



Australian Government

**Rural Industries Research and
Development Corporation**

STEVIA

– An intense, natural sweetener

Laying the groundwork for a new rural industry

A report for the Rural Industries Research and Development Corporation

by Andrew H Rank, David J Midmore

May 2006

RIRDC Publication No 06/020
RIRDC Project No UCQ-17A

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ISBN 1 74151 283 2
ISSN 1440-6845

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Published in May 2006
Printed on environmentally friendly paper by Canprint

Foreword

Australian rural producers are constantly on the lookout for new farm production opportunities to help them survive the never ending cost pressures of farming. Over the years many new enterprises have been tried; some have developed successfully, while others have floundered due, frequently, to inadequate research and false or misleading claims by proponents of the new industry. Most farmers are now more cautious about embarking on new, unproven ventures.

One of RIRDC's prime purposes is to support research into promising new enterprises so farmers can avoid costly mistakes when venturing into a new industry. It generally takes up to ten years of groundwork, investigation and research and 'trial and error' before a new industry is successfully established even on a small scale.

This report covers the early stages of research and trial for one of these new crop enterprises. It covers three of the essentials for a new venture: marketing, processing and agronomy; it then establishes a base for these elements on which to proceed further, so as to be able to add stevia as a new crop for some Australian farmers. The crop's market potential, and therefore appeal, has been enhanced over the last year or so by recognizing its potential to play a significant role in combating the current national, social problem of obesity and its related health issues.

This project was funded from RIRDC Core Funds, which are provided by the Australian Government, with some contributions from CQU and significant (mainly in-kind) support from farmers and industry.

This report, an addition to RIRDC's diverse range of over 1500 research publications, forms part of our New Plant Products R&D program, which aims to encourage innovation in thinking about plant products and their use in an economical and environmentally sustainable manner.

Most of our publications are available for viewing, downloading or purchasing online through our website:

- downloads at www.rirdc.gov.au/fullreports/index.html
- purchases at www.rirdc.gov.au/eshop

Peter O'Brien

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Acknowledgements

This project could not have proceeded without the considerable contribution from co-operation of farmers who provided land, irrigation and equipment and significant amounts of labour to grow plots of this hitherto foreign crop.

A number of people from various parts of the food and beverage industry have also contributed their comments and ideas freely, especially in relation to the application to FSANZ for approval of the use of steviol glycosides as a commercial food ingredient in Australia.

Also gratefully acknowledged are the contributions made from overseas towards the FSANZ application, particularly from Professor J Geuns, of the European Stevia Association and Catholic University of Leuven, Belgium, and from Mr T Katabami, representing the Japanese Stevia Industry and Japanese Ministry of Health and Welfare.

We thank the following for seed sources: K. Sahn, Zaden B.V. - Alphen aan den Rijn - The Netherlands; Mr Feng Xiaohong at Zibo, PRC.

Finally, we thank the Sugar Research Institute (Mackay) for instructive discussions on extraction of steviosides, the CQU School of Biomedical Sciences (especially Graham Boyle and Laurence Hearn) for development of the HPLC extractions and quantification process, and the CQU Non-invasive Quality Assessment Group for assistance with the analyses with NIR.

Abbreviations

ADI	Acceptable Daily Intake (food intake rate)
CQU	Central Queensland University
DPIWE	Department of Primary Industry, Water and Environment (Tasmania)
FSANZ	Food Standards Australia New Zealand (formerly ANZFA Australia & New Zealand Food Authority)
HPLC	High Performance Liquid Chromatography
JECFA	WHO/FAO Joint Expert Committee on Food Additives
mg/kgbw/d	milligrams per kilogram body weight per day (food intake rate)
NIRS	Near Infra-Red Spectroscopy
PSG	Plant Sciences Group of CQU
R-A	Rebaudioside A
SRI	Sugar Research Institute
St	Stevioside
TIAR	Tasmanian Institute of Agricultural Research

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Executive Summary

The leaves of the stevia plant (*Stevia rebaudiana* Bertoni) contain steviol glycosides, which are natural, intense sweeteners with no calories and can be used in a wide range of foods and beverages in place of sugar or other sweeteners (including man-made). Steviol glycosides have been extensively used in Japan, China, Brazil and other countries for more than thirty-five years. This project investigated the key elements of marketing, processing and agronomy to evaluate further the potential for establishing a stevia growing industry in Australia. The activity follows on from an earlier scoping study, funded by RIRDC, and reported at: <http://www.rirdc.gov.au/reports/NPP/w02-022.pdf>

Strong interest has been expressed in the growing of stevia, processing it and using the extracted glycosides by a range of individuals and groups, including farmers, farm organisations, food processors and manufacturers, ingredient suppliers and consumers. Interest has also come from overseas groups including growers, researchers, existing processors and buyers and sellers.

The research investigations undertaken by the project have been wide-ranging. They covered potential growing sites in eastern Australia from Cairns to Tasmania and have been done under near commercial growing conditions rather than as laboratory/glasshouse experiments. The contribution by farmers in growing trial areas (500 – 5,000 plants) has been considerable.

Significant advances have been made by the project in the area of marketing. The objective was to determine if registration of stevia extracts, as a food ingredient, was likely. After assembling and reviewing data (primarily from overseas), the project prepared and submitted a full application to FSANZ for the registration of steviol glycosides as a commercial food ingredient (intense sweetener). This application is now in the process of being assessed and final approval (if successful) is possible by the end of 2006. Applications for approval in other countries, e.g. Europe, are expected to be made over the next year, which could increase worldwide demand for stevia products.

In regard to processing, preliminary investigations undertaken by the Sugar Research Institute (SRI) indicate that the use of Australian sugar extraction and other food processing technology and equipment could be applied to the design of an efficient stevia mill. Various groups have expressed interest in taking equity in a future mill. In the short term, Australian grown stevia could be exported for processing in China or Japan, where mills have expressed an interest in importing leaves from Australia.

Although agronomic investigations have confirmed the potential to grow stevia in Australia, further work is required on some key issues, including weed control, variety selection and regrowth performance. Until potential growers can be assured of adequate yields over two or three years, the high cost of establishing seedlings poses an economic risk few can afford to take.

Further external support for more agronomic and analytical research and trials is required before an industry will develop and become self-sustaining.

1. Introduction

In 1999 the Plant Sciences Group of CQU undertook a desk study and literature review of the plant *Stevia rebaudiana* (Bertoni). This review concluded that stevia could in principle be grown in Australia and could have a place in providing a natural, no calorie, intense sweetener for the food and beverage industry in an era of growing diet consciousness, rapidly rising incidence of Type II diabetes and juvenile obesity.

Following the review, RIRDC provided some limited assistance to extend this preliminary study to make contact with the stevia industry in China and Japan, assess the availability and sources of stevia seed and to contact individuals and groups in Australia who may be interested in contributing to further evaluation studies. A report of this work and the earlier review is available as a RIRDC research report (UCQ 16A- <http://www.rirdc.gov.au/reports/NPP/w02-022.pdf>).

This current report covers the next stage: a two and a half year research project, that has run to three and a half years, which set out to lay a firm base for evaluation of the crop's potential from the growing, processing and marketing points of view. The funding provided for a modest project, with only part-time and casual research personnel, relying heavily on the co-operation of farmers, who contributed field sites and significant in-kind-costs (especially for labour).

The stevia plant is a native of Paraguay on the Tropic of Capricorn. Its leaves have been used by local people for many hundreds of years to sweeten local teas etc, to make medicines more palatable and also just as a sweet treat to chew on. In the 1930's and 1940's stevia was introduced to European countries (e.g. UK and Russia) as a possible sugar substitute (in war time) and Japan has used it extensively since the early 1970's. Traditionally it is cultivated using very labour intensive methods, being propagated by cuttings, dried by laying out in the sun at harvest and the leaves being hand stripped from the branches before packing, storage and processing. In cold areas, too cold to allow for over-wintering, e.g. northern China, it is grown as an annual; otherwise it ratoons as it can survive temperatures as low as -9 degrees Centigrade.

The principles of processing are quite simple: soaking the leaves in hot water to dissolve out the sweet glycosides, then filtering the solution and evaporating it to produce a white powder (or fine crystal) that is 250 – 300 times sweeter than sugar.

The plant in its native area grows as a short-lived perennial, growing at altitude in swampy areas in mountain valleys. It has been taken to many countries and shows a wide climatic adaptation and variability in plant type and also glycoside content. It has been successfully grown in tropical areas such as Indonesia, Thailand and Brazil as well as in temperate and cold zones at latitudes of up to 50 degrees North, including northern Russia, north China, Korea and Canada.

Various names have been used for the purified leaf extract of the plant *Stevia rebaudiana*, including stevia, stevioside (or steviosides), stevia sugar (or powder) and, more recently, steviol glycosides. In 2004 JECFA established that the common name to be used should be steviol glycosides, which will be a mixture of some, or all, of the ten individual glycosides but usually with Stevioside and Rebaudioside A making up most (70% or more) of the glycosides present.

2. Objectives & Approach

The specific objectives of the project were “to lay the basis for a rural industry that will replace imported artificial sweeteners with a risk free, natural sweetener”. The two key activities to achieve these objectives were to “assess information on food safety issues and determine the likely acceptance of steviol glycosides by FSANZ for use in foodstuffs” and “to assess germplasm from diverse sources and test them under a range of environmental conditions leading to quantifiable production outputs on which to evaluate the viability of the industry”.

To undertake this, the project had four main streams of activity:

Agronomy - testing and evaluating all aspects of growing from seed sources to harvesting

Processing - considering all likely processing methods (including storage and handling) and identifying possible processors

Marketing - assessing possible acceptance and uptake by the food and beverage industry (primarily in Australia) as well as identifying the requirements for, and assessing the likelihood of, approval as a commercial food additive

Plant and Product Analysis - identifying, or developing, a reliable method for rapid analysis of plant material to determine the steviol glycosides content and the quantities or ratios of the main sweet glycosides in the leaves (there is a mixture of up to ten slightly different glycosides). The traditional (overseas) method of analysis has been HPLC. For rapid analysis the development of an NIRS procedure was identified as a desirable alternative method. Reliable and rapid analysis is necessary for plant selection, to determine optimal time for harvest and other management practices and as an equitable basis for payment for the crop.

In undertaking this work, larger trial plantings under near commercial conditions rather than small replicated experiments were chosen and a wide range of environments were covered. For marketing and registration investigations, it was decided to seek all possible data from overseas studies, utilising university and other contacts and to include studies not only in English but also for example in Chinese, Japanese and Spanish before considering undertaking food safety trials in Australia.

3. Agronomy

3.1 Seed & Sources

Traditional propagation of stevia plants is by planting cuttings, which readily take root but the procedure is labour intensive. For Australian conditions mechanisation is required and propagation from seed was seen as the preferred method. Seed production, under its natural conditions, is often limited and of very poor quality (low germination, <50 percent). Where management is carefully directed to producing high quality seed, very high seed yields of good quality (>80 percent germination) can be obtained. Seed matures progressively and is shed as it ripens, requiring frequent seed collection for good yields. Some specialist seed producers in China can produce seed crops of over 200 kg/ha (15 kg/mu). As seeds are very small, often 1.5 – 2.0 million/kg, this represents a very high multiplication rate, of more than 1,500:1.

As stevia is a highly heterozygous species, there is much plant-to-plant variation. It frequently does not grow true to type, and constant re-selection for type is required in mother-seed plots. Seed maintains good germination for a limited period (6 – 12 months) when stored at 4 degrees C, and should not be kept longer than this.

Using the skills of specialist seed producers to produce seed for use in Australia is seen as an economic and reliable source of seed for a new Australian industry. With the small seed size, freight will not be a significant cost.

At present there is a limited number of varieties for which seed is available. Selection and breeding of varieties most suitable for Australia is still required. Any very small quantities of seed of new varieties identified could be sent to China for rapid multiplication. There is only one known plant breeder undertaking any varietal selection work in Australia.

The cost for seed imported in bulk is expected to be able to be kept down to the equivalent of less than one tenth of a cent a seedling, for large orders placed on a regular basis in advance. This is a low cost when compared to the 10 cents a seedling cost of producing and planting them out.

3.2 Seedling Production

Testing of seed from a range of sources showed variable and often very low germination. Although reasonable germination could be obtained from small seed samples germinated under laboratory conditions, large scale, commercial production is required. Seed purchased via the internet (usually from South America) can produce less than 5 plants from 1,000 seeds and sometimes none. Four lines of fresh good seed gave over 80% germination under commercial conditions and up to 90% in the laboratory. Older seed samples from a 'poor season' gave a laboratory germination of only 30-50% and commercial germination rates of 10-40%. Very poor germination rates also tended to produce seedlings with poor survival to transplant size.

In the first months of the project seed was forwarded to a commercial specialist seedling producer for testing. Seedling production was found to be difficult, slow and therefore expensive, at 10 cents/seedling without including transplanting costs.

Subsequent testing and trial work by another specialist nursery (Withcott Seedlings) improved the success rate and so reduced the likely cost of commercial quantities (70 – 80,000 seedlings/ha are recommended). They found that close control of temperature (24 to 25 degrees C) and moisture (keeping the soil wet, but not saturated, and not allowing the soil to dry out) are required for two weeks to obtain high germination rates. In addition, seedling growth for the first month is extremely slow and requires good nursery hygiene to prevent seedling losses in this period. This slow germination and initial growth makes direct field planting of seed not feasible.

From these initial trials, which produced more than 100,000 seedlings, transplanting at approximately two months after germination (when 10 cm high) appears to be desirable. If planting in the cooler months (under 20 degrees C temperatures), 'hardening' of the seedlings before transplanting is necessary.

3.3 Site Selection

Factors to consider in site selection that have been identified during this project are:

- Availability of irrigation water. Any moisture stress is likely to induce premature flowering and will reduce leaf production, although, once established, plants are quite hardy and can survive a dry summer without irrigation.
- Temperatures. Above 20 degrees C is necessary for reasonable growth but temperatures in excess of 35 degrees C are likely to induce heat stress and reduce growth. Light frosts can be tolerated (not below minus 10 degrees C). Low humidity with high temperatures is not tolerated well.
- Soils. Lighter acidic, rather than alkaline soils seem preferred. Water-logging in heavy soils can be tolerated once plants are established.
- Latitudes. Southern latitudes, with longer days in summer, encourage vegetative and inhibit reproductive (flower) growth but this is offset by the shorter growing season due to low (winter) temperatures.

3.4 Weeds, Pests and Diseases

Weed control has been identified as the biggest field management concern. With a crop expected to last three years, on-going weed control will be required. Traditionally weed control has been by hand labour. Overseas, little use has been made of herbicides, Trifluralin being the only chemical reported as being used. Some experimentation with other herbicides has been undertaken with mixed results and no recommendations can be made at this stage. There are no herbicides or fungicides of any nature approved for off-label use in Australia. Even with good mature crops weed control will be required, as the crop will not be dense enough to shade out weeds.

Clean crops will be required before harvest to prevent contamination of the leaf material before processing. Inter-row spraying and knock-down sprays will probably have a role. Plastic ground cover to control weeds has been tried with some success but the plastic appears to interfere with the production of secondary shoots and ratooning, but research into orifice size/pleating of plastic may overcome this. Any plastic used should be white on the top side, as in summer all-black plastic predisposes plants to excessive reflective and radiative heat from the mulch.

Insect pests have only been a problem at seed germination and when seedlings are very small. Little other damage has been observed from insects.

Fungal diseases are reported overseas and have caused problems here. *Sclerotinia*, in particular, has caused two crops to fail. One of these was a site following kenaf and peanut crops which were known to have been infected and so increased soil-borne *Sclerotinia*. Management will be required, by avoiding contaminated planting sites and possibly by periodic preventative sprays when infection, especially via cut or broken stems, is possible, e.g. at harvest time.

3.5 Irrigation and Crop Management

Plant densities of 70 – 80,000 per hectare are seen as the minimum required for reasonable yields; higher densities will assist weed control but add to per hectare cost. Growing on low beds with row spacing of 350 – 500 mm and plant spacing of 200 – 250 mm will be required. Final spacing will be determined by machinery wheel spacing. Wheels need to be kept off plants, as they are relatively brittle and too easily damaged.

Irrigation by overhead sprays, furrow or trickle can be used. Trickle is the preferred option as it allows more frequent (and smaller) irrigation and does not unnecessarily irrigate inter-row weeds.

Fertiliser requirements are modest, although full fertiliser trials have not been undertaken. The suggested application rate for NPK is 50:25:25 applied in two or three dressings. No particular additional requirement for any other element has been identified.

3.6 Harvesting

Mechanical harvesting has not been undertaken on any scale yet. Traditional harvesting is by hand: stripping of leaves or hand cutting stems and removal for drying elsewhere (on roadsides etc). Sun drying of cut plants can be quite rapid, 24 hours or less, when humidity is low.

Harvesting should take place prior to flowering (as first flowers appear), as glycoside content in leaves falls after flowering. Mowing has been successfully done by reciprocating mower (at Bowen), slasher (at Mareeba and Bundaberg), hand clipping and ride-on mower (at Gordonvale). Slashing is generally too severe on stems and will encourage fungal infection. In the long term a disc mower with a roller conditioner is seen as the preferred option to allow field drying. In very wet areas (wet tropics) cutting and removal while green, for drying under cover, may be necessary to avoid rain damage.

Collection after drying for transport has not yet been undertaken using machinery. Conventional square baling will probably be most suitable, with adaptation of the pick-up mechanism probably being required to avoid leaf loss in the relatively light crop (estimated at 4 tonnes of dry matter/ha).

Care during handling and transport to minimise leaf loss will be required as the leaf portion is valued at around \$2,000/tonne. When dry, stevia as ‘hay’ can be safely stored for long periods (years) without loss of quality.

3.7 Leaf Quality and Glycoside Content

Stevia is grown for the steviol glycoside content in the leaves (and its other green parts), not just for the leaf mass. Knowledge of the total content and the proportions of the glycosides is essential.

The common (native) varieties of stevia contain a total of 8 – 10% by dry weight of the sweet glycosides in their leaves. There can be up to ten different glycosides, although two usually dominate: Stevioside and Rebaudioside A (St and R-A). R-A has a superior taste profile to St, having no liquorice/metallic after-taste and is slightly sweeter. In the native population of plants St usually makes up 60% of total glycosides and R-A 30%, producing a mixture with a noticeable liquorice after-taste. There can be considerable variation in the content of individual plants and selection for high R-A content (and therefore better taste) has been successfully undertaken over a number of years in various countries resulting in the release of ‘improved’ varieties.

Total glycoside content of stevia leaves has been shown to increase with longer days and is also correlated to leaf size and leaf thickness. When flowering commences the total glycoside content of the leaves can start to fall, so harvesting at the onset of flowering is desirable.

Most lines of stevia appear to flower more readily and frequently in tropical areas than in higher latitudes. When older leaves are mature their glycoside content is highest. Once over-mature and starting to yellow or die, the glycoside content falls, so harvesting is required to prevent loss of content. Selection of varieties for high R-A content and reduced tendency to flower will increase productivity and hence profitability.

Freedom from weeds at harvest is also necessary to maintain leaf, and hence final product, quality. With traditional hand harvesting, removal of weeds during harvest has not been the problem that it could be with fully mechanised harvesting and handling.

The impact of herbicides and fungicides on leaf quality and glycoside content has not yet been investigated and will be required before any chemicals are recommended/ registered for use on stevia.

3.8 Yields

Leaf yields commonly reported overseas are usually in excess of 2 tonnes of leaf/hectare per harvest with some yields reported of twice this in high latitudes (50 degrees N).

Yield data in Australia are limited at present to small sample areas often on a per plant basis. In addition, the row spacings used in most trials have been determined by equipment and requirements of other crops rather than what would be used for stevia alone.

Individual plant yields have varied from 20 to 50 g dry leaf per plant. At a plant population of 75,000/hectare 30 g/ plant would be 2,250 kg/ha, so 2,000 kg/ ha/ year appears easily achievable. Individual harvests of >2000 kg/ha were achieved at Tasmania. In northern areas three harvests have been obtained at intervals down to 8 weeks.

The leaf to stem ratio falls as the individual stem size increases. With small stems, less than 2.5 mm diameter, leaves can be over 55% of leaf and stem weight. With stems about 5 mm, leaf is approximately 50% and with large woody stems or plants with flower heads, leaf can make up less than 40% of the total weight.

The main determinant of yield will be the number of stems per plant. In southern areas (and in pots in the tropics) plants readily produce multiple shoots (forming underground crowns) after cutting. In the north some trials failed to produce new shoots from the crown and only re-grew from the single main stem. Management procedures (such as time of cutting) are being investigated to ensure production of multiple shoots, which is considered essential for adequate production over two or more years.

3.9 Critical Results and Further Work

Plots have been established at a number of locations (see Appendix) over the last three years. These have encountered a range of problems, all of which reflect the possible risks of commercial production. Some problems have been sufficient to cause total failure or abandonment of the trial area and indicate a need for further research. The issues encountered include:

- Weed infestation: A wide range of weeds has been encountered. Planting trials on an 'unused area' sometimes contributed to this; a weed-free area to start with is important. Summer and winter broadleaves and grasses have all been encountered. Hand-weeding has been extensive. Heavy weed growth in the tropical wet season led to abandonment of some plots. Lack of moisture also reduced the effectiveness of some herbicide tests. More work on weed control is needed.
- Drought: Ongoing drought conditions prevented irrigation on two plots and reduced irrigation on others. Plants survived partial irrigation but did not produce. Assured water is essential.

- Flooding: One plot was flooded by storm rain two days after establishment and more than half the plants were lost. Inundation of established plants (at another site) did not result in losses. The main effect of waterlogging has been to hinder weed control.
- Disease: Two trials encountered *Sclerotinia*. One site had had a previous history of infected crops and the trial was ploughed out to prevent further spread of the disease. Avoiding infected areas is desirable or preventative and control spraying may be possible and needs further investigation. No fungicides are currently registered for stevia in Australia.
- Soils: Four sites with neutral to alkaline soils grew plants with limited growth with a tendency to thicker and darker stems and leaves. Re-growth after cutting was very poor in all four. Poor quality water contributed to poor growth at one of the sites. Trials to determine optimum soil pH would be of value.
- Premature flowering: A number of crops started to flower during the expected growing season and so imposed a need to harvest. Some of this flowering was probably caused by stress. Stress at transplanting (temperatures were very high at some sites) probably caused the flowering of plants when only 150 mm high two weeks after transplanting. Heat stress (and related moisture stress) also occurs when temperatures exceed 35 degrees C. Some crops in the north appeared to flower in the September to December period at times unrelated to stress and when growing very strongly. This flowering could have been because the variety (China ZM) was sufficiently induced to flower by short and lengthening days, rather than the usual shortening and shorter days of autumn (the normal time to flower). Further varietal evaluation and selection may be able to offset such photoperiod sensitivity.
- Ratooning: Crops in the south readily produced secondary shoots from the ground, both during initial growth and especially immediately after cutting. By the second year most plants had 10 or more ground shoots. In the tropics, ratoon development was not consistent and sometimes non-existent in the field, even though in pots multiple stem development was reliable. This was most noticeable on the most vigorously growing plants. Earlier cutting, well before flowering, may help. Further testing is required. The use of plastic ground cover for weed control appeared to interfere with the development of new ground shoots. Further varietal evaluation may identify some varieties with stronger ratoon performance.

4. Processing

4.1 Current Technology

Most primary processing of stevia leaves is carried out in China, Japan, Korea, Brazil or Paraguay, where factories are located near original growing areas. Factories in Japan (and Korea) now import leaves for processing, as growing of stevia has almost ceased in Japan.

The most common processes used for extracting steviol glycosides from the leaves consist of:

- soaking leaves in warm/hot water to dissolve the glycosides (in batches)
- filtering the resultant liquid (often after adding a precipitation agent)
- concentration by vacuum evaporation
- resin exchange to separate the glycosides into high and low R-A fractions
- ion exchange purification (sometimes)
- evaporation and spray drying or, less frequently, crystallisation to produce the stevia powder/crystals.

Some secondary processing may be undertaken, especially in Japan, to further separate high R-A fractions or to convert Stevioside to Rebaudioside or some other glycoside to improve taste quality.

This process is very similar to the extraction process of raw sugar from sugar cane.

4.2 Product Quality

Product quality has two elements: purity and absence of contaminants, which are largely determined by processing hygiene and method, and taste quality, which is determined largely by the mix of glycosides present.

Purity levels of commercial steviol glycosides powders vary from 80% to 95%. Sweetness and taste quality will vary with the glycoside content of the leaves processed as well as with the processing method. High taste quality is generally expressed as the percentage of R-A, the higher being the better, with over 50% being enhanced quality and over 80% premium quality. With modern extraction technology and the use of crystallisation rather than spray drying, high purity levels are expected.

4.3 Taste Quality Improvement

There are two avenues available to improve taste quality: further processing or plant breeding and selection.

A number of factories use a range of further processing procedures to improve taste by increasing the level of R-A relative to St. These processes can include further separation or modification (e.g. by enzyme action) of St to produce R-A or a similar tasting glycoside.

Plant breeding and selection, often sponsored by factories, has been used for many years to increase total glycoside content (from 10% to 15% or more) and particularly R-A content (from 4% to 10% or more). A few of these improved varieties could be available and will be tested for suitability in Australia.

The preferred taste improvement approach for Australia is by plant selection, as this will avoid the need for further processing, which can involve the use of chemicals and this could compromise the clean, natural and, possibly, organic status of stevia grown in Australia.

4.4 Processing in Australia and Potential Processors

The extraction process for stevia is similar to that for cane sugar. The Sugar Research Institute (Mackay), a contributor to the project, has considered aspects of sugar extraction technology that could be applied to improve the efficiency of stevia processing. The SRI has undertaken some preliminary processing trials using counter-current diffusion equipment to assess its potential for stevia. Technological developments with filtering, evaporation and crystallisation processes in the sugar industry also have a place in designing a modern, efficient stevia mill. In conjunction with other interested parties the SRI has considered conceptual designs for a stevia mill. Their investigations are ongoing and will be directed at commercial interests as they arise. Issues seen as providing significant improvements over older factories overseas include a continuous flow process using counter-current diffusion rather than batch mixing with water for initial extraction and also minimum water-use principles to reduce evaporation energy costs.

Interest in constructing a stevia extraction mill has been expressed by a number of groups, including grower/co-operative interests as well as food processors, sections of the sugar industry and food ingredient marketing groups. Potential grower interest in a co-operative or equity sharing mill has been strong and would help ensure that the price paid to growers for leaf was equitable and reasonable.

Existing overseas mills have expressed interest in Australia; more than one would consider equity in an Australian mill if leaf supply was assured.

In the short term, there is potential to export leaves for processing overseas (by container load). Factories in China and Japan import leaves for processing and have experienced a shortage of leaf, especially over the last two years. The reduction in leaf supply especially in China may not be temporary, as some past growers switch production to vegetables. An export price for stevia leaf would not be readily given until a guaranteed supply was available, although a 'starting price' of US\$1 – 1.20/kg has been mentioned.

5. Marketing

5.1 Uses

Stevia products and extracts can be and are used in a wide range of foods. Steviol glycosides (the purified extract) are an alternative to sugar in nearly all of its uses and can replace all or some of the sugar. Unlike some man-made chemical sweeteners, they are stable when heated and so can be used in baked goods safely and successfully. Steviol glycosides can be used in conjunction with other sweeteners (e.g., sugar, fructose, intense sweeteners) and they act as a flavour enhancer as well as a sweetener. They have been used in other countries for many years (e.g. Japan for more than 35 years). Food products containing stevia extracts which have been successfully produced and consumed have included:

- aerated soft drinks, mineral waters and cordials etc
- juices and juice drinks
- icecreams, yoghurts, milk and other dairy products
- sauces, chutneys, pickles
- biscuits, cakes and pastries
- preserved fruits, jams and spreads
- processed and frozen vegetables and meats
- confectioneries, chocolate
- cereals and muesli bars.

Other stevia-containing products have included toothpastes, chewing gums and medicinal tablets. It is often recommended for inclusion in weight loss diets and for diabetic foods and as part of diets to combat a range of chronic disorders and infections. The largest consumers of stevia include Japan, China, Korea, Paraguay and Brazil, with both Japan and China having peak consumptions reaching 2,000 tonnes of steviol glycosides per year in the 1995 – 2003 period.

Some beneficial side-effects of steviol glycoside consumption have been demonstrated in a number of scientific studies and papers. These include reducing high blood pressure, reducing high blood glucose levels, a reduction in dental caries bacteria and other bactericidal effects. These effects, combined with the non-calorie sweetness, the ‘all natural’ nature and flavour enhancing properties, add to the attractiveness of using steviol glycosides in foods and beverages.

5.2 Safety, Approval and Registration

Steviol glycosides are not registered for use in all countries. Countries with unrestricted approval include Japan, China, Korea, Taiwan, Indonesia, Thailand, Paraguay, Brazil, Argentina, Israel and Russia. Restricted use is approved in some countries, including USA, where it can be used as a nutritional supplement only (not as a sweetener).

Health and safety aspects have been extensively studied, especially in the main consuming countries, over a long period and safety aspects have been scrutinised by national and international agencies and groups over a number of years.

In 1990 the WHO/FAO Joint Expert Committee on Food Additives (JECFA) reviewed the safety of steviol glycosides and requested additional metabolism and safety studies to be carried out. These studies were undertaken in various countries (Japan, China, Europe) and additional earlier studies (reported in non-English languages) were reviewed and the results submitted to JECFA by Japanese authorities.

As a result, in 2004, JECFA endorsed the safety of the use of steviol glycosides as a food additive and recommended an acceptable daily intake rate, for its use, of 2 mg/kgbw/day, expressed as steviol. This rate is equivalent (in sweetening value) to more than 30% of total sugar consumption in most countries

(and over 50% in some). This JECFA decision is a recommendation only, with the relevant authorities of individual countries having the power to give approval/registration.

The JECFA recommended ADI is temporary, pending further investigation of the possible beneficial effects of large doses for treatment of both hyperglycaemia (diabetes) and hypertension (high blood pressure).

5.3 Australian Status

In Australia at present (2005), steviol glycosides are not approved as a food additive. However stevia leaves, powders and extracts can be used under the “novel foods” provision of food regulations, although no applications under this provision have yet been made. It can also be used for personal home consumption use and stevia extracts in various forms can be purchased from health food stores and by mail order/internet for this purpose.

As part of this project, CQU undertook an extensive review of the extent of existing health and safety literature and concluded that sufficient data were available to support an application to FSANZ (then ANZFA) to have steviol glycosides approved as a food additive (intense sweetener/flavour enhancer). A past application (in 1999) to ANZFA by another group for inclusion of stevioside as a food ingredient lapsed when additional safety metabolism data requested could not be provided. As no-one else appeared prepared at this time to make an application for approval, it was decided that the project could proceed using previously published material to support health and safety issues and no new health and safety studies would be required. The initial part of this application was submitted in May, 2004, with more details submitted to FSANZ in January, 2005.

The approval process within FSANZ includes extensive public risk assessments and other processes and takes at least twelve months to complete before final registration is possible. This process has commenced with an initial assessment report expected to be produced by FSANZ before the end of 2005 (see: <http://www.foodstandards.gov.au/standardsdevelopment/applications/> - application A540). Much of the health and safety data submitted to FSANZ are the same data as submitted to JECFA. The project and PSG gratefully acknowledge the assistance to the application provided by overseas groups in forwarding their data; particularly helpful were the Japanese Stevia Industry representatives.

The application to FSANZ has been made on behalf of future and potential growers, processors, users and consumers of stevia products. If successful the resultant approval will be available for all food processors, manufacturers and consumers to use and benefit from and no one processor or food manufacturer will have exclusive or preferential access or right to the use of steviol glycosides.

5.4 Outlook in Australia

Given the endorsement of the use of steviol glycosides by JECFA, the chances of approval being granted within 12 – 18 months are reasonable. If approved at the rate recommended by JECFA, it would allow replacement of more than 35% of the sugar used (worth \$200 million) in Australia by stevia before the recommended intake rate were to be exceeded, which is an extremely high and unlikely rate of market acceptance and uptake of a ‘new’ food ingredient.

The interest in stevia expressed to the project by various food manufacturers has covered a diverse range of food products – soft drinks, juices, confectionery, cereals, snackbars and dairy and soy products. If approved most initial consumption would have to be provided from imports of steviol glycosides, as it is unlikely that large enough commercial quantities would be grown and processed in Australia at first, although approval would provide a stimulus to production.

Quality concerns about some overseas products may restrict some processors from importing and so further encourage local production, where quality (especially, for example, contamination from heavy metals) could be reliably monitored and controlled.

6. Analysis

6.1 HPLC

The concentration, and therefore content, of steviol glycosides in the leaves of stevia (and in the green stems) varies with plant variety, location, season and growing conditions and time of year. A method of leaf assay is essential to evaluate varietal and management effects and so be able to optimise total glycoside production. A reliable method of analysis is also required to enable farmers to be paid on an equitable basis for the steviol glycosides produced. This has not been the basis of payment in other countries, where weight of leaves alone has been the basis of payment, even though total glycoside content can vary from less than 8% to more than 15% of dry weight.

In addition the 'quality' of the glycosides is largely determined by the ratio of the various (up to ten) glycosides present: it is primarily determined as the percentage that Rebaudioside A is of the total glycosides.

An analysis method using HPLC has been developed for stevia and a standardised approach is used in Japan, China and other countries. This basic procedure was adopted for use by the project, yet proved quite difficult to execute reliably. Minor modifications of the operating procedures were required to suit the specific equipment used. HPLC operation is an acquired skill which was not readily available to the project. Problems encountered included some equipment failure relating to filtration and column contamination that proved hard to overcome until the HPLC unit was replaced. Operator familiarisation with the specific column and mobile phase components needed for stevia analysis required a substantial learning and experimentation period. However, minor adjustments to settings, pH, flow rates and the like have now produced clear and well defined separation of the glycosides enabling identification with confidence and accurate measurement of the quantities of each glycoside.

For calibration of the HPLC, it was necessary to obtain pure, analytical grade samples of the individual glycosides for use as standards. These are not readily available through normal chemical reagent suppliers but the project was eventually able to obtain (at no cost) small quantities from sources in both Argentina and Japan, which the project gratefully acknowledges.

In the long term it may be necessary to isolate and prepare these pure samples in Australia.

CQU now has equipment and procedures in place to be able to analyse plant material as well as purified glycoside samples to confidently determine the total quantities of each glycoside present. Some examples of the resultant chromatograms are attached (Section 9.3) which shows the definition and separation of the glycosides in each analysis. Note the higher Rebaudioside C in the lower sample, which relates to a sample plant collected at a local nursery, compared to a sample from a line provided by the Sahin Seed Company.

6.2 NIRS

For rapid screening analyses of large numbers of leaf samples, it is desirable to develop a mobile, field analysis procedure; NIRS was identified as a potential tool for this. Initial scanning of purified stevia powders showed that NIRS is likely to be suitable for measuring the content of at least the main glycoside components. To use NIRS for leaf analysis, calibration curves need to be constructed by scanning a number of samples of crushed leaf material with a range of known glycoside contents. For rapid screening of powders a different set of calibration curves would be required. The HPLC analysis will be used to provide these samples. The development of an NIRS procedure for leaf analysis has not been done before in Australia or overseas and is likely to require extensive 'trial and error' to refine a procedure and equipment for this purpose. With fresh samples in the field, allowance for water content of plant material will also have to be incorporated within the measurements. The final accuracy of any procedure developed is unlikely to be quite as precise as that possible for HPLC. The development of an NIRS procedure is continuing at CQU.

7. Industry Implications and Economics

Estimates of likely income and expenditure for stevia growing were made early in the project (see table below). Project trials and evaluations have been aimed at improving the accuracy and reliability of these estimates. Work to date has given little reason to alter these initial estimates.

The largest cost, being seedling establishment, could be reduced if growers produced their own seedlings but this would require nursery facilities, skill and experience. It may be a possibility for some potential growers, such as tobacco growers.

It is now more likely that suitable herbicides will be identified and allowance for this should be made, offsetting some labour for weed control.

The ability to spread the cost of seedlings over 3 years is essential and still only probable in the tropics, but further south, where crops produce multiple stems and ratoon more reliably, it seems achievable.

The transition from pots and small plots to commercial field conditions is not yet reliable.

Commercial yields are still to be confirmed in practice. Leaf yields of 4 tonnes/ ha/ year still seem obtainable (Section 3.8). Suitable varieties with higher glycoside content will become available but for initial budget purposes, 10% content should still be assumed.

While the possible export price of leaves offered so far is (without any supply available) less than the low \$20/kg level, it is still expected that a local processor would be able to pay well above \$20/kg (world parity price for stevia powder having risen from \$30/kg to \$50 plus since 2003). The progress with marketing approval and the expectation of growing demand should only strengthen the likely price to growers.

7.1 Income & Expenditure Estimates

Table 1: Crop Costs per Hectare

Item	First Year \$	Second & Third Year(each) \$
Land preparation/cultivation	150	300
Bedding	250	0
Herbicides	0	0
Seedlings & planting out	6,500	0
Fertiliser	500	500
Irrigation, water & pumping	200	250
Labour, weed control etc	300	1,000
Harvesting (per ha contract x 3)	600	600
Freight (4 t leaf = 8 t hay @ \$37/t)	300	300
Sundry	1,300	500
Total cost for the year	10,100	3,450
Total 3 year cost		17,000
Average cost/year		5,667
Note: Excludes cost of irrigation system, any herbicides and artificial drying.		

Table 2: Crop Income/Year/Hectare - Variable Yields & Prices

Yields	Low Price (\$20/kg) \$	Good Price (\$30/kg) \$
2 t leaf @ 10% = 200 kg St/ha	4,000	6,000
2 t leaf @ 15% = 300 kg St/ha	6,000	9,000
4 t leaf @ 10% = 400 kg St/ha	8,000	12,000
4 t leaf @ 12.5% = 500 kg St/ha	10,000	15,000
6 t leaf @ 10% = 600 kg St/ha	12,000	18,000
6 t leaf @ 12.5% = 750 kg St/ha	15,000	22,500
Note: Yields in second & third year are likely to be higher than first year. St = total steviol-glycosides content of leaves.		

8. Conclusions & Further Work

This project has progressed through the early stages of the development necessary for a new stevia growing industry to emerge in Australia. The four areas identified for study - marketing, processing, agronomy and analysis - have all been advanced sufficiently to confirm the potential for a new industry and to significantly reduce the risks facing it.

Marketing

Investigations confirm that there is a significant and growing demand for steviol glycosides both in Australia and overseas. The lack of registration as a food ingredient is the only impediment to its use in Australia and, once registered, steviol glycosides will be used, being obtained by imports pending an Australian source. The application to FSANZ submitted by the project has started the approval process which, if successful, should be completed by the end of 2006.

The endorsement by JECFA of the safety of steviol glycosides gives valuable support to the application and gives cause for optimism. If approval is given, commercial interests of the food and beverage industry will develop the market from there on.

Processing

Investigation and consideration have confirmed the potential to use modern sugar extraction and other food processing technology and equipment, now available in Australia, for stevia processing. Preliminary investigations by the Sugar Research Institute suggest that there could be some limited processing capacity in existing sugar and other food equipment for extraction of steviol glycosides on a pilot basis. An efficient purpose-built extraction mill could be designed and constructed in Australia with existing skills and technology. Interest in providing equity for a future mill has been expressed by a number of groups, including potential stevia growers and overseas stevia processors. As dried stevia can be stored for long periods and is of relatively high value, it could be transported long distances from growers to a mill. In the short term, processing could also be undertaken overseas. Mills in China and Japan import stevia and have expressed interest in Australian grown stevia.

Analysis

After initial difficulties, HPLC analysis of plant and other materials to determine steviol glycoside content can be undertaken at CQU. Refinements to operating procedures and adjustments to equipment settings have produced analysis results which can clearly identify and quantify each of the ten sweet glycosides which may be present in stevia and stevia extracts.

Development of a method of rapid analysis using NIRS has commenced but is not complete. Ability to identify, by NIRS, the main glycosides in powder form has been confirmed. Work to identify and quantify glycosides in crushed leaves, and water soluble leaf extract, is still being undertaken, and results are to be compared with pure samples of Stevioside and Rebaudioside A. The data from these studies will be available at the end of 2006 from the authors of this report, and at the same time in the format of a post-graduate thesis.

Until an NIRS or other rapid analysis/screening procedure is developed, analysis for plant selection, crop management and crop payment purposes will have to rely on the slower (although probably more accurate) HPLC procedure, as has always been the case overseas.

Agronomy

Agronomic work has covered the complete range of issues, such as site selection, seed and seedlings, irrigation, pests and diseases and harvesting. Some significant questions have been resolved, e.g. seedling establishment, but there is still uncertainty as to likely yields, especially over two to three

years. Without certainty of yield, potential growers cannot adequately assess the financial risks and rewards of stevia and so require continuing trials for this data.

Other key items requiring additional research and trial have been identified and include weed control, variety selection and improvement and improved regrowth performance after harvesting. Queensland Department of Primary Industries and Fisheries has expressed some interest, if funded, in continuing this focus of the research for Queensland.

Continued external funding for further agronomic research is required to help establish a stevia growing industry. Even when established a stevia industry will require ongoing research in agricultural issues, such as varietal and yield improvement and pest and disease management as well as in processing. An industry contribution or levy of 1 – 2% of the ex-mill value of the glycosides extracted (powder or crystals) would be appropriate.

Physiology

Some further trials looking at inter-plant variability within three populations of stevia, and the effects of controlled day-length on the ability of stevia to accumulate steviol glycosides, are underway and summary results and the thesis containing the full data set will be available as for those on ongoing research on NIRS.

9. Appendix

9.1 Co-operative Trial & Field Sites

Latitude ° South	Location of Co-operator	02/03	03/04	05/06
16.9	Southedge (Mareeba, Nth Qld)		✓	✓
17.0	Gordonvale (Cairns)		✓	✓
18.0	Euramo (Tully)		✓	✓
20.0	Bowen	✓	✓	✓
20.4	Proserpine		✓	
21.1	Kuttabul (Mackay)			✓
21.2	Oakenden (Mackay)		✓	
23.5	Rockhampton	✓	✓	✓
24.2	Biloela	(✓)	✓	
25.0	Bundaberg		✓	✓
28.6	Burringbar	✓		
28.8	Federal (Lismore)		(✓)	
31.5	Gunnedah		#	✓
31.7	Rowlands Plains (Port Macquarie)	✓	✓	✓
35.8	Canberra		(✓)	
36.3	Kangaroo Island		✓	✓
37.0	Durham Ox (Bendigo)			✓
38.0	Melbourne		(✓)	✓
41.0	Burnie (Tasmania)	✓	✓	

(✓) - plots failed to establish satisfactorily due to extreme drought or other cause
 # - grew stevia but not as part of CQU project

9.2 Contacts

During the project contact has been made with a large number of individuals and organisations to obtain information and exchange ideas. In addition many people also contacted the project seeking information. Most of these contacts came from them finding reference to the project in magazine articles and more usually from the www, in particular the RIRDC website.

In Australia, contacts were made with people representing a wide range of interests including:

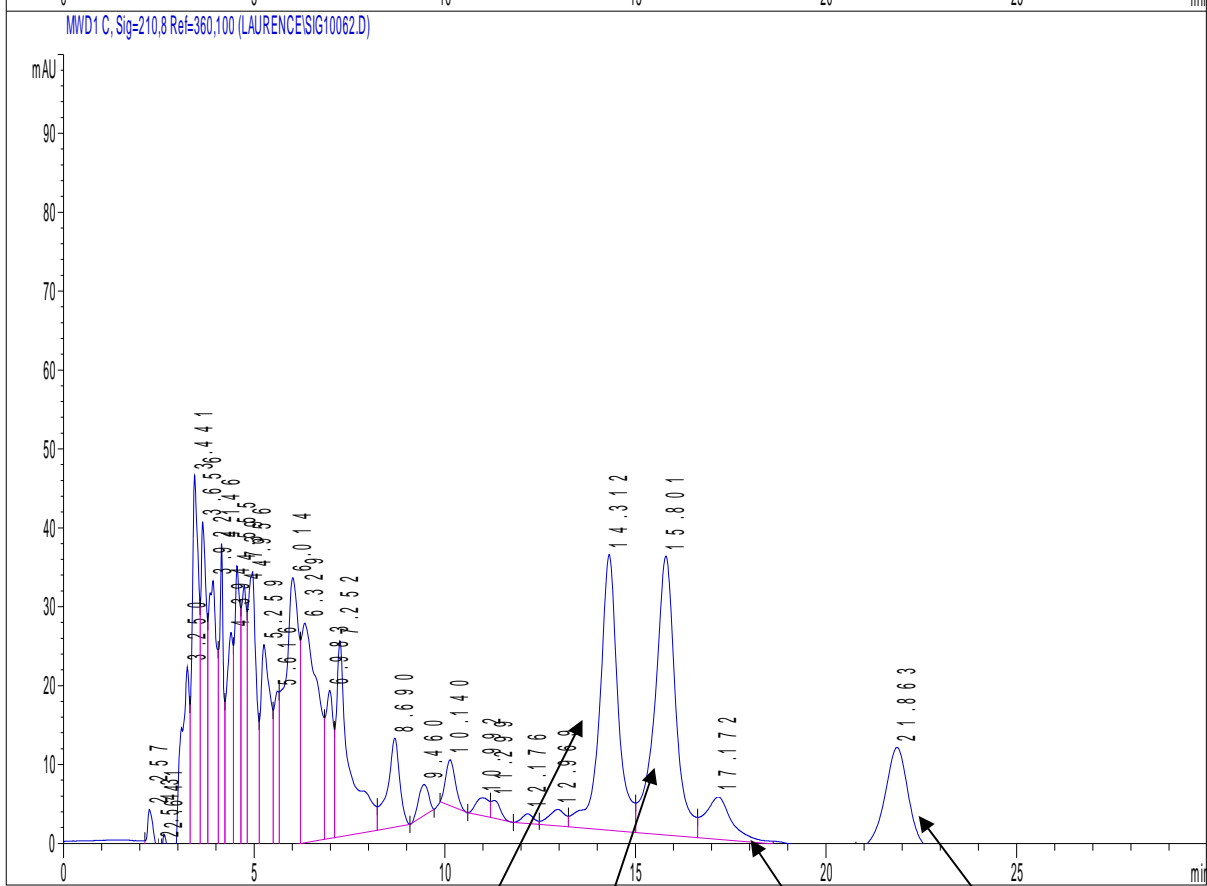
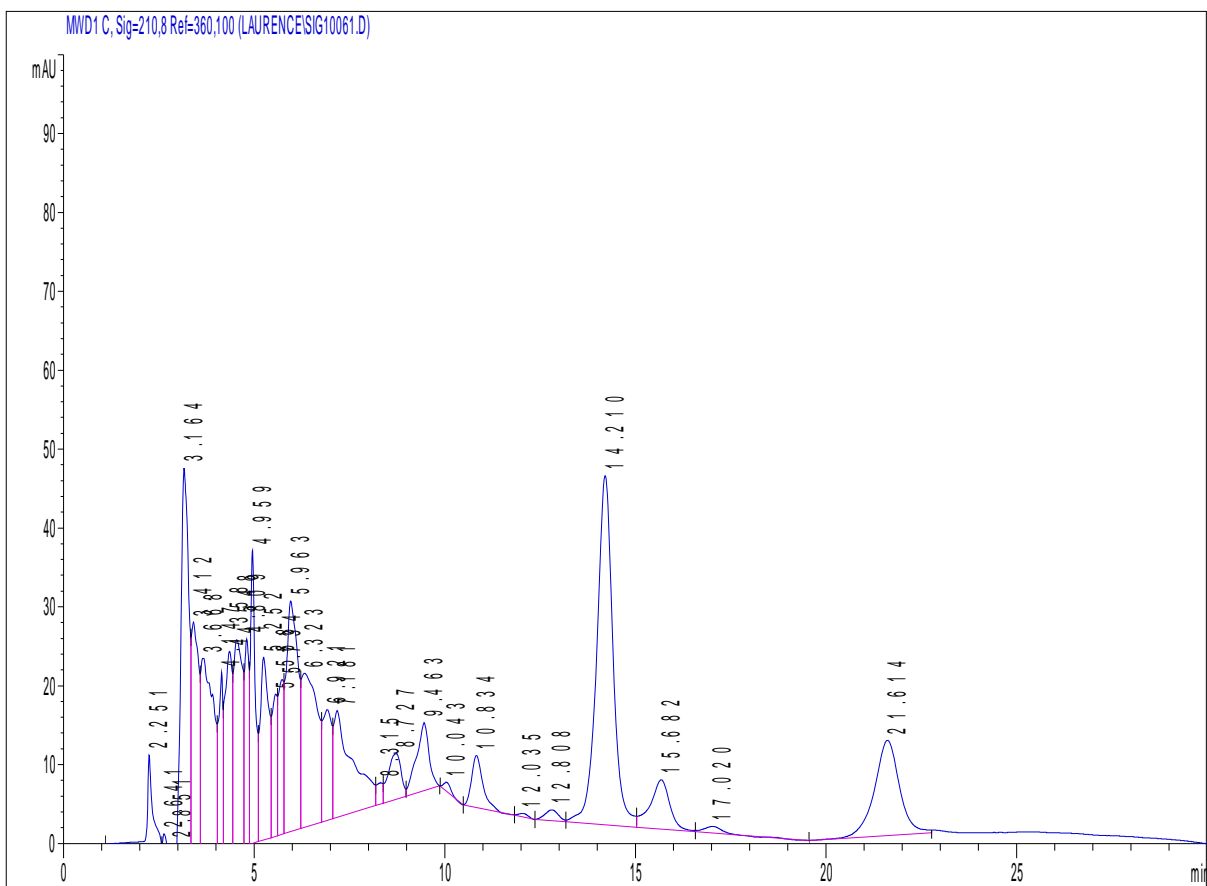
Diabetic groups and individuals, food and beverage manufacturers (covering soft drink, fruit juice, cereal, dairy and soy product, health food and confectionery sectors), food chemists, food processing machinery manufacturers, food ingredient suppliers, organic produce farmers, farmer organisations, farmers and other potential stevia growers, nurseries, general consumers and journalists.

Overseas contacts were initiated by the project and have been maintained with groups in Japan, China, Singapore, Canada and Belgium.

Enquiries and contacts seeking information were received from countries including: India (numerous), Holland, Honduras, Colombia, Argentina, New Zealand, Egypt, Nepal, Canada and USA.

Many of the individuals and organisations contacted wish, for commercial and other reasons, to remain anonymous.

9.3 Chromatograms



stevioside rebaudioside C rebaudioside B rebaudioside A

9.4 Publications

Hawke, J. (2003). The bittersweet story of stevia. Nexus 10 (02)

(http://www.dormanpub.com/articles/PDFs/FFF_March_2003.pdf)

Rank, A. (2004). Stevia – Chapter in The New Crop Industries Handbook, RIRDC Publication No 04/125, September 2004. (<http://www.rirc.gov.au/NewCrops/7%20-%20New%20Crops%20Miscellaneous%20Crops.pdf>)

Maccarone, G. (2005). Natural sugar alternative to be considered by FSANZ. The Independent Monthly, p18. (<http://upload.sjc.uq.edu.au/2005/PDFs/nov2005/018m.pdf>)