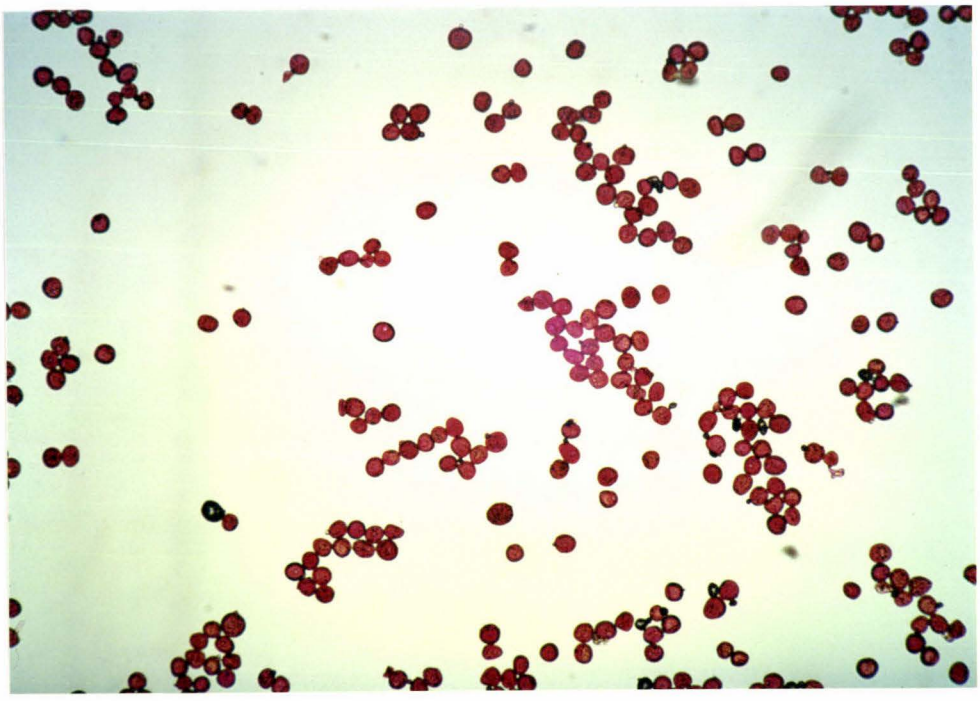
Plate 9a. *Typha orientalis*: *in situ*; flowering spikes in marshland, S. Manawatu.

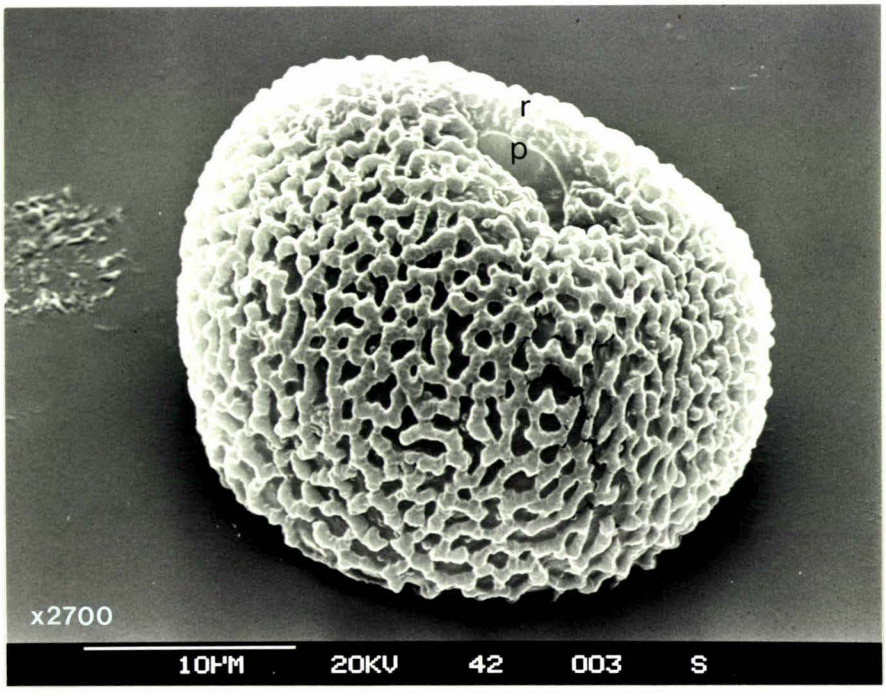
Plate 9b. *Typha orientalis*: male and female catkins; **m**, mature male catkin, yellow with pollen; **f**, female catkin; **fs**, female catkin at stage of seed dispersal; **b**, bare stem separating male and female catkins.


m
b
f
f s

Plate 9c. *Typha orientalis*: pollen grains, stained with basic fuchsin.

Plate 9d. *Typha orientalis*: pollen grain, electron micrograph: **p**, single pore; **r**, rugulate sculpturing of the exine.




r
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003
S

In order to draw any comparisons between the pollen grain structure and the various proximate analyses, it would be necessary to know to what extent the powdered exine has contributed to these measured components. This is suggested as the exine is known to be extremely resistant to breakdown, and withstands high temperatures and treatment with concentrated acids and bases.

The chemical substances (sporopollenins) responsible for exine resistance were shown by Brooks and Shaw (1968) to be formed by oxidative polymerization of carotenes and carotene esters (Faegri and Iversen 1989), and Traverse (1988) considers the compound to have a carotenoid-terpenoid structure.

Additionally, it was found that even after milling the pollen sample, the proportion of intact to broken down grains was approximately 75%. A test for protein was carried out on a freshly-milled pollen sample on a microscope slide, using Coomassie Blue stain, and this gave a positive reaction only for the cell contents from the ruptured cells. This may suggest that the protein content is in fact much higher than shown by proximate analysis. A similar test for lipid, using Sudan Blue stain, showed no apparent colour development in either intact or ruptured cells. A test for starch, using iodine stain, gave a strong positive reaction for this component in the exuded cell contents.

The intine is composed partly of cellulose but has higher proportions than most cell walls of pectic substances, callose and other polysaccharides. This layer, unlike the exine, is very short-lived. However, the carbohydrate levels from the proximate analyses were low except for soluble sugar and a relatively lower level of starch.

Table 9.1 *Typha orientalis*: pollen
Proximate analyses per 100 g dry weight.

A.Moisture:	
oven-dried	30.82
freeze-dried	30.80
B.Lipid	2.81
C.Nitrogen	2.95
D.Protein	15.28
E.Dietary fibre:	
insoluble	3.95
soluble	12.11
F.Soluble sugars	22.82
G.Starch	9.27

(For details of replications and analytical procedures see Part 1, Procedures and analytical methods).

10. *Beilschmiedia tawa* (A.Cunn.) Benth. et Hook.

family: Lauraceae

Tawa

(i) Description and discussion

Beilschmiedia tawa is a tall tree reaching 25 metres in height. It has a spreading crown and a wide trunk, up to 1.2 metres across. The bark is smooth, dark grey, and even-textured. On older trees, which are usually partially covered with lichens and mosses, stout buttressed roots develop. The leaves are narrow and willow-like, up to 10 cm long. The upper surfaces of young leaves are pea-green and are usually covered with silky hairs; the paler undersurfaces are often faintly blue and have distinct midveins. The flowers are small, on panicles up to 8 cm; they arise between the axils of the branchlets towards the tips of the branches. The fruit is a large ovoid drupe, up to 3 cm long, very dark purple with a distinctive bloom. Drupes are produced usually in large numbers, in late spring and summer (October to March).

The distribution of *tawa* is throughout the North Island and the upper part of the South Island. It grows in lowland and montane forest from sea level to 800 m, and is the most common tree in many North Island forests.

References:
Allan 1961; Salmon 1980; Cheeseman 1925; Hooker 1867.

(ii) History

It was the kernels (see Plate 10a, p104) of this tree which were used for food. Colenso (1880:29) recorded the "large, hard, stony seeds of the plum-like drupe of the *tawa* " being used by natives of the interior. The kernel is long and hard, resembling a date stone, and these were collected in large quantities, gathered from beneath the trees, then steamed in the earth ovens for 2 days (Colenso 1880). This, Colenso (1880:29) wrote "does wonders, and makes them serviceable to man". The cooked kernels stored well and were sometimes kept for years. They were one of the staple foods of the Tuhoe (Best 1942). After cooking, kernels were washed and spread out to dry; to eat, dried kernels were steamed again then mashed. Cooked kernels were dark brown, and the

water in which they were soaked was quite thick when the *tawa* was ready to eat (Makereti 1938); the taste of the kernels was likened to potato.

Although the fruit resembles a dark coloured plum, the raw flesh is not very palatable. It was, nevertheless, recorded by Colenso (1880), and other writers last century, as an edible seasonal forest fruit. Called *pokere*, it was collected by children, and described as "very sweet with a slight flavour of turpentine" (Taylor 1848). Allom (1849) wrote of it as "most grateful to the palate and remarkable for the coolness which it imparts to the mouth".

(iii) Collection and organ preparation

Fruits were collected on 24.3.92 from under forest trees in the Esplanade Gardens, Palmerston North (GR323894, NZMS 260 Sheet T24). For the botanical specimen, a whole fruit was preserved. For the analyses, samples were prepared of both raw and cooked material. For the raw sub-samples, berry flesh was removed from the kernels using a scalpel and the kernels were cleaned, rinsed and wiped dry. For freezing, each sub-sample comprised 20 kernels.

For the preparation of a cooked sample, 20 whole fruits were weighed, and cooked by simmering for 2 hours. The flesh was rubbed off when cold, and the kernels washed in distilled water. This preparation was frozen, freeze-dried and weighed prior to milling.

(iv) Results and discussion

For the untreated samples, oven drying was unusually prolonged; kernels continued to lose moisture for 12 days. The freeze-drying was not complete, to constant weight, for 5 days. The measurements for moisture content obtained by these weighing procedures were 57.23 ± 2.75% (three replications), and 50.58% respectively.

The lipid content was relatively low at 2.78 g/100 g. Nitrogen and protein measurements were also relatively low, at 1.66 and 8.37 g/100 g respectively; these values show a high nitrogen to protein ratio, suggesting either the presence of non-protein nitrogen or an incomplete protein extraction. The addition of 3 M sodium hydroxide was required to solubilize mucilage-bound protein in the buffer extraction stage of the Bradford procedure. This extraction was extremely mucilaginous and the residuum poorly defined.

Although the addition of the alkali made the extract apparently homogenous, measurement of the protein in this showed a nil presence. All measured protein was provided by the hydroxide extraction of the former residuum.

The total dietary fibre measured 53.41 g/100 g which was one of the highest values for this component in the study. Insoluble and soluble fibre values were 30.88 and 22.53 respectively.

The measured level of soluble sugar was 4.29 g/100 g, and of starch was 20.91 g/100 g.

Analysis of the cooked kernels showed some dissimilarities from the above results, mainly in the carbohydrate components. The cooking water had not been retained as this included pericarp extract and debris from the pericarp. The decanted water was very thick, brown and mucilaginous.

The moisture content of these samples was 54.46% by freeze-drying. The lipid measurement was slightly raised (per gram dry weight) by cooking, to 3.00 g/100 g. Nitrogen and protein measurements were also both higher (per gram dry weight). The mucilaginous interference in the Bradford procedure was treated as before, and again the protein recovery from this extraction was nil. The values for these components were 1.86 and 9.62 g/100 g for nitrogen and protein respectively, showing a slightly higher figure (5.17 to 1) for the protein to nitrogen ratio. The dietary fibre measurements were considerably raised (per gram dry weight) by cooking. The total fibre increased by 34.13%, and insoluble and soluble components by 37.18% and 29.96% respectively. The soluble sugar level was relatively low at 4.29 g/100 g , and starch was relatively much higher (per gram dry weight) at 20.91 g/100 g.

A summary of proximate analyses is shown in Table 10.1 (p105).

Elemental analysis showed only one outstanding level in the untreated sample; this was nickel which was found at a concentration of 7.8 μg/g. This high level of nickel was lost in the cooked sample, where it was measured to be only 0.95 μg/g. Another metal value which was notably reduced in the cooking was aluminium, which was decreased from 20 μg/g to less than 3.4 μg/g.

Data obtained by PES is shown in Appendix 3.1 (p198).

Examination of the prepared section shows the kernel to contain many hexagonal grain-filled cortical cells, and larger vacuolated cells (see Plate 10b, p104). In the former, the grains are small, round and numerous in most of these cells.

The larger cells are suggestive of mucilage cells, and these are interspersed amongst the ground tissue. Surrounding the cortex is a single-cell layer of epidermis; these cells are characteristically small and isodiametric.

Plate 10a. *Beilschmiedia tawa*: whole kernels.

Plate 10b. *Beilschmiedia tawa*: kernel, transverse section, stained with safranin and "fast green"; **c**, cotyledonous ground tissue; **e**, epidermis; **m**, mucilage cell; **p**, pericarp tissue.


cm 1 2 3

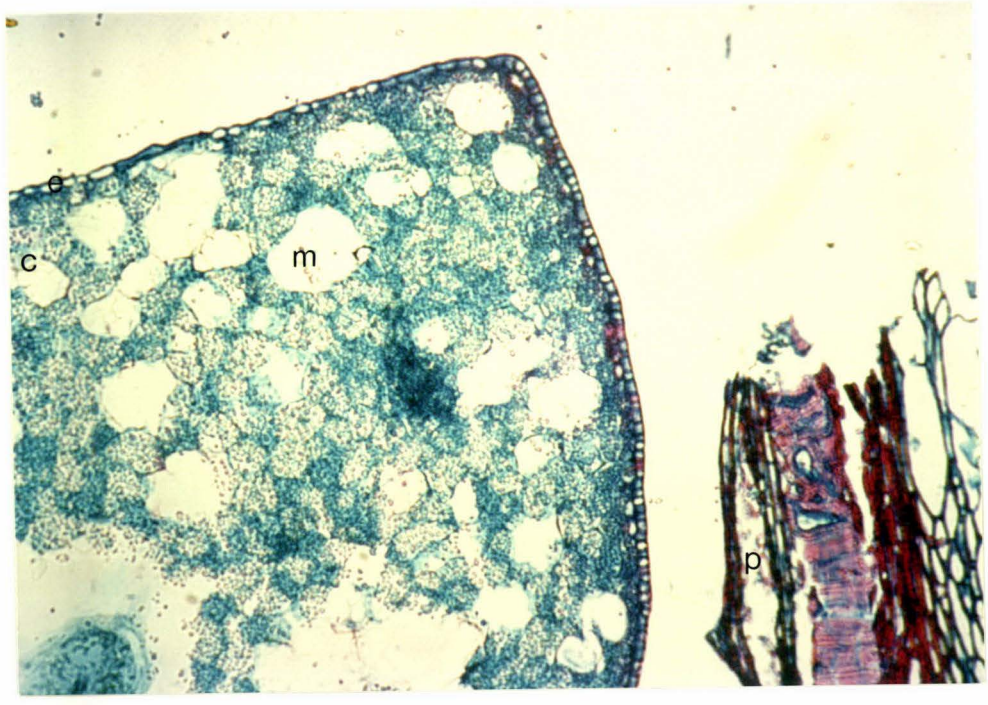

e
c
m
p

Table 10.1 *Beilschmiedia tawa*: kernels
Proximate analyses per 100 g dry weight

	untreated	**cooked**
A.Moisture:		
oven-dried	57.23	-
freeze-dried	50.58	54.46
B.Lipid	2.78	3.00
C.Nitrogen	1.66	1.86
D.Protein	8.37	9.62
E.Dietary fibre:		
insoluble	30.88	42.36
soluble	22.53	24.28
F.Soluble sugar	4.29	3.08
G.Starch	20.91	16.80

(For details of replications and analytical procedures see Part 1, Procedures and analytical methods).

11. *Marattia salicina* Smith

family: Marattiaceae

Horseshoe fern, King fern

Para

(i) Description and distribution

This is the largest herbaceous fern in New Zealand, with stipes up to 100 cm long. The laminae are ovate and 2-pinnate, up to 4 m long and may be 2 metres across. They are dark green and glossy, with a stiff texture. The secondary pinnae are oblong, often about 15 by 2 cm, with tapering apices. The veins are free and parallel, and near the vein endings on the undersides of the secondary pinnae are clusters of contiguous sporangia. Each cluster is boat shaped and forms a synangium, composed of 2 rows of sporangia.

The stipe bases are characteristic in this species. They are articulated with thick, fleshy scales on the rhizome and have two large basal adnate auricles. Each scale (see Plate 11a, p111) is formed by the enlargement of a stipe base, the stipe separating by an articulation above the swelling when the frond is ready to be abscised. The articulated surface of the scale shows a scar mark resembling a horse shoe imprint. The common English name for the fern is derived from this. Buchanan (1876) considered that the root stock approached the scaly bulb more in structure than the fern rhizome, but without a central mass around which the scales might be arranged in order. The continued production of scales upwards and outwards often raises the rhizome above the surface of the ground. The bud swells to a considerable size before the crozier bursts through the cuticle. During this process the latter is split and the edges carried upwards forming the auricles; these remain as a sharp ridge round the scar where the stipe articulated. The new stipe begins to swell at its base at a very early period, spreading over the adjacent surface and forming a new scale. Buchanan (1876) wrote that this form of root stock could be named a scaly sub-aerial rhizome without internodes.

Independent roots only appear from frond buds of scales which are in contact with the soil. These roots are long and fleshy, about 5 mm across (see Plate 11c, p112); they break easily and are slightly mucilaginous.

The growth of the *Marattia* rhizome is remarkably slow, being only about 2 cm in one year. By a process of renewal and movement it lives for an indefinite time, shifting its position in the ground by its outward growth, and the exhausted scales accumulate in a hard mass. In this way rings or detached clumps may be formed concentrically from the original rhizome (Buchanan 1876).

Marattia salicina is sparsely distributed in the northern and eastern parts of the North Island. It grows in lowland forests, usually in rich, damp soils, and is frost tender. The same species is found in Australia, India, South Africa, South America and the Pacific Islands.

References:
Allan 1961; Brownsey and Smith-Dodsworth 1989; Bold *et al.*, 1987; Dobbie 1930; Cheeseman 1925; Buchanan 1876; Hooker 1867.

(ii) History

The edible organ of the fern was the rhizome, described as the "large, scaly bract-like pieces of the big tuberous root" (Colenso 1880:29). He wrote that this was formerly prized as an article of food. Makereti (1938) also recorded that it was an important food of her people. It was formerly cultivated by the Maori near their villages. It has been listed as one of 4 plant species which were planted by Maori before European contact, the others being *Ipomoea chrysorrhiza*, *Caladium esculentum* and *cucurbita* (Reischek 1830).

Makereti (1938) wrote that the Maori referred to this root when he said that he always had potatoes in New Zealand, and the tuberous mass was separated into many parts and planted. The fleshy part was cooked by roasting or boiling. Taylor (1855) recorded that it was a great delicacy, "sweet and mealy, and pronounced excellent".

No preferred harvesting season was specified by Colenso.

(iii) Collection and organ preparation

Specimens of both scales and roots were collected from a plant on Massey University Campus (GR332880, NZMS 260 Sheet T24), on 16.10.91. The rhizome mass rose to about 10 cm above the ground, and the scales appeared as angular brown protrusions, looking as if they were hard and woody. Some scales were cut away for analysis, using a knife, and the tissue cut without any resistance, revealing a pink interior. Roots were also included in this investigation; these were found to run in a lateral and downward direction from the base of the rhizome.

Some scales were partly covered with moss. They were cut to remove the dark outer cuticle, some of which could be pulled off quite easily in strips. Samples of both roots and scales were washed and rinsed with distilled water, and sub-samples taken.

(iv) Results and discussion

The scale sample was measured by oven-drying, to have a moisture content of 75.25 ± 1% (three replications); this moisture loss was not completed for four days. By freeze-drying the calculated figure for moisture content was almost identical, at 75.37%, this procedure requiring three days to reach constant weight.

The lipid content was measured to be 2.18 g/100 g, one of the lowest lipid values in this study.

The nitrogen and protein components were also measured to be very low, at 1.22 g/100 g and 6.62 g/100 g respectively. This shows a relatively low protein to nitrogen ratio of 5.43 to 1. In the protein extraction, the supernatants had appeared colourless to pink, and clear, without obvious mucilage, although the residuum had been poorly defined.

The dietary fibre figures comprised a high soluble fibre component of 23.68 g/100 g and a much lower insoluble fibre component of 17.96 g/100 g.

The starch component was also high at 34.10 g/100 g, this being one of the highest starch values in the study. The soluble sugar content was 6.40 g/100 g.

A summary of proximate analyses is shown in Table 11.1 (p113).

In the elemental analysis of the scales, high levels were found to be present for copper,

aluminium and strontium. The values for copper and aluminium, 26.0 µg/g and 1,692 µg/g respectively, were the highest recorded levels for these elements. The measurement for iron was found to be one of the lowest recorded levels for this mineral.

Data obtained by PES is shown in Appendix 3.1 (p198).

For the root sample, the moisture content was found to be higher than that of the scale. By oven-drying, this was measured to be 80.53 ± 2.4%, requiring five days to reach constant weight. By freeze-drying the moisture content was 87.49%. The oven-dried samples required five days to reach constant weights; replication error was within 2.4%.

The lipid content of this sample was calculated to be 74.23 g/100 g, but this extremely high value must be qualified. After evaporation of the di-ethyl ether, following the extraction process, residual liquid remained in the flask; this was measured to be 1.3 ml. It is suggested that this solvent-extracted component was largely made up of specific volatile plant oils, and this interference has masked the true lipid measurement.

The nitrogen and protein levels in the root sample were both measured to be higher (per gram dry weight) than in the scale sample. These values were 1.55 g/100 g and 7.69 g/100 g respectively, showing a protein to nitrogen ratio of 4.96 to 1, considerably lower than the previous calculated ratio. Again, there was no overt mucilage detected in the protein extraction.

The dietary fibre levels were far higher than those of the scales, with a total NSP value of 58.51 g/100 g. The soluble fibre component was measured to be 30.39 g/100 g which was the highest of all samples analyzed here. The insoluble component was measured to be 28.12 g/100 g.

Both soluble sugar and starch values were considerably lower here than in the scale sample, measuring 3.74 g/100 g and 11.94 g/100 g respectively.

A summary of proximate analyses is shown in Table 11.1 (p113).

Elemental analysis of the root sample showed high levels of copper, iron, chromium, boron, aluminium and strontium. Of these the copper figure was very similar to the high

level recorded in the scale sample. The iron level, notably low in the scale sample at 27.3 μg/g, showed a presence of 444.0 μg/g in the root sample. The high aluminium level was similar to that in the scale sample, and the strontium level was more than doubled in the root sample, at 69 μg/g.

The prepared sections of these two organs are shown in Plates 11b (p111) and 11d (p112). Plate 11b shows part of the scale cortex beneath the layer of bark. The superficial cortical cells were seen to be densely packed with large round grains suggestive of starch grains, but the deeper cortical cells appeared devoid of these. Numerous orange-coloured spots appear throughout the section, suggestive of a resinous fluid. In a fresh hand-cut section of the scale examined microscopically, some cells appeared deep pink and some bright amber. When newly cut, a viscid gum-resinous matter exuded on the surface. An iodine test was carried out to identify the nature of the grains in the superficial cells, and this gave a positive colour development for starch, confirming the high figure obtained by proximate analysis. Vascular tissue appears fairly heavily lignified and stained with safranin.

The root section in Plate 11d (p112) shows the central part of the exarch actinostele which has 16 protoxylem groups. These radiating bundles are distinctly wedge-shaped, and this feature has been considered by Buchanan (1876) to indicate that the root shows a stronger affinity in structure to the Equisetaceae than to the ferns.

Peripheral to the stele, the cortex is composed of parenchymatous cells and very large lacunae. The parenchyma cells contain numerous elliptical inclusions suggestive of either large starch grains or amyloplasts. Also in several cells, both in the cortex and the stele, are dark, often stellate, masses. To identify the presence of starch in the root, a fresh hand-cut section was prepared and iodine applied. The inclusions did not show a positive colour development, further suggesting that they are amyloplasts. This finding is in keeping with the level of starch, which was not notably high, found by proximate analysis. To identify the presence of lipid, Sudan Blue was similarly applied; no colour development was detected suggesting the actual lipid component is low and, as suggested above, the high figure obtained by proximate analysis was caused by volatile oil or resin interference. The section shows abundant bright amber and pink cells which may suggest the presence here of specific plant resins or volatile oils.

Plate 11a. *Marattia salicina*: rhizomal scales, cut from above-ground portion of rhizomal mass.

Plate 11b. *Marattia salicina*: scale, transverse section, stained with safranin and "fast green"; i, inner tissue of scales; r, resin canal.

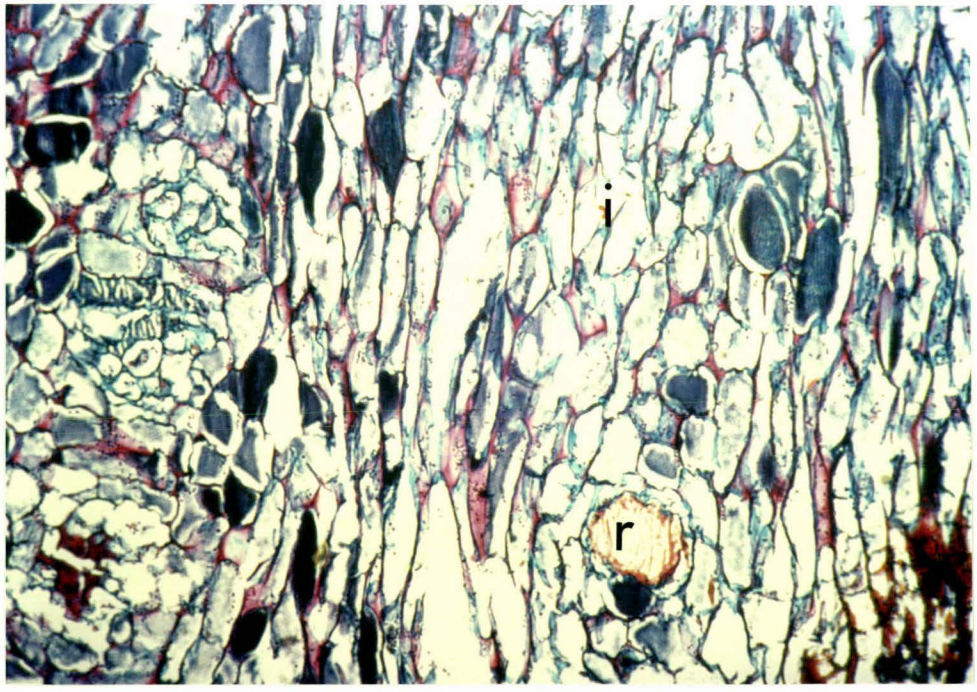

i
r

Plate 11c. *Marattia salicina*: roots.

Plate 11d. *Marattia salicina*: root, transverse section, stained with safranin and "fast green"; **p**, parenchymatous cells of stele; **r**, resin cells; **x**, inner portion of wedge-shaped xylem arm of polyarch stele.

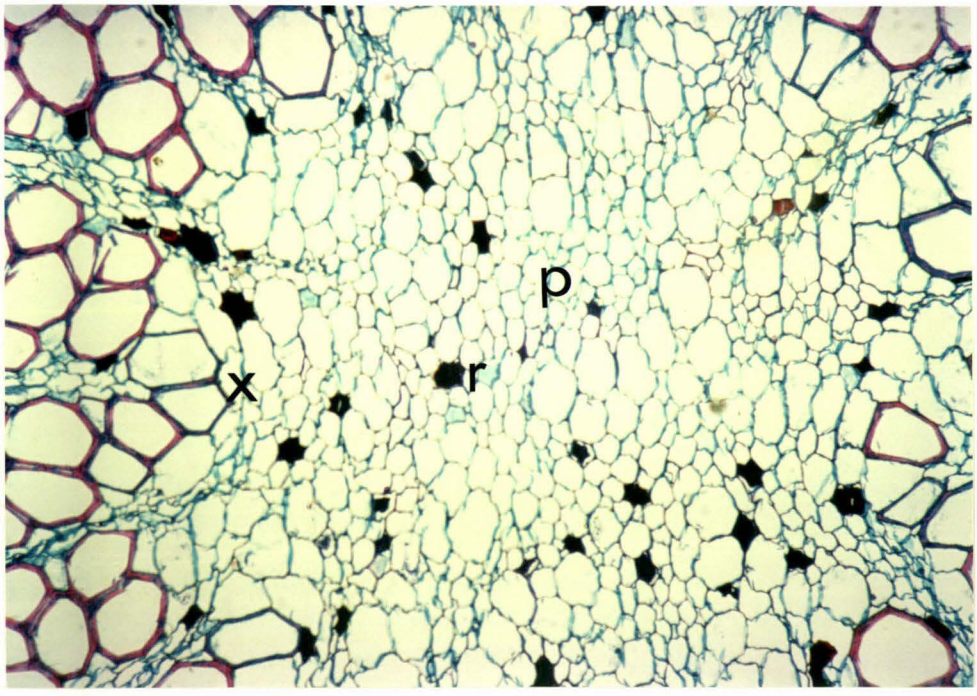

p
x
r

Table 11.1 *Marattia salicina*: rhizomal scales; roots
Proximate analyses per 100 g dry weight

	rhizomal scales	**roots**
A.Moisture:		
oven-dried	75.25	80.53
freeze-dried	75.37	87.49
B.Lipid	2.18	74.23
C.Nitrogen	1.22	1.55
D.Protein	6.62	7.69
E.Dietary fibre:		
insoluble	17.96	28.12
soluble	23.68	30.39
F.Soluble sugar	6.40	3.74
G.Starch	34.10	11.94

(For details of replications and analytical procedures see Part 1, Procedures and analytical methods).

12. *Porphyra columbina* Montagne

family: Bangiaceae

Purple laver

Karengo

(i) Description and discussion

The leafy fronds of *Porphyra columbina* can vary in shape from broad and curved to ribbon-like. Often the edges are wavy and folded, giving the plant a rosette-like appearance. Fronds are usually 10 to 25 cm long but may reach about 70 cm. Frond texture is fairly tough and slightly elastic, and colour varies from greeny brown to purple. On desiccation the fronds stretch like tight membranes over the rough rock surface, and soften again on wetting.

This description applies only to one of the two generations of *Porphyra*; the previously unexplained reproductive life history was investigated by Drew (1949) using a closely related species of this genus, *P. umbilicatus*. Further studies have shown that *P. columbina*, like most other species of *Porphyra*, has a heteromorphic life history, with alternation of a foliose thallus and a filamentous conchocelis phase (Drew 1955; Conway and Cole 1977). The conchocelis generation is filamentous, like strands of fine branched hair up to 200 mm long, growing on shells, whereas the generation described above grows high on rocky shores. These two forms are so dissimilar that each was formerly classified separately. Spores shed from *Porphyra* plants develop into the microscopic or conchocelis stage which survives over the "off-season" when the *Porphyra* blades disappear. Spores develop under favourable conditions to form *Porphyra* plants. Different species of *Porphyra* show variations in the life history pattern and seasonal growth patterns.

Although the foliose generation resembles an *Ulva* in superficial form, *Porphyra* is in fact one of the Protoflorideae, the most primitive of the red algae.

Porphyra columbina grows at mid to high tide levels on most exposed coasts in New Zealand, especially the East Cape area. It favours rough coasts with fairly clean water and does not tolerate estuarine conditions.

References:

Morton 1968; Moore 1963; Nelson 1985.

(ii) History

Colenso (1880:29) wrote "Another peculiar plant was the *karengo* (*Laminaria* sp.), a seaweed found growing in abundance on the flat clayey tidal rocks of the East Coast". The opinions of Dr C O'Kelly (Department of Plant Biology, Massey University) and Dr W Nelson (National Museum of New Zealand) were sought in specifying the plant referred to here by Colenso. W Nelson (pers. comm.) wrote that the term "*karengo*" in Colenso's 1880 paper was far more important and reliable than "*Laminaria*". *Karengo* was only used to refer to species of *Porphyra*, whereas *Laminaria*, in the mid-1800s, was understood to include flat, ribless, bladed algae; therefore Colenso was merely reflecting the understanding of his time about algae rather than providing a name with current meaning. Dr Nelson also suggested that it is safer to consider that *karengo* is equivalent to *Porphyra* spp., rather than *P. columbina*, due to the immense variation between, and within, species.

Porphyra was collected in the winter, when fronds were at their greatest size, long, dark and tender. They were sun dried and closely packed away in baskets (Colenso 1880). *Karengo* was prepared by steaming in the earth oven and was deemed a delicacy. It has been considered to be a good supply of both vitamins and minerals, but with an otherwise low food value (Bradstock 1985). Stack (in Reed 1935) wrote that lightly boiled it tasted like a sweet cabbage, although fresh from the sea the taste was somewhat unpleasant. The dried seaweed was chewed and reported to be refreshing and thirst-quenching.

The related species, *P. laciniata*, is eaten as laver, slack or sloke in Britain, especially in Wales, where it is soaked and boiled. Other species are eaten by people over much of the world, including Japan where *Porphyra* is cultivated as nori .

(iii) Collection and organ preparation

Porphyra specimens were harvested on 3.9.91 from Porirua Bay on the Kapiti Coast (GR684180, NZMS 260 Sheet R26), from rocks below high tide. All plants were small, about 5 cm in length.

Plants were harvested carefully, not contaminated with other seaweeds and free from debris, shells and stones.

The specimens were rinsed in distilled water and drained.

Sub-samples were taken for oven drying and freeze-drying, and one for cooking. A further sample was air-dried and kept for botanical analysis, as FAA is not a suitable preserving medium for algae (C O'Kelly, pers. comm.).

The weighed sample for cooking was boiled gently in a pan with distilled water until the seaweed was soft, about 40 minutes. The hot sample was transferred to a glass dish and left overnight to cool; by morning it had become gelatinous but was not firm. The whole mass was transferred to a pre-weighed bag and frozen, then freeze-dried and weighed prior to milling.

(iv) Results and discussion

The moisture content of the untreated seaweed sample was measured to be 83.03 ± 2.3% (three replications) by oven-drying; the drying time required was 24 hours. The moisture content calculated by freeze-drying was found to be slightly higher, in this case 90.67%, and the required drying time was again 24 hours.

The lipid component was measured to be relatively low, at 2.64 g/100 g.

The nitrogen and protein levels were found to be 2.01 g/100 g and 9.61 g/100 g respectively. The apparent mucilaginous component of the fresh thallus did not present any interference in the protein extraction; the supernatant was clear and colourless but not overtly mucilaginous, and the residuum well-defined. The protein to nitrogen ratio based on these measurements was very low, at 4.78 to 1, suggesting the possibility of the presence of non-protein nitrogen.

The dietary fibre values were very high for the insoluble component and very low for the soluble component. These were measured at 40.37 g/100 g and 10.21 g/100 g respectively.

The soluble sugar level was also very low, at 1.18 g/100 g and starch was marginally higher at 3.18 g/100 g.

A summary of proximate analyses is shown in Table 12.1 (p120).

The elemental analysis of the untreated sample showed extremely high levels of sodium (66,361 µg/g and sulphur (25,991 µg/g); these were both the highest recorded levels for these minerals in this study. Other very high levels were those of boron (46.5 µg/g), iron (235 µg/g), aluminium (177 µg/g), arsenic (32.5 µg/g) and strontium (55 µg/g). No minerals were recorded to be at exceptionally low levels.

Data obtained by PES is shown in Appendix 3.1 (p198).

The samples used for elemental analysis in this case were not from the same collection sample as used for the proximate analyses. Material was obtained from Pacific Harvest Ltd, as dried *karengo*; this is claimed to be hand-harvested from its natural seabed in a remote area of the South Island, and carefully sun-dried.

The proximate analyses of the cooked sample showed a moisture content of 83.22% by freeze-drying, requiring 24 hours drying time.

The lipid measurement was further decreased by cooking, to 2.08 g/100 g.

Nitrogen and protein components were both marginally higher (per gram dry weight) in the cooked samples, measuring 2.12 g/100 g and 10.39 g/100 g respectively. Again no mucilaginous interference was apparent in the protein extraction. The protein to nitrogen ratio was similar to that estimated for the untreated sample, at 4.90 to 1.

The dietary fibre values were notably altered by cooking. The previous high level of insoluble fibre was reduced by approximately 50% to 20.97 g/100 g, and the previous low level of soluble dietary fibre was approximately doubled (per gram dry weight), to 21.48 g/100 g.

Both the soluble sugar level and the starch level were further reduced by cooking, to 0.74 g/100 g and 2.53 g/100 g respectively.

The elemental analysis of the cooked sample showed high levels (per gram dry weight) of copper (10.1 μg/g), sodium (44,917 μg/g), potassium (37,712 μg/g), iron (353 μg/g), silicon (83 μg/g), sulphur (24,051 μg/g), chromium (3.9 μg/g), boron (31.0 μg/g), aluminium (197 μg/g), arsenic (21.5 μg/g) and strontium (48.8 μg/g). The potassium level was twice as high (per gram dry weight) as in the uncooked sample and the sodium level had reduced by approximately one third. Although iodine has not been determined in these analyses, it is well recorded that seaweeds in general have a very high iodine content compared with other plants (Chapman 1979).

Examination of the prepared unstained microscope slide shows a surface view of the monostromatic frond (see Plate 12a, p119). The cell walls are notably thickened. These have been shown to be composed of complex polysaccharides including sulphated polysaccharides and mucilages (Chapman 1979). The cells possess one or two stellate chloroplasts with a pyrenoid, and the pink colour is due to the presence of the red-algal pigment r-phycoerythrin. Galactose has been shown to be the principal sugar residue together with porphyran, a galactose derivative (Boney 1966). The pink colour of the cell contents is due to the presence of the red-algal pigment r-phycoerythrin.

Plate 12a. *Porphyra* sp.: frond, surface view, showing thick-walled cells.

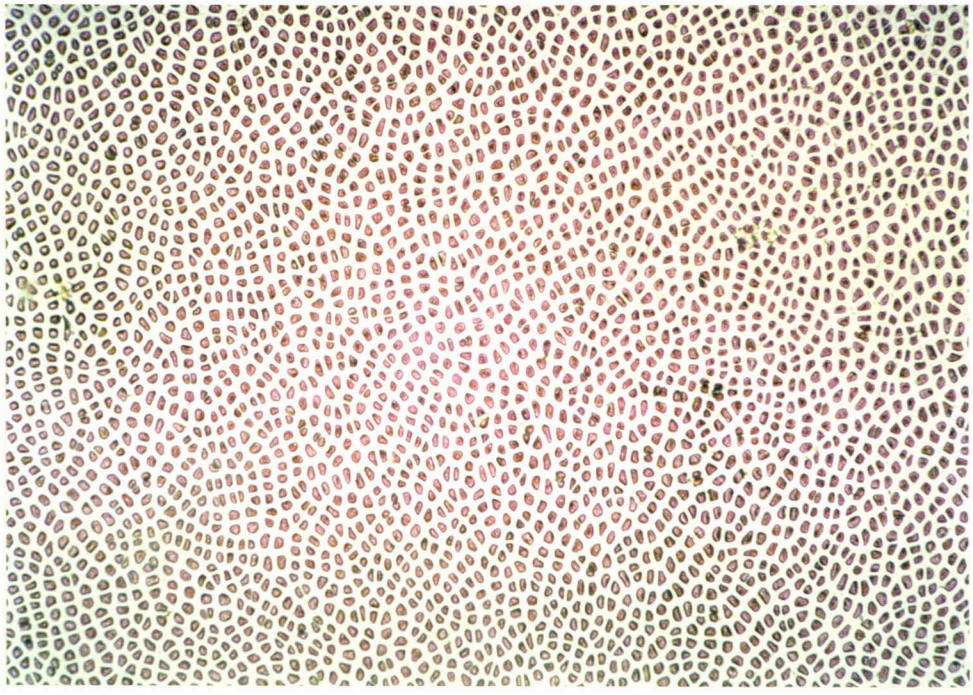


Table 12.1 *Porphyra* sp.: thallus
Proximate analyses per 100 g dry weight.

	untreated	**cooked**
A:Moisture:		
oven-dried	83.03	-
freeze-dried	90.67	83.22
B.Lipid	2.64	2.08
C.Nitrogen	2.01	2.12
D.Protein	9.61	10.39
E.Dietary fibre:		
insoluble	40.37	20.97
soluble	10.21	21.48
F.Soluble sugar	1.18	0.74
G.Starch	3.18	2.53

(For details of replications and analytical procedures see Part 1, Procedures and analytical methods).

13. *Auricularia polytricha* (Montagne) Saccardo.

family: Auriculariaceae

Ear fungus

Hakekakeka, Hakeke

(i) Description and distribution

The basidiocarp of this fungus is a thin ear-like growth, 10 to 50 cm across; it is brown on one side and a dull whitish grey on the other, or convex surface. The latter is composed of short, hair-like hyphal protrusions, giving the fungus its specific name *polytricha* (many-haired). When young, the flesh is soft and pliable, and this becomes hard and tough when older (see Plate 13b, p124). The colour also changes, becoming much darker on the inner or concave surface. This is the basidia-forming surface. The basidia produce long cylindrical epibasidia which extend to the surface of the hymenium. Each terminates in a conical sterigma bearing a basidiospore. When mature these account for the darkened concave surface. The type of basidium in *Auricularia* is the phragmobasidium, with one or more vertical septa. Between these two surfaces is a central gelatinous layer containing narrow hyphae.

This fungus lives on both live and dead trunks of forest trees, most frequently on *Melicytus ramiflorus* (see Plate 13a, p124), *Corynocarpus laevigatus*, and *Hoheria populnea*. It also occurs on *Beilschmiedia tawa*.

The allied European species, *A. auricula-judae*, is distinguished by its pinkish tint and mucilaginous concave surface. The latter species is quite rare in New Zealand; it is more distinctly ear-shaped than the rather cup-shaped young growth of *A. polytricha*.

References:

Kirk 1879; Gaumann 1928; Bold *et al.*, 1987; Hood 1992.

(ii) History

Colenso (1880) elaborated very little on this species or any of the fungi he listed. He wrote that *A. polytricha* was found plentifully in the woods but was not greatly esteemed. Best (1942) recorded many species of fungus that were eaten in former times by the

Maori, and included eleven regional Maori names for this species. Kirk (1879) wrote about the importance of *A. polytricha* as a food item, and of its plentiful distribution on decaying timber in all forest districts. He recorded that from 1872 to 1878 a total of 838 tons of the dried fungus was exported to China; this was the only species marketed and China the sole recipient.

The whole fungus was eaten, cooked usually with other vegetables.

The very closely related *A. auricula-judae* of Europe is eaten there as an ingredient of soups to which it imparts an excellent flavour.

(iii) Collection and organ preparation

Specimens were collected on 2.5.91 from Bledisloe Reserve, Manawatu (GR324882, NZMS 260 Sheet T24). The fungi were growing on the trunk of a dead tree, *Melicytus ramiflorus*, the colony extending from 30 cm to 200 cm above the ground along the upper aspect of the reclining tree. All samples collected were young and pliable.
Sub-samples were taken for oven drying and freeze-drying.
A portion of the basidiocarp was cut and preserved for botanical analysis.

(iv) Results and discussion

For complete moisture loss four days were required for oven drying the samples, and the moisture percentage was measured to be 78.62 ± 3.8% (three replications). This high deviation was considered to be due to possible variation in age of the basidiocarps although they were apparently similar. It was found that older basidiocarps had a much lower moisture percentage, calculated at 57.38%. The measured moisture content by freeze-drying was 82.71%

The lipid component was only 0.77 g/100 g, the lowest in this study.

Values for nitrogen and protein were 1.86 and 8.64 g/100 g respectively showing a very low protein to nitrogen ratio of 4.65 to 1. No mucilage was apparent in the buffer extraction of the protein and the pellet was clearly defined. However, only a very small proportion of the protein was supplied by this extraction, the greater proportion being obtained from the residuum by extracting with the 1 M alkali.

The carbohydrate measurements showed interesting extremes, with total and insoluble dietary fibre figures being the highest in this study, and soluble sugar being the lowest. The insoluble fibre measured 54.88 g/100 g, and the soluble fibre measured 28.27 g/100 g. The poorly represented soluble sugar was measured at only 0.09 g/100 g.

A value of 70.5% was recorded for the digestible carbohydrates in the dried fungus (A H Church quoted in Colenso 1880). This measurement does not match the results obtained here; it is more closely matched to the total carbohydrate figure, as the sum of non-starch polysaccharides, soluble sugar and starch.

A summary of proximate analyses is shown in Table 13.1 (p126).

Of the various minerals known to be essential for fungal nutrition, *viz.* nitrogen, phosphorous, potassium, sulphur, iron, magnesium, manganese and boron, these were all found, by elemental analysis, to be present in moderate proportions. Silicon was noted to be very low, and there were no high levels of any of the toxic metals measured.

For the microscopy section the cutting plane was medial to the basidiocarp, giving a longitudinal section through the tissues.

The upper and lower surfaces can be clearly distinguished although safranin has not been retained (see Plates 13c and d, p125).

Basidia are not apparent on the hymenial surface (see Plate 13d, p125). The specimen had been collected dry and preserved immediately in FAA, and spore-production would not have been active except under water-moistened conditions. The dark blue stained band along the hymenial surface, however, would be representative of discrete inactive basidia (P Long pers. comm.).

The central area of hyphae is the gelatinous portion.
The surface which macroscopically appears grey can be seen in the section to be covered with hair-like hyphal ends projecting at right angles to the surface (see Plate 13c, p125).
Data obtained by PES is shown in Appendix 3.1 (p198).

Plate 13a. *Auricularia polytricha*: basidiocarps on wood (*Melycitus ramiflorus*).

Plate 13b. *Auricularia polytricha*: mature basidiocarp; **Hm**, darker hymenial surface; **Hp**, grey hyphal surface.

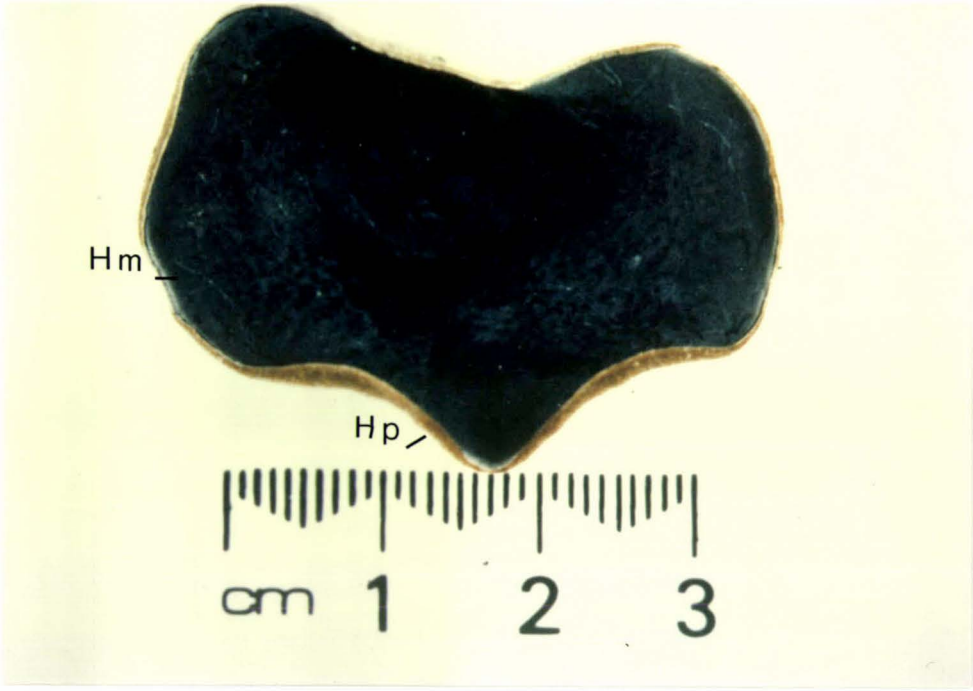
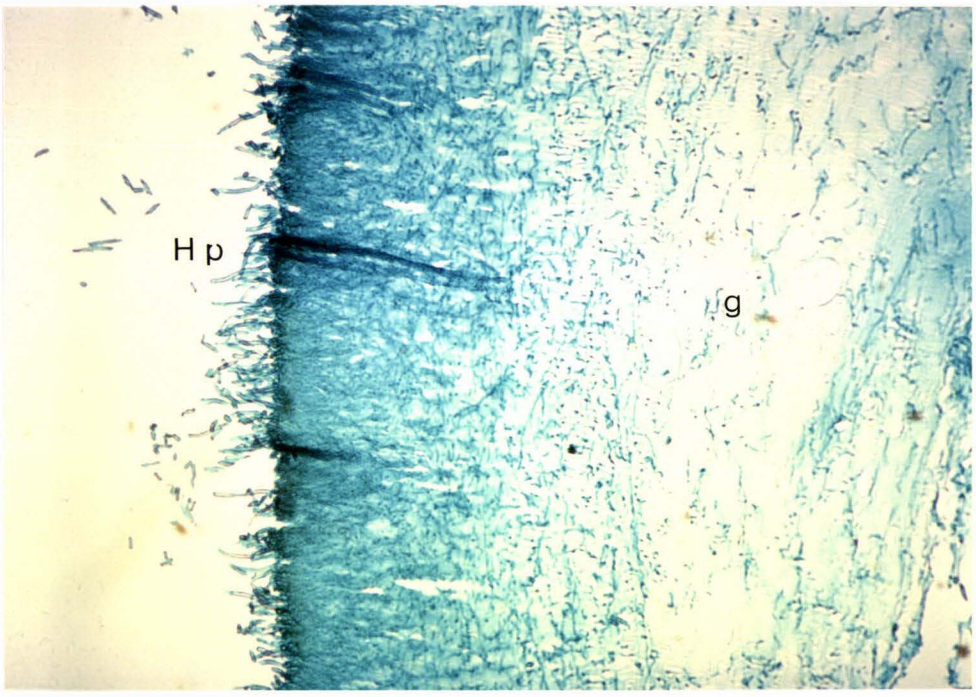

Hm
Hp
cm 1 2 3

Plate 13c. *Auricularia polytricha*: basidiocarp, longitudinal section, stained with safranin and "fast green"; **Hp**, hyphal surface showing "hair-like" hyphal ends; **g**, gelatinous central portion.

Plate 13d. *Auricularia polytricha*: basidiocarp, longitudinal section, stained with safranin and "fast green"; **Hm**, hymenial surface; **g**, gelatinous central portion.


H p
g

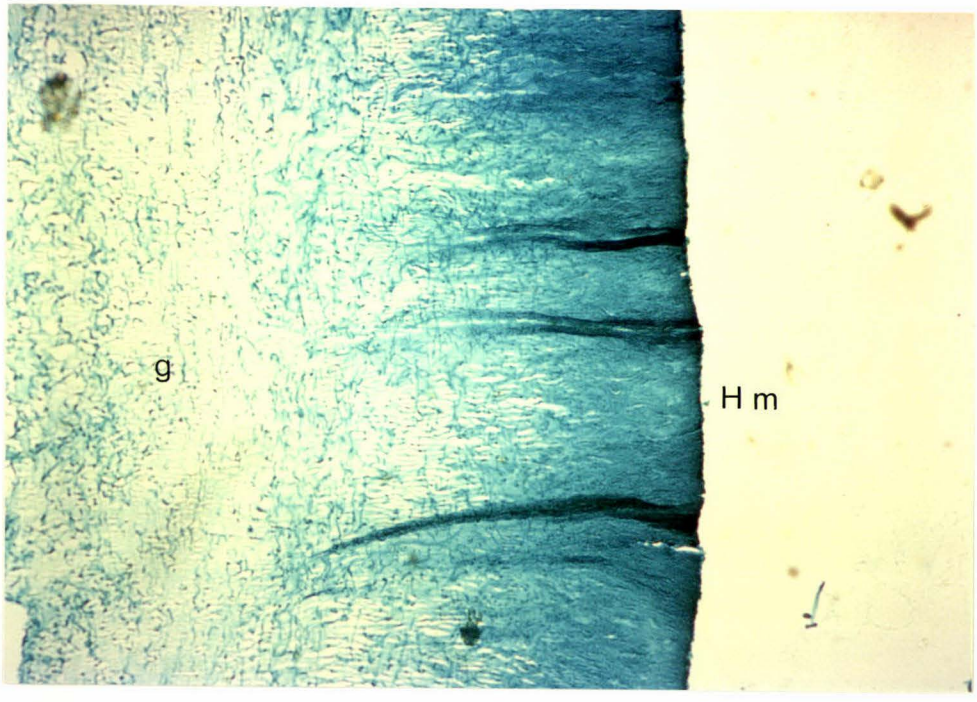

g
H m

Table 13.1 *Auricularia polytricha*: basidiocarp
Proximate analyses per 100 g dry weight

A.Moisture:	
oven-dried	81.97
freeze-dried	79.89
B.Lipid	0.77
C.Nitrogen	1.86
D.Protein	8.64
E.Dietary fibre:	
insoluble	54.88
soluble	28.27
F.Soluble sugar	0.09
G.Starch	3.11

(For details of replications and analytical procedures see Part 1, Procedures and analytical methods).

14. *Arthropodium cirratum* (Forst. f.) R.Br.

family: Liliaceae

Rock lily

Rengarenga

(i) Description and distribution

Arthropodium cirratum is a perfectly glabrous herb. It has many narrow-lanceolate leaves with fleshy bases, rising from stout fleshy rhizomes (see Plate 14a, p130). The flower stem is long, stiff and naked, usually well-exceeding the leaves. The panicle is large and branched, and flowers are white with 6 petals. The outer members of the perianth are narrow and the inner ones broader and thicker; 1 - 3 flowers appear together in uneven-aged groups along the panicle branches. Stamens do not exceed the perianth, and are filiform at the base and thickened in the middle. The anthers are tri-coloured: anther tails are purplish at the top, white in the middle, and golden yellow at the free curled ends.

In its natural habitat, *A. cirratum* grows to about 30 cm in height. It forms localised colonies in rock crevices. It is found in North and South Islands as far south as Greymouth and Kaikoura at latitude 42° 30' S. Flowers are produced in November and December. This attractive lily is also widely cultivated in gardens and grows to a robust plant of up to 100 cm. The rhizome is similarly much larger and fleshier when grown in a richer soil.

References:
Moore and Edgar 1976; Cheeseman 1925; Hooker 1867.

(ii) History

Colenso (1880) wrote that the thick, fleshy roots (*sic*) of the New Zealand lily were formerly eaten, cooked in the earth oven. He noted that the plant grew to a very large size in suitable soil and, as it was to be found around old Maori homes, he assumed that it was probably also cultivated.

(iii) Collection and organ preparation

Cultivated specimens were used in this study and were collected on 5.4.92 from a herbaceous border in the grounds of Massey University (GR324877, NZMS 260 Sheet T24).

The rhizomes were cut off, cleaned and rinsed. Rhizomes were measured to be about 8 cm long and 2.5 cm across. They were fibrous and hard to cut; laterals and roots were cut away and not included. The bases of the stems were very mucilaginous (clear and viscid), but the rhizomes appeared dry with no obvious mucilage.

Sub-samples were taken for proximate analyses and a portion preserved in FAA.

(iv) Results and discussion

The measurement for moisture loss from the *Arthropodium* rhizome samples by oven drying was 87.12 ± 0.76%; this drying was completed in 24 hours. By freeze-drying, the moisture content was slightly lower, measuring 78.01%, and a prolonged drying time of 72 hours was required.

The lipid component was measured at 3.88 g/100 g.

The nitrogen and protein values were both very low. For nitrogen this was only 0.85 g/100 g, and for protein 5.31 g/100 g, showing a protein to nitrogen ratio of 6.25 to 1.

The carbohydrate measurements showed the total dietary fibre component to be very similar to those of most other rhizomes in the study. The insoluble and soluble fibre components showed values of 16.64 and 19.80 g/100 g respectively. There was a relatively high recording for the soluble sugar content, calculated at 16.28 g/100 g. The starch component was the lowest in the study, at 1.22 g/100 g.

A summary of proximate analyses is shown in Table 14.1 (p131).

The elemental analysis revealed some unusually high mineral levels, notably of calcium (13,643 μg/g), zinc (203 μg/g), silicon (91 μg/g) and strontium (37.5 μg/g). No silica bodies have been identified in the section to explain the high level of this element. There

were also some unusually low mineral levels, as of sulphur, phosphorous and sodium.

Data obtained by PES is shown in Appendix 3.1 (p198).

The preparation of a satisfactory microscopy section proved technically difficult. The consistency of the cortex was particularly fragile, and examination of tissues is only possible internal to the damaged cortex. Safranin staining was also very poorly retained (see Plate 14b, p130).

The asymmetrical stele is seen to comprise an incomplete ring of between 10 and 15 vascular bundles. These are peripherally enclosed by a peristellar sheath but no starch sheath is present, confirming the findings in the above starch determination. Protoxylem and metaxylem both show minimal but discernable wall thickening.

Plate 14a. *Arthropodium cirratum*: rhizomes, intact; showing leaf bases and roots.

Plate 14b. *Arthropodium cirratum*: rhizome, transverse section, stained with safranin and "fast green"; **c**, cortex; **ps**, central parenchymatous cells of stele; **st**, stelar region.

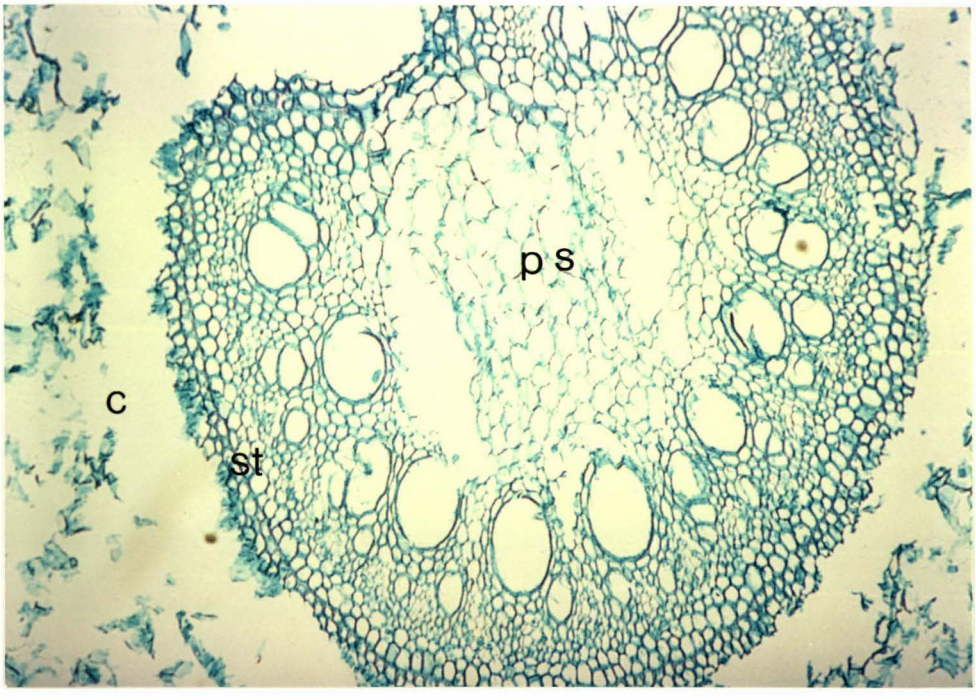

p s
c
st

Table 14.1 *Arthropodium cirratum*: rhizome
Proximate analyses per 100 g dry weight.

A.Moisture:	
oven-dried	87.12
freeze-dried	78.01
B.Lipid	3.88
C.Nitrogen	0.85
D.Protein	5.31
E.Dietary fibre:	
insoluble	16.64
soluble	19.80
F.Soluble sugar	16.28
G.Starch	1.22

(For details of replications and analytical procedures see Part 1, Procedures and analytical methods).

15. *Typha orientalis* C.B.Presl.

family: Typhaceae

Bulrush

Raupo

(i) Description and distribution

Descriptive details of *Typha orientalis* have already been given (see Plant 9, page ?). The underground organ is a thick, fleshy rhizome, 3 - 4 cm across, bearing numerous long fibrous roots (see Plate 15a).

(ii) History

Colenso (1880) wrote that the inner part of the white succulent root (*koreirei*) of *raupo* was eaten, largely raw, especially by children in the summer. He described the taste as being mild, cooling and refreshing, and not unpleasant.

Makereti (1938) also recorded that the succulent roots were a favoured food, generally eaten during the summer season. Crowe (1990), however, has written that these organs are most nutritious in autumn and early winter. Stack (Reed [Ed] 1936) spoke highly of the plant and likened the taste of the "white starchy matter" to arrowroot. Best (1942) also described the taste as rather pleasant; he added that these organs were reputedly high in both starch and sugar. Hildreth (1962) has recorded that rhizomes contain up to 46% starch and 11% sugar, but has given no citation for these analyses.

(iii) Collection and organ preparation

Typha orientalis rhizomes were collected from Gullery's Swamp in the south Manawatu (GR246809, NZMS 260 Sheet S24) on 3.4.92. The specimens were cleaned and sub-sampled. A length of rhizome was cut longitudinally and preserved in FAA. This was later embedded and positioned at right angles to the cutting plane to give a transverse section.

(iv) Results and discussion

The moisture loss by oven drying was 94.34 ± 1.4% (three replications), requiring 48 hours for constancy of weight. By freeze-drying, the moisture content was measured at 93.54%.

The lipid level was within the range for most other rhizomes in this study, at 4.96 g/100 g. A similar measurement, 3.71 to 4.91 g/100 g, was recorded by Claasen (1919).

Nitrogen and protein levels were both low, measuring 1.43 and 8.04 g/100 g respectively, showing a nitrogen to protein ratio of 5.62 to 1.

The total and insoluble dietary fibre components were high, but that of the soluble fibre was very low. Insoluble and soluble fibre values were 37.41 and 7.55 g/100 g respectively.

The soluble sugar component was also high, at 29.02 g/100 g, but starch was measured at only 2.82 g/100 g.

A summary of proximate analyses is shown in Table 15.1 (p135).

High elemental values were recorded for potassium (48,147 μg/g), iron (3362 μg/g), manganese (124 μg/g), silicon (187 μg/g), aluminium (412 μg/g), and strontium (28.5 μg/g). Manganese is often abundant in the epidermis of aquatic plants (Winton 1919), but no storage inclusions have been identified in these sections.

Data obtained by PES is shown in Appendix 3.1 (p198).

Examination of the prepared section shows the cortex to be composed of an aerenchyma of loosely packed, irregular cells, with intercellular spaces. The sheath surrounding the stele comprises a single column of small regular cells, finely thickened on their medial and lateral walls. The vascular bundles in the stele are numerous, arranged in an irregular row close to its periphery, with the phloem characteristically outermost, and the thickened vessels of the xylem well defined (see Plate 15b, p134).

Between the vascular bundles is a parenchymatous ground tissue, rather damaged in the section but still displaying readily identifiable cell inclusions. Notable among these are the raphides which are large and prominent. Small spherical starch grains are also present. The sparse distribution of these could be seen to reflect the low starch measurement in the above analysis.

Plate 15a. *Typha orientalis*: rhizomes, intact; showing leaf bases and roots.

Plate 15b. *Typha orientalis*: rhizome, transverse section, stained with safranin and "fast green"; **ac**, aerenchyma of cortex; **as**, aerenchyma of stele; **v**, vascular bundle.

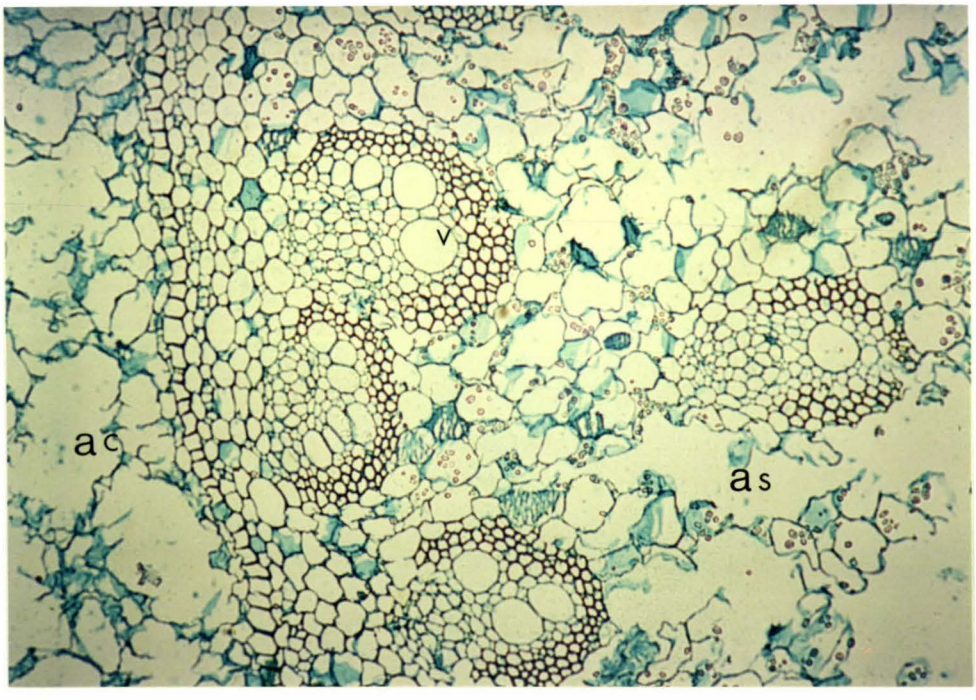

v
ac
as

Table 15.1 *Typha orientalis*: rhizome
Proximate analyses per 100 g dry weight

A.Moisture:	
oven-dried	94.34
freeze-dried	93.54
B.Lipid	4.96
C.Nitrogen	1.43
D.Protein	8.04
E.Dietary fibre	
insoluble	37.41
soluble	7.55
F.Soluble sugar	29.02
G.Starch	2.82

(For details of replications and analytical procedures see Part 1, Procedures and analytical methods).

16. *Bolboschoenus fluviatilis* (Torrey) Soják

family: Cyperaceae

Tall sedge, Purna grass

Riiriiwaka, Riwiwaka, Kukurahu

(i) Description and distribution

Bolboschoenus fluviatilis is a marsh herb with long grass-like leaves. These arise from an underground system of rhizomes with tubers. The creeping rhizome stock is tough and fibrous, forming globose, ligneous tubers at frequent intervals of 50 to 100 cm. These tubers measure up to 40 cm across, have numerous fine, tough, hair-like roots on all aspects particularly on the underside, and from the upper surface the leaves and culms are produced, at right angles to the creeping rhizome (see Plate 16b, p141). New rhizomal growth develops laterally in one or several directions; further tubers are formed along the creeping stock so forming a dense, often stellate, underground network.

The leaves are keeled, harsh and erect, up to 2 m long and 5 to 15 cm wide. The culms are triquetrous and striated, very tall and erect up to 2 m in height. The basal sheathes are loose and membranous. The inflorescence is terminal; it is a compound irregular umbel with 6 to 9 unequal rays bearing a cluster of from 1 to 6 spikelets, with a sessile glomerule of spikelets at the base of the rays.

Bolboschoenus fluviatilis is found from the far north down to latitude 38° S, and also on the west coast between Wanganui and Foxton. It grows in fresh water, brackish swamps and on river margins, never very far from the coast except for recorded sites at Rotorua and Palmerston North, and along the Waikato as far as Lake Taupo (Cheeseman 1925). It attains a much greater size in the warmer regions of the far north (see Plate 16a, p141). Hooker (1867) recorded this as abundant, but it is now more localized. Esler (1978) wrote that it had been recorded from a number of places in the Manawatu by Cook (1947) and Mason (1950), and was locally abundant in drains and on the margins of lagoons. The previously recorded sites in the Palmerston North area, including Longburn, (Esler 1967) have since been drained and the species has apparently disappeared.

References:

Hooker 1867; Cheeseman 1925; Esler 1967, 1978; Moore and Edgar 1976.

(ii) History

The edible portion is the underground rhizomal tuber. Colenso (1880:31) described this as a globular nut-like root of which the kernel-like inner part was eaten. He wrote:

> "it was amusing to witness the half-wild pigs of the modern Maori in the summer season - before the arrival of the European settlers -when the littoral swamps were drying up, how they would go into them, and dig up and crack and munch these roots, concealed in the sedges of the swamps; they were often detected by the sound of their cracking and munching!"

Best (1942) wrote that the edible root (*sic*) had a mealy nature. However, this was only considered to be of value as a vegetable food in times of scarcity (Colenso 1880).

Corms and tubers of related species in the Cyperaceae have been, and are still, used in other parts of the world, particularly in the East, in India and North America. The corms of *Eleocharis tuberosa* (*Scirpus tuberosum*), the water chestnut, are grown in China and Japan; they are eaten raw and are also a source of commercial starch (Winton and Winton 1935). The edible tubers of *Cyperus esculentus* (chufa) are used in the southern states of America; these tubers are very similar morphologically to those of *Bolboschoenus fluviatilis*.

(iii) Collection and organ preparation

Tubers of *Bolboschoenus fluviatilis* were collected on 25.4.92 from the estuarine banks of the Northern Wairoa River, south of Dargaville, near Ruawai (GR970724, NZMS 260 Sheet P08). They were dug up from the upper 20 cm of mud and the whole intact specimens kept moist in a container pending laboratory sample preparation two days later.

Tubers were rinsed and separated into two groups, old and new specimens, as these were distinctly different. It was found that the older tubers were too hard to cut with a knife or scalpel, nor could the outside layers, which were densely covered with adventitious roots, be pared off.

For proximate analyses, young tubers were weighed, freeze-dried and milled. It was intended to use a cooked sample of the older tubers as these in their untreated state were too hard to be milled. Cooked solids and reduced cooking liquids were separately bagged, frozen and freeze-dried. However, even after cooking, the milling proved unsatisfactory as some hard portions remained like small marbles. Of a total of 31.82 g, the unmillable portion of cooked solids was as high as 17.95 g, the remainder comprising 10.64 g from the cooked solids and 3.23 g residue from the reduced cooking liquid.

It was felt that the use of the millable portions alone would be unfairly representative of the whole sample, and consequently no analytical work was carried out using this material.

For the botanical specimen, a young tuber was cut in medial and sagittal vertical planes and the segments preserved in FAA. Embedded segments were later positioned at right angles to the cutting plane to give transverse sections.

(iv) Results and discussion

The young tubers required 12 days of oven-drying to reach constant weight, and the moisture content was calculated to be 79.18 ± 1.5% (three replications). The majority of this loss was during the first 24 hours, but there was a continued gradual loss over the remaining period. By freeze-drying, the measured loss was very similar, at 79.41%.

A sub-sample of older tubers was also oven-dried for comparison, and this showed a moisture content of 51.18%. Similarly by freeze-drying the older tubers gave a comparatively low moisture measurement, 59.21%.

The lipid content of the young tuber samples was very low, at only 0.89 g/100 g, this being the lowest of the angiosperm samples in the study.

The levels of nitrogen and protein were rather higher than those of other rhizome samples, measuring 2.08 and 13.0 g/100 g respectively. These figures show a protein to nitrogen ratio of 6.25 to 1.

The carbohydrate measurements showed high levels of insoluble dietary fibre and starch, and low levels of soluble dietary fibre and soluble sugars. The dietary fibre values were 37.30 and 8.88 g/100 g for insoluble and soluble dietary fibre respectively. The measurements for soluble sugar and for starch were 4.52 and 21.63 g/100 g respectively.

Yanovsky and Kingsbury (1938) recorded proximate analyses for tubers of the closely related species *Scirpus validus*. These values were 26.19 g starch/100 g, 40 g sugar/ 100 g and 19.05 g protein/100 g.

For tubers of *Cyperus esculentus*, mentioned above, Luna (quoted in Winton and Winton 1935) found 28% fat, 14% sucrose, 29% starch, and small amounts of protein; this species is known to be atypical in its high fat content. In *Eleocharis tuberosa* Adolph (quoted in Winton and Winton 1935) found 1.84% protein and 0.18% fat.

A summary of proximate analyses is shown in Table 16.1 (p143).

Very high elemental levels of silicon (64 μg/g) and nickel (6.0 μg/g) were recorded, and also low values for calcium, magnesium, zinc and copper. The high level of silicon would suggest the presence of siliceous cell inclusions, and this is probably also reflective of the extreme hardness of the older tubers.

Data obtained by PES is shown in Appendix 3.1 (p198).

The preparation of a satisfactory section of the young tuber was technically difficult. This shows a damaged cortex of loosely-packed cells (see Plate 16 c and d, p142). Beneath this is a well-defined star-shaped endodermis enclosing the central cylinder. This tissue arrangement is very similar to that described by Winton and Winton (1935) for *Cyperus esculentus*. In that species, the cortical cells have rounded pores and in the central cylinder the parenchymatous cells are also porous walled, with starch grains present in both tissues. It is suggested that , as cortical porosity may be a feature of marshland tubers of the Cyperaceae, this feature may have been a factor in the distortion and degradation of the cortex during histological procedures.

Vascular bundles do not appear to be well represented and are seen as small groups of close, narrow vessels; comparatively, the microscopic structure of *Cyperus esculentus* shows that vascular bundles are neither numerous nor conspicuous.

Plate 16a. *Bolboschoenus fluviatilis*: plants *in situ*.

Plate 16b. *Bolboschoenus fluviatilis*: rhizomal tubers.

Plate 16c. *Bolboschoenus fluviatilis*: rhizomal tuber, transverse section, stained with safranin and "fast green"; **ac**, aerenchyma of tuber cortex; **ar**, aerenchyma of young emergent root cortex; **v**, vascular trace.

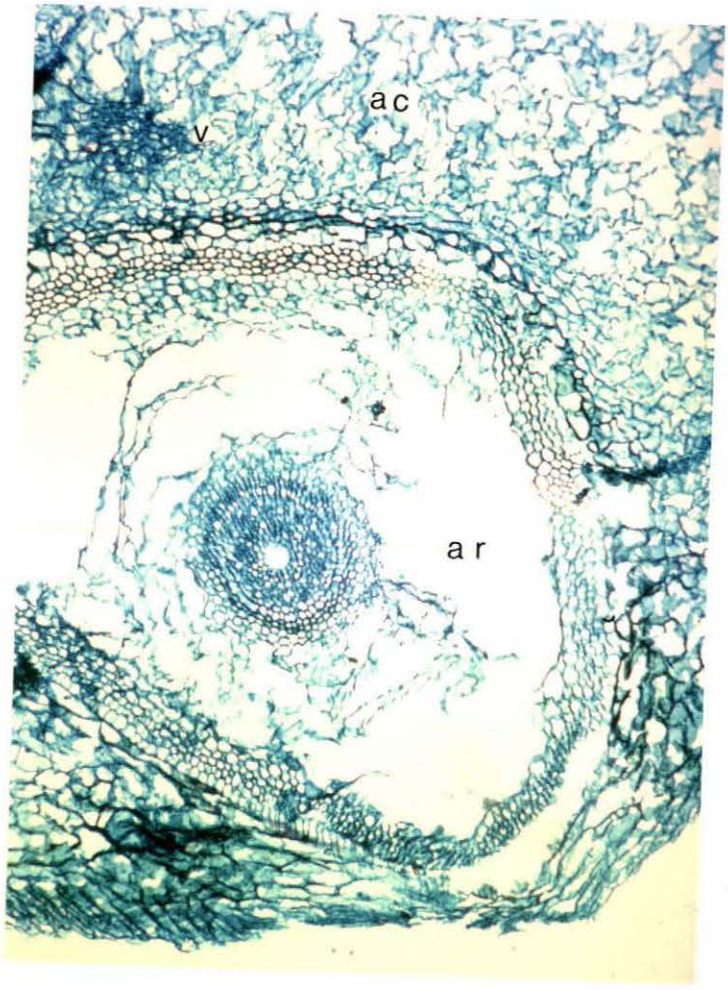

ac
v
ar

Table 16.1 *Bolboschoenus fluviatilis*: rhizomal tuber
Proximate analyses per 100 g dry weight.

A.Moisture:	
oven-dried	79.18
freeze-dried	79.41
B.Lipid	0.89
C.Nitrogen	2.08
D.Protein	13.0
E.Dietary fibre:	
insoluble	37.30
soluble	8.88
F.Soluble sugar	4.52
G.Starch	21.63

(For details of replications and analytical procedures see Part 1, Procedures and analytical methods).

17. *Gastrodia cunninghamii* Hook. f.

family: Orchidaceae

Perei, Huperei, Maukuuku

(i) Description and distribution

The rhizomes of *Gastrodia cunninghamii* are extensively branched; they are fleshy and swollen and break easily. Each rhizomal segment is constricted where it is attached to the adjacent or more deeply-placed segment. They have the irregular remnants of scale leaves, but lack roots (see Plate 17b, p145). New rhizomes arise laterally on existing ones and may give rise to flowering stems. The latter are erect and unbranched, 30 to 100 cm in height with widely spaced scale leaves. The raceme is often long and may contain 10 to 40 flowers; these are a brownish green colour, marked with paler spots (see Plate 17a, p145). The perianth is tubular, swollen at the base and split half-way down on the anterior face. There are 5 short, broad, acute lobes, and the labellum is trowel-shaped with involute margins.

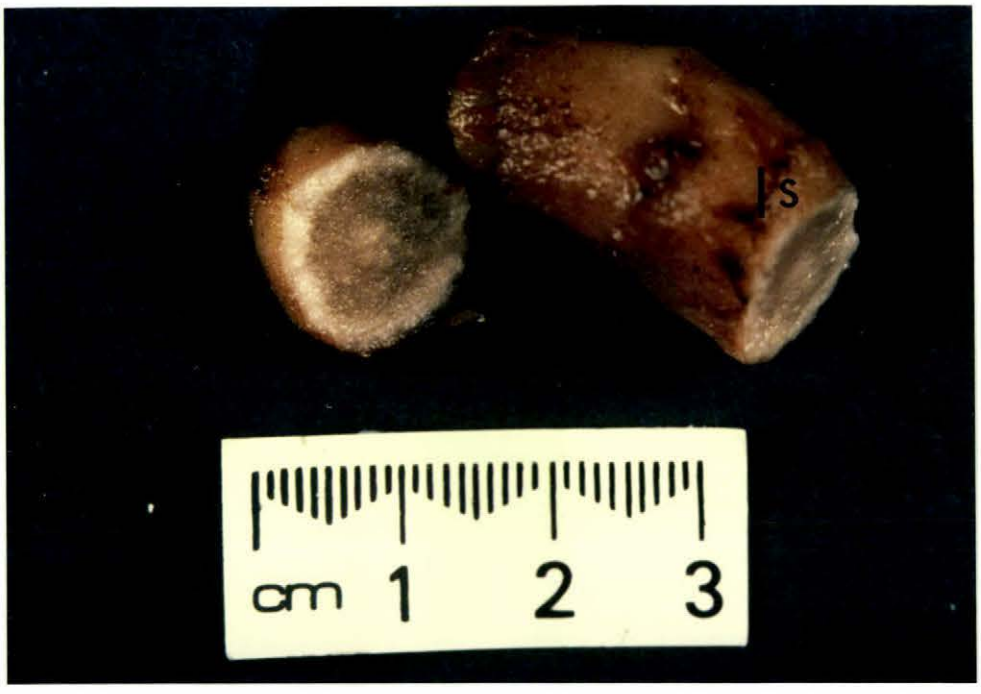
This orchid is leaf-less and lacking in chlorophyll, living in association with a soil fungus which in turn is parasitic on tree roots. The fungus is *Armillaria mellea*, and fungal rhizomorphs are extended from within the root of an infected tree to penetrate the basal rhizomes of the orchid. Fungal cytoplasm is released into the rhizomal cells. The fungal rhizomorphs form a network in the soil around the rhizomes to which they are attached at intervals, and also typically penetrate an adjacent superficial tree root (Gaumann 1928).

The orchid flowers in summer, from December to January, but will only do so when the fungal infection has become established and the rhizomes have adequate stores of carbohydrate.

Gastrodia cunninghamii grows in damp shaded woods in both North and South Islands, but is less frequent north of the East Cape. In the South Island it is commonly associated with species of *Nothofagus*, these being prone to infection with *Armillaria mellea*. It is also found on Stewart Island, and in the Chatham Islands.

Plate 17a. *Gastrodia cunninghamii*: panicle of flowers.

Plate 17b. *Gastrodia cunninghamii*: rhizome showing cut surface; **ls**, leaf scale.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The effective colour camouflage and short flowering season of this species obviously result in an imprecise knowledge of its distribution.

References:
Hooker 1867; Cheeseman 1925; Moore and Edgar 1976; Gaumann 1928; Campbell 1962.

(ii) History

Colenso (1880) referred to the rhizome of *Gastrodia cunninghamii* as a tolerably large fleshy root. He described this as being as big as a middling-sized *kumara* tuber, or of a stout long radish root. He wrote that although this rhizome was eaten by the early Maori, it was rather scarce and found only in the dense forests. Hooker (1867) had earlier recorded this as not uncommon but easily overlooked. Rhizomes were collected in the winter and steamed for eating. Hooker (1867) wrote that they were full of starch and mucilage, and Taylor (1855) that they were sweet and mealy. Best (1942) recorded that the thick, starchy rhizomes were used particularly by the Tuhoe Maori of the Urewera district. According to Colenso (1880) the odour of the plant was disagreeably aromatic.

The related species *G. sesamoides* is recorded as having been eaten by the Tasmanian Aborigines and tasting like beetroot (Maiden 1889); Isaacs (1987) likens the taste to roasted potatoes.

(iii) Collection and organ preparation

Several fine specimens of *Gastrodia cunninghamii* were located in the Esplanade Gardens in Palmerston North (GR326893, NZMS 260 Sheet T24). The orchids were in full flower, growing under mixed evergreens, and apparently associated with an old elm stump. The colony comprised about 30 flowering stems. Some of the rhizomes were dug on 20.12.91 from between 10 and 15 cm below the soil surface. The soil was densely matted with fine tree roots and there was abundant evidence of the fungus *Armillaria*. Larger tree roots, 2 to 5 cm in diameter, were closely associated with the orchids. The rhizomes were very brittle and fragile, and interwoven with the mass of fine tree roots. The elm tree stump, about 2 metres away from the edge of the orchid colony, was alive

and sprouting from the base.
Harvested rhizomes were prepared for proximate analyses, and one segment preserved in FAA.

The traditional harvesting season was not observed for this plant species; location of specimens during the previous flowering season had been unsuccessful and consequently rhizome collection during the winter season had not been possible.

(iv) Results and discussion

Moisture loss by oven drying from *Gastrodia* rhizome samples was measured as 89.57 ± 2.82% (three replications), and the drying time was 48 hours. The measurement by freeze-drying was 88.94%.

The lipid component was calculated to be 1.38 g/100 g, which was one of the lowest lipid values in the study.

The nitrogen and protein levels were relatively high, measuring 2.90 and 13.86 g/100 g. These values, however, give a rather low protein to nitrogen ratio of 4.78 to 1. The supernatant in the buffer extraction was opaque and slightly mucilaginous, over a poorly defined residuum, but satisfactory, well replicated readings were obtained without the addition of concentrated alkali. The relatively lower level of protein may indicate that it was not, in fact, all solubilized. Alternatively, some nitrogen may be present as non-protein nitrogen in the *Gastrodia* rhizome.

The insoluble dietary fibre level was low, measuring 13.10 g/100 g. Soluble dietary fibre was more abundant, at 15.53 g/100 g, and the soluble sugar level was 11.13 g/100 g. A high level was recorded for starch, at 26.70 g/100 g.

A summary of proximate analyses is shown in Table 17.1 (p150).

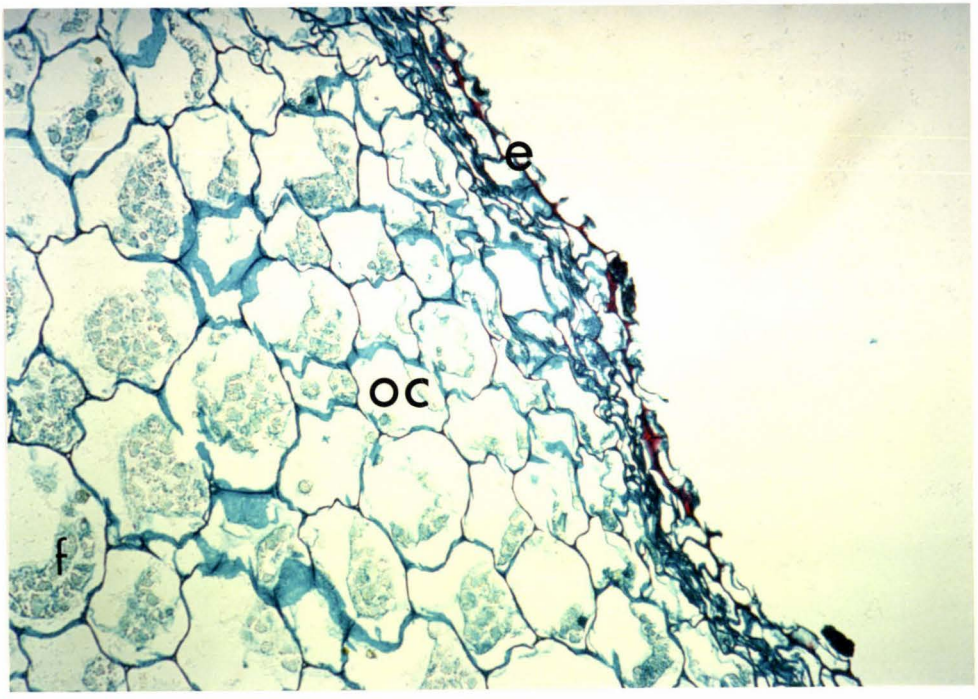
High elemental levels were found of copper (10.9 μg/g), iron (490 μg/g), cadmium (1.4 μg/g), and aluminium (416 μg/g).

Data obtained by PES is shown in Appendix 3.1 (p198).

Examination of the prepared botanical section shows a peripheral region of the flattened suberized cells (see Plate 17c, p149). Below this the cortex is composed of a parenchymatous ground tissue. Green-stained masses are seen in these cortical cells, throughout the cortex but less abundant centrally (see Plate 17d, p149). These masses apparently represent the infecting fungus. Fresh hand-cut sections were prepared to verify the identity of these cell contents, and the fungal hyphae were clearly distinguishable. Iodine stain was also applied to check for the presence of starch and this gave a positive colour development, showing small spherical grains in masses in some cells but these were very irregularly distributed through the cortex. The central region appeared to contain less starch. The vascular bundles appear as small, apparently unthickened cells and some large crystals (or raphides) can be seen adjacent to these cells. Very large raphides also appear in the outer cortical cells, as dark stacks of fine crystals occupying most of the intracellular space. These were seen in hand-cut sections, not shown. The elemental components of these have not been identified. Small vascular bundles also appear as columns of much narrower cells interspersed infrequently through the cortex. These again show no cell wall thickening. Safranin stain has only been retained in this section by the suberin of the epidermis.

Plate 17c. *Gastrodia cunninghamii*: rhizome, transverse section, stained with safranin and "fast green"; **e**, epidermis (showing safranin staining of suberized cell walls); **oc**, outer cortex; **f**, fungal infection (*Armillaria mellea*), stained green.

Plate 17d. *Gastrodia cunninghamii*: rhizome, transverse section, stained with safranin and "fast green"; **ic**, inner cortex; **v**, vascular bundle.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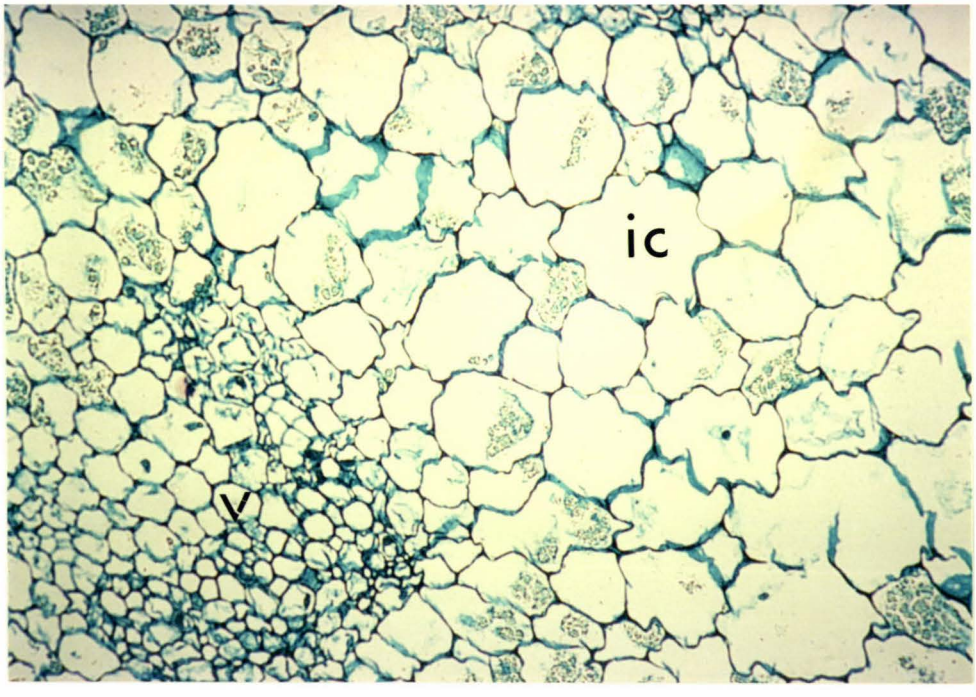
ic
v

Table 17.1 *Gastrodia cunninghamii*: rhizomes
Proximate analyses per 100 g dry weight

A.Moisture:	
oven-dried	89.57
freeze-dried	88.94
B.Lipid	1.38
C.Nitrogen	2.90
D.Protein	13.86
E.Dietary fibre:	
insoluble	13.10
soluble	15.53
F.Soluble sugar	11.13
G.Starch	26.70

(For details of replications and analytical procedures see Part 1, Procedures and analytical methods).

18. *Asplenium bulbiferum* Forst. f.

family: Aspleniaceae

Hen and chicken fern

Mouki, Mouku, Moku

(i) Description and distribution

This is a handsome fern, with the typical frond bearing on it's surface a number of young plants or bulbils, hence the specific name. The stipes are scaly, brown and green above, and the laminae may attain a length of 120 cm or more. The latter are 2 - 3 pinnate, dark green, scaly, elliptic and about 30 cm across. The primary pinnae are up to 35 cm by 12 cm in size and the secondary pinnae have margins divided sometimes to the midrib. The sori are elongate, from 0.2 to 0.4 cm long.

The rootstock of this fern is short and stout, crowned with brown scales.

The characteristic bulbils develop directly from cells on the upper surface of the frond. These latter fall to the ground and produce new ferns, or they take root when the old frond falls over.

Asplenium bulbiferum hybridizes with a wide range of other species, especially with *A. flaccidum*, and many of the hybrids are perpetrated by bulbils.

The distribution of this fern is throughout New Zealand, in North and South Islands, except in drier parts of South Canterbury and inland Otago; also in Stewart Island and the Chatham Islands. It thrives in damp shaded places and is common in lowland to lower montane forests, particularly beside streams.

References:

Allan 1961; Dobbie 1930; Hooker 1867; Brownsey and Smith-Dodsworth 1989.

(ii) History

Colenso (1880) described the edible part of *Asplenium bulbiferum* as the very young succulent and mucilaginous shoots. These were collected, as "fiddleheads", when the shoots were young and curled. They were steamed and eaten as a form of greens prior to the introduction by Cook of *Brassica oleracea*, the "Maori cabbage" which then became a favourite (Colenso 1880). *Mouki* was regarded as one of the best of the fern shoots, and superior to bracken. The Tuhoe tribe continued to eat it with potato after the introduction to New Zealand of the latter (Best 1942).

Young fern shoots were broken off where the stipe was still tender, and the hairs rubbed off. After steaming, the young shoots tasted like asparagus and were perfectly palatable, although sticky and unpleasant to eat raw (Hildreth 1962). Makereti (1938) recorded that these were eaten as very young fronds, also citing another edible species, *A. obtusatum*. Hildreth (1962) considered that young fiddleheads of all ferns could be eaten.

(iii) Collection and organ preparation

Asplenium bulbiferum fronds were collected from the Tiritea Reserve, Palmerston North (GR358842, NZMS 260 Sheet T24), on 5.12.91. There were very many specimens at this site, growing thickly on the forest floor. Very young soft specimens were selected for analysis (see Plate 18a, p154).

The cutting of the fronds released copious clear, sticky mucilage. Stipes were wiped free of hairs and were cut into small pieces for sub-sampling. A young pinna was preserved for botanical investigation.

(iv) Results and discussion

The moisture content of the *Asplenium* sample was 79.19 ± 1.4% (three replications) by oven-drying, this having taken two days for complete moisture loss. By freeze-drying, the moisture content was calculated to be 84.79%.

The lipid content was measured at 3.95 g/100 g.

The nitrogen value was very high in terms of this study, as also was the protein value. Measurements for these two determinations were 3.48 and 19.94 g/100 g respectively, with a protein to nitrogen ratio of 5.73 to 1.

The insoluble dietary fibre level was high, at 34.10 g/100 g, but the soluble component was low, at 11.73 g/100 g. Soluble sugar and starch were both found to be present in relatively low amounts; these were 4.67 and 4.55 g/100 g respectively.

A summary of proximate analyses is shown in Table 18.1 (p155).

The elemental analysis showed high levels of copper (11.5 µg/g), potassium (51,942 µg/g), boron (42.3 µg/g), and strontium (29.0 µg/g).

Data obtained by PES is shown in Appendix 3.1 (p198).

The prepared botanical section shows a transverse section of a young pinna (see Plate 18b, p154). Epidermal cells and stoma are seen to be on both surfaces. Between these the undifferentiated spongy mesophyll is composed of regular cells containing chlorophyllous plastids, and many intercellular spaces. The mid-vein is prominent and enclosed by a single cell sheath. The vein is elliptical and surrounded by the photosynthetic parenchymatous cells.

Plate 18a. *Asplenium bulbiferum*: immature frond ("fiddlehead").

Plate 18b. *Asplenium bulbiferum*: pinna, transverse section, stained with safranin and "fast green"; **e**, epidermis; **sm**, spongy mesophyll; **v**, vascular bundle.


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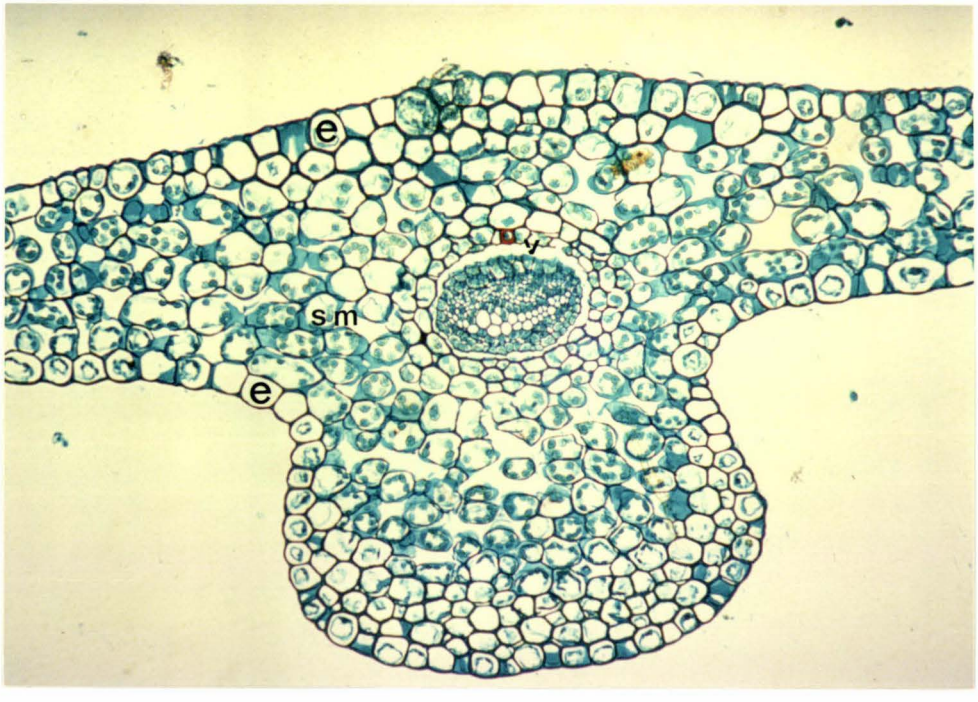
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Table 18.1 *Asplenium bulbiferum*: uncurled fronds
Proximate analyses per 100 g dry weight

A.Moisture:	
oven-dried	74.19
freeze-dried	84.79
B.Lipid	3.95
C.Nitrogen	3.48
D.Protein	19.94
E.Dietary fibre:	
insoluble	34.10
soluble	11.73
F.Soluble sugar	4.67
G.Starch	4.55

(For details of replications and analytical procedures see Part 1, Procedures and analytical methods).

Part 3
Discussion and conclusions

Discussion

The various discussion points arising from the findings in the previous section will be presented here in their botanical, analytical, nutritional and ethnobotanical contexts. Each of these four disciplinary aspects is seen to be an integral part of the overall dimension of this research. As the specific results of individual plant investigations have already been discussed, this section will deal with these findings on a broader level, allowing for comparisons between species and anomalies between the botanical examinations and analytical results. The discussion of the nutritional value of these food-plants is based on the findings of the analytical investigations. Finally the ethnobotanical perspective is viewed in the light of the analytical results and nutritional assessment of the edible plant organs.

Of the various botanical specimens examined, a high proportion of these are underground organs, and, in particular, rhizomes. The paucity of botanical literature on these types is notable, particularly in relation to the volume of literature on the aerial vegetative and reproductive systems. The instance of *Pteridium esculentum* has already been mentioned in the context of its classification and relationship to *P. aquilinum*. However, the differential identification of these is based on laminal components, to the exclusion of rhizomal features. It has not been within the scope of this research to compare and contrast rhizomes of these two species, but a preliminary examination of the *P. esculentum* rhizome at 60 cm depth has shown specific morphological features. The records in early literature of the substantial measurements of edible rhizome, in Northland and Hawkes Bay, have not been matched by the specimens collected in the sandy loam of the Manawatu, but these latter have, nevertheless, distinctly exhibited the macroscopic variation between the distal and proximal portions as detailed by Colenso (1880), and their increased size at this depth. As already described, mucilage was abundantly released when the prepared rhizome sample was digested with a pH phosphate buffer. However, mucilage was not apparent in the distal specimen of the rhizome either on macroscopic examination or on examination of a hand-cut section.

The one other member of the Pteridophyta, *Marattia salicina*, of which the rhizome has been examined, also showed the presence of mucilage in this organ. The rhizomal scale, referred to here, has apparently received little attention in cryptogamological literature, with the exception of the early morphological investigations carried out by Buchanan

(1877). More recently, investigations into the features of the Marattiales have included other *Marattia* species but not *M. salicina* (Bierhorst 1971). The root of this fern has also been included in both analytical and botanical examinations, and has exhibited distinctive features. Botanically, the polyarch stele with long thin xylem arms is characteristic of Marattialean roots (Bierhorst 1971). The number of xylem arms in the prepared section was 18, although Buchanan (1877) showed 16 of these in a hand-drawn section of a root of this species. Bierhorst (1971) in comparison, found 9 xylem arms in a prepared section of *M. alata*, and Bold *et al.* (1987) recorded 12 in that same species, noting that this was many more than occur in most ferns. The root of *M. salicina* examined here appeared to be more mucilaginous than the rhizomal scale. Large mucilaginous cells were seen amongst the smaller ground tissue cells of the cortex, unlike the cortical ground tissue homogeneity seen in *Pteridium esculentum*. The presence of the presumed volatile oil or resin-containing cells has already been related to the unusual finding of a large volume of ether-soluble compound for this organ. No identification has been made of this substance and no literature on its chemical characterization has been sighted.

The rhizome of *Calystegia sepium* exhibited a similar finding of an apparent ether-soluble component. However, there are no outstanding or apparent resin or oil-containing cells in the section of this species. As in the case of the *Marattia* root, this (compound) has not been identified. Mucilage cells are apparent in the *Calystegia* section but these are only marginally larger than other cortical cells. The rhizome of this species was not found to be of the enlarged proportions suggested in the early literature. Again, it was outside the scope of this research to explore the possibilities of rhizomal enlargement with geographical and seasonal variation. The additional consideration of species variation and hybridization has already been mentioned, but as was noted in the case of *Pteridium esculentum* taxonomy, the pertinent distinguishing characteristics relate solely to the aerial organs and not to rhizomal morphology.

The rhizome of *Arthropodium cirratum* was seen to exhibit extreme variation in size relative to site. The microscopically examined section, from the rhizome of a cultivated plant, may represent an atypical morphological picture. However, although the cultivated rhizome was very much larger and fleshier, this was not paralleled by a high starch content; no starch grains were detectable either in the prepared section or in a fresh

hand-cut section, and minimal starch was estimated by analysis. The size of this storage organ, under cultivation, related poorly to its measured storage components, all of which, with the exception of soluble sugar, were extremely low. The leaves and fleshy leaf-bases of this species were noted to be very mucilaginous, but the rhizome was not overtly, or apparently so. Mucilage was, however, released from the prepared sample by the addition of pH 7 phosphate buffer, as was the case in *Pteridium esculentum*. The thin-walled cortical cells, seen in a fresh hand-cut section, show large cells suggestive of mucilage cells.

The rhizome of *Typha orientalis* also shows apparently fragile-walled cells of the cortical and stelar parenchyma. Both this species and *Bolboschoenus fluviatilis* are semi-aquatic monocotyledons, the former in a non-saline and the latter in a saline or brackish water environment. The respective rhizomes exhibit reciprocal levels of soluble sugar and starch, both at fairly extreme levels; the rhizome of *Typha orientalis* has apparently high stores of soluble sugar and very little starch, and the tuberous rhizome of *Bolboschoenus fluviatilis* has high stores of starch and very little soluble sugar. Iodine staining of fresh hand-cut sections has confirmed the relative presence of starch in the respective organs. Unlike the other rhizomatous species, in both these organs mucilage was not overt, and was also not released by the pH 7 buffer. However, its presence was made apparent in both samples by the addition of alkali; although in the section no specific mucilage cells or canals have been identified.

Lastly of the rhizomatous organs is that of *Gastrodia cunninghamii*. This fragile organ showed no presence of mucilage either macroscopically, by tissue examination, or during analytical extraction. The contribution made to the overall storage component of the rhizome by the abundant cellular presence of the fungus *Armillaria mellea* can only be tentatively measured here by specific staining. This shows abundant starch grains in cells largely or wholly devoid of the fungus, consistent with the high starch measurement by proximate analysis. Protein was detected, by staining, to be associated with the enlarged fungal hyphae in the cells; lipid was not detected either in the fungal masses or in the plant cell components.

Of these several rhizomes detailed above, a large proportion are seen to store mucilaginous compounds, either overtly or discretely. Other non-rhizomatous organs also

show the presence of mucilage. These include the kernels of *Beilschmiedia tawa*, the frond stipes of the two pteridophytes *Cyathea medullaris* and *Asplenium bulbiferum*, both the leaf-bases and roots of *Cordyline australis*, the red seaweed *Porphyra* sp. and the fungus *Auricularia polytricha*.

Of the remaining plant species, only one is herbaceous; this is *Sonchus asper*. This is the only plant of which the edible organ is largely photosynthetic. The unfolding frond of *Asplenium bulbiferum* is partially photosynthetic, and the frond of *Porphyra* sp. is photosynthetic by means of the red pigment phycoerythrin in addition to chlorophyll. These three species all show low levels of both soluble sugar and starch in the respective organs. The cotyledonous kernels of the two tree species, *Corynocarpus laevigatus* and *Beilschmiedia tawa* exhibit very little similarity in their storage components although both have relatively high reserves of starch.

In the above paragraphs, the term mucilage has been used in the broadest sense. The wide range of polysaccharides which are included in the category of mucilages are structurally related to the non-cellulose polysaccharides of the plant cell walls. Types of mucilage polysaccharide include the galactomannans, glucomannans, arabinoxylans and xyloarabinans (Southgate 1976). It has already been noted that those species which were not overtly mucilaginous on examination of the fresh section, showed no presence of mucilage cells or mucilage canals on microscopic examination. The release of mucilage was only apparent during extraction procedures using either pH 7 phosphate buffer or alkali. No mucilage tests were performed although the Periodic acid-Schiff (PAS) reagent might have been useful here; this is specific for aldehyde groups in sugar residues and has been used for detecting glycoproteins (Fountain 1985). Mucilage tests were considered by Johansen (1940) to be unsatisfactory.

In some underground organs starch was abundant, as seen in the rhizomes of *Calystegia sepium*, rhizomal scales of *Marattia salicina*, rhizomal tubers of *Bolboschoenus fluviatilis*, and the rhizomes of *Gastrodia cunninghamii*. In others it was low, as in the rhizomes of *Pteridium esculentum*, *Arthropodium cirratum* and *Typha orientalis*. In many organ sections, some of the grain-like cellular inclusions have given a positive iodine test for starch and others not. This frequent finding that apparent starch grains give a negative starch test has suggested that these may be amyloplasts which

at the time are not actively synthesizing and storing starch. These are notable in the rhizomes of *Pteridium esculentum* and *Typha orientalis*. The abundance of iodine stained grains related in every case to the starch abundance as determined by proximate analysis. This may suggest that the starch contents of several organs is very much higher at other seasons, judging by the abundance of these plastids. In most of these organs it is seen that high and low levels of starch are fairly uniformly reciprocated by low and high levels of soluble sugar.

There are a few aspects of the analytical procedures which require discussion; although all procedures were carefully chosen for their suitability and reliability with plant specimens, there is an enormous variation in consistency, organ type and composition which may have allowed for imperfect extractions in some cases.

The calculated moisture contents of the organ sub-samples by oven-drying and freeze-drying gave very close values in the majority of samples. In those where there was some degree of discrepancy in these two percentages the organ was identified as being mucilaginous. In these cases the moisture percentage calculated by freeze-drying was lower than that calculated by oven drying, suggesting the former to be a less satisfactory method of moisture removal, where mucilaginous polysaccharides are present. This was also a problem with the high lipid-containing specimens, notably the kernels of *Corynocarpus laevigatus* and the frond stipe of *Cyathea medullaris*. These both required further freezing, by immersion in a freezing bath, for satisfactory freeze-drying. It is interesting to note that the other samples in that estimation which gave high lipid figures, *Calystegia sepium* rhizome and *Marattia salicina* root, gave no problems with freeze-drying, presumably due to the very different freezing points of the component volatile oils or resins which caused this interference.

No problems were encountered with the solvent extraction of lipid except for the assumed interferences of volatile components in the two species already mentioned,the root sample of *Marattia salicina* and the rhizome sample of *Calystegia sepium*. Some doubt may remain, however, that other discrete amounts of volatile substances may have been present in other samples. No variation in procedure was found which would eliminate this error. The readings therefore must be taken to read as lipid determination inclusive of specific volatile oils and resins where present.

The nitrogen measurement by micro-Kjeldahl was considered a satisfactory procedure, although in some cases the digestion time was prolonged. In order to assess whether there was any loss of ammonia during the digestion stage, six samples were further checked (results not shown) in an automatic micro-Kjeldahl process. These comparative values showed that there was no loss of nitrogen as ammonia during the digestive stage of the manual procedure.

The presence of mucilage in many of the plant organs caused an interference in the Bradford procedure for protein extraction. Solubilization of the formed mucilage seemed to be achieved satisfactorily by the addition of 3 M NaOH. In some instances this gave a high measurement for protein, as in the rhizome of *Pteridium esculentum* and the root of *Cordyline australis*. In other samples the protein measurement was much lower, as in the frond stipe of *Cyathea medullaris* and the leaf-bases of *Cordyline australis*. In both kernel samples of *Beilschmiedia tawa* the protein extraction with the addition of 3 M alkali was nil.

In those samples which showed a low protein content, and which were mucilaginous, the figures obtained were critically assessed against the calculated nitrogen percentage. The conversion factor of 6.25 is the value used by McCance and Widdowson (1978) for protein calculation from the total nitrogen value. Some plants containing urea, purine and pyrimidine derivatives have a lower calculated conversion factor due to the higher proportion of non-protein nitrogen (McCance and Widdowson 1978). In many of the plant sample analyses for nitrogen and protein, the conversion factor is seen to be low, but for others the value is close to 6.25. Appendix 2.1 (p197) gives the factors for converting total nitrogen in foods to protein, suggested by the FAO/WHO Committee on Energy and Protein Requirements (FAO/WHO 1973, in McCance and Widdowson 1978).

In those samples where this ratio is low, the presence of nitrogen as non-protein nitrogen is considered. A second possibility is that protein extraction has not been complete in mucilage-containing samples. All these specimens which have shown mucilage either in their macroscopic or microscopic examination, have also shown a low ratio of protein to nitrogen. It may, however, be a characteristic of mucilage-containing tissues that there is also a surfeit of molecular non-protein nitrogen. No literature has been sighted which relates mucilage content of plant tissues to the presence of non-protein nitrogen.

Figures 1 - 6 (p168-173) show the comparative abundance of storage components measured by proximate analyses for all plant organs examined. Reference plant foods are included for comparison, this data taken from McCance and Widdowson (1978) and converted to percentage dry weights. The proximate analyses are also presented in tabular format in the Appendices (1.1 - 1.6, p191-196). Figures 7 - 20 (p174-187) show the comparative abundance of most of the elements measured by PES. Not included here are those trace minerals and some heavy metals which were predominantly measured as "less than" (<) values. These are recorded in the full elemental tables in Appendix 3.1 (p198). The figures here also include the 8 reference plant foods as above for 9 of the more conventionally important nutritional minerals (McCance and Widdowson 1978). Data on the remaining 5 elements is not available from that source.

From the analytical results obtained, a nutritional assessment of the dietary value of each plant organ can be presented. Comparative terms are used which relate to the nutrient assessment of commonly used plant foods. A selection of 8 plant foods is included in each table as a reference. For brevity in these descriptions the assessments are presented factually; all specific data can be referred to in the appropriate tables.

The rhizome of *Pteridium esculentum* appears to be a good source of soluble sugar, a fair source of protein and dietary fibre, and a poor source of lipid and starch. Of the minerals, iron, manganese, silicon and chromium are well supplied, and none of the other essential minerals are deficient. There are high levels of the toxic metals aluminium and strontium.

The cooked kernels of *Corynocarpus laevigatus* are a good source of lipid, protein and starch, a fair source of dietary fibre, and a poor source of soluble sugar. They are a good source of boron, and a fair source of phosphorous, but otherwise a poor source of nutritional minerals. There is a high level of copper but apparently no toxic or heavy metals. The uncooked kernels contain high levels of lipid, protein and starch, and a fair supply of dietary fibre and soluble sugar. Copper and nickel levels are high.

The cooked pericarp of *Elaeocarpus dentatus* appears to be an excellent source of starch, a fair source of lipid, and a poor source of soluble sugar, protein and dietary fibre. It is a poor source of all minerals except for a high level of copper. The lipid and starch

levels are lower in the uncooked pericarp, and dietary fibre is higher. Silicon and copper levels are both increased by cooking, and potassium decreased.

The cooked herbaceous tissues of *Sonchus asper* are an excellent source of protein, a good source of dietary fibre and lipid, and a poor source of starch and soluble sugar. There are very high levels of calcium, copper, potassium, manganese, silicon and boron, high levels of magnesium, zinc, phosphorous, sulphur and sodium, and a fair level of iron. There is also a very high level of strontium, and the levels of aluminium and cadmium are both high. All nutrient components are marginally higher in the untreated herb apart from lipid which increased with cooking. Minerals notably increased with cooking are calcium, manganese and silicon. Strontium, aluminium, cadmium and copper are all lower in the untreated organ.

The uncooked rhizome of *Calystegia sepium* is a good source of starch and dietary fibre, a fair source of protein, and a poor source of soluble sugar. The level of dietary lipid is indeterminate due to apparent plant oil interference. This is a good source of silicon and a fair source of potassium and boron. There is a higher level (per microgram dry weight) of strontium.

The cooked frond stipe of *Cyathea medullaris* is an excellent source of soluble sugar, a fair source of protein and dietary fibre, and a poor source of lipid and starch. It is an excellent source of potassium and a fair source of boron, phosphorous and silicon. There is a higher level (per microgram dry weight) of aluminium. The raw untreated frond stipe is a far superior source of lipid than the cooked organ, but other storage nutrients are comparable. Aluminium is also high in the untreated organ.

The leaf-bases of *Cordyline australis* are an excellent source of soluble sugar, a good source of dietary fibre, a fair source of protein, and a poor source of lipid and starch. They contain a high level of calcium, potassium, manganese, zinc and phosphorous, but iron is deficient. There is a high level of strontium and a raised level of nickel. The root of *Cordyline australis* is a good source of soluble sugar and dietary fibre, and a poor source of starch, protein and lipid. No nutritional minerals are well supplied.

The apical shoot of *Rhopalostylis sapida* is a good source of protein and soluble sugar,

a fair source of lipid and dietary fibre, and a poor source of starch. It is an excellent source of magnesium, zinc and potassium, and a good source of calcium, silicon, boron, copper and phosphorous. It has an extremely high level of strontium.

The pollen of *Typha orientalis* is an excellent source of protein, a good source of soluble sugar, a fair source of starch, and a poor source of dietary fibre and lipid. It is an excellent source of phosphorous, and a good source of boron, chromium, potassium and sulphur. There are no raised levels of toxic elements.

The cooked kernels of *Beilschmiedia tawa* are a good source of dietary fibre, a fair source of starch and protein, and a poor source of lipid and soluble sugar. They are a poor source of all minerals except for a relatively high level of copper. The untreated kernels have a lower level of dietary fibre and a higher level of starch, otherwise component levels are comparable. There is a higher silicon level in the untreated kernels, but other minerals are similar.

The rhizomal scales of *Marattia salicina* are a good source of starch and dietary fibre, a fair source of protein and soluble sugar, and a poor source of lipid. They are a fair source of calcium, magnesium, zinc, potassium and boron. The level of strontium is moderately high, and of aluminium and copper excessively high. The root of *Marattia salicina* is a good source of dietary fibre, a fair source of protein and starch, and a poor source of soluble sugar. The level of available lipid is indeterminate due to apparent plant oil interference. This is a good source of calcium, iron, potassium, boron and chromium. There are very high levels of copper, aluminium and strontium.

The cooked fronds of *Porphyra* sp. are a good source of protein and dietary fibre, and a poor source of lipid, starch and soluble sugar. There are high levels of potassium, silicon, boron, chromium, copper, phosphorous and sulphur, and a very high level of sodium. There are also high levels of aluminium, arsenic and strontium. The uncooked fronds have comparable nutritional levels except for dietary fibre which is lower in the uncooked organs. They are a good source of magnesium, chromium and boron, and a fair source of potassium, silicon and phosphorous. Sodium and sulphur are abundant. There are high levels of aluminium, arsenic and strontium.

The basidiocarps of *Auricularia polytricha* are an excellent source of dietary fibre, a fair source of protein, and a poor source of lipid, starch and soluble sugar. They are a fair source of phosphorous and calcium, but other nutritional minerals are at low levels or deficient. There are no raised levels of heavy metals.

The rhizomes of *Arthropodium cirratum* are a fair source of dietary fibre and soluble sugar, and a poor source of lipid, protein and starch. They are an excellent source of calcium, copper, zinc and silicon. They also contain raised levels of aluminium and strontium.

The rhizomes of *Typha orientalis* are a good source of dietary fibre and soluble sugar, a fair source of protein, and a poor source of lipid and starch. They are an excellent source of iron, potassium and silicon, and a good source of manganese, boron and phosphorous. Aluminium and strontium levels are raised.

The tuberous rhizomes of *Bulboschoenus fluviatilis* are a good source of protein, starch and dietary fibre, and a poor source of soluble sugar and lipid. They are an excellent source of silicon, and a good source of phosphorous, potassium and chromium. There is a high level of nickel.

The rhizomes of *Gastrodia cunninghamii* are a good source of protein and starch, a fair source of dietary fibre and soluble sugar, and a poor source of lipid. They are a good source of potassium, iron, phosphorous, boron, chromium, silica and copper, and none of the essential minerals are deficient. Levels of aluminium and cadmium are raised.

The stipe fronds of *Asplenium bulbiferum* are a good source of protein and dietary fibre, and a poor source of lipid, starch and soluble sugar. They are high in calcium, magnesium, potassium, boron, phosphorous and copper. There is a raised level of strontium.

It is apparent from the foregoing summaries, together with the tabulated data, that many of these plant foods supply adequate levels of essential dietary components. According to several of the early writers, including Banks (Beaglehole [Ed] 1962), Taylor (1855) and Colenso (1880), the pre-European diet of wild or uncultivated plants was supplemented

by the cultivated *kumara* (*Ipomoea chrysorrhiza*), *taro* (*Colocasia antiquorum*, or *Caladium esculenta*) and the gourd *hue* (*Cucurbita* sp.). It was suggested by Taylor (1855) and Colenso (1880) that at some seasons, when cultivated crops were not yet harvested or had failed, there was sole reliance on the stores of bracken root, *karaka* kernels and other dried plant organs. Variations between dietary recordings reflect geographical and tribal dissimilarities and the relative availability of species. Also included in the diet, to some degree, were birds, fish and shellfish, and the use of these was again seasonally dependent.

The introduction to New Zealand of grain crops and the potato played an important role in diminishing the traditional use of native plant foods (Makereti 1938). Sweetness in food was formerly supplied by the several high sugar foods including stipe fronds of *Cyathea medullaris*, the root, stem and leaf-bases of *Cordyline australis*, and the apical shoot of *Rhopalostylis sapida*. The introduction of cane sugar again diminished the requirement to gather wild plant organs.

Of the plants examined here, those which have not lost their importance in traditional dietary esteem are *Porphyra* sp. and *Sonchus* sp. The *puwha* used presently is largely *S. oleraceus*, being a softer and less bitter herb. The seaweed is a valued food in the East Cape area where the species is abundant on the coastal rocks. Bracken and the other ferns have become largely historical items although *Asplenium bulbiferum* fronds are still used, cooked as greens, in some areas. *Karaka* kernels are also still occasionally used. *Cordyline australis* may no longer be used as a traditional plant food, but this has recently been investigated by Fankhauser (1985) as a possible commercial source of fructose which is abundant in both stem and root.

The availability of many species has declined over the last two centuries, and some plants described by early writers as widespread or abundant are now rare or localized. These include *Gastrodia cunninghamii* and *Marattia salicina*, and the very slow growing *Rhopalostylis sapida*.

The unusually high levels of toxic metals seen in some of the organs may be cause for concern. In this respect, as these may implicate recent environmental pollution, the present nutritional assessment cannot be compared to that of the plants prior to last century. The figures do suggest that some species may be specific metal accumulators.

Figure 1: Comparative abundance of LIPID in plant organs and reference plant foods

For Legend refer to page 187

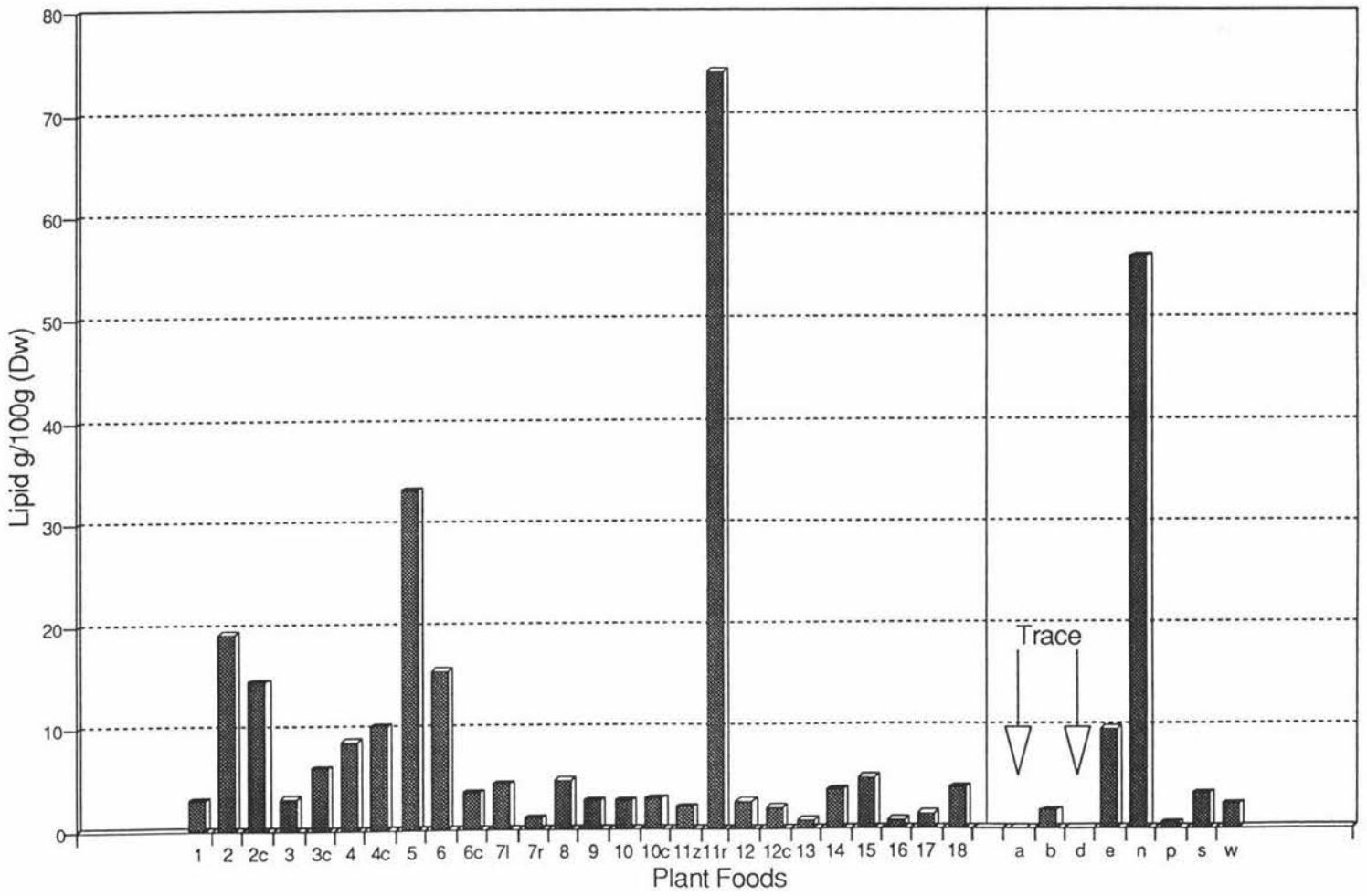


Figure 2: Comparative abundance of NITROGEN in plant organs and reference plant foods

For Legend refer to page 187

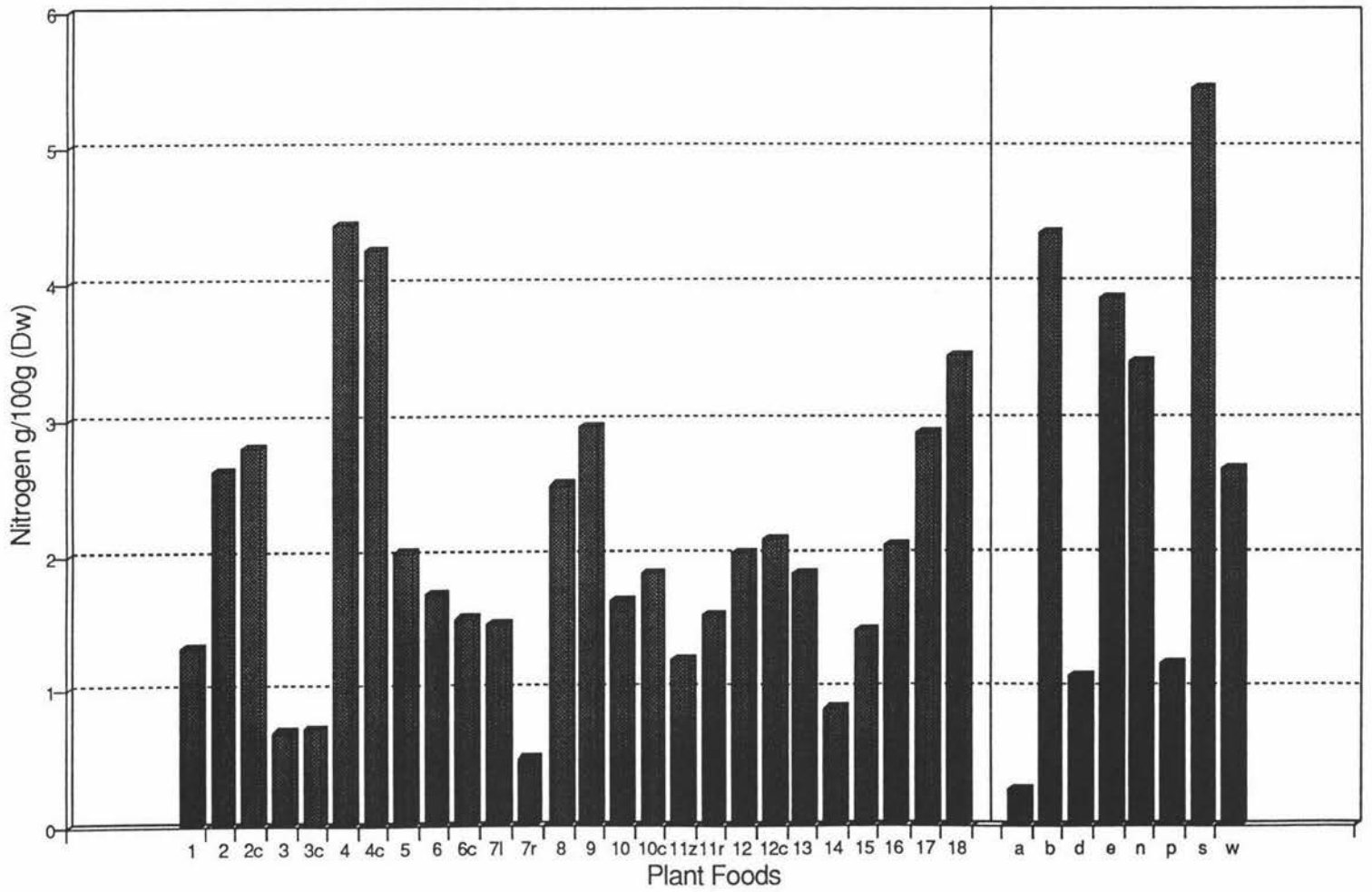


Figure 3: comparative abundance of PROTEIN in plant organs and reference plant foods

For Legend refer to page 187

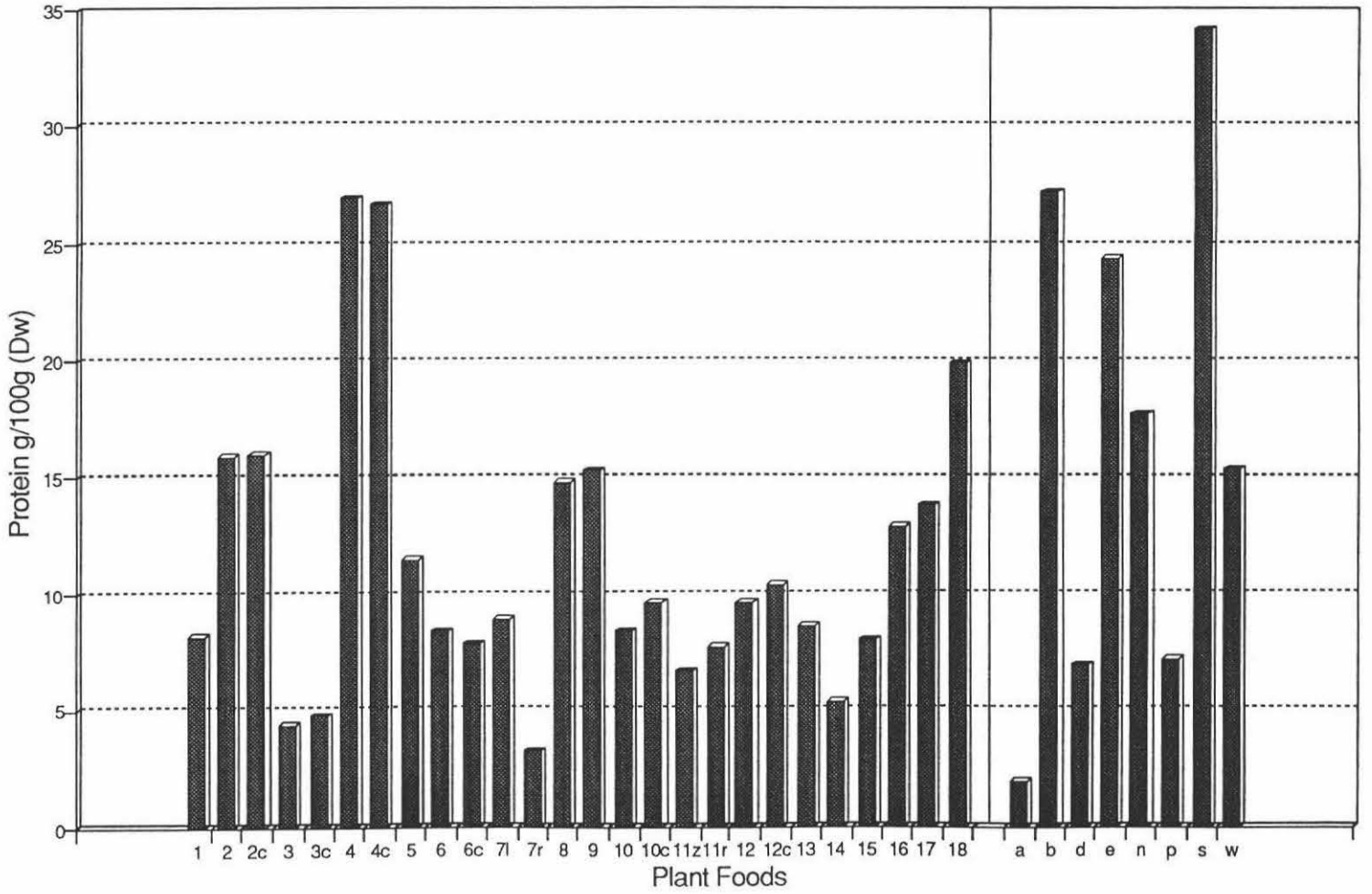


Figure 4: Comparative abundance of DIETARY FIBRE in plant organs and reference plant foods

For Legend refer to page 187

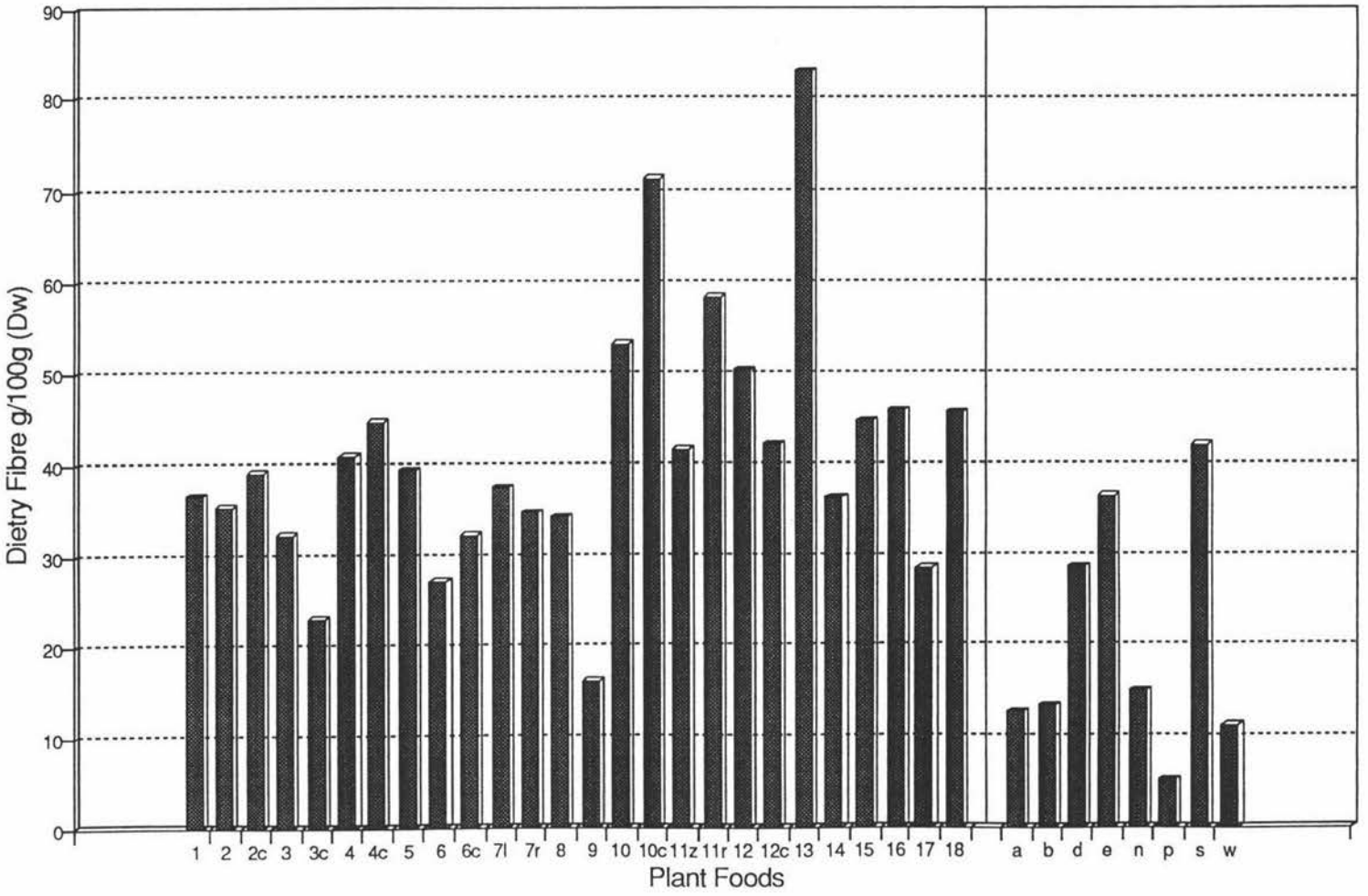


Figure 5: Comparative abundance of SOLUBLE SUGAR in plant organs and reference plant foods

For Legend refer to page 187

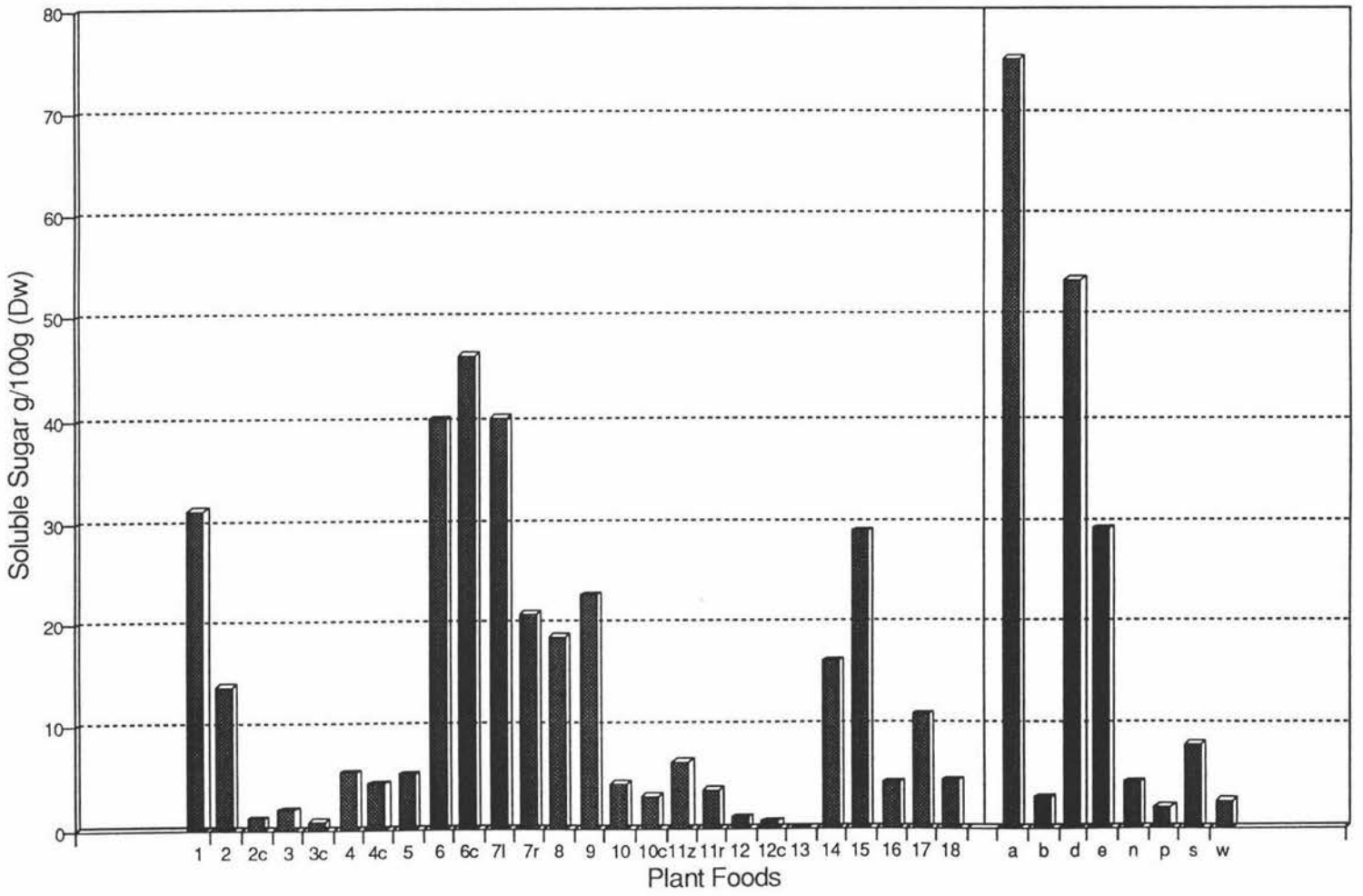


Figure 6: Comparative abundance of STARCH in plant organs and reference plant foods

For Legend refer to page 187

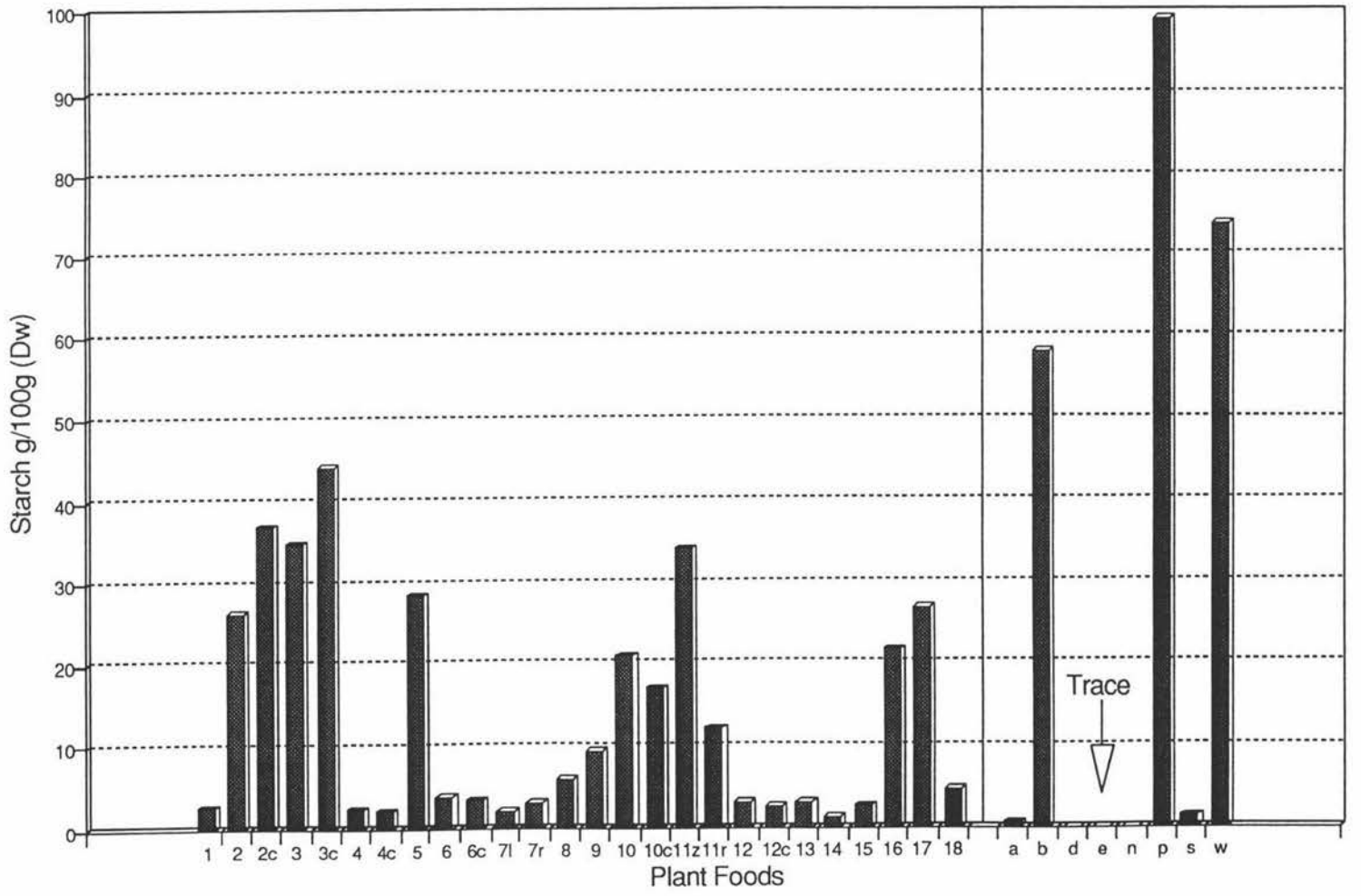


Figure 7: Comparative abundance of CALCIUM in plant organs and reference plant foods

For Legend refer to page 187

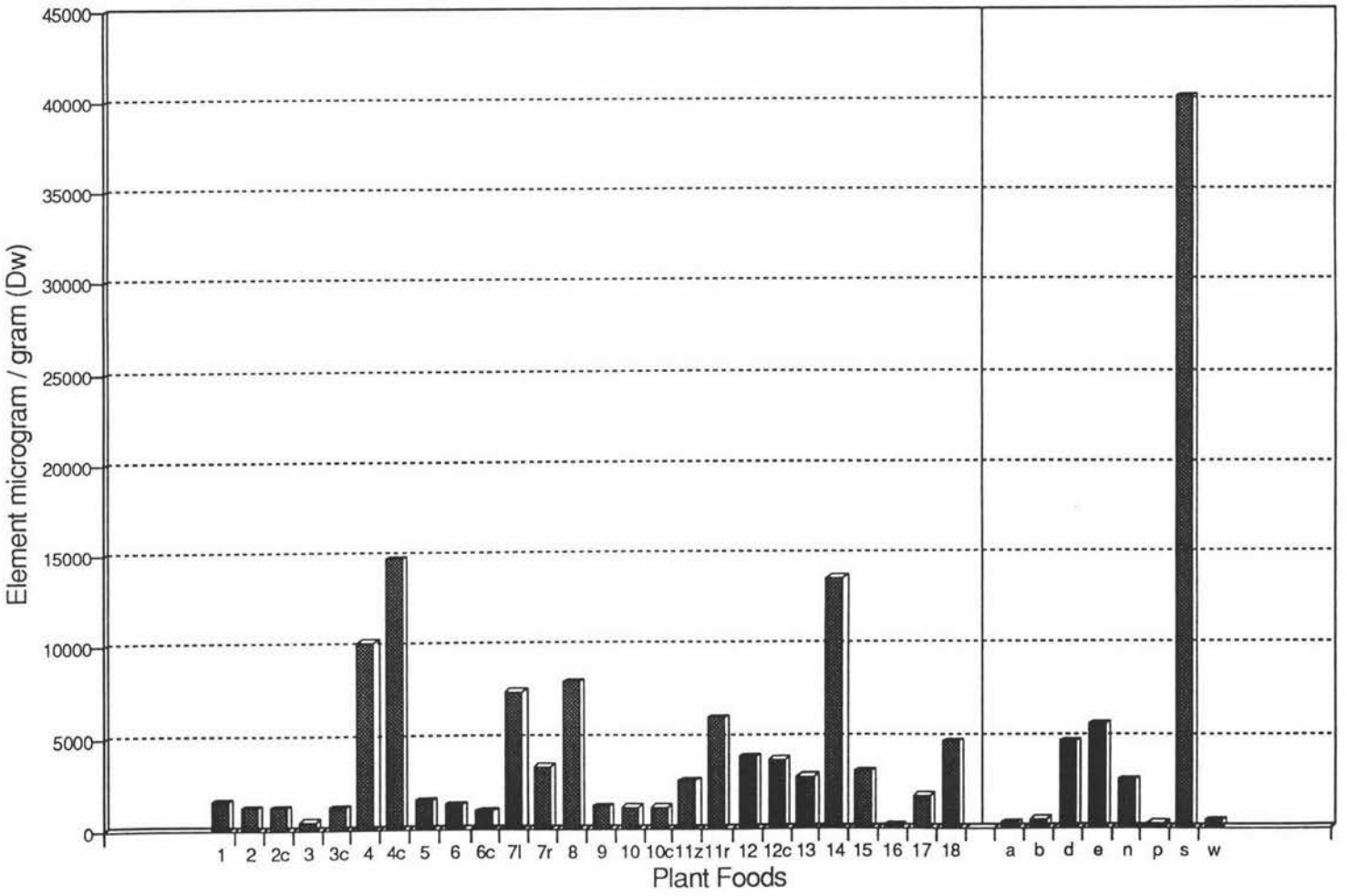


Figure 8: Comparative abundance of MAGNESIUM in plant organs and reference plant foods

For Legend refer to page 187

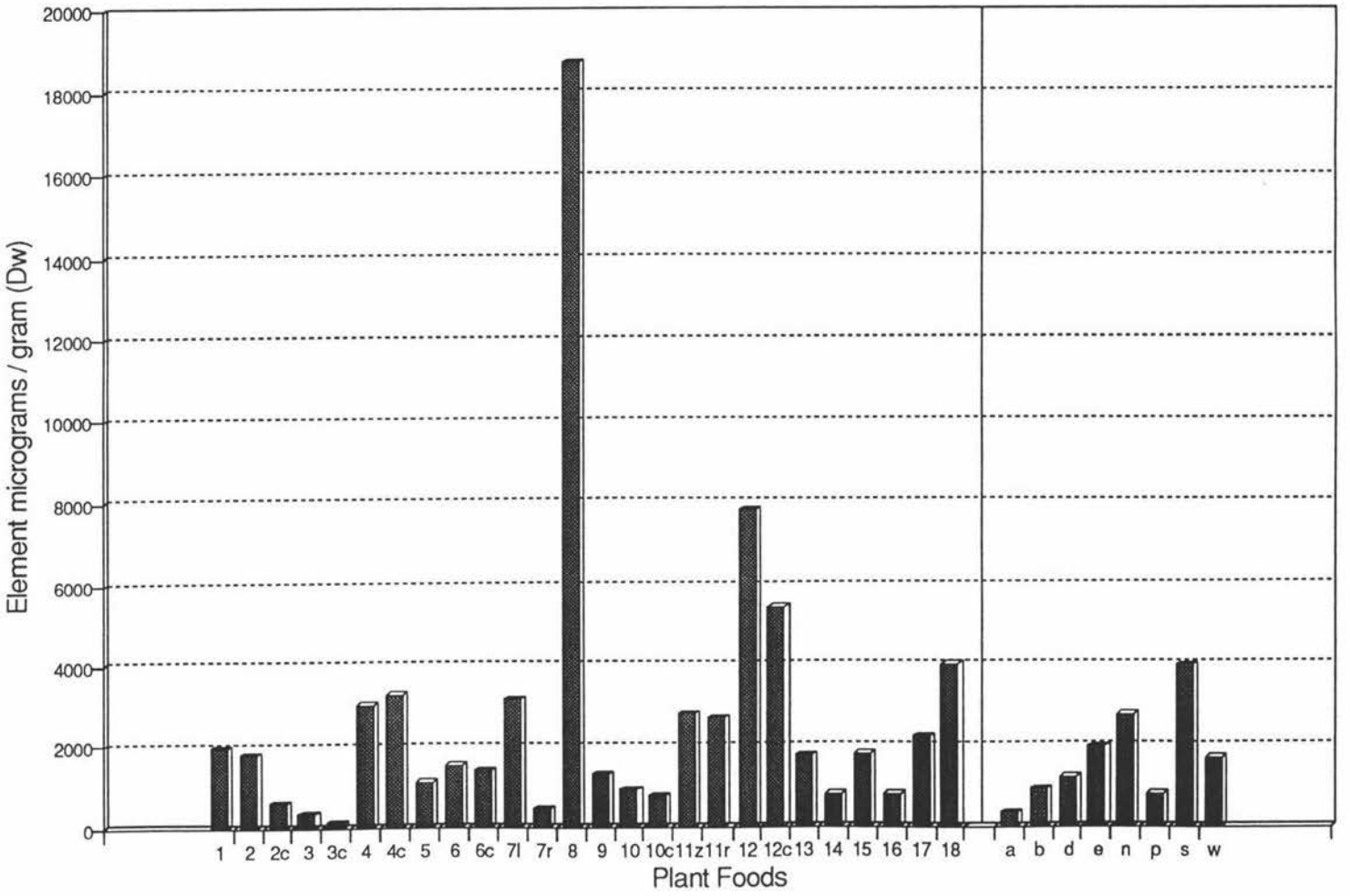


Figure 9: Comparative abundance of ZINC in plant organs and reference plant foods

For Legend refer to page 187

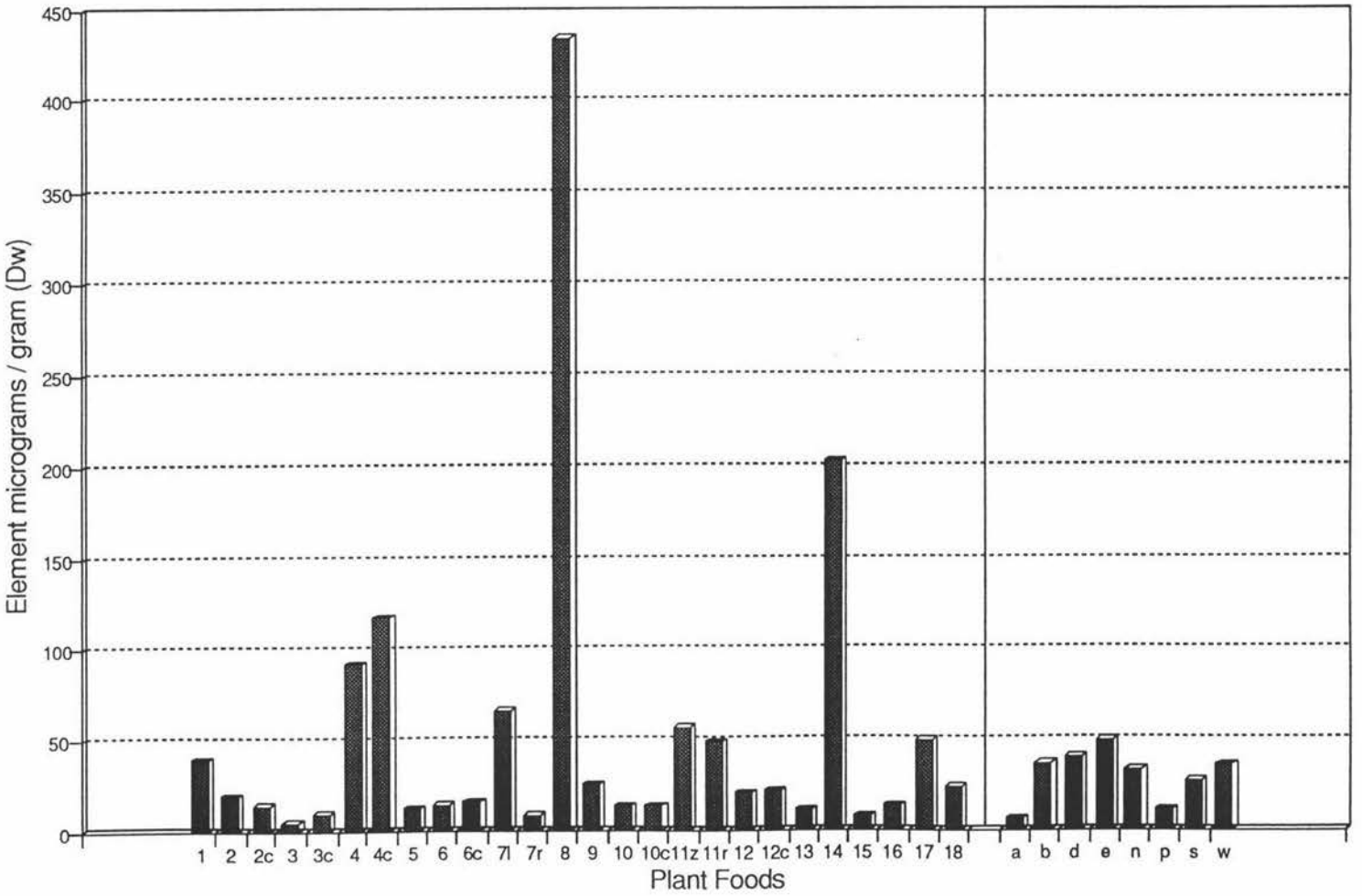


Figure 10: Comparative abundance of IRON in plant organs and reference plant foods

For Legend refer to page 187

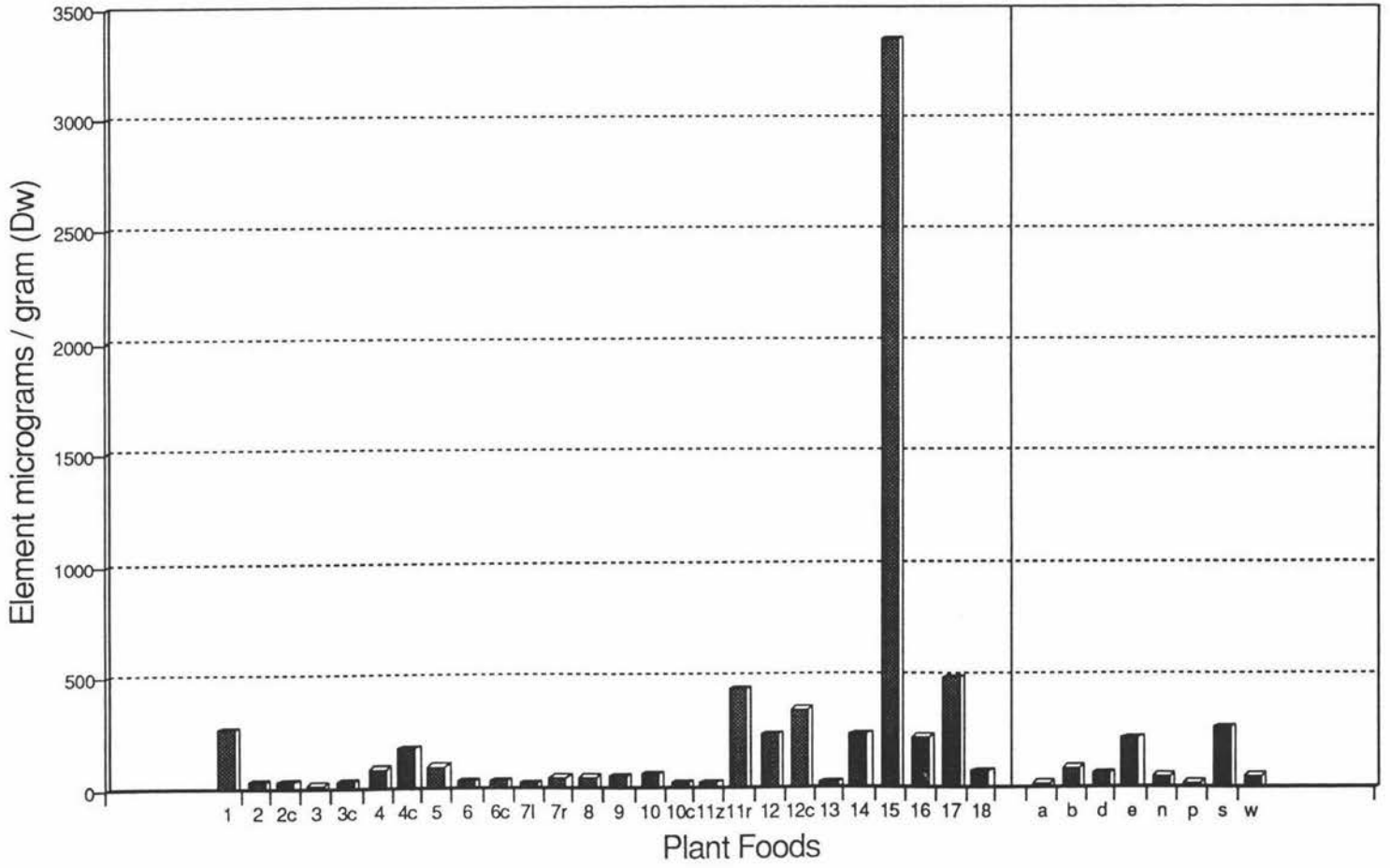


Figure 11: Comparative abundance of POTASSIUM in plant organs and reference plant foods

For Legend refer to page 187

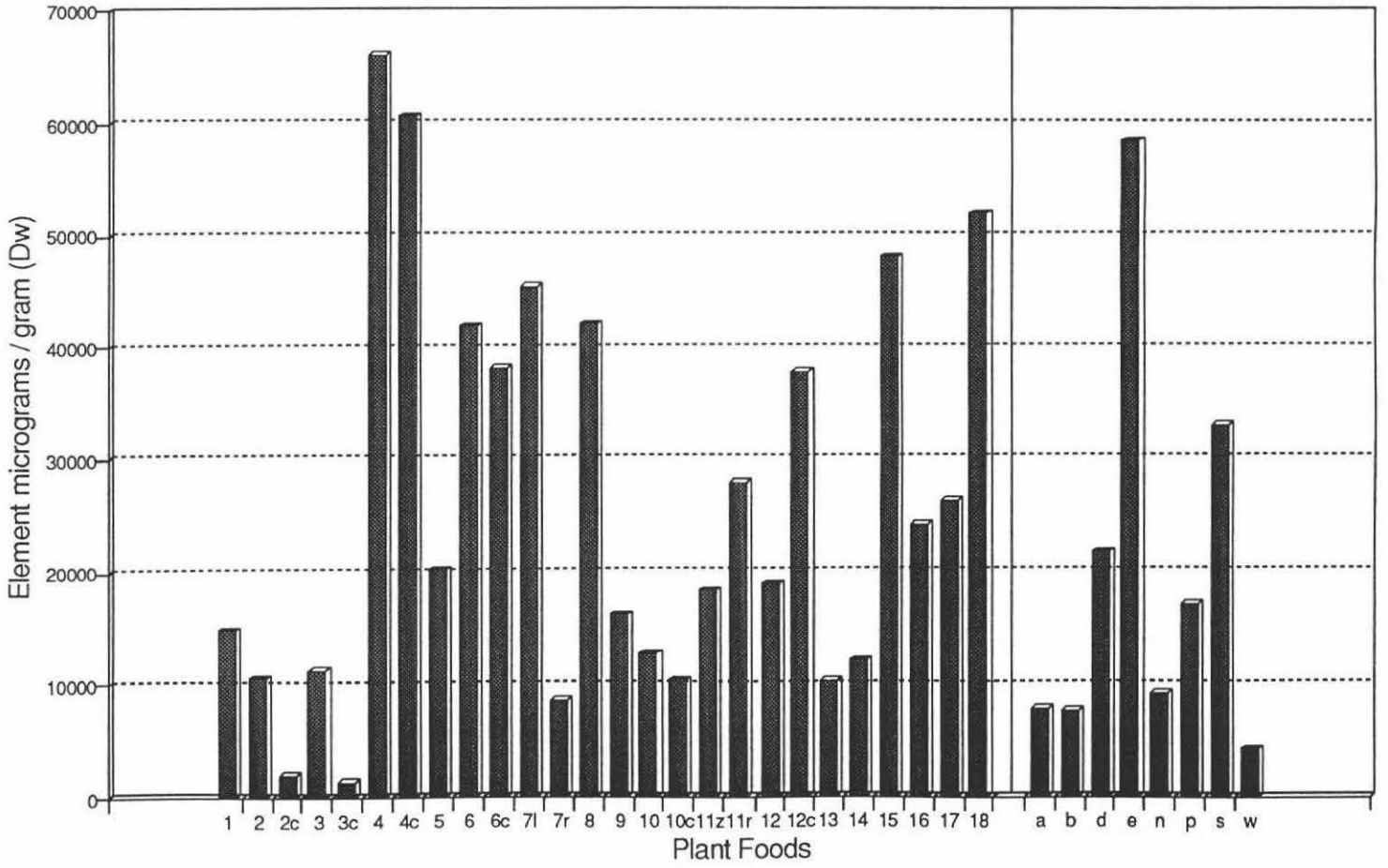


Figure 12: Comparative abundance of MANGANESE in plant organs

For Legend refer to page 187

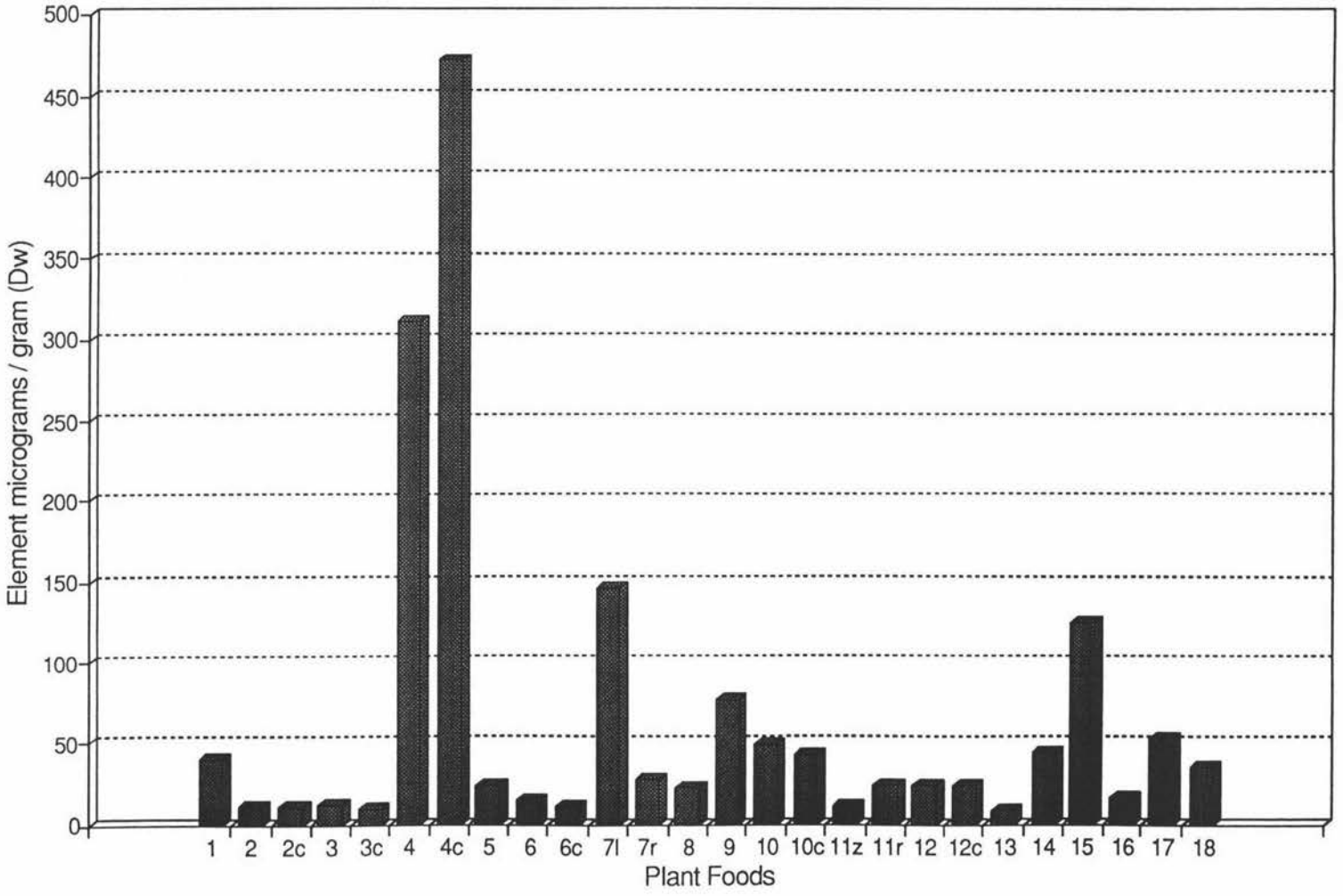


Figure 13: Comparative abundance of SILICON in plant organs

For Legend refer to page 187

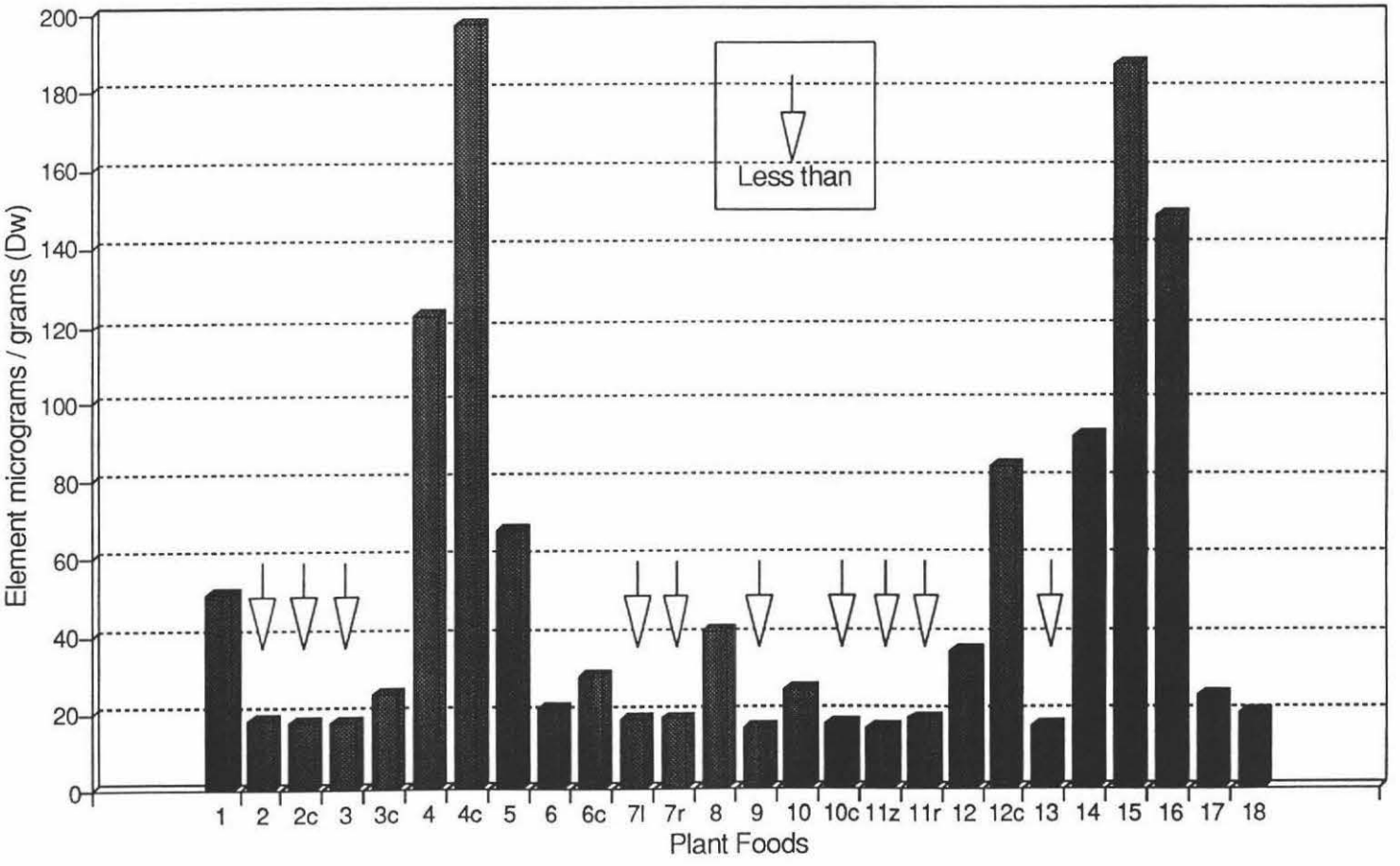


Figure 14: Comparative abundance of BORON in plant organs

For Legend refer to page 187

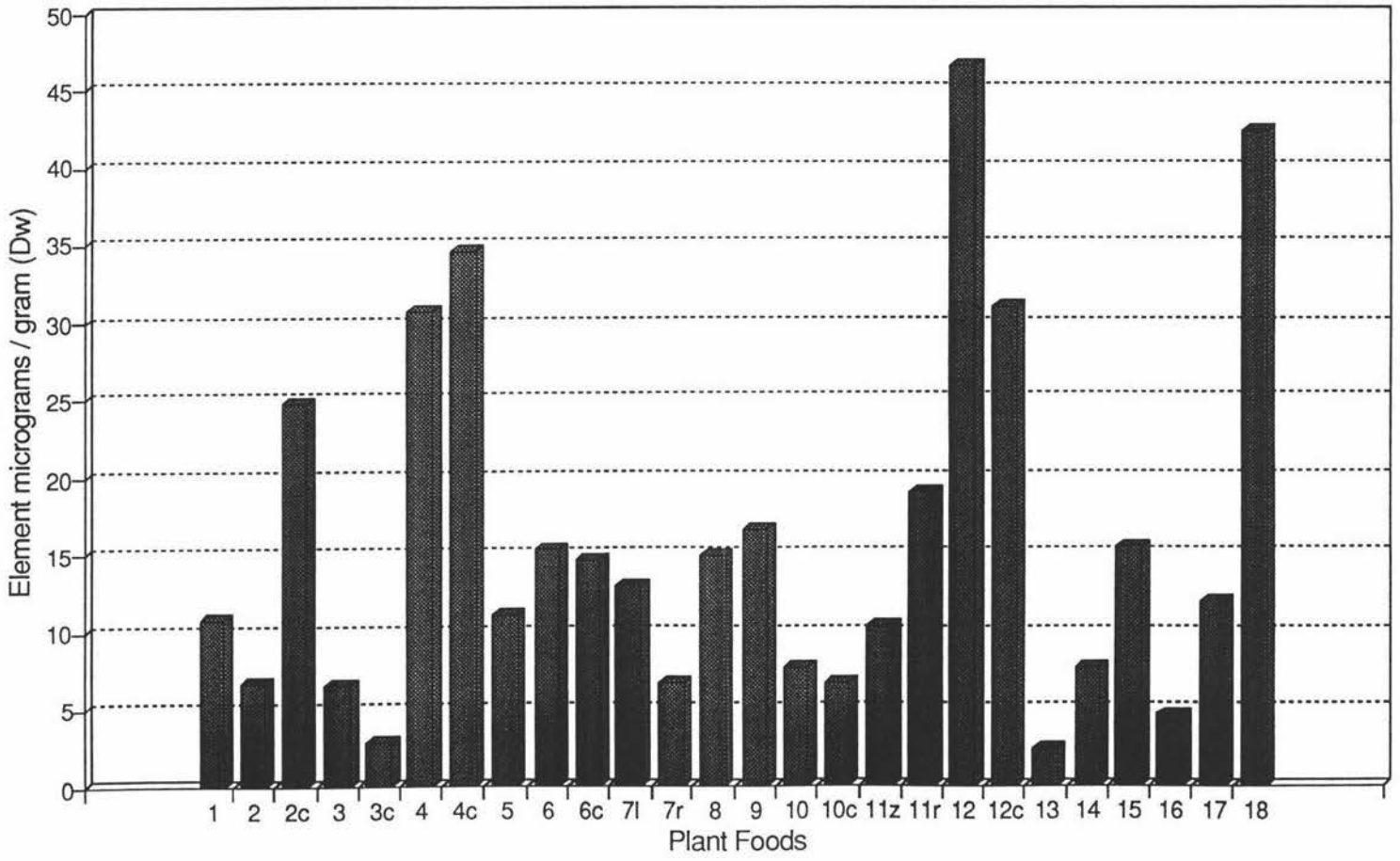


Figure 15: Comparative abundance of PHOSPHOROUS in plant organs and reference plant foods

For Legend refer to page 187

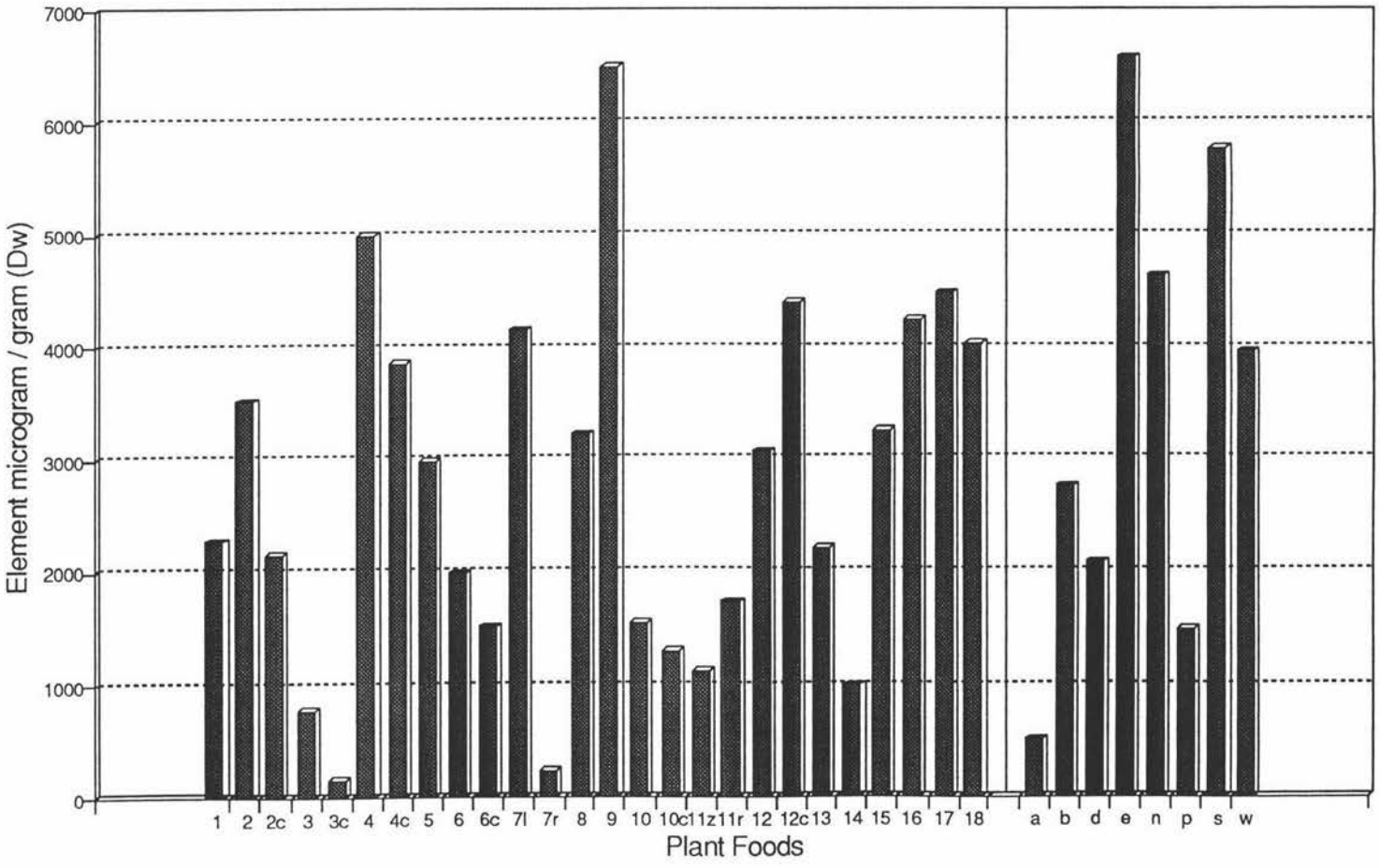


Figure 16: Comparative abundance of SULPHUR in plant organs and reference plant foods

For Legend refer to page 187

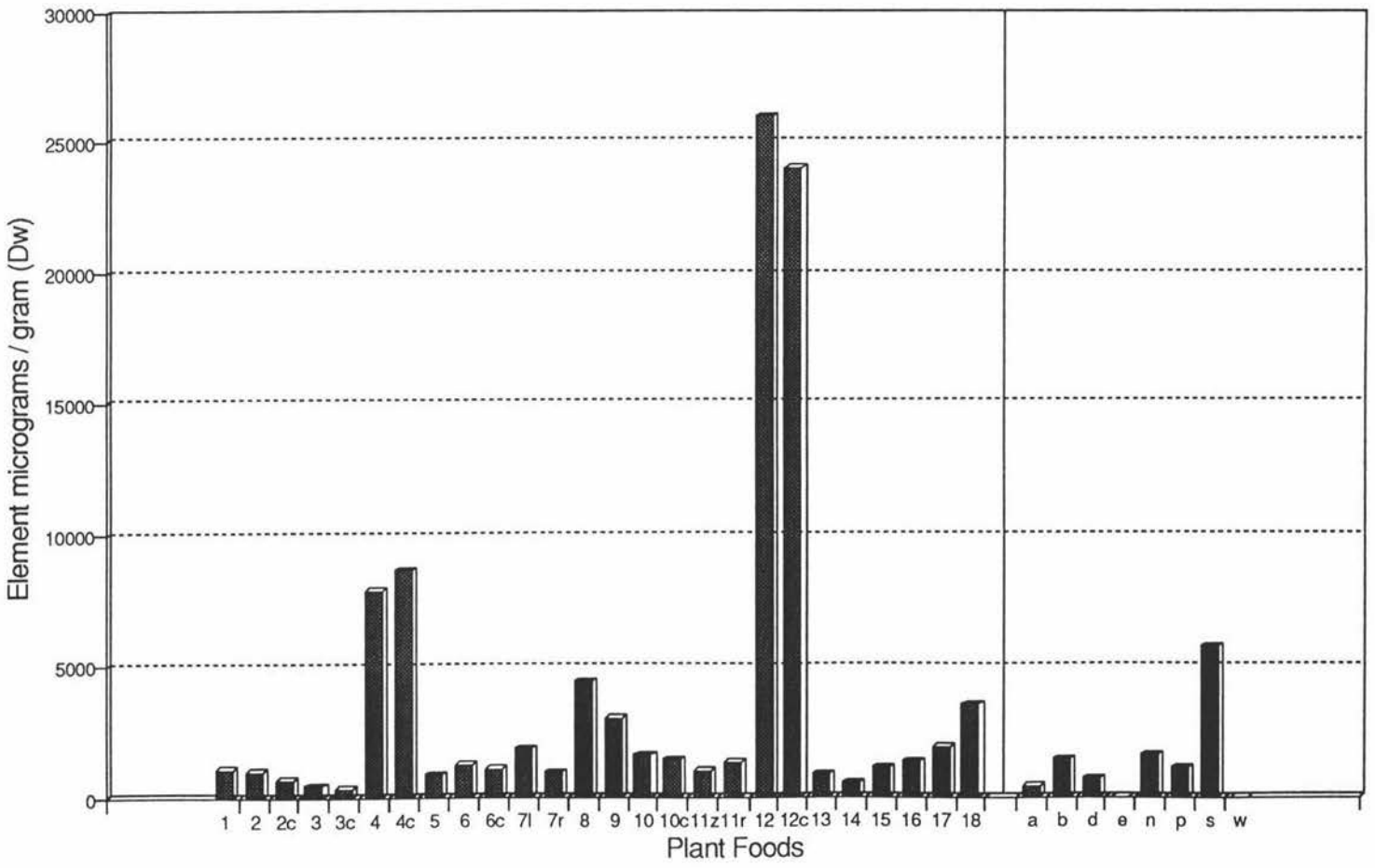


Figure 17: Comparative abundance of SODIUM in plant organs and reference plant foods

For Legend refer to page 187

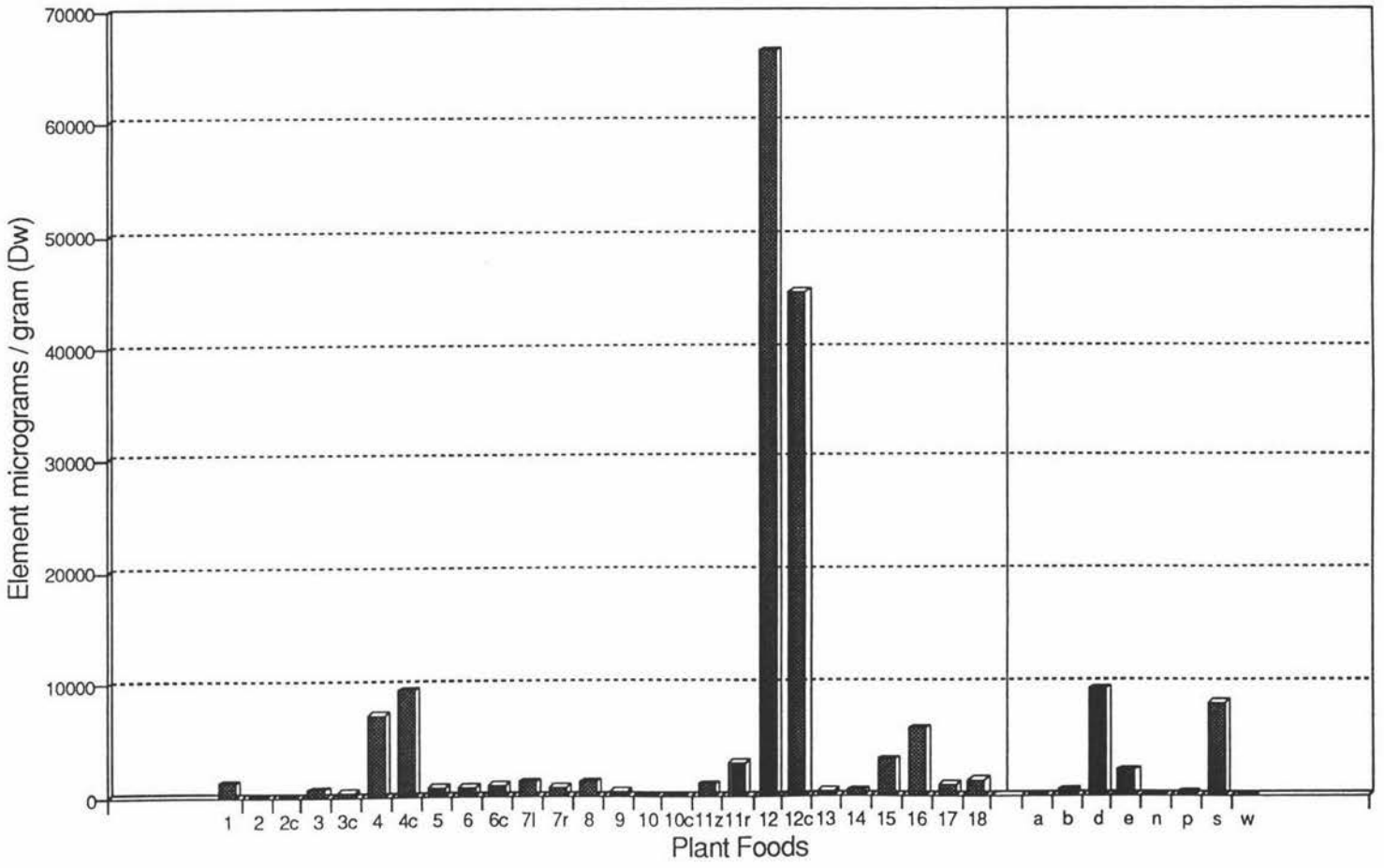


Figure 18: Comparative abundance of COPPER in plant organs and reference plant foods

For Legend refer to page 187

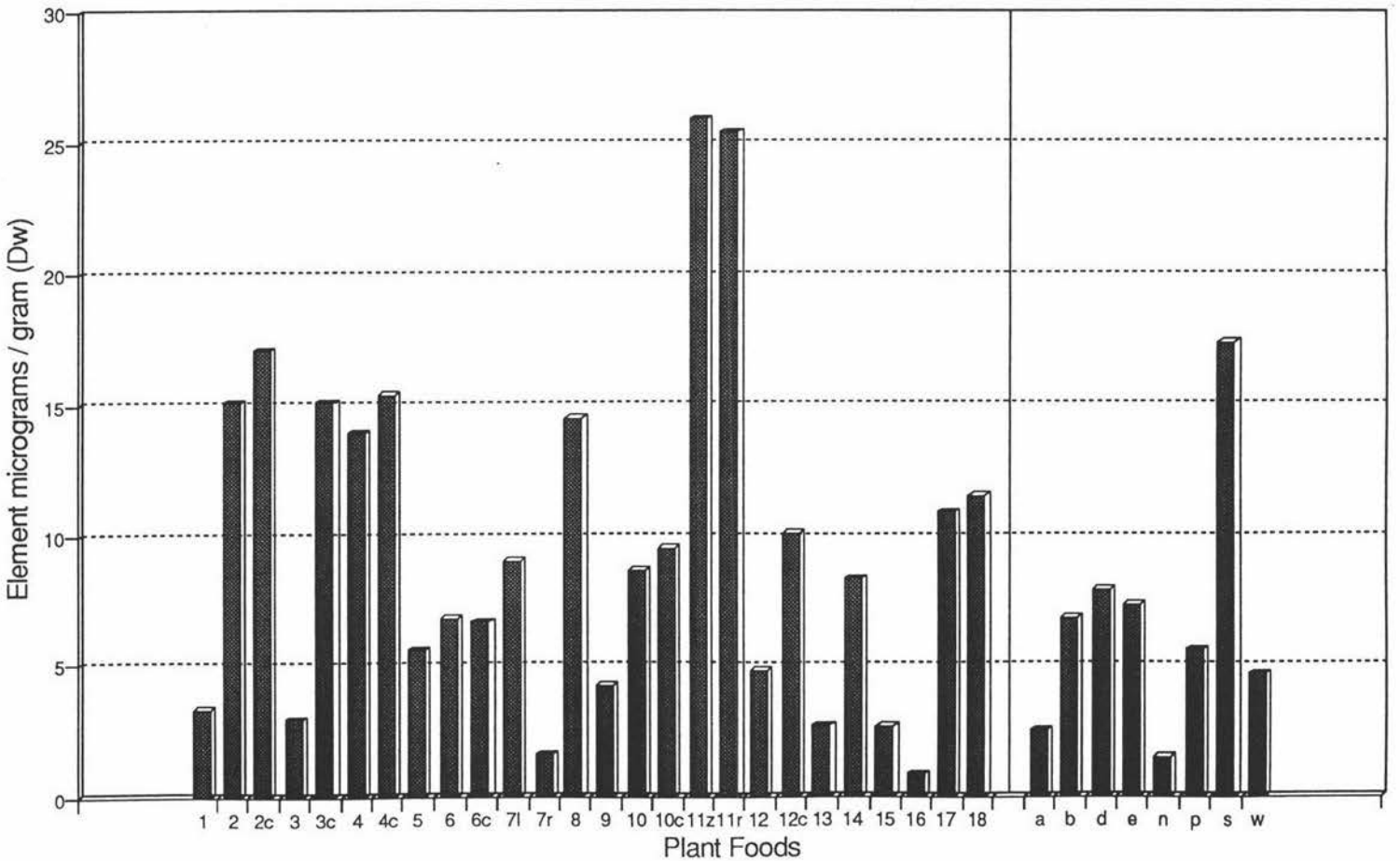


Figure 19: Comparative abundance of ALUMINIUM in plant organs

For Legend refer to page 187

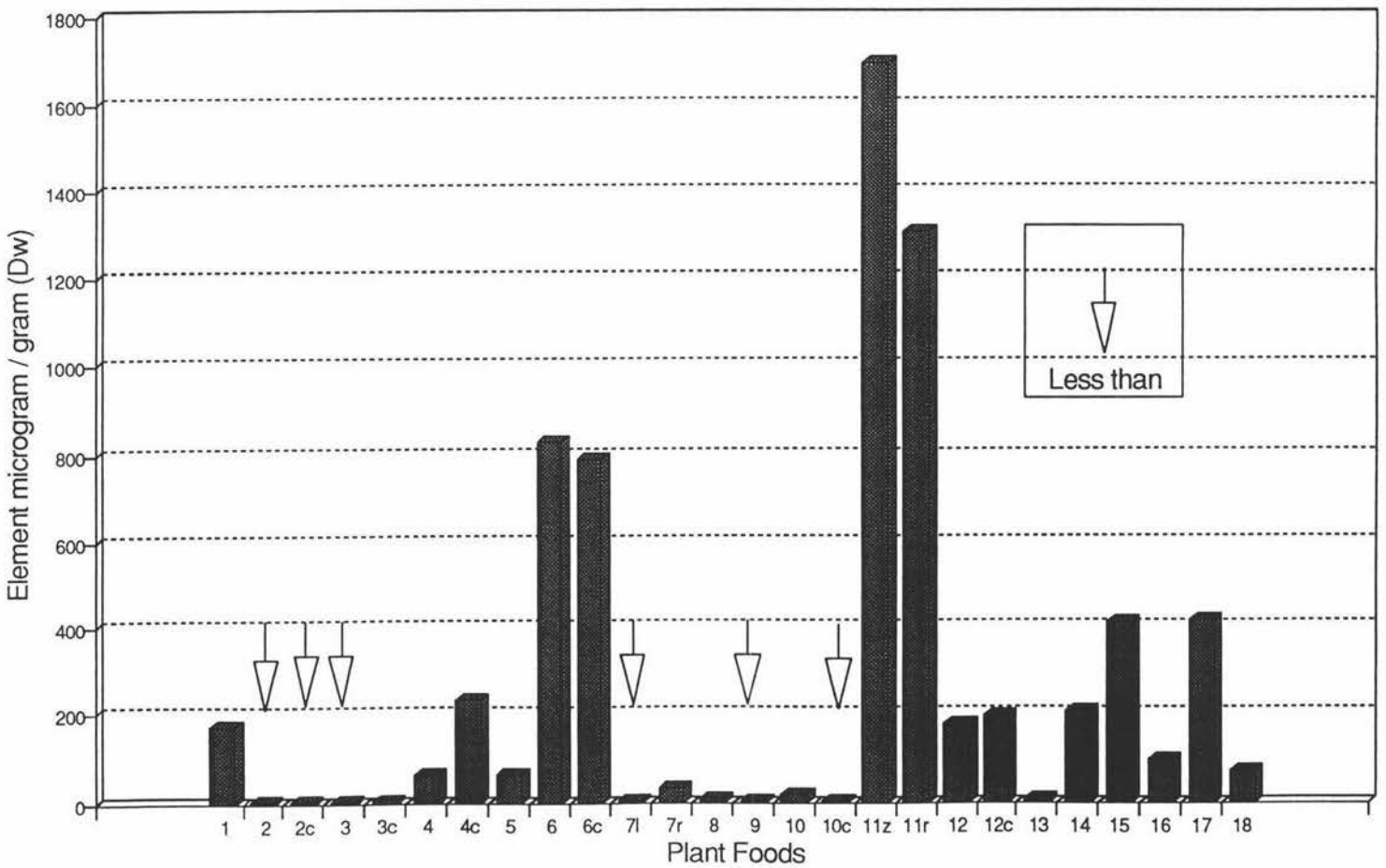
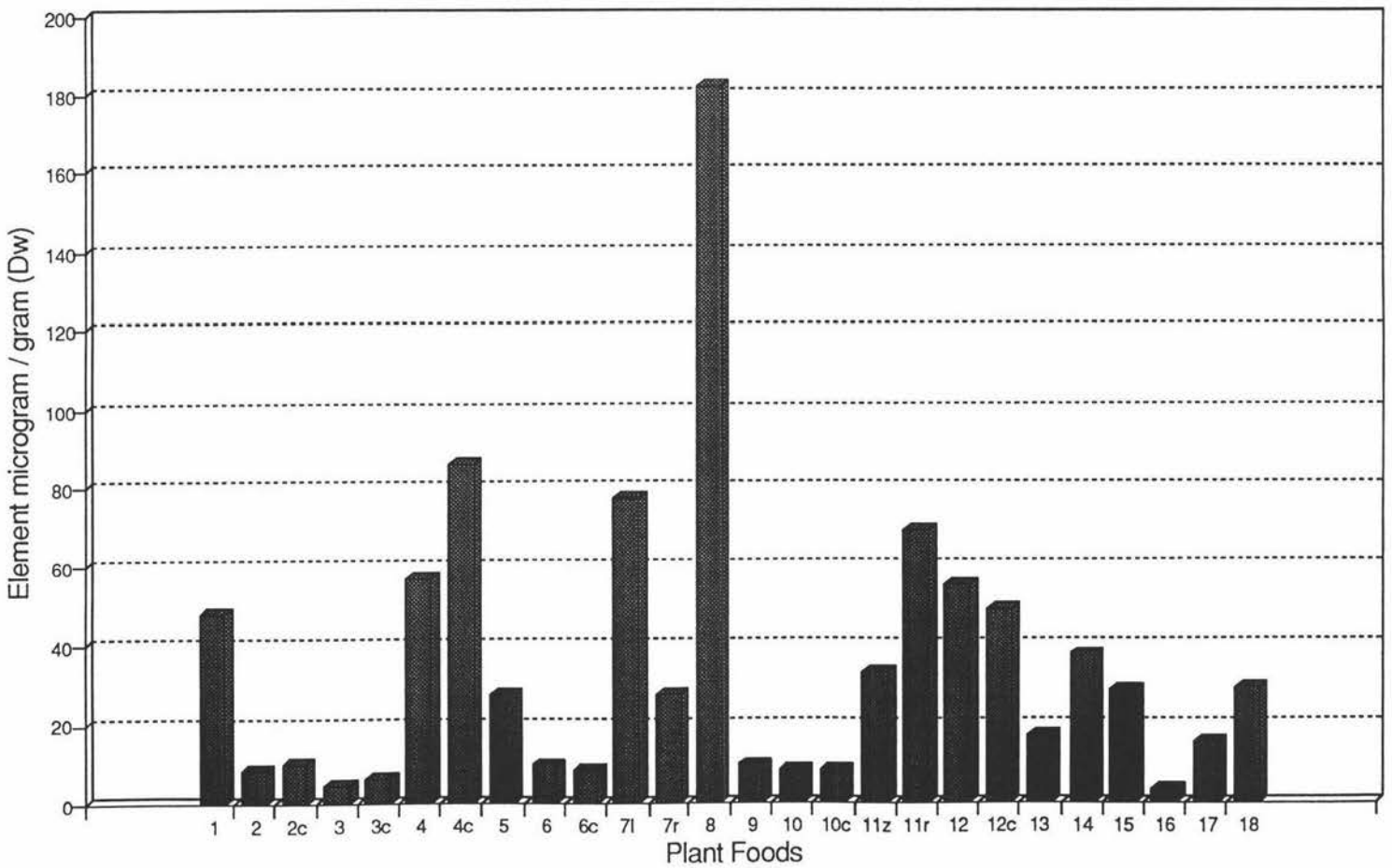


Figure 20: Comparative abundance of STRONTIUM in plant organs

For Legend refer to page 187



Legend for figures

1 *Pteridium esculentum*: rhizome
2 *Corynocarpus laevigatus*: kernel
2c *Corynocarpus laevigatus*: kernel, cooked
3 *Elaeocarpus dentatus*: pericarp
3c *Elaeocarpus dentatus*: pericarp, cooked
4 *Sonchus asper*: herb
4c *Sonchus asper*: herb, cooked
5 *Calystegia sepium*: rhizome
6 *Cyathea medullaris*: frond stipe
6c *Cyathea medullaris*: frond stipe, cooked
7l *Cordyline australis*: leaf bases
7r *Cordyline australis*: root
8 *Rhopalostylis sapida*: apical shoot
9 *Typha orientalis*: pollen
10 *Beilschmiedia tawa*: kernel
10c *Beilschmiedia tawa*: kernel, cooked
11z *Marattia salicina*: rhizomal scale
11r *Marattia salicina*: root
12 *Porphyra columbina*: frond
12c *Porphyra columbina*: frond, cooked
13 *Auricularium polytricha*: basidiocarp
14 *Arthropodium cirratum*: rhizome
15 *Typha orientalis*: rhizome
16 *Bolboschoenus fluviatilis*: rhizomal tuber
17 *Gastrodia cunninghamii*: rhizome
18 *Asplenium bulbiferum*: immature frond

a, apple; b, lentils (boiled); c, cooked sample; d, carrot; e, lettuce; l, leaf bases; n, almond; p, potatoes (boiled); r, root; s, spinach (boiled); w, wheat flour (100% wholemeal); z, rhizomal scale.

Conclusion

The scope of this research is enormous, encompassing as it does the nutritional and ethnobotanical perspectives in addition to the primary botanical and analytical procedures. This investigation does not, however, attempt to present more than an overview of the subject, hence the many unexplored avenues of research into the more complex aspects of plant storage. Speculation on these has, where appropriate been discussed briefly, but not extended beyond the reasonable bounds of an overview presentation. Also no attempt is made here to analyze the nutritional assessment of these plants in terms of Western food standards and recommended dietary intakes. The importance of these plants is rather seen in the context of a pre-European diet which was exclusive of introduced grains, cane sugar, reared meat, dairy foods and alcohol. Reischek (1830) wrote that while he was in New Zealand there were no signs of anything suggesting the least shortage of food. More recently Makereti (1938) wrote that there never was a poor or hungry Maori before the days of the Europeans. In support of this claim she described the three cultivated crop plants and the many wild foods available, which included those species described here. Banks (Beaglehole [Ed] 1962:21) wrote in March 1770, in the context of the largely fern-root diet, that

> "so simple a diet accompanied with moderation must be productive of sound health, which indeed these people are blessed with in a very high degree. Among them I have seen many very healthy old men, and in general the whole of them are as vigorous a race as can be imagined".

Such high praise from one of the first Europeans privileged to witness the aboriginal dietary custom allows little room for its condemnation. The overall findings in this study offer substantial support of this claim.

Appendices

Appendix 1.1

Estimated lipid content of plant organs, determined by solvent extraction; measured in grams per 100 grams dry weight[a].

Plant organ	**lipid g/100 g**
Pteridium esculentum: rhizome	2.95
Corynocarpus laevigatus: kernel	19.23
Corynocarpus laevigatus: kernel[c]	14.51
Elaeocarpus dentatus: pericarp	3.04
Elaeocarpus dentatus: pericarp[c]	6.08
Sonchus asper: herb	8.75
Sonchus asper: herb[c]	10.25
Calystegia sepium: rhizome	33.30[b]
Cyathea medullaris: frond stipe	15.40
Cyathea medullaris: frond stipe[c]	3.53
Cordyline australis: leaf bases	4.38
Cordyline australis: root	1.17
Rhopalostylis sapida: apical shoot	4.75
Typha orientalis: pollen	2.81
Beilschmiedia tawa: kernel	2.78
Beilschmiedia tawa: kernel[c]	3.00
Marattia salicina: rhizomal scale	2.18
Marattia salicina: root	74.23[b]
Porphyra sp: frond	2.64
Porphyra sp: frond[c]	2.08
Auricularia polytricha: basidiocarp	0.77
Arthropodium cirratum: rhizome	3.88
Typha orientalis: rhizome	4.96
Bolboschoenus fluviatilis: rhizomal tuber	0.89
Gastrodia cunninghamii: rhizome	1.38
Asplenium bulbiferum: frond stipe	3.95

[a] - for details of replication and analytical procedure see Part 1, Procedures and analytical methods.

[b] - may contain large proportion of volatile ether-extracted oils

[c] - denotes cooked sample

Appendix 1.2:

Estimated nitrogen content of plant organs, determined by micro-Kjeldahl digestion; measured in grams per 100 grams dry weight[a].

Plant organ	**Nitrogen g/100 g**
Pteridium esculentum: rhizome	1.31
Corynocarpus laevigatus: kernel	2.62
Corynocarpus laevigatus: kernel[c]	2.79
Elaeocarpus dentatus: pericarp	0.69
Elaeocarpus dentatus: pericarp[c]	0.71
Sonchus asper: herb	4.44
Sonchus asper: herb[c]	4.25
Calystegia sepium: rhizome	2.02
Cyathea medullaris: frond stipe	1.70
Cyathea medullaris: frond stipe[c]	1.52
Cordyline australis: leaf bases	1.49
Cordyline australis: root	0.48
Rhopalostylis sapida: apical shoot	2.52
Typha orientalis: pollen	2.95
Beilschmiedia tawa: kernel	1.66
Beilschmiedia tawa: kernel[c]	1.86
Marattia salicina: rhizomal scale	1.22
Marattia salicina: root	1.55
Porphyra sp.: frond	2.01
Porphyra sp.: frond[c]	2.12
Auricularia polytricha: basidiocarp	1.86
Arthropodium cirratum: rhizome	0.85
Typha orientalis: rhizome	1.43
Bolboschoenus fluviatilis: rhizomal tuber	2.08
Gastrodia cunninghamii: rhizome	2.90
Asplenium bulbiferum: frond stipe	3.48

[a] - for details of replication and analytical procedure see Part 1, Procedures and analytical methods.

[c] - denotes cooked sample

Appendix 1.3:

Estimated protein content of plant organs, determined by Bradford procedure; measured in g per 100 g dry weight[a].

Plant organ	**Protein g/100 g**
Pteridium esculentum: rhizome	8.18
Corynocarpus laevigatus: kernel	15.91
Corynocarpus laevigatus: kernel[c]	15.94
Elaeocarpus dentatus: pericarp	4.32
Elaeocarpus dentatus: pericarp[c]	4.72
Sonchus asper: herb	27.00
Sonchus asper: herb[c]	26.68
Calystegia sepium: rhizome	11.55
Cyathea medullaris: frond stipe	8.33
Cyathea medullaris: frond stipe[c]	7.81
Cordyline australis: leaf bases	8.91
Cordyline australis: root	3.22
Rhopalostylis sapida: heart	14.75
Typha orientalis: pollen	15.28
Beilschmiedia tawa: kernel	8.37
Beilschmiedia tawa: kernel[c]	9.62
Marattia salicina: rhizomal scale	6.62
Marattia salicina: root	7.69
Porphyra sp.: frond	9.61
Porphyra sp.: frond[c]	10.39
Auricularia polytricha: basidiocarp	8.64
Arthropodium cirratum: rhizome	5.31
Typha orientalis: rhizome	8.04
Bolboschoenus fluviatilis: rhizomal tuber	13.00
Gastrodia cunninghamii: rhizome	13.86
Asplenium bulbiferum: frond stipe	19.94

[a] - for details of replication and analytical procedure see Part 1, Procedures and analytical methods.

[c] - denotes cooked sample

Appendix 1.4:

Estimated dietary fibre (NSP) content of plant organs, determined by Englyst procedure; measured in g per 100 g dry weight[a].

Plant organ	**Dietary fibre g/100 g**	
	insoluble	soluble
Pteridium esculentum: rhizome	24.35	12.33
Corynocarpus laevigatus: kernel	8.26	27.09
Corynocarpus laevigatus: kernel[c]	11.09	28.02
Elaeocarpus dentatus: pericarp	15.88	16.53
Elaeocarpus dentatus: pericarp[c]	11.39	11.62
Sonchus asper: herb	24.60	16.33
Sonchus asper: herb[c]	27.51	17.20
Calystegia sepium: rhizome	16.41	23.09
Cyathea medullaris: frond stipe	11.73	15.49
Cyathea medullaris: frond stipe[c]	18.43	13.94
Cordyline australis: leaf bases	34.08	3.58
Cordyline australis: root	20.27	14.57
Rhopalostylis sapida: apical shoot	21.78	12.67
Typha orientalis: pollen	3.95	12.11
Beilschmiedia tawa: kernel	30.88	22.53
Beilschmiedia tawa: kernel[c]	42.36	29.28
Marattia salicina: rhizomal scale	17.96	23.68
Marattia salicina: root	28.12	30.39
Porphyra sp.: frond	40.37	10.21
Porphyra sp.: frond[c]	20.97	21.48
Auricularia polytricha: basidiocarp	54.88	28.27
Arthropodium cirratum: rhizome	16.64	19.80
Typha orientalis: rhizome	37.41	7.55
Bolboschoenus fluviatilis: rhizomal tuber	37.30	8.88
Gastrodia cunninghamii: rhizome	13.10	15.53
Asplenium bulbiferum: frond stipe	34.10	11.73

[a] - for details of replication and analytical procedure see Part 1, Procedures and analytical mehtods

[c] - denotes cooked sample

Appendix 1.5:

Estimated soluble sugar content of plant organs, determined by hydrolysis; measured in g per 100 g dry weight[a].

Plant organ	soluble sugar g/100 g
Pteridium esculentum: rhizome	31.38
Corynocarpus laevigatus: kernel	13.82
Corynocarpus laevigatus: kernel[c]	1.13
Elaeocarpus dentatus: pericarp	1.85
Elaeocarpus dentatus: pericarp[c]	0.89
Sonchus asper: herb	5.44
Sonchus asper: herb[c]	4.43
Calystegia sepium: rhizome	5.28
Cyathea medullaris: frond stipe	40.11
Cyathea medullaris: frond stipe[c]	46.23
Cordyline australis: leaf bases	40.20
Cordyline australis: root	20.86
Rhopalostylis sapida: apical shoot	18.64
Typha orientalis: pollen	22.82
Beilschmiedia tawa: kernel	4.29
Beilschmiedia tawa: kernel[c]	3.08
Marattia salicina: rhizomal scale	6.40
Marattia salicina: root	3.74
Porphyra sp.: frond	1.18
Porphyra sp.: frond[c]	0.74
Auricularia polytricha: basidiocarp	0.09
Arthropodium cirratum: rhizome	16.28
Typha orientalis: rhizome	29.02
Bolboschoenus fluviatilis: rhizomal tuber	4.52
Gastrodia cunninghamii: rhizome	11.13
Asplenium bulbiferum: frond stipe	4.67

[a] - for details of replication and analytical procedure see Part 1, Procedures and analytical methods.

[c] - denotes cooked sample

Appendix 1.6:

Estimated starch content of plant organs, determined by hydrolysis and enzymatic digestion; measured in g per 100 g dry weight[a].

Plant organ	**Starch g/100 g**
Pteridium esculentum: rhizome	2.67
Corynocarpus laevigatus: kernel	26.19
Corynocarpus laevigatus: kernel[c]	36.93
Elaeocarpus dentatus: pericarp	34.89
Elaeocarpus dentatus: pericarp[c]	44.01
Sonchus asper: herb	2.39
Sonchus asper: herb[c]	2.10
Calystegia sepium: rhizome	28.42
Cyathea medullaris: frond stipe	3.39
Cyathea medullaris: frond stipe[c]	3.41
Cordyline australis: leaf bases	1.93
Cordyline australis: root	3.20
Rhopalostylis sapida: apical shoot	5.95
Typha orientalis: pollen	9.27
Beilschmiedia tawa: kernel	20.91
Beilschmiedia tawa: kernel[c]	16.80
Marattia salicina: rhizomal scales	34.10
Marattia salicina: root	11.94
Porphyra sp.: frond	3.18
Porphyra sp.: frond[c]	2.53
Auricularia polytricha: basidiocarp	3.11
Arthropodium cirratum: rhizome	1.22
Typha orientalis: rhizome	2.82
Bolboschoenus fluviatilis: rhizomal tuber	21.63
Gastrodia cunninghamii: rhizome	26.70
Asplenium bulbiferum: frond stipe	4.55

[a] - for details of replication and analytical procedure see Part 1, Procedures and analytical methods.

[c] - denotes cooked sample

Appendix 2.1

Factors for converting total nitrogen in foods to protein.

Foods	**conversion factor[a] (per gN)**
Cereals	
Wheat flour	
100% wholemeal	5.83
other wheat flours	5.70
wheat bran	6.31
Rice	5.95
Barley, Oats, Rye	5.83
Soya	5.71
Nuts	
Peanuts, Brazil nuts	5.41
Almonds	5.18
All other nuts	5.30
Milk and milk products	6.38
Gelatin	5.55
All other foods	6.25

[a] - The factors used are those suggested by the FAO/WHO Committee on Energy and Protein Requirements (FAO/WHO 1973 in McCance and Widdowson 1978).

Appendix 3.1 Estimated element content of plant organs, determined by Plasma Emission Spectrometry, measured in micrograms per gram dry weight.

For details of replication and analytical procedure see Part 1, Procedures and analytical methods.

Element	1	2	2c	3	3c
Ca	1606.00	1266.00	1260.00	507.00	1127.00
Mg	1955.00	1787.00	603.00	303.00	142.00
Zn	38.80	19.10	14.00	4.40	9.10
Fe	267.00	29.50	34.00	19.80	34.00
K	14681.00	10489.00	1865.00	11141.00	1274.00
Mn	40.50	11.10	10.20	11.40	8.90
Si	50.50	<17.80	<17.40	<17.40	25.00
B	10.80	6.60	24.80	6.50	2.80
P	2280.00	3515.00	2144.00	761.00	152.00
S	1051.00	997.00	640.00	466.00	355.00
Na	1262.00	28.00	79.00	662.00	299.00
Se	<11.30	<7.10	<0.70	<0.70	<7.10
Cr	4.10	<0.70	<0.70	<0.70	<7.10
Co	<1.16	<.71	<0.70	<0.70	<7.10
Mo	<1.16	<.71	<0.70	<0.70	<7.10
Cu	3.30	15.10	17.10	2.90	15.10
Ni	1.70	4.00	0.87	<0.70	2.20
Al	173.00	<3.60	<3.5	<3.50	4.40
Pb	<5.90	<3.60	<3.5	<3.50	<3.60
Cd	<1.16	<0.71	<0.70	<0.70	<.71
As	<11.80	<7.40	<0.70	<7.00	<7.10
Sr	48.00	8.20	4.30	4.30	5.90
Sn	<1.16	<0.69	<0.70	<0.70	<.71

Appendix 3.1 cont. Estimated element content of plant organs, determined by Plasma Emission Spectrometry, measured in micrograms per gram dry weight.

For details of replication and analytical procedure see Part 1, Procedures and analytical methods.

Element	4	4c	5	6	6c
Ca	10147.00	14759.00	1568.00	1282.00	1008.00
Mg	3003.00	3257.00	1082.00	1509.00	1367.00
Zn	91.00	117.00	12.40	14.10	15.70
Fe	91.00	178.00	93.00	32.30	34.80
K	65934.00	60597.00	20183.00	41856.00	38112.00
Mn	311.00	472.00	22.30	14.10	11.00
Si	123.00	197.00	67.00	21.00	29.50
B	30.80	34.50	11.10	15.30	14.60
P	4987.00	3849.00	2984.00	1974.00	1512.00
S	7860.00	8644.00	856.00	1246.00	1062.00
Na	7159.00	9313.00	771.00	746.00	846.00
Se	<7.50	<7.2	<6.80	<7.90	<6.80
Cr	<.75	0.96	<0.68	<0.69	<0.68
Co	<.75	<.72	<0.68	<0.69	<0.68
Mo	<.75	<.72	<0.68	<0.69	<0.68
Cu	14.00	15.40	5.60	6.80	6.70
Ni	3.20	<.72	<0.68	<0.69	<0.68
Al	65.00	231.00	64.00	830.00	789.00
Pb	<3.80	<3.6	<3.40	<3.50	<3.40
Cd	1.90	3.30	<0.68	<0.69	<0.68
As	<7.50	<7.2	<6.80	<6.90	<6.80
Sr	57.00	86.00	27.00	9.30	8.40
Sn	<.75	<.72	<0.68	<0.69	<0.68

Appendix 3.1 cont. Estimated element content of plant organs, determined by Plasma Emission Spectrometry, measured in micrograms per gram dry weight.

For details of replication and analytical procedure see Part 1, Procedures and analytical methods.

Element	7l	7r	8	9	10	10c
Ca	7437.00	3359.00	7951.00	1254.00	1128.00	1107.00
Mg	3101.00	435.00	18741.00	1266.00	880.00	728.00
Zn	65.00	8.20	435.00	24.80	13.10	13.10
Fe	26.00	46.00	42.80	49.80	60.50	27.00
K	45460.00	8503.00	42020.00	16079.00	12587.00	10332.00
Mn	146.00	27.50	21.80	76.00	48.80	42.00
Si	<17.80	<18.30	40.50	<15.90	25.60	<17.2
B	12.80	6.60	14.80	16.50	7.70	6.60
P	4149.00	226.00	3222.00	6501.00	1549.00	1284.00
S	1816.00	921.00	4449.00	2993.00	1606.00	1391.00
Na	1235.00	769.00	1160.00	355.00	117.00	68.00
Se	<7.10	<7.40	<6.90	<6.40	<6.40	<6.90
Cr	<0.71	<0.74	0.96	<0.66	0.97	<0.69
Co	<0.71	<0.74	<0.69	<0.64	<0.63	<0.69
Mo	<0.71	<0.74	<0.69	<0.64	<0.64	<0.69
Cu	9.00	1.60	14.50	4.20	8.60	9.50
Ni	7.20	<0.73	2.00	<0.64	7.80	0.95
Al	<3.60	36.00	8.30	<3.2	20.10	<3.40
Pb	<3.60	<3.70	<3.50	<3.2	<3.20	<3.40
Cd	<0.71	<0.74	<0.69	<0.64	<0.64	<0.69
As	<7.10	<7.30	<6.90	<6.40	<0.64	<0.69
Sr	77.00	27.50	182.00	9.30	8.20	7.80
Sn	<0.71	<0.74	<0.69	<0.64	<0.64	<0.69

Appendix 3.1 cont. Estimated element content of plant organs, determined by Plasma Emission Spectrometry, measured in micrograms per gram dry weight.

For details of replication and analytical procedure see Part 1, Procedures and analytical methods.

Element	11z	11r	12	12c	13
Ca	2598.00	6005.00	3848.00	3707.00	2757.00
Mg	2783.00	2678.00	7795.00	5389.00	1707.00
Zn	56.00	47.80	19.80	21.80	10.60
Fe	27.30	444.00	235.00	353.00	25.50
K	18187.00	27854.00	18817.00	37712.00	10174.00
Mn	10.60	23.30	23.50	23.00	7.80
Si	<15.90	<18.30	35.80	83.00	<16.20
B	10.40	19.00	46.50	31.00	2.40
P	1124.00	1721.00	3058.00	4387.00	2204.00
S	1003.00	1323.00	25991.00	24051.00	874.00
Na	1075.00	2904.00	66361.00	44917.00	421.00
Se	<6.40	<7.30	<7.40	<7.00	<6.50
Cr	<0.64	1.20	3.20	3.90	0.84
Co	<0.64	<0.73	<0.74	<0.70	<0.65
Mo	<0.64	<0.73	<0.74	<0.70	<0.65
Cu	26.00	25.50	4.80	10.10	2.70
Ni	<0.64	2.20	3.00	2.20	1.10
Al	1692.00	1305.00	177.00	197.00	8.40
Pb	<3.20	<3.70	<3.70	<3.50	<3.20
Cd	<0.64	<0.73	<0.74	<0.70	<0.65
As	<0.64	<0.73	32.50	21.50	<6.50
Sr	32.80	69.00	55.00	48.80	17.00
Sn	<0.64	<0.73	<0.74	<0.70	<0.65

Appendix 3.1 cont. Estimated element content of plant organs, determined by Plasma Emission Spectrometry, measured in micrograms per gram dry weight.

For details of replication and analytical procedure see Part 1, Procedures and analytical methods.

Element	14	15	16	17	18
Ca	13643.00	3092.00	164.00	1714.00	4745.00
Mg	786.00	1758.00	790.00	2212.00	3996.00
Zn	203.00	7.30	12.90	48.80	23.30
Fe	238.00	3362.00	222.00	490.00	74.00
K	12112.00	48147.00	24112.00	26293.00	51942.00
Mn	43.00	124.00	15.10	52.50	34.30
Si	91.00	187.00	148.00	24.00	20.00
B	7.60	15.40	4.60	11.90	42.30
P	994.00	3251.00	4231.00	4477.00	4025.00
S	512.00	1145.00	1340.00	1903.00	3519.00
Na	460.00	3132.00	2396.00	864.00	1398.00
Se	<6.9	<6.50	<6.50	<7.30	<7.10
Cr	0.78	<0.65	1.05	1.15	0.81
Co	<0.74	<0.65	<0.67	<0.73	<0.71
Mo	<0.69	<0.65	<0.65	<0.73	<0.71
Cu	8.30	2.60	0.90	10.90	11.50
Ni	<0.69	<0.65	6.00	1.10	<0.74
Al	207.00	412.00	98.00	416.00	74.00
Pb	<3.50	<3.30	<3.20	<3.70	<3.60
Cd	<0.69	<0.65	<0.65	1.40	<0.71
As	<6.90	<6.50	<6.50	<7.30	<7.10
Sr	37.50	28.50	2.90	15.40	29.00
Sn	<0.69	<0.65	<0.65	<0.73	<0.71

Appendix 3.1

Legend

1	*Pteridium esculentum*: rhizome
2	*Corynocarpus laevigatus*: kernel
2c	*Corynocarpus laevigatus*: kernel, cooked
3	*Elaeocarpus dentatus*: pericarp
3c	*Elaeocarpus dentatus*: pericarp, cooked
4	*Sonchus asper*: herb
4c	*Sonchus asper*: herb, cooked
5	*Calystegia sepium*: rhizome
6	*Cyathea medullaris*: frond stipe
6c	*Cyathea medullaris*: frond stipe, cooked
7l	*Cordyline australis*: leaf bases
7r	*Cordyline australis*: root
8	*Rhopalostylis sapida*: apical shoot
9	*Typha orientalis*: pollen
10	*Beilschmiedia tawa*: kernel
10c	*Beilschmiedia tawa*: kernel, cooked
11z	*Marattia salicina*: rhizomal scale
11r	*Marattia salicina*: root
12	*Porphyra columbina*: frond
12c	*Porphyra columbina*: frond, cooked
13	*Auricularia polytricha*: basidiocarp
14	*Arthropodium cirratum*: rhizome
15	*Typha orientalis*: rhizome
16	*Bolboschoenus fluviatilis*: rhizomal tuber
17	*Gastrodia cunninghamii*: rhizome
18	*Asplenium bulbiferum*: immature frond

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